Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2224–2228, April 1989
Cell Biology

The *ras* and *myc* oncogenes cooperate in tumor induction in many tissues when introduced into midgestation mouse embryos by retroviral vectors

(*ras–myc* cooperation/*in utero* infection with oncogenic viruses/tumor induction in embryos)

SALLY J. COMPERE*, PATRICIA BALDACCI*†, ARLENE H. SHARPE*‡, TIMOTHY THOMPSON§¶, HARTMUT LAND§, AND RUDOLF JAENISCH*

*Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142; ‡Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115; and §Imperial Cancer Research Fund, Lincoln Inns Fields, London, England

Communicated by Salome Gluecksohn Waelsch, December 14, 1988

ABSTRACT **Midgestation embryos were infected with replication-defective retroviral vectors that either transduced the *myc* oncogene, the *ras* oncogene, or both oncogenes simultaneously. The *myc* virus induced tumors in diverse organs at a very low frequency and with a long latency period, while ≈20% of the mice derived from embryos infected with the *ras* virus developed tumors in the skin with a latency of 4–8 weeks. In contrast, infection of embryos with the *ras/myc* double oncogene virus resulted in 27% of the animals developing rapidly growing and malignant tumors in a great variety of tissues after a median latency period of 2–3 weeks. All tumors were of monoclonal origin, as shown by Southern analysis using the provirus as a molecular marker. Our results are consistent with the hypothesis that the *ras* and *myc* oncogenes cooperate in transforming cells, but that additional alterations are necessary for realization of the fully malignant phenotype. Our observations also suggest that a much wider range of cell types become targets for malignant transformation when the embryos are exposed to the *myc* and the *ras* oncogenes simultaneously than when exposed to the same oncogenes separately. Infection of mouse embryos with vectors carrying different oncogenes or oncogene combinations may be an efficient and rapid method for evaluating the spectrum of cell types at risk for malignant conversion following mutation of a protooncogene to a transforming gene.**

Development of cancer is a multistep process involving multiple alterations of the cellular genome (1–3) and current models suggest that mutations occur in protooncogenes, resulting in the generation of oncogenes. Although oncogenes are expressed in numerous tumor types, the activity of the proteins encoded by these genes is still unclear. Land *et al.* (4, 5) and Ruley (6) established a system to distinguish between the functions of several oncogenes. While the *myc* oncogene allowed primary cells to be permanently established in culture, introduction of the *ras* family of oncogenes elicited a transformed phenotype in cells without prolonging their life-span. Primary fibroblasts containing only one oncogene were not tumorigenic upon injection into nude mice but became tumorigenic when expressing both types of oncogenes. More recently, Sinn *et al.* (7) generated two strains of mice carrying the *myc* and *ras* genes, respectively, with each oncogene transcribed from the mouse mammary tumor virus promoter. When interbred, tumor formation was much more rapid in the F_1 generation than in the parent strains carrying a single oncogene. Tumor development was, however, still stochastic and clonal. This observation led to the important conclusion that although myc and ras proteins cooperated *in vivo*, additional alterations were needed to induce tumor formation.

In this study, we have introduced the *ras* and/or *myc* oncogene into midgestation mouse embryos. The genes were under the transcriptional control of the viral long terminal repeat, which is active in most or all cell types of the midgestation mouse embryo (8–10). This experimental approach allowed us to evaluate the types of cells that are susceptible to malignant transformation when exposed to the *ras* or *myc* oncogenes individually or when exposed to both oncogenes simultaneously.

MATERIALS AND METHODS

Derivation of Viral Vectors. The construction and characterization of the replication-defective retroviruses used in these experiments are detailed elsewhere (11, 12) and are illustrated in Fig. 1. All of the vectors are based on the transcriptional regulatory sequences of Moloney murine leukemia virus (13). The v-*myc* oncogene was derived from the MC29 chicken leukemia virus (14).

Titration of Virus Supernatants. Virus supernatants were concentrated and titered as described (12). Those viruses that did not carry a neomycin-resistance marker were titered by focus-forming assays (colony-forming units) or by extracting RNA from the concentrated virus stocks followed by dot blot analysis. Viruses containing a *neo* marker were used to standardize the assay (15).

Injection of Mouse Embryos *in Utero*. Females of three inbred strains (C57BL/6J, BALB/c, and FVB/N) were mated with males of the same strain and the day of the vaginal plug was counted as day 0 of gestation. Embryos were infected by microinjection of either ψ-2 cells producing virus or concentrated viral stocks directly through the uterine wall as described (8). The virus producer cells were pretreated with mitomycin C at 20 μg/ml for 2 hr to prevent cell division while permitting virus production.

Histology and Immunohistochemistry. Lesions were fixed in 10% buffered formalin and then dehydrated in graded alcohols and xylenes (16). The tissues were embedded in paraplast and 4-μm sections were prepared and stained with Harris' hematoxylin and eosin (16). Immunohistochemistry was performed as described (12). Rabbit antibodies to bovine muzzle keratins (1:80 dilution; Dako Corporation, Santa Barbara, California), myoglobin (1:500; Dako), S100 (1:150; Dako), Desmin (1:500; Dako) (1:80; Sigma), and factor VIII (1:200; Dako) were used in the studies. Goat primary anti-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: cfu, colony-forming unit.

†Present address: Institute Pasteur, 25 Rue du Dr. Roux, 75724 Paris Cedex 15, France.
¶Present address: Department of Urology and Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

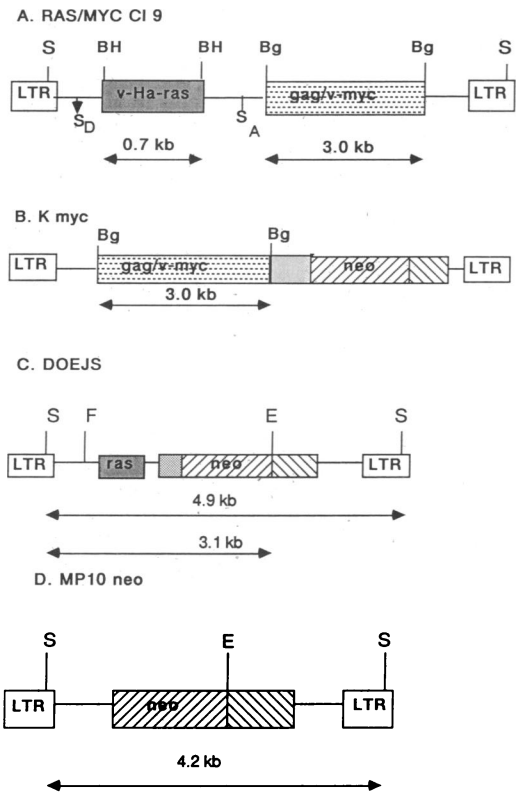

FIG. 1. Structure of replication-defective retroviruses transducing oncogenes. LTR, long terminal repeat; S_D/S_A, splice donor/acceptor; S, *Sst* I; BH, *Bam*HI; Bg, *Bgl* II; F, *Bgl* II/*Bam*HI fusion; E, *Eco*RI; arrows show the fragment sizes after enzyme digestion. The *neo* gene is flanked by sequences of the simian virus 40 and pBR322 origin at the 5′ and 3′ ends, respectively (12).

bodies to vimentin (1:80; Polysciences) (1:60; Sigma) were also used.

Detection of Viral RNA and DNA Sequences. Nucleic acid was purified from tumor tissues and subjected to Southern and Northern analysis as described (12).

RESULTS

Transduction of *ras* and *myc* Oncogenes into Midgestation Mouse Embryos. Mouse embryos were infected at day 8 of gestation with the *ras/myc* virus (either as a concentrated virus stock or as mitomycin C-treated ψ-2 producer cells) by microinjection. Previous studies with a retrovirus transducing the human c-*HRAS1* oncogene had established that injection of concentrated virus stocks was the most efficient method to infect embryos and to induce tumors in the postnatal animal (12). Similar results were obtained in this study. A high concentration of the *ras/myc* virus was found

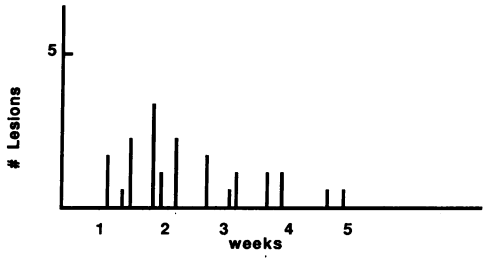

FIG. 2. Incidence of lesions after infection of midgestation embryos with the *ras/myc* virus.

to be lethal to the developing embryo because only 1% of the fetuses survived beyond birth when 5000 colony-forming units (cfu) were injected. Live animals, however, were obtained when the virus inoculum was diluted to 1000 cfu or when producer cells were used for the injection. Table 1 shows that tumors developed in 27% of the surviving animals. The tumors developed rapidly with 20% appearing by 1.5 weeks of age and 77% prior to 4 weeks of age with a median latency of 3 weeks (Fig. 2).

A total of 39 tumors, which arose in different organs and were associated with a variety of phenotypes, were detected in 138 mice derived from injected embryos. Thirty-two percent of the animals with lesions appeared to have a loss of normal motor function exhibiting a circular motion or partial limb paralysis. Upon autopsy, most of these animals were found to have brain lesions. In addition, 15 tumors involved the skin. Five tumors occurred in the peritoneal or pleural cavity and involved liver, kidney, lung, or the rib cage.

To examine whether the rapid tumor induction after injection of the *ras/myc* virus required simultaneous expression of both the *ras* and the *myc* oncogene in the embryonic cells, embryos were injected with retroviral constructs that transduced either the *ras* or the *myc* virus (Fig. 1). Previous studies (12) had shown that injection of a vector transducing the human c-*HRAS1* oncogene was not lethal but led to skin lesions in 20% of the animals after a latency period of 4 weeks (Table 1). After injection of embryos with a retroviral construct transducing the *myc* oncogene, only 5 of 286 animals developed tumors and these occurred in the eye, ovary, liver, lung, or gastrointestinal tract after a latency period of 15–22 weeks (Table 1).

Our results allow the following conclusions. While transduction of the *myc* oncogene alone into midgestation embryos induced tumors very inefficiently, the *ras* oncogene caused skin lesions but not other tumors with a high efficiency (12). In contrast, tumor formation in a great variety of tissues was induced when both oncogenes were jointly transduced into the developing embryo. The *ras/myc* virus was much more effective in causing tumors than the *ras* virus, which induced mainly skin tumors with a latency of 4 weeks after injection of 5000 cfu into embryos. When the same dose of the *ras/myc* virus was used, few of the injected embryos

Table 1. Infection of midgestation embryos with vectors carrying the *ras* and/or *myc* oncogenes

Gene introduced	Method of infection	No. of embryos injected	No. of animals alive at 1 wk	No. of animals with lesions	Age, weeks
v-*myc*/*neo*	1000 cells	363	179	1 (0.5%)	15–22
v-*myc*/*neo*	5000 cfu	222	107	4 (1%)	
c-Ha-*ras*/*neo*	1000 cells	258	118	17 (14%)	≈4
c-Ha-*ras*/*neo*	5000 cells	421	234	57 (25%)	
v-*ras*/v-*myc*	5000 cfu	228	3		
v-*ras*/v-*myc*	1000 cfu	70	40	11 (27%)	≈3
v-*ras*/v-*myc*	1000 cells	338	98	28 (28%)	

Embryos were injected either with virus-producing and mitomycin C-treated cells or with concentrated virus stocks. The results of injection with the human c-*HRAS* virus (DOEJ) have been described by Compere *et al.* (12) and are included in this table for comparison.

survived to birth. Only when the virus stock was diluted were live animals obtained that developed tumors, with a median latency of 3 weeks.

Histologic Analysis. All tumors were examined by light microscopy. Five lesions arose in mice infected with the *myc* virus. Two of these were benign. One was a benign hamartoma in a lung and the other was an inflammatory lesion in the gastrointestinal tract. The three malignant lesions were an ovarian stromal tumor, a poorly differentiated carcinoma of unknown origin, and malignant spindle cell tumor replacing an eye. Previous microscopic analyses of lesions arising in mice infected with c-Ha-*ras* indicated that the majority were benign cutaneous neoplasms (80%) and the remainder were subcutaneous malignant tumors (12).

In contrast, light microscopic analysis of the *ras/myc* lesions revealed that the majority consisted of malignant neoplasms (18/35), while the rest were either benign neoplasms (12/35) or lesions consisting of foci of hemorrhage and/or acute and chronic inflammation, without evidence of malignancy (5/35). Because only a portion of each lesion was examined microscopically, it is possible that the latter 5 lesions represent areas of hemorrhage and inflammation adjacent to a tumor that was not represented in the available tissue section but was used for molecular analysis. The histologic classification of the *ras/myc* lesions is summarized in Table 2 and representative lesions are shown in Fig. 3.

The malignant lesions were diverse in origin. A large number (7/18) occurred in the brain. Six of these tumors involved the cerebellum and had virtually identical histologic features (Fig. 3*a*). These tumors exhibited a prominent angiocentric pattern, as well as a pattern in which tumor cells were arranged in lobulated groups of epithelial-like cells. These tumors behaved aggressively, invading the cerebellar parenchyma. Their histologic features are most consistent with a meningeal origin. The other brain tumor was a primitive malignant neoplasm that was highly cellular and composed of hyperchromatic round cells with a scant amount of cytoplasm and had metastasized to the liver.

Another large group of lesions (7/18) exhibited remarkably similar histologic features and consisted primarily of elongated spindle-shaped cells with elongated nuclei arranged in clusters and whorls (Fig. 3*b*). In addition, nodular areas of epithelioid polygonal cells were present in four of these tumors and the other two had a prominent vascular component, reminiscent of angiosarcoma. Immunohistochemical analyses indicated that three of the lesions were epithelial in origin and ruled out a neuroectodermal, a smooth muscle, or skeletal muscle cell origin for the other lesions. The results of immunohistochemical studies suggested that the other spindle cell lesions are high grade sarcomas. The remainder of the malignant *ras/myc* tumors included three squamous cell carcinomas in the skin (Fig. 3*c*) and a poorly differentiated malignant neoplasm that replaced the renal medulla.

In summary, our results indicate that the *ras* and *myc* oncogenes, when jointly introduced into midgestation em-

Table 2. Summary of histologic analysis of lesions after *ras/myc* double infection

	No. observed
Benign (all skin)	
Surface epithelial hyperplasia with severe dysplasia, premalignant	9
Mixed appendage tumor	2
Mesenchymal proliferation	1
Malignant	
Brain	
Neoplasm exhibiting prominent angiocentric pattern and epithelial papillary configurations (consistent with meningeal origin)	6
Primitive neoplasm (? neural, lymphoid, germ cell origin)	1
Skin	
Squamous cell carcinoma	3
Kidney	
Malignant neoplasm replacing renal medulla	1
Spindle cell neoplasms in heart, skin, and subcutaneous tissue, chest wall (poorly differentiated carcinomas, high grade sarcomas)	7

Immunohistochemical studies were used to determine the origin of the spindle cell malignant neoplasms. Three of the spindle cell neoplasms stained with keratin antibodies, indicating an epithelial origin for the tumors. The other spindle cell neoplasms did not stain with antibodies to keratin, myoglobin, or desmin, excluding a smooth skeletal muscle cell origin, or to S100, excluding a neuroectodermal origin. The latter neoplasms stained only with vimentin. Taken together, the immunohistochemical analyses suggest that spindle cell lesions are most probably sarcomas. No factor VIII antibody that recognized mouse endothelial cells was available, precluding an analysis of these tumors for a vascular origin.

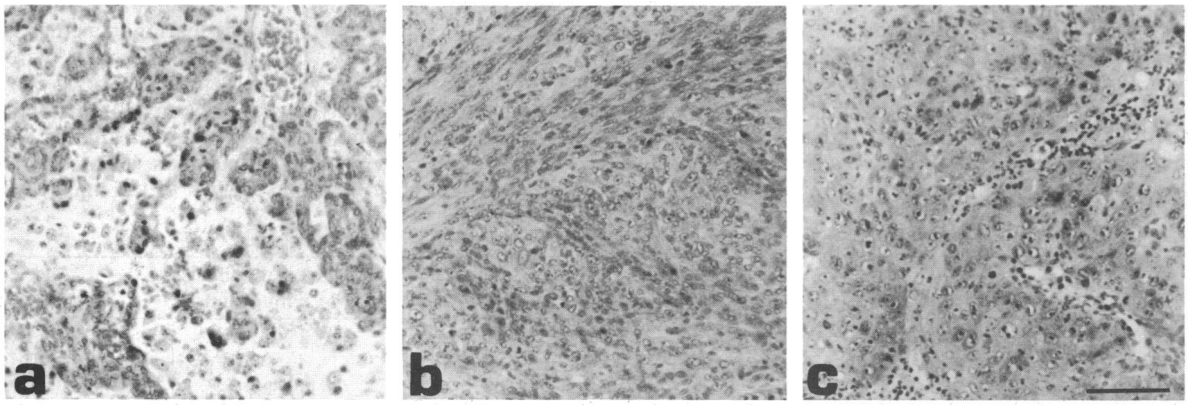

FIG. 3. Histology of representative lesions obtained from mice infected with the *ras/myc* virus. (*a*) Malignant brain tumor. The tumor exhibits two distinct microscopic patterns. In this photograph, the angiocentric pattern, consisting of cells with poorly defined cytoplasm or oval nuclei lying adjacent to capillary-like channels, is most prominent. The aggressive behavior of this component is seen here with tumor cells invading the brain parenchyma. The other pattern, consisting of tumor cells arranged in lobulated groups of epitheloid cells, is not seen in this field. (*b*) Malignant subcutaneous tumor. The tumor consists of anaplastic spindle-shaped cells with bizarre nuclei and many mitoses. (*c*) Squamous cell carcinoma from shoulder region. This tumor invaded to the level of skeletal muscle. The tumor shows squamous differentiation. Irregular masses of dysplastic cells with variable keratinization proliferate downward, invading the dermis. (Bar = 100 μm.)

bryos induce a wide spectrum of neoplasms, the majority (90%) of which are malignant or premalignant. In contrast, exposure of embryos to *myc* or *ras* alone results in either inefficient tumor induction or induction of primarily benign lesions, respectively.

Detection of Viral DNA and RNA. To correlate tumor formation with virus infection, DNAs were isolated from 12 lesions and the presence of the provirus was detected by Southern blotting analysis and probing with v-*ras* or v-*myc* radiolabeled probe (Fig. 1). For v-*ras*, digestion of the DNA samples with *Bam*HI generated an internal fragment carrying the v-*ras* sequences of 700 base pairs, indicating no gross rearrangement of the viral structure (Fig. 4*A*, lanes 2–10). Endogenous mouse *ras* sequences were also detected (the two larger fragments present also in the negative control; lane 1) and served as an internal control for digestion and transfer of the DNA. The presence of the v-*myc* sequences was determined by Southern blotting analysis of the DNA samples after digestion with *Bgl* II, which generates a 3.0-kilobase (kb) internal v-*myc* fragment (Fig. 4*B*, lanes 2–10). The endogenous mouse *myc* genes were not detected under the hybridization conditions used with a chicken v-*myc* radiolabeled probe (lane 1). To determine whether the tumors contained one or multiple proviral structures the Southern blot of DNAs that had been digested with *Bgl* II (from Fig. 4*B*) was denatured and reprobed with the v-Ha-*ras* oncogene to detect the flanking sequences (Fig. 4*C*). This analysis indicated the presence of only a single provirus in each of the tumors induced by infection of midgestation embryos, suggesting that a single viral particle promoted each hyperplasia.

When RNA was isolated from 11 tumors and tested for viral transcripts by dot blot analysis, 8 of the samples were positive for viral RNA. Our inability to detect viral RNA in all samples is likely due to many of the tumors being necrotic or composed of tumor and normal tissue, which would result in RNA degradation or a lower sensitivity of detecting viral RNA. Fig. 5 shows that the expected full-length viral transcripts of 6 kb, as well as the spliced subgenomic RNA, were seen on Northern blots of 4 RNA samples extracted from *ras/myc* virus-induced tumors. These results support the conclusion that the embryonic cells carried intact proviruses that transcribed functional viral mRNA.

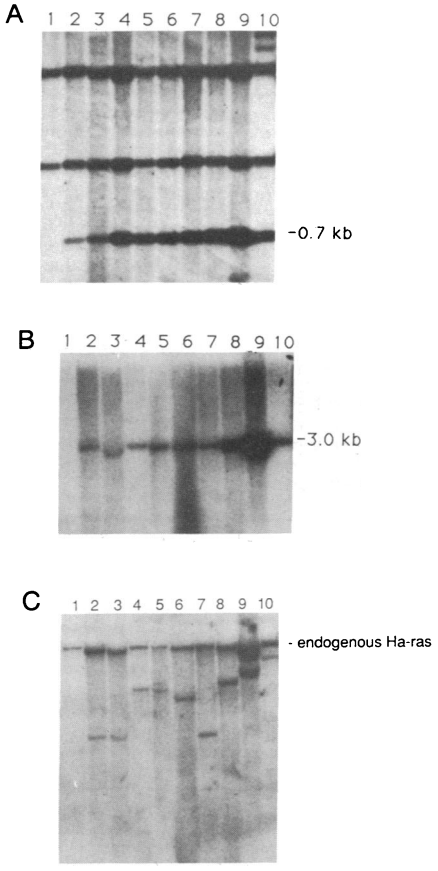

FIG. 4. Detection of the virally transduced oncogenes. Southern blotting analysis of tumors induced by infection of animals with the *ras/myc* virus. The samples include brain lesions (lanes 2 and 7), subcutaneous growths (lanes 3–6, with lanes 4 and 5 representing different parts of the same lesion), a liver tumor (lane 8), a subcutaneous growth produced from subcutaneous injection of *ras/myc* virus into a young animal (lane 9), the *ras/myc* producer cell line (lane 10), and uninfected tissue (lane 1). (*A*) The DNA samples were digested with *Bam*HI and probed with the v-Ha-*ras* gene. (*B*) The DNA samples were digested with *Bgl* II and probed with the v-*myc* gene. (*C*) The nitrocellulose filter from *B* was washed under denaturing conditions to remove the v-*myc* probe and was reprobed with the v-Ha-*ras* gene to analyze the site of integration.

DISCUSSION

The aim of this study was to assess the transforming potential of the *ras* and *myc* oncogenes in the developing embryo. For this, replication-defective retroviral vectors that transduced either the *ras*, the *myc*, or both oncogenes were microinjected into midgestation mouse embryos. Because previous studies had shown that retroviruses are expressed in cells of most or all tissues of the midgestation embryo (8, 9), we expected that the virus would be expressed in every infected cell and that the spectrum of tumors arising in animals derived from injected embryos would reflect the oncogenic spectrum of the oncogenes.

The neoplasms induced by the *myc* virus alone were rare and resulted in a low incidence of diverse tumors that developed after a latency period of 15–22 weeks. When midgestation embryos were exposed to a vector transducing the human c-*HRAS* oncogene, tumor formation was more efficient and predominantly benign skin neoplasms appeared in 24% of the animals with a median latency of 4 weeks (12). In contrast to the results obtained with the single oncogene carrying viruses, the *ras/myc* double oncogene virus had a dramatic effect on the fetuses. When concentrated virus with a titer comparable to that used for the single oncogene viruses was injected, only 1% of the injected fetuses survived to birth. Only when the virus stock was diluted prior to injection were live animals obtained, 27% of which developed rapidly growing tumors in a variety of tissues. Animals infected with the *ras* and *myc* oncogenes frequently developed lesions in the nervous system involving the brainstem, cerebellum, and

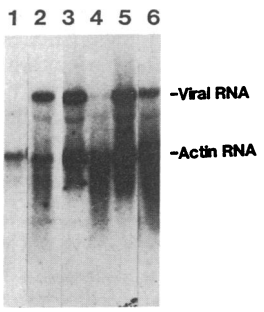

FIG. 5. Detection of viral transcripts. RNA from three subcutaneous tumors (lanes 3, 4, and 6), and a cell culture of one tumor (lane 5; from tumor in lane 3), a ψ-2 cell line without *ras* (lane 1) and from the *ras/myc* ψ-2 producer cell line (lane 2) were analyzed by Northern blotting with sequential hybridization to the *ras* gene and then the actin gene. *ras*-specific and actin-specific RNA species are indicated.

cerebrum, whereas no brain lesions were promoted upon infection with the *ras* or *myc* oncogene alone. The cell types transformed by infection with both *ras* and *myc* oncogenes were diverse and included cells of neural, connective, and epithelial tissues but not of the hemopoietic system. It is possible that these are the fastest growing tumors, which kill the animal before slower tumors of other tissues become evident. Our results indicate that many cells of the developing embryo are highly susceptible to the combined expression of both oncogenes and are consistent with the hypothesis that the *ras* and *myc* oncogenes act synergistically *in vivo* in cellular transformation. It should be noted that the *ras* oncogene in the two *ras*-transducing vectors had a different origin. While the *ras/myc* double oncogene virus carried the v-Ha-*ras* gene, the DOEJ vector transduced the human c-*HRAS* gene (12). We therefore do not have direct evidence that the v-Ha-*ras* oncogene causes a similar phenotype as the human c-*HRAS* gene when injected into midgestation embryos. However, because both oncogenes have been shown to have identical biological effects when infecting tissue culture cells or when injected into newborn mice (17), our data strongly suggest that the combination of the *ras* and *myc* oncogenes not only significantly enhances the overall efficiency of tumor induction but also dramatically expands the range of cells that are vulnerable to transformation.

The oncogenes transduced by the vectors were transcriptionally controlled from the viral long terminal repeat, which has been shown to be efficiently expressed in cells of most or all tissues of the midgestation embryo (8, 9, 18). The spectrum of tumors, therefore, does not reflect tissue-restricted expression of the oncogenes but rather their intrinsic oncogenic potential. The oncogenic potential of an oncogene may be affected by characteristics that are particular for certain tissues. Thus, while the combination of *ras* and *myc* induced tumors in many tissues, transformation induced by the *ras* oncogene alone was predominantly restricted to the skin. This is consistent with the hypothesis that tissue-specific factors such as expression of the cellular *myc* gene, which is elevated in skin (19), cooperate with the *ras* oncogene in tumor promotion. Cooperation of both oncogenes has also been seen in prostate organ culture (11). When the *ras* or *myc* oncogene was introduced singly, only premalignant lesions were induced. In contrast, infection of these cultures with the *ras/myc* virus resulted in aggressively growing and malignant tumors of various types.

The *ras* and *myc* oncogenes have been expressed in transgenic mice from a variety of different tissue-specific promoters (7, 20–25). While joint expression of both oncogenes resulted in accelerated tumor formation, single tumors appeared after a variable latency period, suggesting that although the *ras* and *myc* oncogenes cooperate in tumor formation, coexpression of both oncogenes was not sufficient, but that additional events were necessary for malignant progression (7). Similarly, the latency between infection of embryos with the *ras/myc* virus and the development of lesions is consistent with the premise that other alterations are necessary for malignancy to occur.

Since normal cells can, in fact, repress the transformed phenotype of cells expressing oncogenes (26, 27), part of the progression to malignancy is believed to be the capacity of a mutant cell to grow in the presence of normal tissue. Because in transgenic animals the promoter controlling the oncogenes is activated in many or all cells of a given tissue, the neoplastic proliferation may not be due to clonal expansion of a single transformed cell, but rather to abnormal growth of a group of cells. Because of the lack of suitable markers, the clonality of the tumors could not be assessed in studies with transgenic animals. In contrast, after injection of virus into midgestation embryos, only single cells become infected and a tumor must be the consequence of clonal outgrowth of a transformed cell. In our studies, Southern blots using the provirus as a convenient molecular marker indicated that all *ras/myc*-induced tumors were of monoclonal origin.

Human carcinomas have been shown to be of clonal origin (28–30). The infection of midgestation embryos with oncogenic viruses may mimic the pathogenesis of naturally occurring tumors where a cell with a spontaneous mutation expands clonally to form a tumor in the presence of normal tissue. The exposure of mouse embryos to different oncogenes or combinations of oncogenes may, therefore, provide a useful and rapid means to assess their oncogenic potential within the developing organism.

We thank Philip Chassler for superb technical assistance in the histological preparations. We thank Drs. Michael Imber, Janina Longtine, and James Morris for helpful discussions regarding the pathology of the lesions and Dr. Geraldine Pinkus for helpful discussions and for generously providing antibodies for our analyses. S.J.C. is a fellow of the Cancer Research Institute. P.B. is a visiting scientist from the Pasteur Institute. A.H.S. is a Lucille P. Markey Scholar. This research was funded by National Institutes of Health Grants 5RO1 HD-19015 and (OIG) 5R35CA44339.

1. Bishop, J. M. (1987) *Science* **235**, 305–310.
2. Weinberg, R. A. (1985) *Science* **230**, 770–776.
3. Konstantinidis, A., Smulow, J. B. & Sonnenschein, C. (1982) *Science* **216**, 1235–1237.
4. Land, H., Parada, L. F. & Weinberg, R. A. (1983) *Nature (London)* **304**, 596–602.
5. Land, H., Parada, L. F. & Weinberg, R. A. (1984) *Science* **222**, 771–778.
6. Ruley, H. E. (1983) *Nature (London)* **304**, 602–606.
7. Sinn, E., Muller, W., Pattengale, P., Tepler, I., Wallace, R. & Leder, P. (1987) *Cell* **49**, 465–475.
8. Jaenisch, R. (1980) *Cell* **19**, 181–188.
9. Jähner, D., Stuhlmann, H., Stewart, C. L., Harbers, K., Löhler, J., Simon, I. & Jaenisch, R. (1982) *Nature (London)* **298**, 623–628.
10. Stuhlman, H., Cone, R., Mulligan, R. C. & Jaenisch, R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7151–7155.
11. Thompson, T. C., Southgate, J., Kitchener, G. & Land, H. (1989) *Cell*, in press.
12. Compere, S. J., Baldacci, P. A., Sharpe, A. H. & Jaenisch, R. (1989) *Mol. Cell. Biol.* **9**, 6–14.
13. Mann, R., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **33**, 153–159.
14. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (1982) in *Molecular Biology of Tumor Viruses*, eds. Weiss, R., Teich, N.,Varmus, H. & Coffin, J. (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Cone, R. D., Weber-Benarous, A., Baorto, D. & Mulligan, R. C. (1987) *Mol. Cell. Biol.* **7**, 887–897.
16. Sheehan, C. & Hrapchak, B. B. (1980) *Theory and Practice of Histotechnology* (Mosby, Saint Louis, MO).
17. Tabin, C. J. & Weinberg, R. (1985) *Virology* **53**, 260–265.
18. Sharpe, A. H., Jaenisch, R. & Ruprecht, R. (1987) *Science* **236**, 1671–1674.
19. Dotto, G. P., Gilman, M. Z., Maruyama, M. & Weinberg, R. A. (1986) *EMBO J.* **5**, 2853–2857.
20. Groner, B., Schonenberger, C.-A. & Andres, A. C. (1987) *Trends Genet.* **3**, 306–308.
21. Andres, A. C., Schönenberger, C. A., Groner, B., Hennighausen, L., LeMeur, M. & Gerlinger, P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1299–1303.
22. Quaife, C. J., Pinkert, C. A., Ornitz, D. M., Palmiter, R. D. & Brinster, R. L. (1987) *Cell* **48**, 1023–1034.
23. Compere, S. J., Baldacci, P. A. & Jaenisch, R. (1988) *Biochim. Biophys. Acta* **948**, 129–149.
24. Suda, Y., Aizawa, S., Hirai, S., Inoue, T., Furuta, Y., Suzuki, M., Hirohashi, S. & Ikawa, Y. (1987) *EMBO J.* **6**, 4055–4065.
25. Leder, A., Pattengale, P. K., Kuo, A., Stewart, T. A. & Leder, P. (1986) *Cell* **45**, 485–495.
26. Land, H., Chen, A. C., Morgenstern, J. P., Parada, L. F. & Weinberg, R. A. (1986) *Mol. Cell. Biol.* **6**, 1917–1925.
27. Dotto, G. P., Weinberg, R. A. & Ariza, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6389–6393.
28. Vogelstein, B., Fearon, E. R., Hamilton, S. R. & Feinberg, A. P. (1985) *Science* **227**, 642–645.
29. Fearon, E. R., Hamilton, S. R. & Vogelstein, B. (1987) *Science* **238**, 193–197.
30. Forrester, K., Almoguera, C., Han, K., Grizzle, W. E. & Perucho, M. (1987) *Nature (London)* **327**, 298–303.