Plasmid-induced “hit-and-run” tumorigenesis in Chinese hamster embryo fibroblast (CHEF) cells

(plasmid DNA/transfection/chromosomal aberrations)

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ABSTRACT The Chinese hamster embryo fibroblast cell line CHEF/18 is readily transfected by plasmid DNA. In the present transfection studies with CHEF/18 cells, focus formation induced by plasmids containing the mutant human c-Ha-ras gene *EJ* was compared with that of control plasmids without the *EJ* insert. The focus-forming activity of the transfected plasmid J132, a recombinant of the Harvey murine sarcoma virus LTR and the normal human c-Ha-ras1 in pBR322, also was assessed. Foci were recovered after transfection with either pSV2gpt or pSV2neo at about 10% the frequency obtained with the *EJ*-containing plasmids, and J132 gave a similar frequency, all well above background obtained with salmon sperm DNA. Whereas foci from transfection with *EJ*-containing plasmids contained the *EJ* DNA, no plasmid DNA was detected in either tumorigenic or tumor-derived cells from foci transfected with pSVgpt, pSVneo, or J132. Evidence that genomic changes were induced by plasmid transfection is based on finding chromosomal aberrations in all expanded foci and tumor-derived cells examined. The results suggest the occurrence of “hit-and-run” tumorigenesis induced by transient plasmid transfection.

The introduction of foreign DNA into mammalian cells, principally by the calcium phosphate/DNA co-transfection procedure (1), has played a central role in the identification of genes with transforming effects. This procedure led to the recognition that some retroviruses contain transforming-gene sequences, which were identified by their transforming effect when integrated into the genomes of suitable recipient cells (2). The continuing role of the transforming gene in producing a protein essential for maintenance of the transformed state was established with the use of temperature-sensitive viral mutations.

Transfection studies were initiated in this laboratory with CHEF/18 cells to identify the genetic events underlying tumorigenesis (3). A comprehensive study (4) established the genetic independence of tumor-forming ability from cell culture transformation traits, such as growth in low-serum and semisolid media; it also was established that multiple genetic steps were required to convert the cells of the CHEF/18 line into tumor-forming cells. We wondered, therefore, whether introduction of cloned oncogenes could induce CHEF/18 cells to form tumors. As previously reported (5, 6), the cloned human mutant c-Ha-ras gene *EJ* was found to be tumorigenic when introduced into CHEF/18 cells by transfection, and the tumor-derived cells contained the *EJ* gene. However, in chromosome studies of the transfected cells and tumor-derived subclones, chromosome aberrations of various kinds were found to accompany the tumorigenic transformation (unpublished data). These observations

raised the possibility that chromosome aberrations may regularly accompany transfection and, therefore, that unidentified genetic changes in gene expression may contribute to tumorigenicity following transfection with a defined DNA sequence.

Recently, mutations were described that occurred in the bacterial *i* gene during transfection, mainly by the DEAE-dextran sulfate method, with specially constructed shuttle vectors (7, 8). Both point mutations and rearrangements were detected, at a combined frequency of about 1%, in monkey, mouse, and Syrian hamster cells. These findings provide a further cautionary note in the interpretation of transfection experiments.

We report here a different consequence of transfection: namely, tumorigenic transformation following transient exposure of normal CHEF/18 cells to plasmids that induce permanent genomic changes—i.e., “hit-and-run” tumorigenesis. Similar results were obtained with three plasmids: pSV2gpt (9) and pSV2neo (10), which are widely used for many purposes, and J132 (11), which contains the Moloney murine leukemia virus long terminal repeat (LTR) promoting transcription of the normal human cellular proto-oncogene c-Ha-ras. These plasmids were introduced by transfection into normal diploid Chinese hamster embryo fibroblasts (CHEF/18), and foci were recovered. When grown up for testing, the focus cells appeared transformed and produced tumors in nude mice, but no transfected DNA was detected either in the transformed cells or in their tumor derivatives.

These results are reminiscent of the hit-and-run model of tumorigenesis proposed by Temin (12), according to which a virus may induce some permanent changes in the cellular genome that lead eventually to malignant transformation. More recently, examples of hit-and-run transformation have been described following infection with herpes simplex virus (13) and with adenovirus 12 (14). In transformation by herpes simplex virus DNA, no unique set of viral genes seems to be consistently retained or expressed in the transformed cells, and similar results have been obtained with human cervical carcinoma, suggesting that viral DNA is not needed to maintain the transformed phenotype (13). Furthermore, there is no conclusive evidence that initiation of neoplasia is mediated by a viral protein (15). In the case of adenovirus, tumorigenicity can still be maintained despite the loss of all viral DNA from tumor-derived adenovirus-transformed cells (14).

Another possible avenue of explanation for our results lies in the promoter-insertion model of tumorigenesis. Hayward *et al.* (16) found that infection of chickens with a chronic leukemia virus could lead to delayed tumorigenesis, as a consequence of insertion of a viral LTR adjacent to the potentially transforming cellular gene *c-myc*. In the experi-

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Abbreviations: LTR, long terminal repeat; *EJ*, mutant form of human c-Ha-ras oncogene; kb, kilobase(s).

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ments to be described here, however, no analogous promoter/enhancer sequences of vector origin were detected.

One characteristic of the CHEF hit-and-run cells is the presence of chromosome aberrations. This finding has led us to postulate that the plasmids induce genomic rearrangements, which in turn activate cellular genes that promote transformation, as well as inhibit expression of genes that suppress tumor-forming ability in normal cells (17). Since plasmid DNA was not detectable by hybridization at early times after transfection, it is not known whether integration into the cellular genome is prerequisite for this destabilizing effect.

MATERIALS AND METHODS

Cells. The origin and properties of CHEF/18 cells have been described (3). Briefly, CHEF/18 is a stably diploid fibroblast cell line derived from a Chinese hamster male embryo and recloned twice. These cells are non-tumorigenic in nude mice (3, 4). NIH/3T3 cells were a gift from M. Wigler. CHEF/18 and BS21-2 (4) cells were grown in alpha minimal essential medium; NIH/3T3 cells were grown in Dulbecco's modified Eagle's medium. Both media were supplemented with 10% fetal bovine serum, L-glutamine, penicillin, and streptomycin. All cell lines were free of mycoplasma as judged by the assay of Schneider *et al.* (18). For tumorigenicity assays, congenitally athymic (nude) BALB/c mice were injected subcutaneously in the flank with $4-10 \times 10^6$ cells (3). Chromosomes were prepared for Giemsa-binding analysis as described (19).

Plasmids. pEJ contains the 6.6-kilobase (kb) *Bam*HI fragment of the human c-Ha-*ras* mutant gene *EJ*, inserted into plasmid pBR322 (20). J132 is a recombinant plasmid, containing the Harvey murine sarcoma virus LTR and human c-Ha-*ras*1 in pBR322, with a transforming activity of 1.3×10^2 focus-forming units per μ g of insert DNA in the NIH/3T3 cell transfection assay (11). pSV2gpt (9) and pSV2neo (10) were obtained from Bethesda Research Laboratories; they contain the simian virus 40 (SV40) origin of DNA replication and the early region promoter as well as either the gene *Ecogpt*, which confers resistance to mycophenolic acid, or *neo*, which confers resistance to neomycin (G418, GIBCO). pSVgptEJ is a recombinant of pSVgpt into which the 6.6-kb *Bam*HI fragment of *EJ* was inserted (6).

Transfection and Microinjection. Transfection was carried out essentially as described (5), with slight modifications. Twenty-four hours before transfection, 18-ID-3 (a subclone of CHEF/18) cells were seeded at 10^6 cells per 100-mm dish in alpha minimal essential medium plus 5% fetal bovine serum and refed 4 hr before DNA was added. DNA-calcium phosphate coprecipitate was prepared by the method of Wigler *et al.* (21); 20 μ g in 1 ml was added per dish. After 16–18 hr of incubation, cells were shock-treated with dimethyl sulfoxide (final concentration 9%) for 30 min, washed, and refed. Approximately 72 hr later and then twice weekly, cultures were refed with medium containing 3% fetal bovine serum. Foci were scored 4 weeks after DNA exposure.

For microinjection (22), cells were plated at 200 cells per 100-mm tissue culture plate in medium supplemented with 10% fetal bovine serum. In pre-marked colonies with 20–200 cells, each cell was injected with plasmid DNA dissolved at 30 μ g/ml in an isotonic injection buffer. After cells reached confluence, they were fed twice weekly with medium supplemented with 3% fetal bovine serum for 4 weeks. Foci that developed from microinjection colonies were picked and analyzed.

Detection of Plasmid DNA Sequences in Foci and Tumors. DNA was prepared from transformed cell lines and their tumor derivatives as described (22). Southern blot analysis of restriction endonuclease-digested high molecular weight

DNA was carried out as described (22). Briefly, samples of genomic DNA (10 μ g) were digested to completion with *Bam*HI or *Eco*RI under reaction conditions recommended by the supplier (New England Biolabs), electrophoresed in 1% agarose gels, transferred to nitrocellulose filters, and hybridized to *EJ*, LTR, or pSVgpt 32 P-labeled probes. Hybridized filters were exposed to x-ray film (Kodak XR-5) with an intensifying screen (Kodak X-Omat) at -80°C .

For dot blot analysis, high molecular weight cellular DNAs were denatured in 57 mM Tris Cl, pH 7.4/0.2 M NaOH/1 M NaCl/0.1 M sodium citrate, pH 7, at 80°C for 10 min and then neutralized with 2 M Tris Cl before they were spotted onto nitrocellulose in a 96-well microsample filtration manifold (Schleicher & Schuell). The filter was hybridized with 32 P-labeled probes and processed as described above for Southern blots.

Hirt supernatant was prepared essentially as described (23). Briefly, exponentially dividing cells were harvested from roller bottles and were lysed at room temperature with 25 mM Tris Cl, pH 7.5/10 mM EDTA/0.6% NaDodSO₄. After 30 min, NaCl was added to 1 M, and the lysate was kept at 4°C for at least 2 hr. Mixtures were spun at $35,000 \times g$ for 30 min at 4°C , supernatant was collected, and DNA was extracted.

RESULTS

Origin of Hit-and-Run Foci. The occurrence of hit-and-run tumorigenesis in CHEF cells was initially detected on control plates, in experiments designed to establish conditions for use of Mulligan-Berg vectors. CHEF/18 cells were transfected with either pSV2gptEJ or pSV2neoEJ, and each vector without the *EJ* insert was included among the controls. In addition, transfection with the plasmid pEJ was included for comparison of yields. The results, summarized in Table 1 (Exps. 1–3), show that each of the pSV2 plasmids without the oncogene insert induced foci at frequencies well above the background level obtained with salmon sperm DNA transfection. Similar frequencies were obtained with two different recipient cell lines: the standard CHEF/18-ID-3 and the CHEF-derived, anchorage-independent, non-focus-forming mutant BS21 (4). The yields without the *EJ* insert averaged about 10% the yields obtained with the oncogene.

This result is consistent with other data showing that the ratio of morphologically transformed foci to mycophenolic acid-resistant colonies after pSV2gpt transfection is typically 8% (data not shown). Also, the pSV2 plasmids with *EJ* insert were more effective than pEJ itself, suggesting that the simi-

Table 1. Origin of hit-and-run foci

Exp.	Target cells	Transforming efficiency, foci per μg of DNA per 10^6 cells			
pSV2 plasmids					
		Control	pEJ	pSV2gpt	pSV2gptEJ
1	CHEF/18	0.2	39.4	12.2	81.4
2	CHEF/18	0.02	—	2.8	57.4
				pSV2neo	pSV2neoEJ
3	BS21-2	0.2	76	14	148
J132 plasmid					
		Control	pEJ	J132	
4	CHEF/18	0.02	26.2	3.4	
5	CHEF/18	0.01	16.0	2.2	
6	CHEF/18	0	26.2	2.8	

In each experiment, five or more plates were treated with 1 μ g of plasmid DNA plus 19 μ g of salmon sperm DNA. Control plates received 20 μ g of salmon sperm DNA.

an virus promoter/enhancer sequence in the pSV2 plasmids improved transfection over that obtained with the native promoter from *EJ*-cells. In other experiments, the J132 plasmid, containing the normal *c-Ha-ras* gene under the regulation of a strong promoter (Moloney murine leukemia virus LTR), was tested to find out whether the transforming effect described with NIH/3T3 cells (11) could be reproduced with CHEF cells. In these experiments, summarized in Table 1 (exps. 4–6), foci were recovered at frequencies about 10% that obtained with pEJ.

In both sets of experiments, foci in experimental plates were first detected microscopically during the second week after DNA was added. Foci on control plates that received salmon sperm DNA, on the other hand, arose at a later time and were consistently smaller than the foci on experimental plates at the time of staining. The foci on experimental and control plates also differed in morphology. J132- and pSV2gpt-induced foci were morphologically very transformed, with multiple layers of highly refractile cells growing in a disoriented criss-cross manner. Within the foci, cells no longer resembled typical fibroblasts but instead were either round or short spindle-shaped cells. Background foci from control plates, however, were merely several layers of orderly overgrowth with cells normally aligned. Foci were obtained at a similar frequency by microinjection of plasmid DNA into CHEF/18 cells (data not shown).

Tumorigenicity of Hit-and-Run Foci. Both transfected and background foci were picked and grown to be tested for tumorigenicity in nude mice. Most foci from plasmid-treated cultures formed tumors in a relatively short time (Table 2). Several foci from control plates were also picked, and the cells subsequently were injected into nude mice. After more than 6 months, none of these mice had developed tumors, thus confirming that the foci from experimental plates were qualitatively different from those few in the control plates.

DNA from Hit-and-Run Foci and Tumors. Genomic DNAs from J132 and pSV2gpt foci as well as from tumors derived from these foci were tested for the presence of plasmid DNA. When J132 foci and tumor-derived DNA were probed with the cloned 6.6-kb *Bam*HI fragment of the *EJ* gene, only the hamster homologous sequences were detected (Fig. 1A). This result shows that the human *Ha-ras*1 gene in the original J-132 plasmid was not present in these cells. To test whether the viral LTR was integrated adjacent to a hamster cellular oncogene, as in the promoter-insertion model (16), these DNAs were probed with the viral LTR. Again the results were negative (Fig. 1B).

Two endogenous fragments that hybridized weakly with the LTR plasmid probe were noted in CHEF cell DNAs in previous experiments (unpublished data). Since the bands are faint they probably represent heterologous LTR-like sequences present in one or a few copies. As such, they provide a control in the present experiments, because a homologous sequence of transfected DNA should provide a stronger hybridization signal than endogenous heterologous sequences. We conclude that, because no transfected se-

FIG. 1. (A) Southern blot hybridization of the *EJ* (*Bam*HI 6.6-kb fragment) probe to genomic DNAs from J132-induced foci and tumors. Genomic DNAs were extracted, digested with *Bam*HI, electrophoresed in 1% agarose, transferred to nitrocellulose paper, and hybridized to ³²P-labeled nick-translated (10⁷ cpm) *EJ* DNA. Genomic DNAs were from J132-induced tumors derived from foci 3-2 and 1-2 and a microinjected focus (lanes 1–3, respectively); from independent foci 3-1, 3-2, 3-3, and 1-2 (lanes 4–7); from CHEF/18 cells (lane 8); and from a pEJ-induced tumor (lane 9). Standards (lanes S) are a mixture of λ (*Hind*III), ϕ X(*Hae* III), and psvgptEJ cut with *Eco*RI, *Bam*HI, *Sst* I, and *Kpn* I. (B) Blot hybridization of LTR plasmid probe to genomic DNAs from J132-induced foci and tumors. Genomic DNAs were digested with *Eco*RI, electrophoresed, transferred, and hybridized to ³²P-labeled nick-translated (10⁷ cpm) LTR DNA [Harvey murine sarcoma virus LTR clone p14 (11)]. DNAs were from foci 3-1, 3-2, 3-3, 3-4, 1-1, and 1-2 (lanes 1–6, respectively); from three tumors derived from focus 1-2 (lanes 7–9); from two tumors derived from focus 3-2 (lanes 10 and 11); and from CHEF/18 cells (lane 12). Standards (bands not shown) are as in A.

quences were detectable by these methods, neither the viral LTR nor the human *Ha-ras*1 sequences were present in these cells. Furthermore, no hybridizing DNA was detected when the cellular DNAs were probed with the entire J132 plasmid (data not shown). Similarly, when pSV2gpt foci and tumor-derived DNAs were probed with the pSV2gpt plasmid, no plasmid DNA could be detected (Fig. 2). In contrast, plasmid DNA was present in tumors from foci transfected with pSVgptEJ (Fig. 2, lanes 15–18).

There remained the possibility that the plasmids were not integrated but were self-replicating in the cytoplasm. To test this possibility, DNA was prepared from the Hirt supernatant (23) of J132 tumor cells and again probed with either the LTR or the 6.6-kb *Bam*HI fragment of the *EJ* gene. No hybridizing sequences were detected in the Hirt supernatant

Table 2. Tumors induced by hit-and-run focus cells

Cell line	Tumors formed/ foci tested	Latency
pSV2gpt foci		
Exp. 1	6/6	1–7 weeks
Exp. 2	6/8	2–10 weeks
J132 foci		
Exp. 4	6/6	1–4 weeks
18-1D-3 foci (background)	0/3	>6 months

Each cell line was derived from a focus of independent origin; experiment numbers refer to Table 1. For each cell line, 4–6 sites were injected with 6–10 $\times$ 10⁶ cells per site.

FIG. 2. Blot hybridization of pSVgpt probe to genomic DNAs isolated from pSVgpt-induced foci and tumors and digested with *Bam*HI. Lanes 1–6: foci SVgpt-1–6. Lanes 7 and 8: tumors derived from SVgpt-2 and SVgpt-3, respectively. Lane 9: CHEF/18 cells. Lanes 10–12: tumors derived from SVgpt-4. Lanes 13 and 14: tumors derived from SVgpt-6. Lanes 15–18: tumors derived from foci transfected with pSVgptEJ. Standards (lanes S) are same as in Fig. 1.

(data not shown). The absence of the pSVgpt plasmid in the pSVgpt-transformed cells was confirmed by their lack of resistance to mycophenolic acid (data not shown).

Finally, it was considered that the failure to detect plasmid DNA could be due to rearrangements of plasmid DNA such that the integrated sequences were not in a homologous position in all cells and, therefore, would not be detected as a discrete band in a Southern blot. We tested this possibility by means of dot blot hybridizations of genomic DNA of foci and tumor-derived cells, using various probes (Fig. 3). The results confirm that none of the transformed or tumor cells carried any detectable plasmid DNA.

Secondary Transfections with DNA from Hit-and-Run Derived Tumors. To determine whether a cellular oncogene activated in these tumors could be detected, we tested genomic DNAs in transfection assays, using both NIH/3T3 and CHEF/18 cells as recipients. DNAs were from five tumor lines derived from two independent J132 foci and six tumor lines derived from four independent pSV2gpt foci. All of the pSV2gpt tumor lines produced foci at a frequency well above background in NIH/3T3 cells, ranging from 0.1 to 0.35 focus-forming unit per μ g of DNA. (Control plates transfected with CHEF/18 DNA did not contain a significant number of foci.) Yields were about one-fifth as great but still significant in transfections into CHEF/18 cells, except for one line with a yield of 0.72 focus-forming unit per μ g of DNA. Poorer yields of foci were obtained with J132-induced tumor lines: only two were positive with CHEF/18 cells, and none, with NIH/3T3. These results suggest that the different plasmids may have induced tumorigenesis by activation of different genes. Further evidence supporting this suggestion comes from the different patterns of chromosome aberrations found in the two sets of transfectants.

Chromosome Changes in Transformants and Tumor Cells. The chromosome constitutions of six expanded foci from pSVgpt transfection (Table 1, Exp. 1), six from J132 transfection (Table 1, Exp. 4), and numerous tumor lines from them were examined by analysis of Giemsa-banded karyotypes. The findings are summarized here and will be reported in detail elsewhere.

All six J132 lines contained changes in chromosome 3, either an extra copy of 3q or a translocation involving an extra 3q. Three of the six lines had changes in one copy of chromosome 9 as well, involving loss of 9p and translocation of 9q. In the tumor-derived cells from J132 foci, few additional changes had occurred.

The pSV2gpt foci and tumor-derived cells had more complex rearrangements than the J132 lines. The cells of all six pSVgpt-transformed lines contained an extra copy of chromosome 3q as well as other changes. The distal part of chromosome 7q was missing in three of the six lines and was translocated to chromosome 1q in the fourth line. One line also had a reciprocal 4;8 translocation. The tumor-derived lines from pSV2gpt foci had numerous additional rearrangements as well.

Thus, all of the hit-and-run transfected lines contained chromosome aberrations, but in general those from J132 transfections were less rearranged than those from pSV2gpt-induced foci. In addition, the specific rearrangements differed in the two sets of cell lines, although all were trisomic for chromosome 3q or part of it. Some changes in chromosomes 7 and 9 probably included amplifications.

DISCUSSION

The Mulligan-Berg plasmid vectors pSV2gpt and pSV2neo and the LTR-containing plasmid J132 have been shown to induce hit-and-run tumorigenesis in diploid CHEF/18 cells. In all, 19 foci from pSV2gpt treatment and 8 foci from J132 were grown up, tested, and found to lack detectable plasmid DNA. Seven out of seven J132 lines and 14 out of 19 pSVgpt lines tested in nude mice were tumorigenic, but no plasmid DNA was detected in their tumor-derived cells. Focus formation with the pSV2 plasmids occurred at 5–10% the frequency observed with the same plasmids containing the *EJ* oncogene. In contrast, most foci from pEJ, pSV2gptEJ, and pSV2neoEJ transfections contained the plasmid DNA; only those harboring the DNA were tumorigenic, with one exception (data not shown).

FIG. 3. Dot blot hybridization of *EJ* probe to DNA from J132-transfected foci and tumors. Five micrograms of DNA was loaded into each well on top row, and 1 μ g of DNA into each well in the bottom row. Columns a–f: DNA from foci 3-1, 3-2, 3-3, 3-4, 1-1, and 1-2. Columns g–i: DNA from tumors derived from focus 1-2. Columns j and k: DNA from tumors derived from 3-2. Columns l and m: DNA from a focus microinjected with J132 plasmid and its tumor derivative, respectively. Column n: DNA from a tumor derived from another microinjected focus. Column o: DNA from CHEF/18 cells. Column p: 50 pg (upper well) and 10 pg (lower well) of J132 plasmid (equivalent to 5 and 1 copies per genome, respectively).

These results are not attributable to spontaneous transformation for the following reasons: (i) Transformation frequencies in plates transfected with J132 or pSV2gpt were always higher than in control plates. Since both experimental and control plates had salmon sperm DNA as carrier, the only difference between them was the presence of the plasmids. (ii) Foci on control plates arose later than those on J132- or pSV2gpt-transfected plates. In addition, the differences in size and morphology of foci between control and experimental plates suggest that spontaneous foci on control plates are weakly transformed compared to foci on experimental plates. (iii) In the tumorigenicity studies, the J132- and pSV2gpt-induced foci formed tumors readily in nude mice, whereas foci from control plates did not. (iv) The hit-and-run phenomenon was observed with three different plasmids (J132, pSV2neo, and pSV2gpt). (v) Hit-and-run transformation was also observed in CHEF/18 cell microinjected with J132, further indicating that the phenomenon is not the result of transformation *per se*. (vi) Similar frequencies of hit-and-run transformation were observed with two recipient cell lines, CHEF/18-1D-3 and BS21-2 (Table 2).

We conclude that the hit-and-run foci were induced by the plasmids themselves and that during subsequent growth, the plasmid DNA was lost from the transformed cells.

A telling feature of the hit-and-run process is the presence of chromosomal aberrations in the transfected cells. In previous studies with diploid CHEF cells, the occurrence of aberrations has been a consistent property of tumorigenic and tumor-derived cells (19, 24, 25) but not of transformed cells lacking tumor-forming ability (25). It was inferred from these and other findings that the transformed cells may have undergone single-gene mutations, whereas multiple genetic changes were involved in tumorigenesis. The presence of chromosomal aberrations is a signal that unidentified changes in gene expression may have occurred.

The finding of trisomy for chromosome 3q associated with tumorigenicity in the hit-and-run cells is consistent with previous evidence that an extra copy of chromosome 3 or 3q is frequently present in tumorigenic cells recovered after treatment with mutagens (25) or with 5-azacytidine (19). In some tumors from azacytidine-treated cells, trisomy for chromosome 3q was the only abnormality observed. It was suggested that other changes in gene expression requisite for tumor formation may have occurred as a result of associated hypomethylation (26). In the present study, additional chromosome changes were found in all tumor-derived lines.

If one accepts genetic changes resulting from chromosomal aberrations as causal in tumorigenicity of the hit-and-run cells, one may then ask how the plasmids have induced the aberrations. The possibility that seems most likely is plasmid-induced DNA damage. Since the plasmids are capable of integrating into the host genome, they must induce endonucleolytic cleavage either directly or indirectly. Although no excision functions have been identified in the Moloney murine leukemia virus LTR or in the SV40 promoter/enhancer sequences, they may promote or enhance gene expression of host endonucleases.

In this connection, the observed differences between J132 and pSV2 foci in the types of chromosome changes induced and in transfectability are noteworthy. The LTR and simian virus sequences may enhance different genes. The presence

of a third copy of chromosome 3q seems to be characteristic of tumorigenic CHEF cells, although additional changes are seen in tumor-derived material. Here for example, partial loss of chromosome 7 was seen in the pSV2gpt-induced lines but not in the J132-induced lines, where losses of part of chromosome 9 were frequent.

The relative ease of inducing foci in CHEF/18 cells with tumor-derived DNA from the pSV2gpt transfectants provides a clear direction for further research, designed to recover transforming genes from hit-and-run cell lines. A second aim is to identify the plasmid sequences responsible for the hit-and-run phenomenon.

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