Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 383–387, January 1983
Biochemistry

Isolation and preliminary characterization of the transforming gene of a human neuroblastoma cell line

(DNA-mediated gene transfer/human oncogene/tRNA suppressor)

KENJI SHIMIZU, MITCHELL GOLDFARB, MANUEL PERUCHO*, AND MICHAEL WIGLER†

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Communicated by Barbara McClintock, October 12, 1982

ABSTRACT **DNA from the human neuroblastoma cell line SK-N-SH is capable of inducing foci of transformed NIH 3T3 cells after DNA-mediated gene transfer. Using genetic selection with the *Escherichia coli sup F* gene, we have isolated human sequences from mouse cells responsible for the oncogenic transformation. These sequences are present in all human DNAs surveyed and no gross rearrangements of these sequences are found in SK-N-SH cells. Although clearly distinct from two other human transforming genes present in bladder, lung, and colon carcinoma cell lines, all three transforming gene sequences may be related members of the *ras* gene family.**

Several types of experiments have indicated the existence of oncogenes of cellular origin. One set, the oncogenes present in the rapidly transforming retroviruses (the v-*onc* genes), has been detected by their homology to cellular genes (1–6). These cellular genes (c-*onc*) presumably have normal physiological functions when residing unperturbed in their usual chromosomal setting. Another set of oncogenes, or cellular transforming genes, has been detected in the DNA of tumors and tumorigenic cell lines by their ability to induce foci of transformed cells after DNA-mediated gene transfer into NIH 3T3, a normal growth-controlled mouse fibroblast cell line (7–11). These transforming genes presumably arise from normal cellular genes by mutation (12–14). Several different human transforming genes have been reported and recently the transforming gene of a human bladder carcinoma cell line has been isolated by molecular cloning (12–14). We report here the isolation and preliminary characterization of another transforming gene, that present in the human neuroblastoma cell line SK-N-SH.

MATERIALS AND METHODS

Animal Cells. NIH 3T3 cells (15) were obtained from R. Weinberg and co-workers (7) in 1978. These cells have undergone several subclonings in our laboratory since that time to reduce spontaneous transformation. They are maintained at low cell densities in Dulbecco's medium containing antibiotics (streptomycin at 50 μg/ml and penicillin at 50 units/ml) and 10% calf serum (Flow Laboratories). Human tumor cell lines used, which have been described (10), were: neuroblastomas, SK-N-SH and IMR-32; lung carcinomas, SK-LU-1 and Calu-1; colon carcinoma, SK-CO-1; hepatoma, SK-HEP-1; and bladder carcinoma, T24. GM 2998 and XPVA-Het are low-passage human fibroblast lines and were provided by L. Grossman. PR371 is a human lung tumor that has been maintained in *nude* mice and was provided by J. Fogh. The various NIH 3T3 transformants are described below.

Preparation of DNA, Hybridization, and Restriction Endonuclease Digestion. High molecular weight DNA was prepared from animal cells as described (10). DNA was prepared from λ phage Charon 4A and its derivatives by phage banding in CsCl followed by phenol extraction (16). Plasmid DNA was prepared from chloramphenicol-amplified cultures by detergent lysis (17). DNA restriction endonuclease fragments were purified by agarose gel electrophoresis and extracted from the agarose by the KI/glass powder method (18). Agarose gel electrophoresis and Southern blotting were carried out as described (19). ^{32}P-Labeled probes were prepared by nick-translation (20) and blot hybridization was carried out at 71°C in 0.90 M NaCl/0.09 M Na citrate, pH 7.0. The final blot wash was 75 mM NaCl/7.5 mM Na citrate, pH 7.0 at 71°C. Restriction endonuclease digestions were in buffers recommended by vendors (New England BioLabs, Bethesda Research Laboratories) and restriction endonuclease mapping was done by analysis of double digestion products.

The bacterial plasmids used were blur 8 and pK5. Blur 8 contains a 300-base-pair *Bam*HI insert in pBR322 that is a member of the human *Alu* family repeat (21) and was provided by W. Jelinek. pK5 is a pBR322 derivative containing the *sup F* gene. Its complete restriction map will be published in another paper. The *sup F* gene was originally provided by I. Kudo and U. RajBhandary.

Suppressor Rescue. DNA from NP-1, an NIH 3T3 transformant derived from SK-N-SH DNA (see Fig. 1), was partially digested with *Eco*RI (0.1 unit of enzyme per μg of DNA) at 37°C for 30 min; 150 μg of DNA was loaded onto 10–40% sucrose gradients in 100 mM NaCl/50 mM Tris·HCl/10 mM EDTA, pH 8.0, and centrifuged at 24,000 rpm in a SW27 rotor for 24 hr at 20°C. Gradients were fractionated, and the 12- to 30-kilobase-pair (kbp) fractions were pooled and ethanol precipitated. DNA was dissolved, mixed (1:1, mol/mol) with a 1.5-kbp *Eco*RI-cleaved *sup F*-containing fragment from pK5 (see above), ligated at 100 μg/ml with T4 DNA ligase (Bethesda Research Laboratories) in the buffer recommended by vendor. Ligated DNA was mixed (1:3) with NIH 3T3 carrier DNA and used to transfect NIH 3T3 cells by a modification of the calcium phosphate method (22) as described (10). Transformed foci were picked after 3 wk and expanded for analysis. DNA was prepared from NIH 3T3 transformants containing *sup F*, partially digested with *Eco*RI, and size fractionated as described above. DNA in the 15- to 25-kbp range was mixed (1:1, mol/mol) with *Eco*RI "arms" of Charon 4A and ligated as described above. Ligated product was packaged into phage particles (23) and titered on the *sup E sup F E. coli* strain BNN45. To select *sup F*-containing phage derivatives, 10^6 packaged phage were plated onto a suppressor-free *E. coli* host strain, KS624. Phage yielding plaques on KS624 were picked and tested for the pres-

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: kbp, kilobase pair(s).

* Present address: Dept. of Biochemistry, State Univ. of New York, Stony Brook, NY 11794.
† To whom reprint requests should be addressed.

ence of suppressor by plating on a *lac* Z^{am} strain, JG139, in the presence of the β-galactoside indicator dye X-gal (5-bromo-4-chloro-3-indoyl β-D-galactoside) (24). Phage containing the *sup F* gave blue plaques and were picked and expanded for further analysis. A detailed description of these techniques will be given in another paper.

Recombinant DNA experiments were conducted under EK1/P2 containment conditions as specified by the National Institutes of Health guidelines.

RESULTS

Molecular Cloning of Sequences with Transforming Activity from Transformed Cells. DNA from SK-N-SH, a cell line derived from a human neuroblastoma, can efficiently transform NIH 3T3 cells (10). DNA from the NIH 3T3 transformants can in turn transform fresh NIH 3T3 cells, due to the serial passage of a transforming principle by DNA-mediated gene transfer. To isolate this transforming principle, we began with DNA from a line of NIH 3T3 cells transformed with DNA from SK-N-SH, NP-1, that had a higher specific activity of transformation than DNA from SK-N-SH itself.

We used the same strategy for gene isolation that we had used in isolating the transforming gene of the human bladder carcinoma cell line T24 (12). This method (suppressor rescue), which has been described briefly (12) and will be described in greater detail in another paper, entails ligating restriction endonuclease-digested DNA from NP-1 to a restriction endonuclease fragment containing a bacterial tRNA amber suppressor gene (*sup F*). Because the transforming activity of NP-1 DNA was sensitive to cleavage with *Eco*RI, *Bam*HI, and *Hin*dIII (10), we partially digested NP-1 DNA with *Eco*RI and then ligated size-fractionated DNA to a 1.5-kbp *Eco*RI fragment containing *sup F*. The ligated DNA was used to transform NIH 3T3 cells and DNA from the resulting transformants was used in a second round of gene transfer to yield cells containing only a single copy of *sup F* flanking the transforming gene. We used DNA from these transformants to construct *Eco*RI partial libraries in the bacteriophage λ vector Charon 4A, which contains two amber mutations. Only those phage that have undergone mutation at their amber loci or contain *sup F* can grow in a suppressor-free bacterial host. We selected in this way several phages that contained *sup F* and flanking DNA from the NIH 3T3 transformants. The lineage of four of these phages and the NIH 3T3 transformants used is shown in Fig. 1.

DNA was prepared from all four λ phages and analyzed by *Eco*RI digestion. In addition to the Charon 4A arms, all phage DNA had a 1.5-kbp *Eco*RI fragment containing *sup F* (see Fig. 2). Also, all four contained a common 7.0-kbp *Eco*RI fragment and two phage, λ NPS-1-1-1 and λ NPS-1-1-2, derived from NPS-1-1, contained a common 5.8-kbp *Eco*RI fragment. DNA from all four phages were assayed for transforming activity on NIH 3T3 cells. Only DNA from two phages, λ NPS-1-1-1 and λ NPS-1-1-2, were active. They had a specific activity of transformation (about 10^3 foci per μg of phage DNA) equal to that of DNA from phage containing the transforming gene of the bladder carcinoma cell line T24. These results suggested that we had obtained a biologically active clone of the transforming sequences of SK-N-SH.

Use of Transforming Sequences as Hybridization Probes. Restriction endonuclease sites in λ NPS-1-1-1 DNA were mapped, and the presence and positions of human or mouse repeated sequences in this DNA were determined (Figs. 2 and 3). DNA digests were electrophoresed in agarose gels, blotted onto nitrocellulose filters, and hybridized with ^{32}P-labeled nick-translated total human DNA or total mouse DNA. λ NPS-1-1-1 and λ NPS-1-1-2 sequences hybridized exclusively with human DNA (Fig. 2), consistent with an origin from the human SK-N-SH cell line.

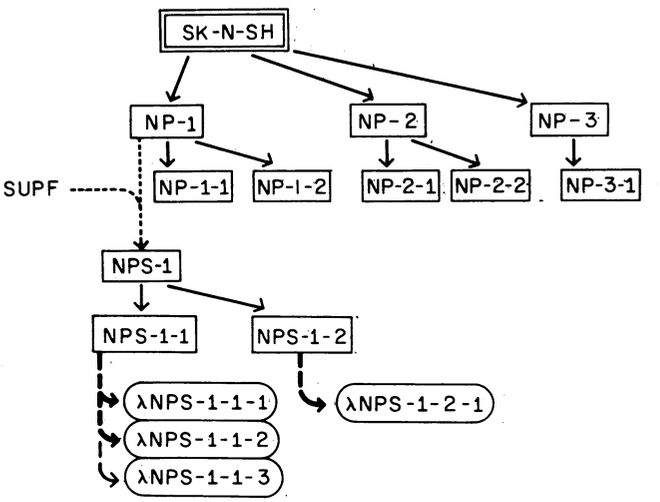

FIG. 1. Lineage of transformants and phage derivatives. DNA from the human cell line SK-N-SH was used to induce foci of transformed NIH 3T3 cells. Three foci were picked and expanded to yield the primary transformants, NP-1, NP-2, and NP-3. DNAs from each of these were then used to derive secondary transformants. In addition, DNA from NP-1 was used to isolate the transforming sequences. DNA from NP-1 was ligated to *sup F* DNA and the ligated DNA was used to induce transformed foci. DNA from one of these, NPS-1, was used to derive the additional transformed foci NPS-1-1 and NPS-1-2. From these, we derived λ phage containing *sup F*. →, DNA transfection; ⟶, ligation and transfection; ⇢, suppressor rescue.

The structure of λ NPS-1-1-1 DNA does not necessarily reflect the structure of the transforming sequences as they occur in SK-N-SH. Flanking sequences that are not essential to gene function are often lost during DNA-mediated gene transfer and linkage between previously unlinked sequences is established (25, 26). Nevertheless, we conclude that the transforming gene of SK-N-SH is probably composed of two *Eco*RI fragments because (*i*) the transforming activity of SK-N-SH is destroyed by *Eco*RI cleavage and (*ii*) two *Eco*RI fragments comprise the entire transforming sequence of λ NPS-1-1-1. To investigate the structure of the transforming gene of SK-N-SH further, we used two restriction fragments (denoted L and R in Fig. 3), one from each *Eco*RI fragment, as probes in Southern hybridizations. Neither fragment L nor fragment R contain human repetitive sequences and therefore they are good probes for unique sequence DNA.

In the first Southern hybridization experiment (Fig. 4), fragment R was used as a probe against *Eco*RI-cleaved DNA from NIH 3T3 (lane A), SK-N-SH (lane B), one NIH 3T3 secondary transformant from the human bladder carcinoma cell line T24 (lane L), one NIH 3T3 secondary transformant from the human lung carcinoma cell line Calu-1 (lane K), a series of NIH 3T3 transformants of various lineage but all ultimately derived by transfection from SK-N-SH (lanes C–J); a series of human cell lines (lanes P–Y), and human placenta (lane M). Fragment R hybridized strongly to a 9.2-kbp *Eco*RI fragment in SK-N-SH DNA, to single fragments of various sizes in each NIH 3T3 transformant derived from SK-N-SH, and to single *Eco*RI fragments in each of several human DNAs. These fragments comigrate with the 9.2-kbp fragment present in SK-N-SH. No strong hybridization was seen in either NIH 3T3 cells or NIH 3T3 cells transformed with other human transforming genes.

In the second Southern hybridization experiment (Fig. 5), a 1:1 mixture of fragments L and R was used as probe to survey

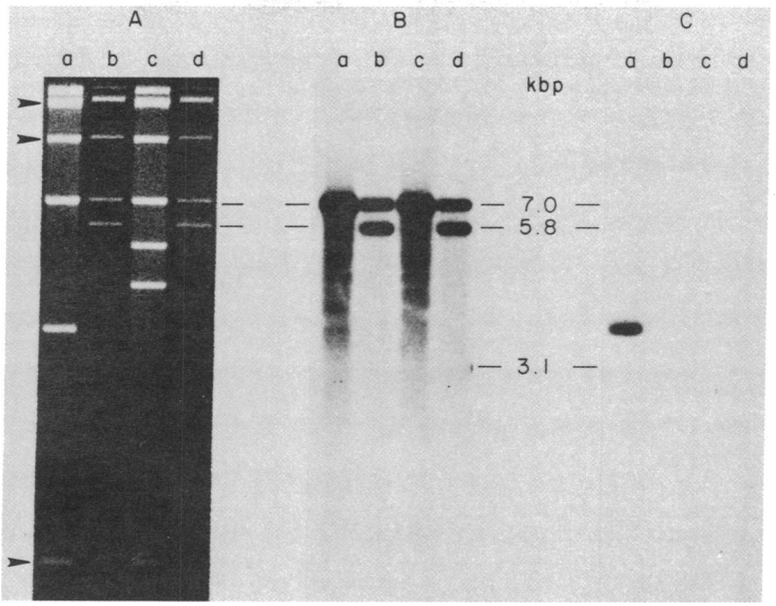

FIG. 2. Analysis of λ phage containing *sup F*. DNA (0.2–1.0 μg) from the four phage λ NPS-1-2-1 (lanes a), λ NPS-1-1-2 (lanes b), λ NPS-1-1-3 (lanes c), and λ NPS-1-1-1 (lanes d) was digested with *Eco*RI and electrophoresed in 1.0% agarose gels. Each phage is an independent isolate. (*A*) Ethidium bromide-stained gel. (*B* and *C*) DNA was denatured, transferred to nitrocellulose filters, and hybridized with total ^{32}P-labeled nick-translated human DNA (*B*) or mouse DNA (*C*). Digested DNAs contain in common 19.9- and 11.0-kbp λ arms, a 1.5-kbp fragment containing *sup F*, and a 7.0-kbp fragment hybridizing to repetitive human DNA. The 1.5-kbp fragment is not readily visible in lanes b and d, which contain less DNA than lanes a and c. In addition, λ NPS-1-1-1 (lanes d) and λ NPS-1-1-2 (lanes b) contain a common 5.8-kbp fragment that hybridizes to repetitive human DNA. λ NPS-1-2-1 contains a 3.7-kbp fragment that hybridizes to repetitive mouse DNA. Arrowheads indicate the 19.9-, 11.0-, and 1.5-kbp fragments.

the same DNAs. In this experiment, two *Eco*RI fragments appeared in all human DNAs: the 9.2-kbp fragment observed previously and a 7.0-kbp fragment. Two *Eco*RI fragments were also seen in most NIH 3T3 cells transformed with SK-N-SH DNA. The apparent exceptions, NP-1 and NP-1-2 (lanes C and E), contained two coincident *Eco*RI fragments that were separately hybridizable to fragments L and R (data not shown). Again, no strong hybridization was seen in either NIH 3T3 cells or NIH 3T3 cells transformed with other human transforming genes. Taking these two experiments together, the results imply that the transforming sequences of SK-N-SH are contained in *Eco*RI fragments of 9.2 and 7.0 kbp, that these transforming sequences are human in origin, and that the transforming gene of SK-N-SH is distinct from the transforming genes of T24 and SK-LU-1 (10–12).

We have inferred the restriction pattern of the chromosomal transforming gene of SK-N-SH (Fig. 3). We show that the gene coincides with the sequences of λ NPS-1-1-1 from the left-most *Eco*RI site to the left-most *Bgl* II site. We are confident of the left-most *Eco*RI site because the left-most *Eco*RI/*Xba* 1 fragment of λ NPS-1-1-1 hybridizes to the same *Eco*RI DNA segments as does fragment L (data not shown) and of the *Bgl* II site because *Bgl* II cleavage destroys the transforming activity of λ NPS-1-1-1 DNA (data not shown).

Distantly Related Sequences in Mice and Humans. When fragment R was used as the probe in Southern blots, weakly hybridizing *Eco*RI fragments were observed in all mouse and human DNAs. In human DNAs, three *Eco*RI fragments, of approximate sizes 4.5, 10.5, and 15.0 kbp, were observed but only a single weakly hybridizing fragment (7.0 kbp) was observed in

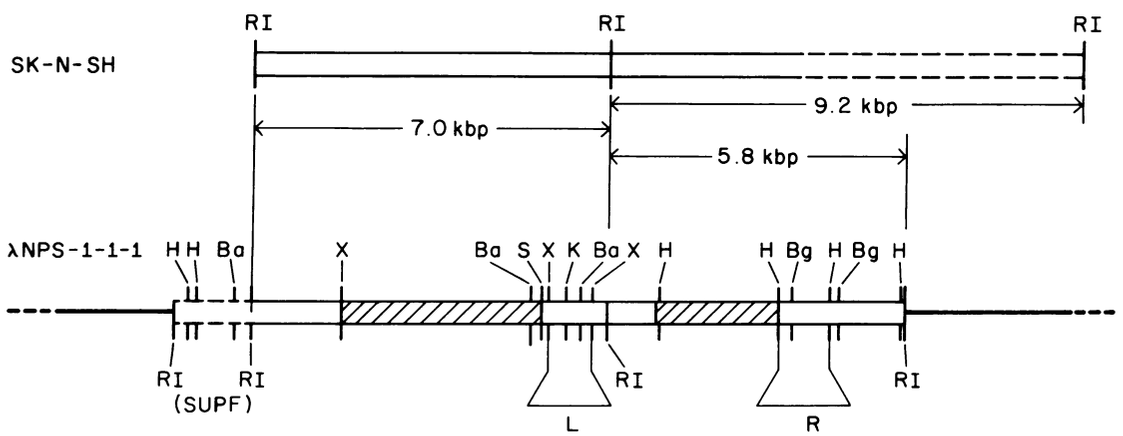

FIG. 3. Structure of the transforming sequences in SK-N-SH cells and λ NPS-1-1-1. The restriction endonuclease sites of phage λ NPS-1-1-1 insert were mapped. ▨, Regions containing human *Alu* sequences as determined by blotting with plasmid blur 8; L and R, fragments used in Southern hybridization experiments. The structure of the transforming sequences in SK-N-SH cells as inferred from the structure of λ NPS-1-1-1 and the hybridization experiments is also shown. RI, *Eco*RI; H, *Hin*dIII; Ba, *Bam*HI; X, *Xba* I; K, *Kpn* I; Bg, *Bgl* II; S, *Sma* I.

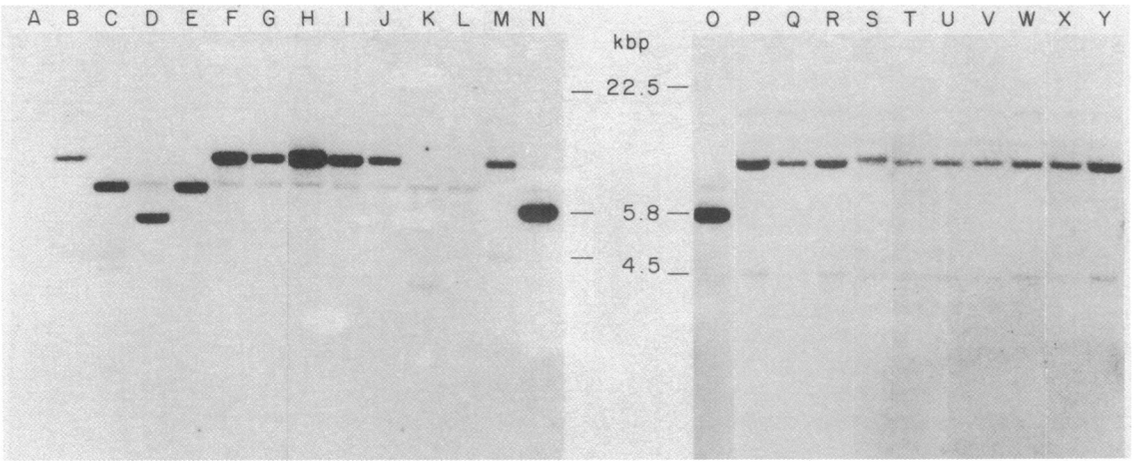

FIG. 4. Hybridization of transformant and human DNAs with SK-N-SH transforming sequences (fragment R). Five-microgram samples of DNA (lanes: A, NIH 3T3; B, SK-N-SH; C, NP-1; D, NP-1-1; E, NP-1-2; F, NP-2; G, NP-2-1; H, NP-2-2; I, NP-3; J, NP-3-1; K, an NIH 3T3 secondary transformant from Calu-1; L, an NIH 3T3 secondary transformant from T24; M, human placenta; P, SK-LU-1; Q, Calu-1; R, SK-CO-1; S, PR371; T, GM 2993; U, XPVA-Het; V, IMR-32; W, SK-N-SH; X, T24; Y, SK-HEP-1) were digested with *Eco*RI, electrophoresed in 1.0% agarose gels, and analyzed by blot hybridization with ^{32}P-labeled (3×10^8 cpm/μg) fragment R (Fig. 3) as probe. Lanes N and O contain an *Eco*RI digest of a mixture of 120 pg of λ NPS-1-1-1 DNA and 5 μg of NIH 3T3 DNA carrier.

mouse DNA. We tentatively conclude from this that the R fragment, which spans the *Bgl* II site disrupting transforming activity, contains sequences that are conserved in evolution and that a single mouse homolog of this human gene exists. In humans, as many as three additional homologous genes may exist.

Finally, we note that, in NIH 3T3 cells transformed with DNA from the human lung carcinoma cell line Calu-1, an additional weakly hybridizing *Eco*RI fragment, 3.6 kbp in size, is observed. Curiously, a comigrating species is not detected by hybridization in human DNA. We therefore examined two additional NIH 3T3 transformants for the presence of this band: NIH 3T3 cells transformed with DNA from SK-LU-1, another human lung carcinoma cell line, and NIH 3T3 cells transformed with DNA from SK-CO-1, a human colon carcinoma cell line. DNA from these transformants also contained a 3.6-kbp *Eco*RI fragment that hybridized to fragment R (data not shown). Previous work has shown that SK-LU-1, SK-CO-1, and Calu-1 contain a common transforming gene (10). We interpret this result to mean that the transforming gene common to these three human carcinoma cell lines is distantly related to the transforming gene of SK-N-SH. We attribute our ability to observe this fragment in transformants, but not in human DNA, to an amplification of the transforming sequences in NIH 3T3 cells that raises the hybridization signal above background levels. Although other interpretations are possible, other work justifies this conclusion (unpublished data).

DISCUSSION

Several human transforming genes have been identified by using DNA-mediated gene transfer and focus induction in NIH 3T3 cells as an assay. Transforming genes have been isolated by molecular cloning from T24 (12, 14), a human bladder carcinoma cell, and from EJ (13), another human bladder carcinoma cell. The two genes appear to be identical to each other and also to the transforming gene of yet a third human bladder carcinoma cell line, J82 (27). However, all three cell lines used in those studies were probably derived from a common cell line and represent only a single instance of activation of that gene (unpublished data). A second human transforming gene has been identified that is common to a colon carcinoma cell line and several other lung and colon carcinoma cell lines (10, 11). Several other

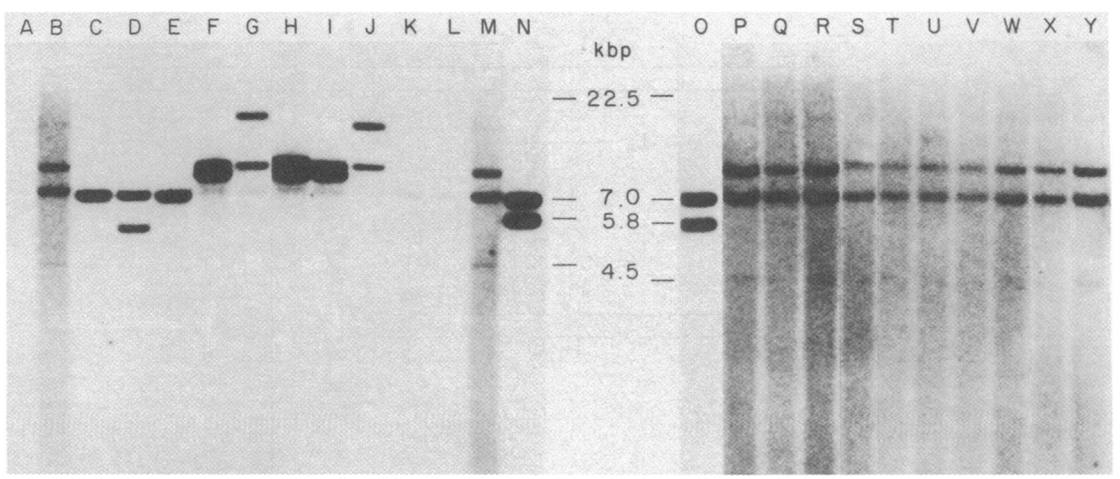

FIG. 5. Hybridization of transformant and human DNAs with SK-N-SH transforming sequences (fragments R and L). *Eco*RI-digested DNAs are as in Fig. 4. In this experiment, a 1:1 mixture of fragments R and L (Fig. 3) was ^{32}P-labeled by nick-translation to 3×10^8 cpm/μg and used as the hybridization probe.

distinct human transforming genes are associated with a breast carcinoma cell line, MCF-7 (28), a leukemic cell line, HL60 (11), and a neuroblastoma cell line, SK-N-SH (10).

In this paper, we have reported the isolation by molecular cloning of the transforming gene from SK-N-SH. Biologically active transforming sequences were obtained by genetic selection. Hybridization studies showed that these sequences were found in each NIH 3T3 cell transformed by SK-N-SH DNA. Two *Eco*RI fragments, of approximate sizes 9.2 and 7.0 kbp, comprise the gene and are found in every human cell line we have examined. Thus, like the transforming gene of T24, the transforming gene of SK-N-SH is likely to have arisen without gross sequence rearrangement from a normal cellular gene.

The SK-N-SH transforming sequences hybridize to other human sequences. In particular, a 1.0-kbp *Hin*dIII fragment (fragment R) hybridizes to *Eco*RI fragments in human DNA of sizes 4.5, 10.5, and 15.0 kbp. Thus, as many as three other human genes may be closely related to the SK-N-SH gene. In addition, the 1.0-kbp *Hin*dIII fragment hybridizes, although less strongly, to the human sequences present in NIH 3T3 cells transformed with the common lung/colon carcinoma transforming gene. Thus, the transforming gene of SK-N-SH may belong to a still larger gene family. Other work (unpublished) confirms this conclusion and shows that this family contains at least three distinct transforming genes: the transforming gene of SK-N-SH, the transforming gene common to lung and colon carcinoma cell lines, and the transforming gene present in the bladder carcinoma cell line T24. Recent work also indicates that these genes are related to Kirsten and Harvey *ras* genes (refs. 27 and 29; unpublished data), the oncogenes of Kirsten and Harvey sarcoma viruses (30).

We thank C. Fraser and C. Lama for their excellent technical help and J. D. Watson for his generous support. This work was also supported by grants from the American Business Cancer Research Foundation, the National Institutes of Health (GM 27643), and the National Cancer Institute (CA 29569). K.S. was a Robertson Research Fellow and M.G. is a Damon Runyon–Walter Winchell Fellow.

1. Ellis, R. W., DeFeo, D., Margak, J. M., Young, H. A., Shih, T. Y., Ghang, E. H., Lowry, D. R. & Scolnick, E. M. (1980) *J. Virol.* **36**, 408–420.
2. Oskarsson, M., McClements, W. L., Blair, D. G., Maizel, J. V. & van de Woude, G. F. (1980) *Science* **207**, 1222–1224.
3. Rousell, M., Saule, S., Lagrov, C., Rommens, C., Bevy, H., Graf, D. & Stehelin, D. (1979) *Nature (London)* **281**, 452–455.
4. Scherr, C. J., Fedele, L. A., Donner, L. & Turek, L. P. (1979) *J. Virol.* **32**, 860–875.
5. Spector, D. H., Varmus, H. E. & Bishop, J. M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4102–4106.
6. Witte, O. N., Rosenberg, N. & Baltimore, D. (1979) *Nature (London)* **281**, 396–398.
7. Shih, C., Shilo, B.-Z., Goldfarb, M. P., Dannenberg, A. & Weinberg, R. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5714–5718.
8. Krontiris, T. G. & Cooper, G. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1181–1184.
9. Shih, C., Padhy, L. C., Murray, M. & Weinberg, R. A. (1981) *Nature (London)* **290**, 261–264.
10. Perucho, M., Goldfarb, M. P., Shimizu, K., Lama, C., Fogh, J. & Wigler, M. H. (1981) *Cell* **27**, 467–476.
11. Murray, M., Shilo, B., Shih, C., Cowing, D., Hsu, H. W. & Weinberg, R. A. (1981) *Cell* **25**, 355–361.
12. Goldfarb, M. P., Shimizu, K., Perucho, M. & Wigler, M. H. (1982) *Nature (London)* **296**, 404–409.
13. Shih, C. & Weinberg, R. A. (1982) *Cell* **29**, 161–169.
14. Pulciani, S., Santos, E., Lauver, A. B., Long, L. K., Robbins, K. C. & Barbacid, M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2845–2849.
15. Jainchill, J. S., Aaronson, S. A. & Todaro, G. J. (1969) *J. Virol.* **4**, 549–553.
16. Kaiser, A. D. & Hogness, D. S. (1960) *J. Mol. Biol.* **2**, 392–415.
17. Tanaka, T. & Weisblum, B. (1975) *J. Bacteriol.* **121**, 354–362.
18. Vogelstein, B. & Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 616–619.
19. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
20. Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1184–1188.
21. Jelinek, W. R., Toomey, T. P., Leinwand, L., Duncan, C. H., Houck, C. M., Deininger, P. L. & Schmid, C. W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1398–1402.
22. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–467.
23. Steinberg, N., Tiemeier, D. & Enquist, L. (1977) *Gene* **1**, 255–280.
24. Murray, N. E., Brammar, W. J. & Murray, K. (1977) *Mol. Gen. Genet.* **150**, 53–61.
25. Perucho, M., Hanahan, D. & Wigler, M. H. (1980) *Cell* **22**, 309–317.
26. Pellicer, A., Wigler, M. H., Axel, R. & Silverstein, S. (1978) *Cell* **14**, 133–141.
27. Der, C. J., Krontiris, T. G. & Cooper, G. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3637–3640.
28. Lane, M. A., Sainten, A. & Cooper, G. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5185–5189.
29. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. (1982) *Nature (London)* **297**, 474–478.
30. Ellis, R. W., DeFeo, D., Shih, T. Y., Gonda, M. A., Young, H. A., Tsuchida, N., Lowy, D. R. & Scolnick, E. M. (1981) *Nature (London)* **292**, 506–511.