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Three human transforming genes are related to the viral *ras* oncogenes

(human tumor cells/molecular cloning/gene families)

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ABSTRACT **Three distinct transforming genes present in human tumor cell lines are all related to the viral oncogenes of Harvey and Kirsten murine sarcoma viruses, designated v-H-*ras* and v-K-*ras*, respectively. The transforming gene of a bladder carcinoma cell line has been shown to be a human homolog to v-H-*ras* [Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. (1982) *Nature (London)* 297, 474–478; Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S. & Barbacid, M. (1982) *Nature (London)* 298, 343–347]. The transforming gene common to one colon (SK-CO-1) and two lung carcinoma (SK-LU-1 and Calu-1) cell lines is the same human homolog of v-K-*ras* as is the transforming gene previously identified in a lung carcinoma cell line Lx-1 [Der, C. J., Krontiris, T. G. & Cooper, G. M. (1982) *Proc. Natl. Acad. Sci. USA* 79, 3637–3640]. The transforming gene of SK-N-SH neuroblastoma cells is weakly homologous to both v-H-*ras* and v-K-*ras*. NIH 3T3 cells transformed with the SK-N-SH transforming gene contain increased levels of a protein serologically and structurally related to the protein products of the v-H-*ras* and v-K-*ras* genes. Therefore, it represents a third member of the *ras* gene family, which we have called N-*ras*. Based on the homology with the v-*ras* genes, we have established the orientation of transcription and approximate coding regions of the cloned human K-*ras* and N-*ras* genes.**

The progression of a cell from normalcy to malignancy may be due in part to the activation of transforming genes of cellular origin. The existence of cellular transforming genes has been demonstrated by the ability of genomic DNAs from certain tumors and cell lines to induce foci of transformed NIH 3T3 cells after DNA-mediated gene transfer. Transforming genes in rodent (1, 2) and human (3–9) tumor cells have been detected in this way. We have detected three distinct transforming genes in our study of 21 human tumor cell lines: one common to two lung carcinoma (SK-LU-1 and Calu-1) and colon carcinoma (SK-CO-1) cell lines, one in a bladder carcinoma (T24), and one in a neuroblastoma (SK-N-SH) cell line (9).

Several research groups have shown that certain transforming genes detected by transfer to NIH 3T3 cells are related to viral oncogenes. Der *et al.* (5), Parada *et al.* (10), and Santos *et al.* (11) have demonstrated that the transforming gene of T24 and EJ, two human bladder carcinoma cell lines that probably are derived from the same source (unpublished data), is the human homolog of v-H-*ras*, the oncogene of the Harvey sarcoma virus. Der *et al.* (5) have also shown that the transforming gene of Lx-1, a human lung carcinoma cell line, is a human homolog of v-K-*ras*, the oncogene of the Kirsten sarcoma virus. The genes that we have isolated from human tumor cell lines are related also to the viral oncogenes, designated v-*onc*. We demonstrate that the transforming gene common to Calu-1, SK-LU-1, and SK-CO-1, like the transforming gene in Lx-1 characterized by Der *et al.* (5), is a human homolog to v-K-*ras*. We also demonstrate that the transforming gene of SK-N-SH is related to both v-K-*ras* and v-H-*ras* and probably codes for an immunologically crossreactive and structurally related protein. Based on the homology with the v-*ras* genes, we have established the orientation of transcription and probable coding regions of these genes.

MATERIALS AND METHODS

Human Tissue and Tissue Culture Cell Lines. T24, Calu-1 SK-LU-1, SK-CO-1, and SK-N-SH are human tumor cell lines (9). HT14B is a NIH 3T3 cell line transformed by Harvey sarcoma virus unintegrated viral DNA. Other transformed cell lines are described in the text.

Preparation of DNA. DNA was prepared from tissue culture cells by $NaDodSO_4$/proteinase-K lysis and phenol/chloroform extraction as described (9). Plasmid and bacteriophage DNAs were prepared as described (12, 13).

Enzymes. Restriction endonucleases were purchased from New England BioLabs and Bethesda Research Laboratories and used according to suppliers' instructions. *E. coli* DNA polymerase I was purchased from Bethesda Research Laboratories, and pancreatic DNase I was from Worthington Biochemicals.

Southern Filter DNA Blot Hybridization. DNA samples were digested with restriction endonucleases and subjected to agarose gel electrophoresis and filter-blot transfer by the method of Southern (14). Filter-blotted DNAs were hybridized with a nick-translated ^{32}P-labeled DNA probe under two sets of conditions. Stringent hybridization conditions entailed hybridization in a mixture containing 6× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M Na citrate, pH 7.0), Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.2% bovine serum albumin), and denatured salmon sperm DNA (20 μg/ml) for 16 hr at 74°C (15), followed by sequential washing at 74°C with 2×, 1×, and 0.5× NaCl/Cit in 0.1% $NaDodSO_4$. Nonstringent hybridization conditions entailed hybridization in a mixture containing 30% (vol/vol) formamide, 6× NaCl/Cit, 2× Denhardt's solution, *E. coli* DNA (100 μg/ml), yeast RNA (200 μg/ml), 50 mM sodium phosphate (pH 7), and 10 mM EDTA at 37°C for 36 hr, followed by washing at 50°C in 6× NaCl/Cit/0.1% $NaDodSO_4$. Hybridized DNA was revealed by autoradiography.

Immunoprecipitation of Cellular Protein with Rat Anti-*ras* p21 Antiserum. NIH 3T3 normal and transformed cells were

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Abbreviations: NaCl/Cit, 0.15 M sodium chloride/0.015 M sodium citrate, pH 7.0; kbp, kilobase pairs.

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labeled in methionine-free medium containing 20 μCi (1 Ci = 3.7×10^{10} Bq) of [^{35}S]methionine per ml (New England Nuclear) for 18 hr. Labeled cells were lysed in phosphate-buffered saline containing 1% Triton X-100, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, and 2 units of aprotinin per ml, and the lysates were sheared through a syringe and clarified at 100,000 × *g* for 45 min at 4°C. Clarified supernatants were preabsorbed with goat anti-rat IgG and *Staphylococcus aureus* protein A. Immunoprecipitation was performed with anti-v-H-*ras* p21 rat monoclonal antibody Y13-259 (16) (the gift of M. Furth and E. M. Scolnick) for 5 hr at 4°C, followed by addition of goat anti-rat IgG for 1 hr. Immune complexes were absorbed to protein A, and the protein A suspension was washed extensively in lysis buffer. Protein A pellets were boiled in NaDodSO$_4$ sample buffer and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis by the method of Blattler *et al.* (17). Radiolabeled proteins in gels were visualized by fluorography.

Molecular Clones. Clones of avian and mammalian v-*onc* genes are described in Table 1. λT24 and λP3 are clones from λL47.1 genomic libraries containing the transforming gene of the T24 cell line and the nontransforming homologous human sequences, respectively (25). pT24 is a pBR322 derivative with a 6.2-kilobase-pair (kbp) *Bam*HI insert bearing the T24 transforming gene. The SK-N-SH transforming gene is contained on a Charon 4A recombinant phage, λNPS-1-1-1 as described (26). (See also Fig. 4*B*.) λNPS-1-1-1 also contains portions of pBR322 and the *E. coli* tRNA *sup F* gene.

Cloning the Transforming Gene of Calu-1. An initial DNA clone of part of the transforming sequences of the Calu-1 transforming gene was obtained by using the strategy of Gusella *et al.* (27) as described by others (28, 29). DNA was prepared from NIH 3T3 secondary and tertiary transformants containing the transforming gene of Calu-1. Phage libraries were prepared from these DNAs in λ Charon 4A (30) by the method of Hohn and Murray (31) and screened for the presence of human sequence by the method of Benton and Davis (32); the probe was "BLUR8", a clone of the dispersed, repeated human "*Alu*" family sequences (33). One λ phage clone, λL2-34, was isolated this way. Unique sequence DNAs from this clone were then used as probes for isolating "contiguous" DNA from our λ Charon 4A libraries. More than 20 independent phages containing inserts with overlapping restriction endonuclease maps were isolated in this manner. A representative set of five overlapping phage isolates, together with a composite restriction endonuclease map of 26 kbp of cloned DNA, is shown in Fig. 4*A*. pLC3 is the 3.0-kbp *Eco*RI fragment of λL2-11 cloned into the *Eco*RI site of pBR322 (see Fig. 4*A*).

Table 1. Molecular clones of v-*onc* genes

v-*onc* designation	Virus of origin*	Molecular clone	Restriction fragments bearing v-*onc*	Ref. no.
fps†	PRCII SV	pRCII-1B	*Kpn* I 1.5 kbp	M. Bishop‡
yes†	Y73 SV	λY73-11A	*Sst* I 4.0 kbp	18
rel†	ARVT	prel	*Eco*RI 0.8 kbp	19
ski†	SKV	pvski-1	*Xho* I 2.8 kbp	Unpublished data
abl§	Abelson MuLV	pABsub3	*Hin*dIII/*Sst* II 2.0 kbp	20
fes§	Feline SV	pGA-FeSV	*Pst* I 0.5, 0.55 kbp	21
mos§	Moloney MuSV	pmos-1	*Pst* I 0.45 kbp	D. Dina‡
H-*ras*§	Harvey MuSV	pBS-9	*Eco*RI 0.5 kbp	22
		pHB-11	*Eco*RI/*Bam*HI 2.2 kbp	22
K-*ras*§	Kirsten MuSV	pHiHi-3	*Eco*RI 1.0 kbp	22
		pKBE-2	*Eco*RI/*Bam*HI 3.1 kbp	22
fms§	McDonough feline SV	λSM-FeSV	*Kpn* I 2.8, 4.8 kbp	23
sis§	Simian SV	pvsis	*Eco*RI/*Sal* I 2.1 kbp	24

The table lists the v-*onc* sequences that were tested for homology to three human transforming genes.
* SV, simian virus; ARVT, avian reticuloendotheliosis virus T; SKV, Sloan–Kettering virus; Mu, murine.
† Avian.
‡ Personal communication.
§ Mammalian.

RESULTS

Three Human Transforming Genes Have Homology to Viral *ras* Genes. Molecular clones of v-*onc* were cleaved with restriction endonucleases to separate v-*onc* and vector sequences, and triplicate aliquots of these digests were subjected to agarose gel electrophoresis and Southern nitrocellulose filter blotting. The three replica filters were hybridized at low stringency to ^{32}P-labeled recombinant DNAs containing all or part of the three different human transforming genes (Fig. 1). The transforming human genes were those isolated from the bladder carcinoma cell line T24 (Fig. 1*B*), the lung carcinoma cell line Calu-1 (Fig. 1*C*), and the neuroblastoma cell line SK-N-SH (Fig. 1*D*).

All three human transforming genes showed homology to v-H-*ras* and v-K-*ras* (Fig. 1, lanes 8–11). The human transforming genes were not homologous to nine other v-*onc* genes (Fig. 1, lanes 1–7, 12, and 13). The hybridization detected in other lanes of this figure represent hybridization between pBR322 plasmid and λ phage DNA sequences in the probes and on the filters. It is not surprising that each human transforming gene that hybridized with one also hybridized with both v-H-*ras* and v-K-*ras* because these v-*onc* genes share sequence homology and encode immunologically and structurally related proteins (22).

To explore further the homology between these genes, we hybridized each v-*ras* gene separately under conditions of high stringency to Southern blotted DNAs of the T24, SK-N-SH, and Calu-1 transforming genes and to pBR322 clones containing v-H-*ras* and v-K-*ras* (Fig. 2). As expected, v-H-*ras* hybridized well to the T24 transforming gene (Fig. 2*A*, lane c) and to the normal allele of this gene (Fig. 2*A*, lane d). The v-H-*ras* probe hybridized only weakly to a 3.0-kbp *Eco*RI restriction endonuclease fragment of the Calu-1 transforming gene (Fig. 2*A*, lane e) and to two *Eco*RI DNA fragments of the SK-N-SH transforming gene (Fig. 2*A*, lane g). In contrast, the v-K-*ras* probe was most closely related to the Calu-1 transforming gene, hybridizing to 3.1-, 3.0-, and 2.4-kbp *Eco*RI DNA fragments of this gene (Fig. 2*B*, lanes l and m). Longer autoradiography of the filter showed weak hybridization between v-K-*ras* and the T24 transforming gene (Fig. 2*C*, lanes j and k) and the two *Eco*RI fragments that comprise the SK-N-SH transforming gene (Fig. 2*C*, lane n).

In summary, all three human transforming genes shared homology to the v-*ras* genes. The T24 transforming gene was closest to v-H-*ras*, the Calu-1 transforming gene was closest to v-K-*ras*, and the SK-N-SH transforming gene was more distantly related to the v-*ras* genes.

The Lung and Colon Carcinoma Transforming Gene Is a Human Homolog of v-K-*ras*. DNAs from normal and transformed NIH 3T3 cells and from human cells were cleaved with restriction endonuclease *Eco*RI and subjected to gel electrophoresis and filter-blot hybridization, with the pKBE-2 clone

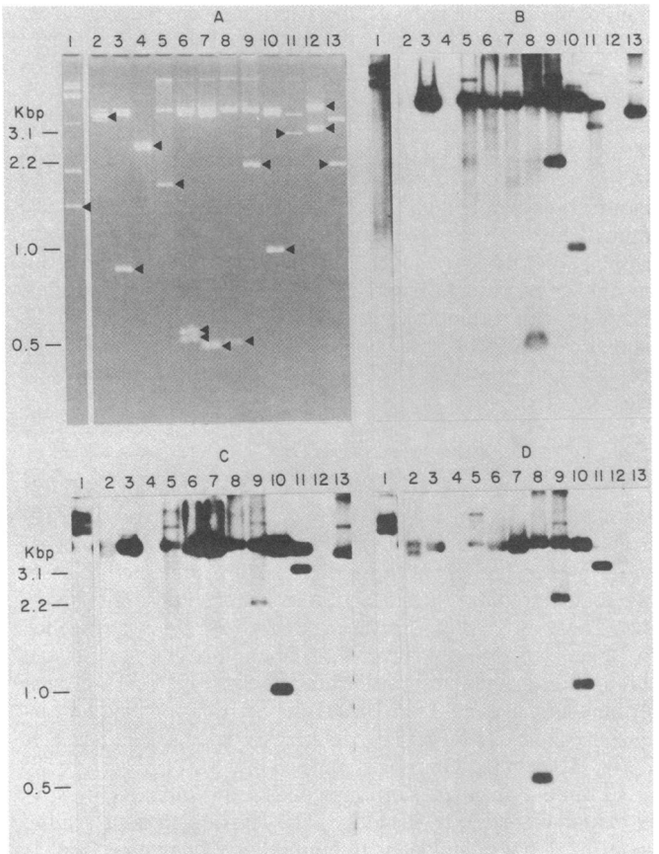

FIG. 1. Southern filter hybridization of three human transforming genes to v-*onc* DNA sequences. Molecular clones of retroviral oncogenes were digested with restriction endonucleases to separate oncogenic sequences from plasmid or bacteriophage DNA vectors. Digests were subjected to electrophoresis through 1% agarose gels, and the DNAs were transferred from gels to nitrocellulose filter papers (14). Filters were hybridized with cloned human transforming gene DNAs, which had been labeled with ^{32}P by nick translation, and filters were subsequently washed under nonstringent conditions. (*A*) Ethidium bromide stain of a gel prior to filter transfer (arrows denote restriction endonuclease fragments containing v-*onc* sequences). (*B–D*) Filter hybridizations with ^{32}P-labeled pT24 DNA (*B*), ^{32}P-labeled pLC3 (*C*), and ^{32}P-labeled λNPS-1-1-1 (*D*). Lanes show the v-*onc* DNA restriction digests: 1, v-*fps Kpn* I digest of pRCII-1B; 2, v-*yes Sst* I purified insert from λY73-11A; 3, v-*rel Eco*RI digest of prel; 4, v-*ski Xho* I purified insert from pvski-1; 5, v-*abl Hin*dIII/*Sst* I digest of pABsub3; 6, v-*fes Pst* I digest of pGA-FeSV; 7, v-*mos Pst* I digest of pmos-1; 8, v-H-*ras Eco*RI digest of pBS-9; 9, v-H-*ras Eco*RI/*Bam*HI digest of pHB-11; 10, v-K-*ras Eco*RI digest of pHiHi-3; 11, v-K-*ras Bam*HI/*Eco*RI digest of pKBE-2; 12, v-*fms Kpn* I purified inserts from λ SM-FeSV; 13, v-*sis Eco*RI/*Sal* I digest of pvsis.

of v-K-*ras* (22) used as the ^{32}P-labeled hybridization probe (Fig. 3). NIH 3T3 cells transformed with DNA from the lung and colon carcinoma cells (Fig. 3, lanes 1, 2, and 3) contain K-*ras*-related sequences not endogenous to NIH 3T3 (Fig. 3, lane 4). The newly acquired K-*ras*-related *Eco*RI fragments in these transformed cells comigrated with v-K-*ras*-related *Eco*RI fragments prominent in human DNA (Fig. 3, lane 5). These *Eco*RI fragments are 2.4, 3.0, 3.1, and ≈6.7 kbp in size. Only one high molecular weight K-*ras*-related *Eco*RI fragment in human DNA was not transferred to NIH 3T3 cells. Similar results were observed by Der *et al.* (5) in NIH 3T3 cells transformed with DNA from Lx-1, indicating that the same human K-*ras* homolog is the transforming gene of these cells.

v-K-*ras* Homologous Regions of the Calu-1 Transforming Gene. A large portion (26 kbp) of the transforming gene of the

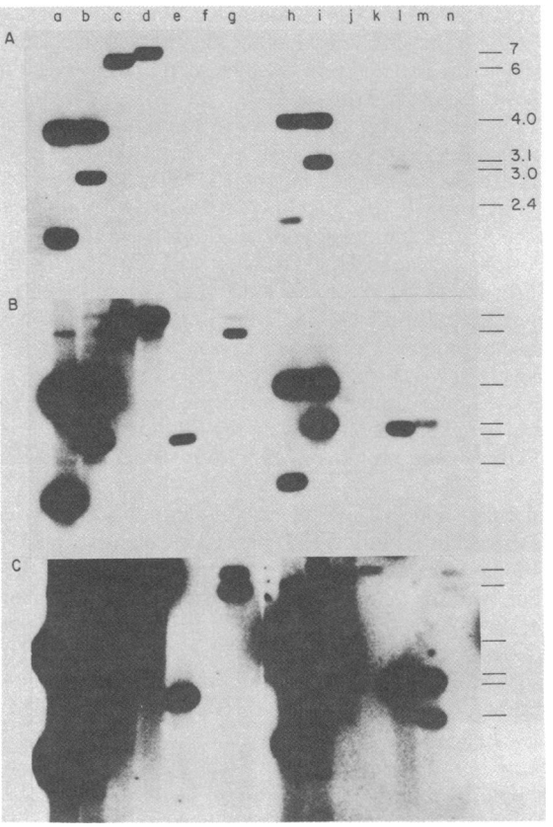

FIG. 2. Hybridization of v-*ras* sequence probes to filter-blotted human transforming gene DNAs. Cloned human transforming gene and v-*ras* gene DNAs were cleaved with restriction endonucleases, and duplicate aliquots were subjected to 1% agarose gel electrophoresis and Southern filter blotting. The filters were hybridized with either ^{32}P-labeled pHB-11 v-H-*ras* (lanes a–g) or ^{32}P-labeled pKBE-2 v-K-*ras* (lanes h–n). The filters were washed under stringent conditions. Autoradiographic exposures were for 2 hr (*A*), 12 hr (*B*), and 72 hr (*C*). Lanes: a and h, *Eco*RI/*Bam*HI pHB-11 (0.1 μg); b and i, *Eco*RI/*Bam*HI pKBE-2 (0.1 μg); c and j, *Bam*HI λT22 (1.0 μg); d and k, *Bam*HI λP3 (1.0 μg); e and l, *Eco*RI λL2-L11 (1.0 μg); f and m, *Eco*RI λL2-R7 (1.0 μg); g and n, *Eco*RI λNPS-1-1-1 (1.0 μg). Size markers are in kbp.

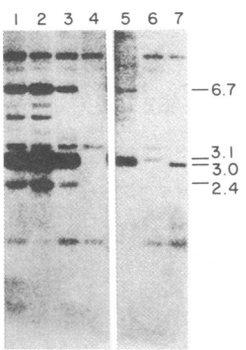

FIG. 3. Identification of lung and colon carcinoma transforming genes as K-*ras* homologs. Six micrograms of *Eco*RI-digested cellular DNA was electrophoresed through 1% agarose gels and subsequently blotted to nitrocellulose. The filters were hybridized and washed under stringent conditions with ^{32}P-labeled pKBE-2 (v-K-*ras*) as probe. Lanes: 1, NIH 3T3 transformed with SK-CO-1 DNA; 2, NIH 3T3 transformed with SK-LU-1 DNA; 3, NIH 3T3 transformed with Calu-1 DNA; 4, NIH 3T3; 5, T24; 6, NIH 3T3 with 50 pg of *Eco*RI-cleaved λL2-R7 DNA; 7, NIH 3T3 with 50 pg of *Eco*RI-cleaved λL2-11 DNA. For structure of λ clones, see Fig. 4*A*. Size markers are in kbp.

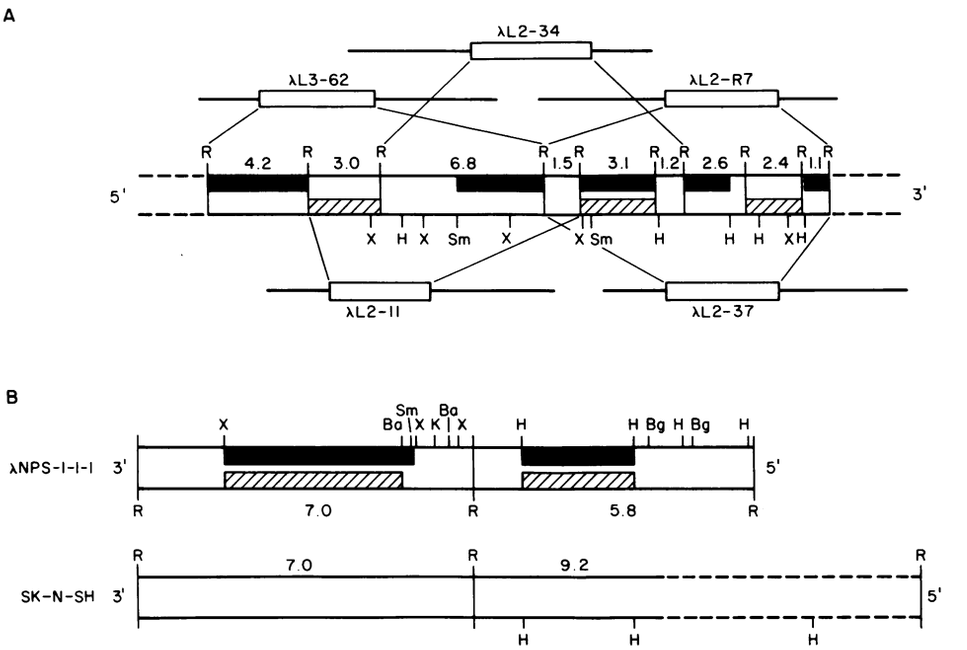

FIG. 4. Maps of *ras*-homologous regions in Calu-1 and SK-N-SH transforming genes. (*A*) Composite restriction endonuclease map of part of the Calu-1 transforming gene. The map was derived from overlapping restriction endonuclease maps of inserts in λ Charon 4A, some of which are shown above and below the map. We estimate that 30% of the gene remains to be cloned. ■, Restriction fragments that hybridize with the BLUR8 clone of human *Alu* repeat sequence DNA; ▨, restriction fragments that hybridize at high stringency to the v-K-*ras* clone pKBE-2. The 5′ and 3′ ends of the transcription unit were deduced by hybridization of the cloned Calu-1 transforming gene with 5′ and 3′ specific restriction endonuclease fragments of pKBE-2. (*B Upper*) Restriction endonuclease map of the SK-N-SH transforming gene contained within λNPS-1-1-1 is taken from Shimizu *et al.* (26). (*B Lower*) 5′ flanking sequences of *B Upper* have undergone rearrangement, showing the restriction map of the genomic SK-N-SH transforming sequence (26). ■, Restriction fragments that hybridize with the BLUR8 clone; ▨, restriction fragments that hybridize at high stringency with the v-H-*ras* clone pHB-11. The 5′ and 3′ orientations of the transcription unit were determined as above. Numbers show distances in kbp. Sites for restriction endonucleases are: Ba, *Bam*HI; Bg, *Bgl* II; H, *Hin*dIII; K, *Kpn* I; R, *Eco*RI; Sm, *Sma* I; and X, *Xba* I.

human lung carcinoma cell line Calu-1 was cloned into partially overlapping λ Charon 4A phages as described. A composite restriction endonuclease map for this gene is shown in Fig. 4*A*. Three separate regions of homology to v-K-*ras* were determined by hybridization analysis, comprising 3.0-, 3.1-, and 2.4-kbp *Eco*RI fragments (see Fig. 2, lanes l and m). All three v-K-*ras*-related *Eco*RI fragments and a fourth 6.7-kbp *Eco*RI fragment, which has not been cloned yet, were present in all NIH 3T3 cells transformed with DNA from various lung and colon carcinoma cell lines (Fig. 3, lanes 1–3, 6, and 7). These Kirsten homologous regions do not arise by tandem gene duplications because they hybridized to discrete regions of the cloned v-K-*ras* gene (data not shown). Indeed, hybridization with specific v-K-*ras* DNA fragments allowed us to make an unambiguous assignment of the direction of transcription (see Fig. 4*A*). In contrast to the small T24 transforming gene, which is entirely contained on a 2.9-kbp *Sac* I fragment (25), the transforming gene of Calu-1 is probably greater than 30 kbp.

The SK-N-SH Neuroblastoma Transforming Gene Is a New Member of the *ras* Gene Family. Although the SK-N-SH neuroblastoma transforming gene was weakly homologous to both v-H-*ras* and v-K-*ras*, we reasoned that it may encode a protein structurally and serologically related to Harvey and Kirsten *ras* gene product. We tested this possibility by using a broadly reactive monoclonal antibody against *ras*-encoded protein to immunoprecipitate [^{35}S]methionine-labeled extracts from three independently derived NIH 3T3 transformants containing the SK-N-SH transforming gene. Immune precipitates from these cells and from NIH 3T3 transformed by Harvey sarcoma virus unintegrated DNA, NIH 3T3 transformed by DNAs from human lung and colon carcinoma cells, and NIH 3T3 itself were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 5). A protein with an apparent M_r of 19,000 was seen in immunoprecipitates of v-H-*ras*-transformed NIH 3T3 (Fig. 5, lane 2) but not in NIH 3T3 controls (Fig. 5, lane 1). A similarly migrating protein was seen in NIH 3T3 cells transformed with either

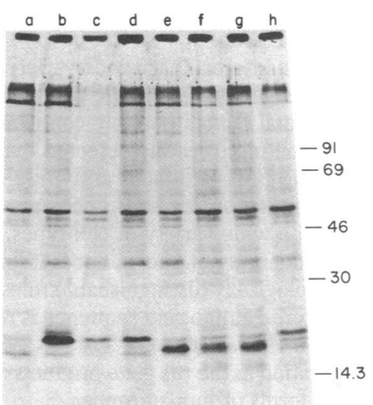

FIG. 5. *ras*-Related proteins in NIH 3T3 transformed cells. Cells (5×10^5) were labeled for 18 hr with 80 μCi of [^{35}S]methionine and extracted with nonionic detergents, and cleared lysates were used for immunoprecipitation with rat anti-*ras* p21 monoclonal antibody Y13-259 (16). Immunocomplexes were collected onto *S. aureus* protein A, dissolved and boiled in NaDodSO$_4$ sample buffer, and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis on a 12.5% polyacrylamide gel. Proteins were visualized by fluorography. Lanes: a, NIH 3T3; b, HT14B; c and d, two NIH 3T3 lines independently transformed with Calu-1 DNA; e–g, three NIH 3T3 lines independently transformed with SK-N-SH DNA; h, one NIH 3T3 line transformed with SK-CO-1 DNA. A ^{14}C-labeled protein mixture (Amersham) provided M_r standards (shown $\times 10^{-3}$).

Calu-1 or SK-CO-1 DNA (Fig. 5, lanes 3, 4, and 8). A uniquely migrating protein with an apparent M_r of 17,500 was seen in each NIH 3T3 transformant containing the SK-N-SH transforming gene (Fig. 5, lanes 5–7). This protein had an isoelectric point similar to that found for the v-H-*ras*-encoded protein (data not shown). Our findings indicate that the SK-N-SH neuroblastoma transforming gene is another member of the *ras* gene family.

We exploited the homology between the SK-N-SH transforming gene and the v-*ras* genes to determine its direction of transcription and approximate regions of homology (see Fig. 4*B*).

DISCUSSION

Three different human transforming genes that can be detected by the NIH 3T3 transformation assay are members of the *ras* gene family. The transforming gene of a bladder carcinoma cell line (T24) is a human homolog of the v-H-*ras* gene (5, 10, 11). The transforming gene of Lx-1, a human lung carcinoma cell line, is a human homolog of v-K-*ras* (5). Comparison of the work of Der *et al.* (5) with ours indicates the presence of the same transforming gene in two lung carcinoma cell lines (SK-LU-1 and Calu-1) and in one colon carcinoma cell line (SK-CO-1). This same gene is also detectable by DNA transfer in human lung and colon tumors maintained in *nude* mice (unpublished data) and in the colon carcinoma cell line SW480 (3, 9). The transforming gene of a human neuroblastoma cell line (SK-N-SH) is related to (but distinct from) the homologs of the v-H-*ras* and v-K-*ras* genes and represents a third branch within the *ras* gene family. Each branch may have more recent evolutionary offshoots. Thus, Chang *et al.* (34) reported two human homologs of v-H-*ras* (H-*ras*-1 and -2) and two homologs of v-K-*ras* (K-*ras*-1 and -2). A comparison of restriction endonuclease maps for these genes with the three human transforming genes we have isolated indicates that the T24 bladder carcinoma-transforming gene is H-*ras*-1, the lung and colon carcinoma-transforming gene is probably K-*ras*-2, and the SK-N-SH neuroblastoma-transforming gene is a heretofore uncharacterized gene. We propose calling the human transforming gene of SK-N-SH the N-*ras*-1 gene.

It is of considerable interest that a wide variety of tumor cells contain activated *ras* genes, detectable by gene transfer into NIH 3T3 cells. Several factors possibly contribute: *ras* transforming genes may be more readily detected than other transforming genes by the NIH 3T3 focus assay; *ras* genes may be easily activated by mutation; and *ras* genes may have critical cellular functions in a wide variety of cell types. The function of the *ras* gene products is not known nor is it known whether they perform physiologically distinguishable roles. However, it is known that an altered amino acid sequence is responsible for the activation of the H-*ras*-1 gene of T24 (25, 35, 36), and we speculate that alteration in the *ras* gene products may be a common step in many forms of human cancer.

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