Three human transforming genes are related to the viral ras oncogenes

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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., Krontris, T. G. & Cooper, C. 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), Parang et al. (10), and Carcin 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 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 is a structurally and 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 NaDodSO4/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. Escherichia 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 32P-labeled DNA probe under two sets of conditions. Stringent hybridization conditions entailed hybridization in a mixture containing 6 X NaCl/Cit (1 X 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 X, 1 X, and 0.5 X NaCl/Cit in 0.1% NaDodSO4. Nonstringent hybridization conditions entailed hybridization in a mixture containing 30% (vol/vol) formamide, 6 X NaCl/Cit, 2 X 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 X NaCl/Cit/0.1% NaDodSO4. Hybridized DNA was revealed by autoradiography.

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

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 \times 10^{10} \text{ Bq}) of \textsuperscript{35}S methionine per ml (New England Nuclear) for 15 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 \times 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. Furrth and E. M. Scollnick) 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\textsubscript{4}/polyacrylamide gel electrophoresis by the method of Blatter 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. AT24 and AP3 are clones from AL7.1 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 (kb) BamHI insert bearing the T24 transforming gene. The SK-N-SH transforming gene is contained on a Charon 4A recombinant phage, ANPS-1-1-1 as described (26). (See also Fig. 4B.) ANPS-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 "BLURS", a clone of the dispersed, repeated human "Alu" family sequences (33). One of three lambda clones, AL2-34, 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. 4A. pLC3 is the 3.0-kbp EcoRI fragment of AL2-11 cloned into the EcoRI site of pBR322 (see Fig. 4A).

**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 \textsuperscript{35}S-labeled recombinant DNAs containing all or part of three different human transforming genes (Fig. 1). The transforming human genes were those isolated from the bladder carcinoma cell line T24 (Fig. 1B), the lung carcinoma cell line Calu-1 (Fig. 1C), and the neuroblastoma cell line SK-N-SH (Fig. 1D).

All three human transforming genes showed homology to v-H-ras and v-K-ras (Fig. 1, lanes 5–11). The human transforming genes were not homologous to nine other v-onc genes (Fig. 1, lanes 2, 7, 12, and 13). The hybridization detected in other lanes of this figure represent hybridization between pBR322 plasmid and A 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. 2A, lane c) and to the normal allele of this gene (Fig. 2A, lane d). The v-H-ras probe hybridized only weakly to a 3.0-kbp EcoRI restriction endonuclease fragment of the Calu-1 transforming gene (Fig. 2A, lane e) and to two EcoRI DNA fragments of the SK-N-SH transforming gene (Fig. 2A, 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 EcoRI DNA fragments of this gene (Fig. 2B, lanes 1 and m). Longer autoradiography of the filter showed weak hybridization between v-K-ras and the T24 transforming gene (Fig. 2C, lanes j and k) and the two EcoRI fragments that comprise the SK-N-SH transforming gene (Fig. 2C, 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 EcoRI and subjected to gel electrophoresis and filter-blot hybridization, with the pKBE-2 clone.

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

<table>
<thead>
<tr>
<th>v-onc designation</th>
<th>Virus of origin*</th>
<th>Molecular clone</th>
<th>restriction fragments</th>
<th>v-onc Ref. no.</th>
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<tr>
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<td>PRCCII SV</td>
<td>pRCl-1B</td>
<td>Kpn 1 1.5 kbp</td>
<td>M. Bishop†</td>
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<tr>
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<td>AY73-1A</td>
<td>St 1 4.0 kbp</td>
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<td>prel</td>
<td>EcoRI 0.8 kbp</td>
<td>19</td>
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<tr>
<td>ski§</td>
<td>SKV</td>
<td>pveki-1</td>
<td>Xho 1 2.8 kbp</td>
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</tr>
<tr>
<td>ab§</td>
<td>Abelson</td>
<td>pAubeb3</td>
<td>HindIII/St II 20</td>
<td></td>
</tr>
<tr>
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<td>pGA-FeSV</td>
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<tr>
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<td>pmo-1</td>
<td>Pst I 0.45 kbp</td>
<td>D. Dina§</td>
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<td>EcoRI 0.5 kbp</td>
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<tr>
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<td>Kirsten</td>
<td>pHHi-3</td>
<td>EcoRI 1.0 kbp</td>
<td>22</td>
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<td>McDonough</td>
<td>ASM</td>
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<td>Simian SV</td>
<td>pvis</td>
<td>EcoRI/Sal 1.21</td>
<td>24</td>
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</table>

* SV, simian virus; AVRT, avian reticuloendotheliosis virus T; SKV, Sloan-Kettering virus; Mu, murine.
† Avian.
‡ Personal communication.
§ Mammalian.
were genes transferred were genic EcoRI stain mide donuclease from digests: 1, 32P-labeled ANPS-1-1-1 and digest was not served by sert by v-K-ras fragments. carcinoma colon digest of v-K-ras DNA; 1, NIH 3T3 transformed with cloned human molecular weight 3.1, v-fins DNA was not cleaved. Similar results were observed by Der et al. (5) in NIH 3T3 cells transformed with DNA from Lx-1, indicating that the same K-ras homolog is the transforming gene of these cells.

**v-K-ras Homologous Regions of the Calu-1 Transforming Gene.** A large portion (25 kbp) of the transforming gene of the

of v-K-ras (22) used as the 32P-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 EcoRI fragments in these transformed cells conigrated with v-K-ras-related EcoRI fragments prominent in human DNA (Fig. 3, lane 5). These EcoRI fragments are 2.4, 3.0, 3.1, and =6.7 kbp in size. Only one high molecular weight K-ras-related EcoRI 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 K-ras homolog is the transforming gene of these cells.

**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 32P 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 32P-labeled pT24 DNA (B), 32P-labeled pLC3 (C), and 32P-labeled ANPS-1-1-1 (D). Lanes show the v-onc DNA restriction digests: 1, v-fps Kpn I digest of pRCH-1B; 2, v-yes Sst I purified insert from λY73-11A; 3, v-rel EcoRI digest of preI; 4, v-ski Xho I purified insert from pski-1; 5, v-abl HindIII/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 EcoRI digest of pBS-9; 9, v-H-ras EcoRI/BamHI digest of pH2-11; 10, v-K-ras EcoRI digest of pHII-3; 11, v-K-ras BamHI/EcoRI digest of pKBE-2; 12, v-fms Kpn I purified inserts from λ SM-FeSV; 13, v-sis EcoRI/Sal I digest of pvsis.

**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 32P-labeled pH-11 v-H-ras (lanes a–g) or 32P-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, EcoRI/BamHI pH-11 (0.1 μg); b and i, EcoRI/BamHI pKBE-2 (0.1 μg); c and j, BamHI AT22 (1.0 μg); d and k, BamHI AP3 (1.0 μg); e and l, EcoRI AL2-L11 (1.0 μg); f and m, EcoRI AL2-R7 (1.0 μg); g and n, EcoRI ANPS-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 EcoRI-digested cellular DNA was electrophoresed through 1% agarose gels and subsequently blotted to nitrocellulose. The filters were hybridized and washed under stringent conditions with 32P-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 EcoRI-cleaved AL2-R7 DNA; 7, NIH 3T3 with 50 pg of EcoRI-cleaved AL2-L11 DNA. For structure of λ clones, see Fig. 4A. Size markers are in kbp.
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. 4A. Three separate regions of homology to v-K-ras were determined by hybridization analysis, comprising 3.0-, 3.1-, and 2.4-kbp EcoRI fragments (see Fig. 2, lanes 1 and m). All three v-K-ras-related EcoRI fragments and a fourth 6.7-kbp EcoRI 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. 4A). 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 [35S]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 NaDodSO4/polyacrylamide gel electrophoresis (Fig. 5). A protein with an apparent Mr 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

![Diagram](image-url)
Calu-1 or SK-CO-1 DNA (Fig. 5, lanes 3, 4, and 8). A uniquely migrating protein with an apparent Mr 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. 4B).

**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 that 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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