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Transformation of human cells by DNAs ineffective in transformation of NIH 3T3 cells

(DNA transfection/anchorage independence/multiple myeloma DNAs/Ha-*ras*/*Blym*)

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ABSTRACT **Neonatal human foreskin fibroblasts can be transformed to anchorage-independent growth by transfection with DNAs inefficient in transforming NIH 3T3 cells. Human cells transfected with DNA from GM 1312, a multiple myeloma cell line, or MOLT-4, a permanent lymphoblast line, grow without anchorage at a much higher frequency than do the parental cells and their DNAs can transform human cell recipients to anchorage-independent growth; they have extended but not indefinite life spans and are nontumorigenic. Human fibroblasts are also transformed by DNAs from two multiple myeloma lines that also transform 3T3 cells; however, restriction analysis suggests that different transforming genes in this DNA are acting in the human and murine systems. These results indicate that the human cell transfection system allows detection of transforming genes not effective in the 3T3 system and points out the possibility of detection of additional transforming sequences even in DNAs that do transform murine cells.**

Rapid advances in understanding the molecular changes in human oncogenesis have been provided by transfection of tumor DNAs into NIH 3T3 cells (1). Of all human tumor DNA tested, however, some 70% are ineffective in 3T3 transformation (2). In developing a DNA transfection system using normal human cells as recipients (3), we found that the cells could be transformed to anchorage-independent growth by MOLT-4 DNA, which transforms 3T3 cells inefficiently (4) or not at all (5). We have used a series of three multiple myeloma cell lines, GM 1312, GM 1500, and GM 2132, to examine transformation of human cells by tumor DNAs that do (GM 1500 and GM 2132) and do not (GM 1312) transform NIH 3T3 cells (5).

We show that DNA of GM 1312 does transform human cells to anchorage-independent growth, yielding transfectants that grow in soft agar at much higher frequencies than do the parental fibroblasts and whose DNA can transform naive recipients to anchorage-independent growth. DNAs of GM 2132 and GM 1500 transform human cells at a higher frequency than does that from GM 1312. Since GM 1500 DNAs transform both human and 3T3 cells, we tested by restriction analysis whether the same gene was acting in the two systems: although restriction by five enzymes gave similar results in the two systems, *Sac* I inactivated the transforming activity of the DNA in 3T3 cells (5) but not in human cells, suggesting the possibility that different genes are recognized by the two cellular recipients.

These results show that the human cell transfection system can detect transforming genes in DNAs ineffective in the 3T3 system and indicate the possibility of detecting additional transforming sequences in DNAs containing oncogenes that can transform 3T3 cells.

MATERIALS AND METHODS

Cells. Neonatal human fibroblasts were obtained from primary cultures established in this laboratory with foreskins from Central Suffolk Hospital (Riverhead, NY) or Brookhaven Hospital (Patchogue, NY). Stock cultures were grown without antibiotics in a Dulbecco's modified Eagle's medium (DMEM) (6) prepared in this laboratory plus 20% fetal bovine serum (Microbiological Associates); they were checked for mycoplasma by the method of Chen (7). NIH 3T3 cells were the gift of H. Ozer (Hunter College) and were grown in DMEM plus 10% fetal bovine serum. Three multiple myeloma cell lines, GM 1312, GM 1500, and GM 2132, were obtained from the Human Genetic Mutant Cell Repository and were grown in RPMI 1640 medium plus 15% heat-inactivated fetal bovine serum. MOLT-4 cells were obtained from E. Henderson (Temple University) and were grown in RPMI 1640 medium plus 20% serum. A murine squamous cell carcinoma cell line (CRL 1453) and a human malignant melanoma cell line (CRL 1424) were obtained from the American Type Culture Collection and were grown in McCoy's 5A medium (Flow) plus 10% fetal bovine serum.

Cells were suspended in buffer A (10 mM Tris·HCl, pH 8/10 mM EDTA/10 mM NaCl) containing 1% (wt/vol) sodium lauryl sulfate and 100 μg of proteinase K (EM Science, Gibbstown, NJ) per ml and were digested overnight at 55°C. The suspension was extracted with redistilled phenol and centrifuged at 500 × *g*. The extraction and centrifugation were repeated until the upper phase was clear. The solution was treated with 2-butanol and dialyzed against buffer C (50 mM Tris·HCl, pH 8/10 mM EDTA/10 mM NaCl) until the A_{270} was <0.05. RNase A (Sigma), treated at 70°C for 30 min, and RNase T1 (Sigma) were added to final concentrations of 50 μg/ml and 2 μg/ml, respectively. After RNase treatment, the DNA was extracted as before and dialyzed exhaustively against buffer C. DNAs were characterized by electrophoresis on neutral 0.4% agarose gels (molecular weights were found to be $\approx 100 \times 10^6$) and by absorption spectroscopy.

A pBR322 plasmid (pT24-c3) containing the T24 bladder carcinoma Ha-*ras* (Ha = Harvey) gene as a 6.6-kilobase (kb) *Bam*HI fragment was obtained from the American Type Culture Collection. It was transfected into *Escherichia coli* and amplified, the plasmid was purified, and the DNA was extracted (8). Plasmid pHu*Blym*-1 (9) contains the 0.95-kb *Eco*RI transforming fragment of λ Hu*Blym*-1 inserted in the *Eco*RI site of pBR322 and was the gift of G. Cooper (Harvard University).

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Abbreviation: kb, kilobase(s).

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DNAs were treated with DNase I (Sigma) or restriction enzyme *Hin*dIII, *Bam*HI (Bethesda Research Laboratories), *Sac* I, *Xho* I, *Pvu* II, or *Eco*RI (New England Biolabs) in buffers recommended by their suppliers. The enzymes were used at 5–40 units/μg DNA; digestions were monitored by removing an aliquot of the digestion mixture, adding an equal amount of suitable monitor DNA (λ or adenovirus 2), and following the digestion of the monitor DNA on agarose gels.

DNA Transfection. Human cells were transfected with DNAs as described (3). In brief, cells were plated at 10^5 per 60-mm dish and allowed to grow overnight. The medium was removed, and cells were rinsed twice with 2 ml of phosphate-buffered saline (P_i/NaCl: 0.171 M NaCl/3.4 mM KCl/10.1 mM Na_2HPO_4/1.8 mM KH_2PO_4) and treated for 2 min with 3 ml of a PEG 6000 insertion mixture [prepared by adding 30 g of PEG (autoclaved for 15 min and cooled to 45°C) to 60 ml of medium without serum at 45°C; the mixture was then cooled to 37°C]. After PEG treatment, the cells were rinsed twice with 3 ml of P_i/NaCl; then 4 ml of complete medium containing DNA as a calcium phosphate precipitate was added. [DNA in Hepes-buffered saline (which contains, per liter, 8.0 g of NaCl, 0.37 g of KCl, 0.125 g of $Na_2HPO_4 \cdot 2H_2O$, 1 g of dextrose, 5 g of Hepes at pH 7.05) was mixed with 1.25 M $CaCl_2$ to a final concentration of 0.125 M $CaCl_2$ and allowed to stand at room temperature for about 20 min; 0.5 ml of the DNA solution was added to 3.5 ml of complete medium for each dish treated.] After 24 hr the medium/DNA mixture was removed, the cells were washed twice with 3 ml of P_i/NaCl, and 4 ml of fresh medium was added.

The cells were allowed to grow for 6 days and were then plated in soft agar as described (3). In brief, cells were rinsed twice with 2 ml of P_i/NaCl, treated with trypsin, and resuspended in complete medium, and viable cell counts were determined by hemocytometer counting in the presence of a vital dye. Then 10^5 cells were plated in 3 ml of a 0.33% soft agar layer over an 8-ml 0.5% soft agar base and the dishes were scored immediately for cell clumps that might later be mistaken for transformed colonies. The cells were allowed to grow for 14 days, fed occasionally with small amounts of complete medium, and anchorage-independent colonies ranging in size from a few hundred to several thousand cells were counted.

***In Vivo* Growth Potential.** Tumorigenicity of parent and transfected cells was tested by injection into nude (athymic NCr-*nu*) mice obtained from the National Cancer Institute–Frederick Cancer Research Facility Animal Resources Program. Cells were collected, washed with P_i/NaCl without Ca^{2+}, and injected (10^7 cells per mouse) subcutaneously into 21- to 28-day-old mice that had been irradiated with x-rays [400 rads (1 rad = 0.01 gray)] 24 hr earlier.

RESULTS

We first examined the transforming ability of DNAs from lymphoblast lines derived from three multiple myeloma patients, GM 1312, GM 1500, and GM 2132. Lane *et al.* (5) found that GM 1312 was ineffective in transforming 3T3 cells, but GM 1500 and 2132 transformed the murine cell line well. We first checked our DNA preparations by transfecting them into NIH 3T3 cells. Table 1, lines 1–3, shows that our results for induction of anchorage-independent growth agree well with those of Lane *et al.* (5) for focus formation in these cells. We next tested the three DNAs in human cell recipients. We found that although GM 1500 and GM 2132 were very effective in transforming the cells to anchorage-independent growth, GM 1312 also mediated transformation (Table 1). DNA from MOLT-4 cells also produced anchorage-independent colonies. In contrast, treatment of the human cells with PEG and $CaCl_2$ (but no added DNA) or transfection with DNAs from normal mammalian cells or tissues yielded only rare anchorage-independent colonies under our conditions. Table 1 also shows that transfection of human cells with a plasmid containing a Ha-*ras* oncogene yielded anchorage-independent clones at about 12 times, whereas pHu*Blym*-1 gave about 20 times, as many transfectants as did genomic GM 2132 DNA.

Table 1. Transformation of NIH 3T3 or human cells with multiple myeloma DNAs

DNA donor	Cellular recipient	Foci, no. per μg of DNA*	Colonies, no. per 10^5 cells per μg of DNA
GM 1312	NIH 3T3	<0.005	4.9 ± 0.51
GM 1500	NIH 3T3	0.10	58.4 ± 3.8
GM 2132	NIH 3T3	0.15	43.4 ± 4.3
None	NIH 3T3	—	3.6 ± 1.1
GM 1312	Human foreskin fibroblast	—	25.8 ± 2.0
GM 1500	Human foreskin fibroblast	—	48.0 ± 3.0
GM 2132	Human foreskin fibroblast	—	41.2 ± 8.9
MOLT-4	Human foreskin fibroblast	—	11.0 ± 2.0
pT24-c3	Human foreskin fibroblast	—	460 ± 6.8
pHu*Blym*-1	Human foreskin fibroblast	—	916 ± 10.7
No treatment	Human foreskin fibroblast	—	0.8 ± 0.2
Human placenta	Human foreskin fibroblast	—	0.36 ± 0.1
Human foreskin fibroblast	Human foreskin fibroblast	—	0.09 ± 0.1

NIH 3T3 cells were treated by standard transfection protocols (5), DNA from one of the multiple myeloma cell lines (GM 1312, GM 1500, or GM 2132) was inserted, and after growth the cells were plated in soft agar (3). For each genomic DNA, transfection were carried out with several DNA concentrations (usually 0.2–5 μg per dish) and the value for transfection efficiency (colonies per 10^5 cells per μg) was taken from the slope in the linear portion of the curve. For the cloned oncogenes, a similar procedure was followed, in the concentration range of 0.05–0.2 μg of DNA per dish. Anchorage-independent colonies were counted after 2 weeks' growth. Human cells were permeabilized by PEG 6000 treatment, and the myeloma or plasmid DNAs were inserted. After 6 days' growth, the cells were plated in soft agar and scored, and colonies were counted after 2 weeks' further growth (3). Data are the average (±SEM) of 10 plates.
*Data from ref. 5.

In the human cell system, transfection by normal eukaryotic and even prokaryotic DNAs yields low frequencies of abortive colonies that grow very poorly when isolated from soft agar (3). Are the soft agar colonies obtained upon transfection of human cells with GM 1312 DNA similar to the abortive colonies seen with normal DNAs? Both the size of the GM 1312-transfected colonies in soft agar and the transfection frequency of GM 1312 argued against such a possibility, but we felt it imperative to test the GM 1312 transfectants directly. Table 2 shows that transfectants of GM 2132 and GM 1500 DNA can be picked individually from soft agar. Most clones attach to the plastic surface of the culture flask, and from 40% to 85% of those picked grow to at least a confluent culture in a T-75 flask. Transfectants of GM 1312 attach at least as well as do those of GM 2132 or GM 1500. Although the success of those clones in growing to fill the T-75 culture flask was somewhat lower than transfectants of the other two DNAs, it was well within experimental

Table 2. Growth of primary transfectants on plastic surfaces and in soft agar

Cell type	Attachment on plastic, %	Growth on plastic, %	Growth in soft agar, no. of colonies per 10^5 cells
NFF (GM 2132)	—		
Clone 58	80	40	400 ± 21
Clone 68	100	80	16 ± 5
NFF (GM 1500)			
Clone 39	90	60	23 ± 3
Clone 48	80	60	119 ± 4
NFF (GM 1312)			
Clone 19	80	30	ND
Clone 24	100	40	159 ± 11
CRL 1424	ND		1400 ± 36
CRL 1453	ND		1169 ± 17
NFF	ND		0

Agar colonies of human neonatal foreskin fibroblasts (NFF) transfected with DNA from GM 2132, GM 1500, or GM 1312 were picked and placed in individual T-25 flasks containing 1 ml of DMEM plus 20% serum. The number of colonies that attached to the plastic surface and the number that grew to fill the T-25 flask and (after trypsining and transfer) a T-75 flask were scored. Cells from the latter flask were trypsined and plated in soft agar at a density of 10^5 per 60-mm dish. Colonies were counted after 14 days' growth. For comparison, the parental foreskin fibroblasts and two fully tumorigenic cell lines (CRL 1424, a human malignant melanoma line, and CRL 1453, a murine squamous cell carcinoma line) were plated in soft agar at the same time. Anchorage-independent growth frequencies are the average (±SEM) of 6 replicate plates. ND, not done.

range of such culture attempts. We also tested the ability of the transfectants to grow in soft agar (without further treatment). Table 2 shows (*i*) that the plating efficiency in soft agar varied widely among individual clones arising from one transfecting DNA and (*ii*) that the frequency of growth upon replating in soft agar of the GM 1312 transfectant is well within the range of the GM 2132 and GM 1500 transfectants. In comparison, the parental neonatal foreskin fibroblasts did not form clones when plated in soft agar (10 plates at 10^5 cells per plate), and a fully oncogenic human malignant melanoma (CRL 1424) and mouse squamous cell carcinoma (CRL 1453) yielded about 1400 and 1200 anchorage-independent colonies per 10^5 cells, respectively. We also tested transfectants of GM 1500 DNA for tumorigenicity in immune deficient mice. No tumors were observed at 20 weeks in any of the five mice injected with primary transfectants of GM 1500 or with the parental human fibroblasts, although all mice injected with human malignant melanoma or murine squamous cell carcinoma cells developed tumors.

DNA of primary transfectants resulting from insertion of MOLT-4 cellular DNA into normal human cells can transform naive human cell recipients to anchorage-independent growth. We wanted to test whether anchorage-independent human transfectants of GM 1500 and of GM 1312 DNA also acquired the transforming ability. Clones growing in soft agar were picked and grown to large cell numbers, and their DNA was extracted; such primary transfectant DNAs, or DNA from the myeloma parents, were inserted into normal human fibroblast recipients. In the case of GM 1500 and its primary transfectant, the frequencies of anchorage-independent colonies were about 19 and 13 colonies per 10^5 cells per μg of DNA, respectively (Table 3). For GM 1312 and its primary transfectant, the rates were 16 and 25, respectively. These data indicate that DNA of these primary transfectants can transform naive human fibroblasts to anchorage-independent growth with an efficiency at least as great as that of the parental myeloma DNAs. The transformation efficiency

Table 3. Transforming activity of DNAs from multiple myeloma lines and their primary transfectants

DNA donor	Cellular recipient	Colonies, no. per 10^5 cells per μg of DNA
GM 1312	Human fibroblast	16.2 ± 3.8
NFF (GM 1312)	Human fibroblast	24.8 ± 3.8
GM 1500	Human fibroblast	18.8 ± 2.2
NFF (GM 1500)	Human fibroblast	13.4 ± 6.3
None	Human fibroblast	0

DNAs from two multiple myeloma lines and from the primary transfectants resulting from transfection of the myeloma DNAs into human neonatal foreskin fibroblasts (NFF) were inserted into human fibroblast recipients. The cells were grown and plated in soft agar, and the number of anchorage-independent colonies was determined after 2 weeks' growth. Data are the average (±SEM) of 10 replicate plates.

of GM 1312 primary transfectants is within experimental range of that seen with GM 1500 (Table 3) and GM 2132 (data not presented) DNAs.

DNAs from GM 1500 and GM 2132, which transform 3T3 cells, also transform human cells to anchorage-independent growth. Is the same transforming gene active in the two systems? The 3T3-transforming oncogene in these DNAs has not been cloned and does not seem to correspond to any currently known oncogene (G. Cooper, personal communication). However, this gene has been characterized by determination of the sensitivity of the transforming activity to inactivation by a series of restriction endonucleases. The right part of Table 4 shows the results of Lane *et al.* (5) for 3T3-transforming activity remaining after restriction of DNA from a typical pre-B-lymphocyte neoplasm (697), DNA from an intermediate B tumor (2 PK3), DNA from a mature-B tumor (GM 2132), and the DNA of 3T3 cells previously transfected with GM 1500. In the left part of Table 4 are our results on restriction of GM 1500 DNA with the enzymes used by Lane *et al.* These data indicate that—like the gene from GM 1500 DNA that transforms 3T3 cells—neither *Eco*RI, *Hin*dIII, *Bam*HI, *Xho* I, nor *Pvu* II inactivates the gene that transforms human cells. However, *Sac* I, which did inactivate in the 3T3 system, did not inactivate the gene that transforms human cells, whether the enzyme was present at 10-fold excess (units of restriction enzyme per μg of DNA) (experiments B–D) or 40-fold excess (experiments E and F).

The data also illustrate several characteristics of human cell transfection experiments: although the absolute frequency of transformation varies among independent experiments, the relative results are consistent. For example, in experiment A of Table 4 transfection with GM 1500 DNA yielded 23 colonies per 10^5 cells per μg of DNA before digestion with *Xho* I and 23.6 (103%) afterwards; in experiment B, 77.6 before and 94.4 (122%) after digestion; in experiment C, 10.6 before and 7.8 (74%) after digestion; and in experiment D, 42 before and 66 (157%) after digestion. All of these experiments clearly indicate lack of inactivation of transforming activity. These data can be compared with those for DNase digestion in experiment D: 42 colonies per 10^5 cells per μg of DNA for untreated DNA and 0.33 for digested DNA. Data for *Xho* I digestion also illustrate a not-infrequent increase in transfection frequency seen in some batches of restricted DNAs; this may reflect an advantage in uptake, integration, or expression of smaller DNA fragments.

DISCUSSION

Human cells can be transformed to anchorage-independent growth by DNAs ineffective in transforming NIH 3T3 cells.

Table 4. Transfecting activity of restriction endonuclease-digested DNA

Human cell-transfecting activity after digestion, clones per 10^5 cells per μg of DNA									NIH 3T3-transfecting activity after digestion, clones per 10^5 cells per μg of DNA*							
Experiment	No enzyme	*Eco*-RI	*Hind*-III	*Bam*-HI	*Xho* I	*Sac* I	*Pvu* II	DNase	DNA	No enzyme	*Eco*-RI	*Hind*-III	*Bam*-HI	*Xho* I	*Sac* I	*Pvu* II
A	23.0	10.3	29.6	21.0	23.6				697	+	+	+	−	−		
B	77.6			81.2	94.4	78.2			2 PK3	+	+	+	−	+		
C	10.6			16.8	7.8	28.2			GM 2132	+	+	+	+	+	−	+
D	42.0	69.3			66.0	119		0.33	NIH (GM 1500)	+	+	+	+	+	−	+
E	86.7					52.8	105.2									
F	294					264	428									
	+	+	+	+	+	+	+	−								

(Left) human cells were transfected with GM 1500 DNA that had been treated with *Eco*RI, *Hin*dIII, *Bam*HI, *Xho* I, *Sac* I, *Pvu* II, or DNase under conditions recommended by their suppliers at 10 units/μg of DNA or 40 units/μg of DNA (for *Sac* I, experiments E and F). Results are shown for six independent experiments. Restriction digestions were monitored by removal of a small portion of the complete digestion mixture, addition of λ or adenovirus 2 DNA at an equal concentration to the myeloma DNA, digestion under the same conditions as the main digestion mixture, and electrophoresis on neutral agarose gels. After digestion, the enzymes were heat inactivated or removed by phenol treatment and centrifugation. The DNA was then precipitated with ethanol, centrifuged, and resuspended. The digested DNAs were inserted into human fibroblasts (3), the cells were grown and plated in soft agar, and the anchorage-independent colonies were determined after 2 weeks' growth. The results of the experiments are summarized in the bottom line. (Right) summary of the data of Lane *et al.* (5) for transfecting activity in NIH 3T3 cells after digestion of DNAs from three lymphoid neoplasms [697 (a pre-B tumor), 2 PK3 (an intermediate B tumor), and GM 2132 (a mature B tumor)] or the NIH 3T3 transfectant of DNA from GM 1500.
*Data from ref. 5.

We found previously that DNA from MOLT-4 cells, a lymphoblast line from a patient with acute lymphocytic leukemia (a neoplasm of intermediate T-lymphocyte origin), could transform human cells to anchorage-independent growth (3). In contrast, DNA from a variety of normal human sources (human placenta or foreskin fibroblasts) or calf thymus produced 1/10th to 1/100th as many colonies in soft agar as did the MOLT-4 DNA. We now show that DNA from the multiple myeloma (a neoplasm of mature B lymphocytes) cell line GM 1312, which does not transform 3T3 cells, is active in human cells. Primary transformants of MOLT-4 or GM 1312 DNA can be picked from soft agar, grown to large cell numbers, and characterized: they are anchorage-independent, grow to higher saturation densities than the parental fibroblasts, and have extended life spans (but are not immortal), and their DNA can transform naive human recipient cells to anchorage-independent growth. These properties remain stable over at least 30 generations after isolation from soft agar (3).

Human cells can also be transformed by DNAs that are effective in the 3T3 system. GM 1500 and GM 2132, multiple myeloma lines derived from different patients, have consistently given high yields of large rapidly growing colonies in soft agar. We have examined by restriction analysis the possible identity of the genes in GM 1500 DNA that transform 3T3 and human cells. Five of the six restriction enzymes give similar results in the two systems: neither *Eco*RI, *Hin*dIII, *Bam*HI, *Xho* I, nor *Pvu* II inactivates the transforming activity. However, *Sac* I digestion does inactivate the transforming activity of GM 2132 and GM 1500 in 3T3 cells (5), whereas digestion at 10 units/μg of DNA or at 40 units/μg of DNA did not inactivate its transforming activity in human cells. The simplest explanation of these data is that different genes in the GM 1500 DNA are transforming 3T3 and human cells. However, other explanations cannot be excluded. There might be more than one locus in GM 1500 DNA able to transform human cells to anchorage-independent growth, and thus cleavage with a restriction enzyme might not inactivate all of these loci. A second possibility is that the same locus is transforming 3T3 and human cells, and *Sac* I cleavage removes an element that is required for integration or expression in 3T3 cells but is not necessary in human cells.

Comparison of the pattern of inactivation of GM 1500 DNA with those for the other lymphoid neoplasms tested by Lane *et al.* in NIH 3T3 cells indicates that the gene in GM 1500 that transforms human cells does not seem to show identity to any of the other classes of transforming genes (Table 4). Are human cells recognizing the same gene in DNAs that are 3T3 transformers and in those that are non-3T3 transformers? For the case of MOLT-4 and GM 1500, two lines of evidence indicate that this is not the case. First, the 10-fold higher transformation frequency of GM 1500 and GM 2132 relative to MOLT-4 suggests at least quantitative differences in the transforming sequences. Restriction analysis data indicate qualitative differences between the transforming genes as well. We found that the transforming activity of MOLT-4 DNA was greatly reduced by DNase I treatment or digestion with *Bgl* I or *Bam*HI (3); our current data show that GM 1312 activity is reduced by DNase I but not by *Bam*HI.

DNA from about 30% of all human tumors tested can transform NIH 3T3 cells, and most of these contain an activated *ras* gene (2). The T24 bladder carcinoma line contains a Ha-*ras* oncogene that has been cloned and sequenced (10, 11). We tested the ability of the cloned Ha-*ras* gene to transform human cells to anchorage-independent growth; Table 1 shows that the pT24-c3 plasmid (containing the Ha-*ras* gene inserted into the *Bam*HI site of pBR322) yields high transformation frequencies. Another transforming gene recognized by 3T3 cells has been cloned from Burkitt lymphoma DNA and identified as a human homologue of chicken *Blym*-1 (9). The plasmid carrying the human *Blym* (pHu*Blym*-1) also yields high transfectant frequencies in the human cell system (Table 1). These data clearly indicate that human cells recognize both the Ha-*ras* and *Blym* oncogenes. Comparison with the two genomic myeloma DNAs, GM 2132 and GM 1500, would imply that the cloned oncogenes are only 10–20 times more effective, but such comparisons do not allow for possible effects of such factors as size, supercoiling, or presence of plasmid sequences on uptake, integration, or expression in human cells.

Sager *et al.* (12) examined the effects of *ras* on a selected strain of human foreskin fibroblasts in a focus-forming assay and found that *ras* was ineffective in production of foci. Since anchorage independence appears at an earlier stage in human cell transformation than does focus formation (and can be produced in one step by treatment with various physical or chemical agents) (13, 14), their results, taken

with ours, could indicate that *ras* can mediate only very early step(s) in the transformation process in human cells. Yoakum *et al.* (15) found that introduction of Ha-*ras* by protoplast fusion into human bronchial epithelial cells followed by selection for resistance to induction of terminal squamous differentiation and extensive cultivation eventually led to the production of oncogenic human cells. The nature of the changes occurring in human cells leading to oncogenic transformation during prolonged cultivation after carcinogen treatment is unknown, but such a progression to oncogenicity has also been observed in human cells treated with chemical (16) and physical (17) carcinogens.

Human cells show a different progression from normal to tumorigenic than do rodent cells (13, 14). They acquire phenotypic markers in a different sequence than do rodent cells. Anchorage independence, for example, is a late marker of transformation in rodent cells, being virtually synonymous with tumorigenicity in most rodent systems (18). In human cells, however, anchorage independence is acquired early, ability to form foci is acquired later, and tumorigenicity usually appears only after additional changes (13, 14). [In neither system is the exact nature of the change required for acquisition of anchorage independence understood, although the data of Peehl and Stanbridge (19) suggest a growth factor requirement.] Thus, the ability to detect in human cells a very early event in the transformation process offers the possibility of detecting oncogenes that are superfluous to transformation of immortal rodent cells and possibly difficult to detect in primary rodent cells. The recognition by normal human fibroblasts of transforming sequences in tumor cell DNAs ineffective in NIH 3T3 transformation points out one such case. The apparent indication of different transforming genes for GM 1500 in 3T3 and in human cells implies the possible existence of additional transforming genes, even in DNAs that do transform 3T3 cells. Delineation of the numbers, classes, and identities of such sequences may offer important information on the oncogenic process in man.

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