pathogenic capsule-forming bacteria such as the pneumococcus (*24, 25*). Newborns do not develop the ability to respond to the capsular polysaccharide of these bacteria until late in ontogeny and are thus extremely susceptible to such pathogens. Administration of an anti-Id vaccine early in life could prime the immune system to produce protective antibodies upon subsequent antigen exposure (*25*). Anti-Id vaccines might also be useful for adults who do not respond to conventional vaccines, such as the small percentage of individuals vaccinated with the current HBV vaccine who fail to produce anti-HBs. The studies reported herein demonstrate the efficacy of such a vaccine for HBV in chimpanzees, and suggest that internal image anti-Id may be candidates for developing vaccines against other infectious agents.

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Primary Rat Embryo Cells Transformed by One or Two Oncogenes Show Different Metastatic Potentials

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Second-passage rat embryo cells were transfected with a neomycin resistance gene and the activated form of the c-Ha-*ras* I gene, or with these two genes plus the adenovirus type 2 E1a gene. Foci of morphologically transformed cells were observed in both cases; however, the frequency of transformation was at least ten times higher with two oncogenes than with the *ras* gene alone. All the transformed cell lines gave rise to rapidly growing tumors when injected subcutaneously into nude mice. All but one of the cell lines transformed by the *ras* oncogene alone formed metastatic nodules in the lungs of animals that had been injected subcutaneously with transformed cells. When transformed cells were injected intravenously, all the *ras* single-gene transformants gave rise to many metastatic lung nodules. In contrast, cell lines transformed with *ras* and E1a did not generate metastases after subcutaneous injection and gave rise to very few metastatic lung nodules after intravenous injection. These data demonstrate that a fully malignant cell with metastatic potential, as measured in an immunodeficient animal, can be obtained from early passage embryo cells by the transfection of the *ras* oncogene alone.

DNA TRANSFECTION EXPERIMENTS have identified dominant transforming genes from the genomes of tumor cell lines and tumor tissues (*1–3*). Studies on the early region transforming genes of polyoma virus and adenovirus indicated that two viral genes are required to stably transform primary rodent cells in culture (*4–7*). The role of cellular oncogenes in the transformation of primary rodent cells has been examined by two groups of independent investigators (*8, 9*), who showed that two so-called cooperating oncogenes are required to stably transform primary cells in culture. Later, the transformation of primary cells with a single oncogene was described (*10, 11*).

We now report the transformation of second-passage rat embryo cells after transfection of the single oncogene c-Ha-*ras*. The frequency of focus formation was found to be ten times greater when a second oncogene, the adenovirus E1a gene, was cotransfected with c-*ras*. We examined the phenotypic differences between cell lines transformed by one or two oncogenes. The most striking difference between the two types of transformants is that cells transformed by the c-*ras* oncogene alone exhibited a greater propensity to form metastatic lesions after injection into nude mice than did cells transformed by two oncogenes.

To study the effects of the *ras* oncogene on early passage rat embryo cells, we constructed the plasmids pSV2ras, pRasEn1, and pRasEn2 (Fig. 1); pSV2ras contains the activated form of the c-Ha-*ras*-I gene, as cloned from the bladder carcinoma cell line T24 (*12*), under the transcriptional control of the SV40 early region promoter-enhancer element. We constructed the plasmid pRasEn1 by inserting a 234-bp DNA fragment containing the SV40 72-bp and 21-bp repeat elements into pEJ at a position roughly 2.5 kb distal to the putative polyadenylation signal of the *ras* gene (*13*). The plasmid

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pRasEn2 is analogous in structure to pRasEn1, except that the 234-bp fragment was inserted in the opposite orientation. Two other plasmids, pRSVneo and pE1a, were also used in the gene transfer experiments described below. Plasmid pRSVneo contains the aminoglycoside phosphotransferase gene, which confers resistance to the antibiotic G418, under the control of the Rous sarcoma virus long terminal repeat (LTR) element (*14*). Plasmid pE1a contains the adenovirus (Ad) E1a gene in approximately 2.4 kb of Ad DNA spanning 0.9 to 7.9 map units of the Ad 2 genome (*15*).

Table 1 shows the results of three separate DNA transfections of secondary rat embryo cells with the cloned oncogenes described in the legend to Fig. 1. Plasmid pRSVneo was included in each transfection (except the control without DNA), and transfected cells were selected with G418. After 2 weeks in selective medium, the transfection frequency was measured as the number of G418-resistant colonies per 1×10^6 cells plated. This averaged about 100, similar to frequencies other investigators have observed for stable transfection of early passage rodent cells using the neomycin (*10*) or *Ecoggpt* (*9*) selectable markers. The transformation frequency for each of the cloned oncogenes was measured as the percentage of G418-resistant colonies that exhibited a transformed morphology characterized by a round shape, loose attachment to the substrate, and reduced cytoplasmic to nuclear ratio (Table 1). No morphologically transformed colonies were observed in the absence of the *ras* oncogene, confirming the low spontaneous transformation rate of second-passage rodent cells. Transfection of the plasmid pE1a alone resulted in immortalization of rat embryo cells, as reported previously (*4, 8*). We carried pE1a-transfected rat embryo cell lines to passage 20 by splitting cell cultures at a 1 to 10 dilution every 4 days with no signs of senescence. Under the same conditions, the growth of nontransfected rat embryo cells is arrested at approximately passage five.

Fully morphologically transformed foci were observed when any of the four c-*ras*–containing plasmids pEJ, pRasEn1, pRasEn2, or pSV2ras were transfected into rat embryo cells, even in the absence of pE1a. The frequency of *ras*-induced transformation was similar (approximately 5 percent of G418-resistant colonies), regardless of the type of *ras* construct used in the transfection (Table 1). The presence of an SV40 enhancer element in pSV2ras, pRasEn1, or pRasEn2 did not increase the frequency of transformation observed with the plasmid pEJ. When pE1a and pEJ were cotransfected into rat embryo cells, the frequency of morphological transformation was increased at least tenfold above that with *ras* alone. Thus, while a single gene, *ras*, was able to induce transformation of rat embryo cells, two genes, *ras* and E1a, transformed cells at a much higher frequency.

To investigate potential phenotypic or molecular differences between the single-gene and two-gene transformants, foci of transformed cells were picked after 2 weeks in G418-containing medium and colonies were expanded to mass cultures. One phenotypic difference between the single- and two-gene transformants was observed immediately. More than 90 percent of the foci picked from two gene–transfected cultures went on to become established in culture. In contrast, the foci derived from cultures transfected with the

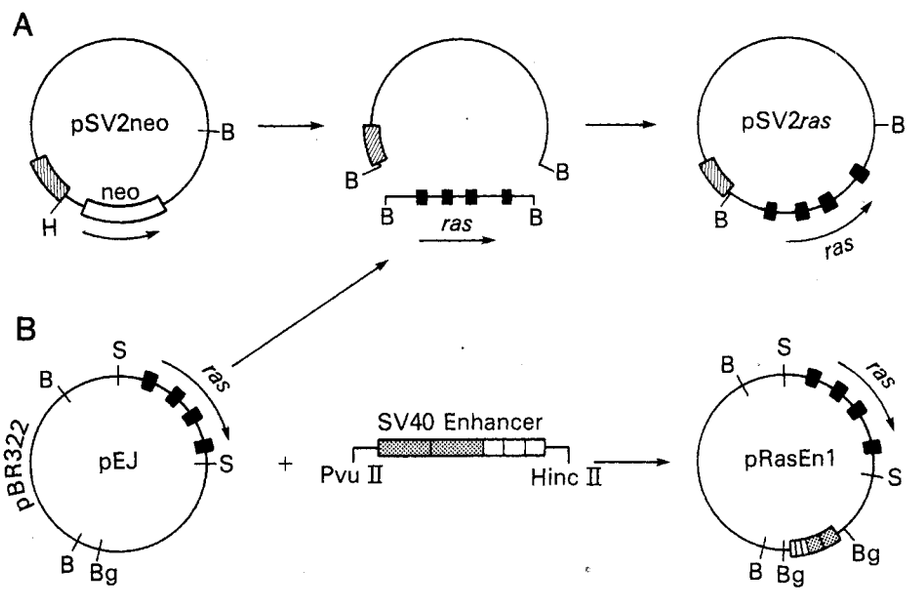

Fig. 1. Construction of plasmids pSV2ras and pRasEn1. (A) To construct pSV2ras, pSV2neo (*24*) was digested with Bam HI and Hind III, and Bam HI synthetic linkers were ligated onto the ends of the vector after blunt ends were produced with T4 DNA polymerase. A 2.9-kb Sac I fragment from pEJ, containing the four coding exons (filled squares) as well as the putative polyadenylation signal of the activated c-Ha-*ras*-I gene (*13*), was also treated with Bam HI linkers and ligated to the pSV2 vector above. (B) The plasmids pRasEn1 and pRasEn2 were constructed by inserting the SV40 enhancer downstream from the c-Ha-*ras*-I coding region in pEJ. A 234-bp Pvu II/Hinc II restriction fragment containing enhancer from the SV40 mutant CS 1096 (*25*) was isolated and synthetic Bgl II linkers were added. This fragment contains both copies of the 72-bp repeat element (stipled region) and all three copies of the 21-bp repeat element (open squares) but lacks the Goldberg-Hogness sequence. The fragment was inserted into the plasmid pEJ at the unique Bgl II site. Plasmid pRasEn1 contains the 234-bp SV40 fragment such that the former Pvu II site is proximal to the *ras* polyadenylation site. Plasmid pRasEn2 has the 234-bp fragment in the opposite orientation. H, B, S and Bg represent restriction sites for Hind III, Bam HI, Sac I, and Bgl II, respectively. The cross-hatched region in pSV2neo and pSV2ras represents the SV40 early region transcription control element.

Table 1. Frequency of transformation of rat embryo cells by transfected oncogenes. Primary rat cells from 17-day-old Sprague-Dawley embryos (Flow) were trypsinized and 1×10^6 cells were placed in each tissue culture flask in DMEM with 10% FCS and antibiotics. Calcium phosphate DNA precipitates were formed in a volume of 2 ml (*23*). Where indicated, 8 μg of pRSVneo and 8 μg of each oncogene were used. Plasmid pBR322 DNA was used as a carrier to bring each transfection mix to 12 μg/ml. Precipitates were applied to the cells for a period of 4 hours, followed by a glycerol shock for 2 minutes in 3 ml of 15% (weight to volume) glycerol containing 1× Hepes buffered saline (*23*). After 40 hours cells were trypsinized, counted, plated at 3×10^5 and 1×10^6 cells per 10-cm petri dish, and fed with medium containing G418 (Gibco) at 700 μg/ml. After 2 weeks in selective medium, colonies were picked or plates were stained with crystal violet and colonies with more than 50 cells were scored.

Donor DNA	Frequency		
	Transfection*	Transformation†	
No DNA	0.0	0/0	(0.0)
	0.0	0/0	(0.0)
pRSVneo	78.5	0/204	(0.0)
	53.5	0/139	(0.0)
	103	0/68	(0.0)
pE1a+	67.8	0/156	(0.0)
pRSVneo	90.0	0/300	(0.0)
pEJ+	53.9	7/140	(5.0)
pRSVneo	59.7	12/215	(5.6)
	63.1	10/101	(9.9)
pEJ+pE1a+	242	280/436	(64)
pRSVneo	192	242/500	(48)
	101	116/263	(44)
pRasEn1+	167	17/384	(4.4)
pRSVneo	78.9	12/205	(5.9)
pRasEn2+	173	20/399	(5.0)
pRSVneo	86.9	7/139	(5.0)
pSV2ras+	86.3	7/138	(5.1)
pRSVneo			

*Number of G418-resistant colonies per 1×10^6 cells plated in G418-containing medium. †Number of morphologically transformed colonies per number of G418-resistant colonies (percentage in parentheses).

ras oncogene alone were successfully established in culture at a rate of approximately 20 percent. This lower rate was observed for foci derived from cultures of cells transfected with any of the four *ras*-containing plasmids. Most foci that did not become established in culture did so at the initial transfer to microtiter tissue culture trays. Thus the overall frequency of stable transformation was 50 times greater with two oncogenes than with *ras* alone.

Once mass cultures of cells were obtained, growth curves were generated for three cell lines in Dulbecco's modified Eagle medium (DMEM) containing 10 percent fetal calf serum (FCS). The doubling times for a two-gene transformant, RE1; a pEJ transformant, 2R; and a pRasEn1 transformant, Ren2 (R, *ras*; RE, *ras* + E1a; Ren, *ras* + enhancer) were 12, 24, and 20 hours, respectively.

Six two gene–transformed rat embryo cell lines, RE1 through RE6, five pEJ-transformed cell lines, 1R through 5R, and three pRasEn1-transformed cell lines, Ren1 through Ren3, were assayed by Northern blot analysis for expression of the transfected *ras* and E1a genes. All six lines transformed with E1a and *ras* showed roughly comparable and abundant levels of the characteristic 1.2-kb *ras* transcript (Fig. 2C). Cytoplasmic RNA from the human bladder carcinoma cell line T24 was included for comparison (lane 9). In this autoradiogram no *ras*-specific transcript is visible in RNA extracted from untransfected rat embryo cells (lane 5). However, on longer exposure a faint band was visible around the position 1.2 to 1.4 kb. A rat-specific c-*ras* probe gave the same pattern of hybridization as did the human c-*ras* probe. In the few transformed cell lines examined, immunoprecipitation of the c-Ha-*ras* gene product showed an abundance of the slightly slower migrating form of p21 characteristic of the point-mutated gene product (*16*).

Expression of E1a transcripts in the same six cell lines, RE1 through RE6, is shown in Fig. 2D. The fastest migrating species of approximately 0.8 to 1.0 kb are the authentic 12*S* and 13*S* E1a transcripts. The slower migrating 3-kb species probably represent E1a-specific transcripts that are not processed properly and contain downstream (E1b) sequences. The cell line RE2 (lane 2) contains two additional bands of approximately 1.8 and 3.5 kb. These transcripts may originate from an aberrant integration event. These results demonstrate that lines RE1 through RE6 all contain *ras* and E1a transcripts of the appropriate sizes.

A similar analysis of the cell lines transformed by the *ras* gene alone (Fig. 2, A and B) shows that all lines contained *ras*-specific transcripts at levels greater than the amount present in the T24 cell line, and are similar to the levels present in the lines transformed by *ras* and E1a (Fig. 2, A and C). The levels of *ras* transcripts in the three cell lines transformed by the enhancer containing plasmid pRasEn1 are only equal to, or even less than, the amounts seen in cell lines transformed by pEJ (compare lanes 8 to 10 with lanes 2 to 6 in Fig. 2). Differences in the number of integrated and expressing *ras* plasmids could explain the apparent inability of the SV40 enhancer element to stimulate levels of *ras* expression in these three cell lines. As expected, expression of the E1a gene is absent in cell lines transformed by the *ras* gene alone (Fig. 2B).

The transformed cell lines (Table 2) formed tumors after injection into 4-week-old nude mice. When 5×10^5 cells were injected subcutaneously, tumors grew to 1 cm diameter in 10 to 14 days. Tumors continued to grow, some becoming 3 cm in diameter by 4 weeks. Consistent with the growth properties observed during in vitro culture, the two-gene transformants gave rise to more rapidly growing tumors than the *ras* transformants. Autopsies were performed 4 to 6 weeks after injection of transformed cells. With the exception of cell line 2R, all the cell lines transformed by pEJ (1R, 3R, 4R, 5R) or pRasEn1 (Ren1, Ren2, Ren3) often gave rise to metastatic nodules in the lungs of animals bearing tu-

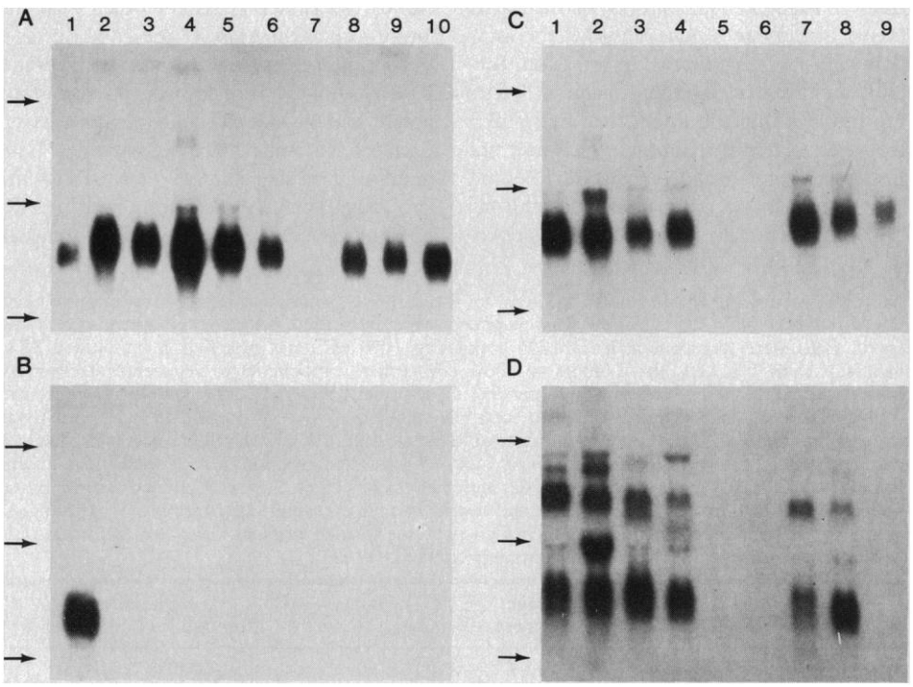

Fig. 2. Characterization of expression of transfected E1a and *ras* genes in transformed rat embryo cell lines. (A) Northern blot analysis of *ras* expression of cell lines transformed by *ras* alone. Lanes 1 to 10 contain cytoplasmic RNA from the human bladder carcinoma cell line T24, 1R, 2R, 3R, 4R, 5R, third-passage untransfected rat embryo cells, Ren1, Ren2, and Ren3, respectively. (B) Northern blot analysis of E1a expression of *ras*-alone transformants. The sample order is identical to that in (A) except lane 1, which contains 3 μg of polyadenylated RNA from a C3H mouse embryo cell line transformed by adenovirus type 5. (C) Northern blot analysis of *ras* expression of cell lines transformed by *ras* and E1a. Lanes 1 to 9 contain cytoplasmic RNA from the cell lines RE1, RE2, RE3, and RE4; third-passage untransfected rat embryo cells; and rabbit β-globin messenger RNA, RE5, RE6, and T24, respectively. (D) Northern blot analysis of E1a expression cell lines transformed by *ras* and E1a. Sample order is identical to that in (C). The three arrows indicate the positions of, from top to bottom, 28*S* ribosomal RNA (4.5 kb), 18*S* ribosomal RNA (1.8 kb), and rabbit β-globin messenger RNA (600 bases). Cytoplasmic RNA was isolated by lysis of cells with 0.5% NP40. Nuclei were centrifuged at 1000*g* and the supernatant was extracted twice with a mixture of phenol and chloroform (1:1). RNA was recovered by ethanol precipitation of the aqueous phase. Precipitated material was dissolved in water, and the amount of nucleic acid was determined by spectrophotometric reading at 260 nm. Unless otherwise indicated, all lanes contain 20 μg of cytoplasmic RNA fractionated on 0.9% agarose gels containing 6% formaldehyde and 20 m*M* 3-[*N*-morpholine]propanesulfonic acid. After electrophoresis, gels were blotted onto nitrocellulose filters for 15 hours in 20× standard saline citrate (SSC). Filters were baked for 2 hours at 80°C, then treated for 5 hours with a solution containing 45% formamide, 5× SSC, 10% dextran sulfate, 5× Denhardt's solution, and salmon sperm DNA (100 μg/ml). The *ras* probe used was a 2.9-kb Sac I fragment from the plasmid pEJ that contained all four *ras* exons. The E1a probe was a 1.5-kb fragment of the left end of the Ad 2 genome up to the Hpa I site. Phosphorus-labeled nick-translated probes with specific activities of at least 5×10^8 cpm/μg were hybridized to the filters for 15 hours at 45°C. Filters were washed with 2× SSC and 0.1% sodium dodecyl sulfate at 68°C and then exposed to Kodak XAR-5 film at −70°C with Dupont Cronex intensifying screens for 20 hours.

mors from subcutaneous injection of transformed cells. The number of metastatic nodules observed varied from a few to more than 100 per animal. None of the cell lines transformed with E1a and *ras* formed metastases in the lungs, heart, liver, kidneys, bladder, ovaries, or spleen when cells were injected subcutaneously.

Our metastasis assay measures the ability of tumorigenic cells that grow at the site of subcutaneous injection to intravasate and extravasate the circulatory system, to invade normal tissue, and to colonize at new locations. This assay requires tumorigenic cells to have many of the metastatic properties associated with naturally occurring metastatic tumors. However, it has some technical limitations. First, since the number of metastatic events that occur is generally low, the assay does not offer quantitative information. Second, rapidly growing tumorigenic cell lines can produce tumor burdens that are lethal 3 to 4 weeks after inoculation, which may not be sufficient time to allow metastases to occur. For these reasons we made use of the tail vein–lung metastasis assay. Single-cell suspensions are injected into the lateral tail veins of 4-week-old nude mice and cells are transported directly to the lungs via the circulatory system. Their ability to extravasate, invade normal tissue, and form lung nodules is measured at autopsy approximately 2 weeks after injection. Results of this assay correlate well with the ability of malignant cells to metastasize from primary sites of injection.

In a control experiment, 20 nude mice were injected with passage-three or passage-four rat embryo cells (Table 2). No metastatic nodules were detected in any organ of these animals. Cell lines transformed by the *ras* gene alone gave rise to many metastatic lung nodules. This was true for lines transfected with pEJ or pRasEn1. Some cell lines infrequently gave rise to metastases in tissues other than the lungs, such as the heart, ovaries, and rib cage. In contrast, cell lines transformed with *ras* and E1a all showed very low or no metastatic potential in the tail vein–lung metastasis assay. Each cell line was assayed at least twice. Cells of different passage number were used for the two assays. With the exception of cell line 4R, no significant change in the number of metastases was observed as a function of passage number. Metastases following subcutaneous injection were observed as early as passage three or four for the cell lines 2R, 5R, Ren2, and Ren3. Cell lines 2R and 3R showed high metastatic potential when assayed by tail vein injection at passages 38 and 40. Therefore, we conclude that the metastatic phenotype is inherently and stably associated with these cells and has not been selected for or enhanced by passaging cells in culture.

We introduced various forms of the c-Ha-*ras* oncogene, with or without the adenovirus E1a gene, into early passage rat embryo cells obtained from 17-day-old Sprague-Dawley embryos. While *ras* alone transformed cells derived from the primary explants, the frequency of morphological transformation scored as the fraction of G418-resistant colonies was at least ten times greater when the E1a and *ras* genes were cotransfected (Table 1). The question of what viral or cellular oncogenes, alone or in combination, are required to transform primary cells in culture has been discussed (*8–11*). Although certain combinations of oncogenes facilitate transformation, some of the data (transformation by a single *ras* oncogene with or without enhancer elements) are difficult to reconcile. Differences in the preparation of the primary cultures, stage of embryos used, and methods used with selectable markers may contribute to the variations observed.

We have extended previous studies by examining the single-gene and two-gene transformants for phenotypic differences that could be correlated with a particular transfected oncogene. The most interesting phenotypic difference between the one- and two-gene transformants is their metastatic potential in nude mice. Cell lines transformed by the *ras* gene alone formed lung metastases after subcutaneous or intravenous injection, whereas cell lines transformed by *ras* and E1a formed very few lung metastases, and then only after intravenous injection.

These results suggest that the E1a gene may have some inhibitory effect on the metastatic phenotype. Cook and co-workers found that hamster cell lines either infected with or transformed by human adenovirus type 2 show an increased susceptibility to lysis by natural killer cells and macrophages relative to hamster cells transformed by adenovirus type 12 (*17, 18*). The specific Ad 2 function responsible for this has been mapped to the E1a gene (*19*). Therefore, it is possible that the low metastatic potential of the two gene–transformed cell lines is due to an increased susceptibility to cytolytic lymphoid cells. The hypothesis that the E1a gene affects the metastatic phenotype is testable. We are currently deriving clones from cell lines 1R, 5R, and Ren2 that have been cotransfected with E1a

Table 2. Formation of metastatic nodules in the lungs of animals injected intravenously with transformed cell lines. Confluent monolayers of cells were trypsinized, counted, and centrifuged at low speed. Cells were resuspended in DMEM containing 10% FCS and placed in a humidified 37°C incubator with 5% CO_2 for 3 hours to allow cell-surface components to regenerate. Cells were centrifuged at 1500 rev/min and resuspended in phosphate-buffered saline without calcium and magnesium at a concentration of 5×10^5 cells per milliliter. A 0.1-ml portion (5×10^4 cells) was injected into the lateral tail vein of 4- or 5-week-old female nude mice. Each cell line was assayed at two different passage numbers. Mice were killed 2 or 3 weeks after injection. Lungs were inflated with Bouin's solution [picric acid, formaldehyde, and acetic acid (15:5:1)] and examined for metastatic nodules. The heart, liver, kidneys, spleen, and ovaries were also examined for metastases. Histological examinations confirmed the presence of malignant tissue in lung nodules. Two hundred metastatic nodules is the upper limit that can be accurately counted.

Cell line	Passage number	Lung nodules from tail vein injection
Normal rat embryo cells	3 and 4	No nodules detected in 20 animals
1R	13	>200, >200, >200, 135
	16	179, 158, 126
2R	30	172, 166, 141, 121
	38	>200, >200, >200, >200, 132
3R	22	150, 125, 64, 27
	26	82, 30, 21, 5
4R	8	21, 12, 10, 9, 6
	13	>200, 168, 147, 132
5R	8	>200, >200, >200, >200, >200
	13	>200, >200, >200, >200
Ren1	9	>200, 157, 136, 117, 100
	15	141, 88, 79, 38
Ren2	8	>200, >200, >200, >200, >200
	14	>200, >200, >200, >200
Ren3	13	>200, >200, >200, >200
	16	>200, >200, >200, >200, >200
RE1	18	0, 0, 0, 0
	22	0, 0, 0, 0, 0
RE2	11	2, 0, 0, 0
	25	1, 0, 0, 0, 0
RE3	8	7, 6, 4, 2
	22	1, 0, 0, 0
RE4	5	0, 0, 0, 0
	25	2, 2, 1, 0
RE6	8	1, 0, 0, 0
	18	1, 1, 0, 0

and the selectable marker *Ecogpt*. The metastatic potential of these lines requires further examination in the presence of E1a expression.

Another explanation for the difference in metastatic potential observed between one- and two-gene transformants is that different populations of rat embryo cells are transformed by the *ras* gene alone, unlike the situation when *ras* is cotransfected with E1a. It is possible that only a small subset of the heterogeneous mixture of rat embryo cells is susceptible to transformation by the *ras* oncogene alone. This subset of cells may also be predisposed toward the metastatic phenotype. Single gene–transformed cell lines show normal diploid karyotypes (*20*), suggesting that gross chromosomal abnormalities or rearrangements are not a feature of cell types that are susceptible to transformation by the *ras* oncogene alone. When two cooperating oncogenes are cotransfected, the increase in the frequency of morphological transformation could be the result of transformation of a broader spectrum of embryo cell types. Cell types predisposed to metastasis should be present in the population of cells transformed by *ras* and E1a, but the percentage of such cells would be low (*21*).

NIH3T3 cells transformed by the *ras* oncogene are metastatic in nude mice (*22*). We have demonstrated that the *ras* oncogene can transform early passage rat embryo cells into tumorigenic cells that are highly metastatic in an immunodeficient animal. Thus, normal cells with a limited potential for growth in culture have become established, have been transformed, and have acquired metastatic potential apparently through the action of a single oncogene. Although the *ras* oncogene can induce morphological transformation, we do not yet know whether *ras* confers or induces the properties of establishment and metastasis or whether they are present in the recipient cell.

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Diverse Gene Expression for Isotypes of Murine Serum Amyloid A Protein During Acute Phase Reaction

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Serum amyloid A protein (SAA) is a precursor for a major component of amyloid fibrils, which, upon deposition, cause secondary amyloidosis in diseases such as rheumatoid arthritis. In mice, SAA is encoded by at least three genes, which show diverse expression during inflammation. Furthermore, in amyloidosis-resistant SJL mice, the gene expression for one SAA isotype, SAA2, is defective, although SAA2 gene expression is normal in amyloidosis-susceptible BALB/c mice. Because only SAA2-derived products deposit in mouse amyloid tissues, the resistance of SJL mice to amyloidosis seems to be due to defective SAA2 gene expression. Thus, the study emphasizes the importance of SAA gene structure in determining susceptibility to amyloidosis.

SECONDARY AMYLOIDOSIS, A SERIOUS complication of chronic inflammatory diseases such as rheumatoid arthritis, juvenile arthritis, and ankylosing spondylitis, is caused by the formation in tissues of amyloid fibrils. The major component of amyloid fibrils is derived from serum amyloid A protein (SAA). SAA is the most prominent acute phase reactant, and high SAA concentrations are frequently found in various inflammatory diseases, thus predisposing the patient to amyloid deposition (*1*). However, the normal physiological function of SAA is not known, although SAA may bind to a lipopolysaccharide portion of bacterial cell walls and may function in clearance of bacteria (*2*). We have recently isolated and characterized complementary DNA (cDNA) clones encoding two major SAA isotypes of BALB/c mice—SAA1 [11.75 kilodalton (kD)] and SAA2 (11.65 kD)—which show a 95 percent homology in their cDNA sequences (*3*). We report here that the mouse genome contains at least three SAA genes and that, while the SAA1 and SAA2 genes are expressed at high levels during acute phase reactions, the gene for the third SAA isotype (SAA3) is expressed only at a very low level. We further show that the SAA2 gene expression is meager in SJL mice. Since only the SAA2-derived product is deposited in mouse amyloid tissues (*4*), the resistance of SJL mice to azocasein-induced amyloidosis is probably due to defective SAA2 gene expression.

Mouse SAA2 cDNA was used to screen the BALB/c mouse Hae III genomic library in phage Charon 4A (*5*), and four positive phage clones were isolated. On the basis of their hybridization with the SAA2- and SAA1-specific synthetic oligonucleotide probes (Fig. 1A), the two clones λ5-12 and λ39-2 were identified as carrying the SAA2 and SAA1 genes, respectively (Fig. 2). In addition, while the λ5-12 clone contained a Xho I site 250 base pairs (bp) downstream of the Eco RI site within the SAA2 coding region, the λ39-2 clone contained a Stu I site near the Eco RI site in the SAA1 coding region: these restriction sites are unique to the SAA2 and SAA1 cDNA's, respectively (*3*). To define a possible linkage of the

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