MOLECULAR AND CELLULAR BIOLOGY, Nov. 1984, p. 2370–2380
0270-7306/84/112370-11$02.00/0

Vol. 4, No. 11

Expression of the Amplified Domain in Human Neuroblastoma Cells

RICHARD W. MICHITSCH, KATE T. MONTGOMERY, AND PETER W. MELERA*

Laboratory of RNA Synthesis and Regulation, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University, New York, New York 10021

Received 22 March 1984/Accepted 17 August 1984

Screening of a partial cDNA library prepared from the human neuroblastoma cell line BE(2)-C with genomic DNA probes containing sequences representative of the amplified domain of that cell line allowed us to identify cloned transcripts from an active gene within the domain. The gene BE(2)-C-59 is amplified ca. 150-fold and encodes a 3.0- and a 1.5-kilobase RNA transcript, both of which are overproduced in BE(2)-C cells. A survey of a large variety of human tumor cell types indicated that this gene is amplified to varying degrees in all neuroblastoma cell lines and a retinoblastoma cell line that exhibit obvious cytological manifestations of DNA sequence amplification, i.e., homogeneously staining regions and double-minute chromosomes. The BE(2)-C-59 gene is not amplified, however, in other nonrelated tumor types, even those containing amplified DNA. Although the functional significance of this specific gene amplification in neuroblastoma cells remains unknown, an indication that it may relate to the malignant phenotype of these cells follows from the remainder of our data which show that the amplified BE(2)-C-59 gene shares partial homology with both the second and third exons, but not the first exon, of the human c-*myc* oncogene.

The chromosomal abnormalities homogeneously staining regions (HSRs) and double-minute chromosomes (DMs), recognized in a wide variety of mammalian cells as structural manifestations of DNA sequence amplification, have been found to be highly prevalent in human neuroblastoma cell lines and tumors (3, 5, 6). Recently, we have been able to directly demonstrate by Southern blotting procedures and hybridization in situ with kinetically purified genomic DNA probes that neuroblastoma cells do indeed contain amplified DNA and that their HSRs and DMs harbor these sequences (27). Additionally, our data have also shown that, among several HSR- or DM-containing human neuroblastoma cell lines established from various patients, the great majority amplify a large array of cross-hybridizing fragments in addition to a number of fragments which may be cell line specific (27). In no case, however, have any of the fragments found to be amplified in neuroblastoma cells also been found to be amplified in other nonrelated human tumor cells known or suspected to contain amplified DNA. Hence, human neuroblastoma cells appear to amplify a number of specific DNA fragments which they maintain in culture and, presumably, in vivo as HSRs or DMs.

The function of these amplified sequences remains unknown, however, and unlike most other amplification systems in which the products of the amplified genes are reasonably well characterized (7, 17, 19, 25), little evidence has yet been provided to indicate the extent to which the amplified DNA sequences in neuroblastoma cells may be expressed and what the gene products may be. We therefore used probes prepared by C_0t fractionation (8) and representative of the amplified DNA from the HSR-containing human neuroblastoma cell line BE(2)-C (27) to screen a partial cDNA library prepared from the polysomal polyadenylated [poly(A)$^+$] RNA of the BE(2)-C cell line. Recombinant plasmids containing inserts hybridizable with components of these heterogeneous amplified DNA probes were then used as probes in Southern blots to demonstrate their homology to amplified neuroblastoma DNA and in Northern blots to identify their corresponding mRNAs. Using this approach, we isolated a plasmid, pBE(2)-C-59, whose insert hybridizes with an amplified 3.8-kilobase (kb) *Eco*RI genomic DNA fragment in a variety of human neuroblastoma cell lines and in the retinoblastoma cell line Y79T and with two different-sized abundant RNA transcripts.

Recently, Schwab et al. (32) and Kohl et al. (20) reported the presence in human neuroblastoma cells of an amplified 2.0-kb *Eco*RI genomic DNA fragment with limited homology to the retroviral transforming gene v-*myc*. Utilization of probes specific for the 5′ and 3′ domains of v-*myc* (32) has indicated that this homology resides within the 5′ domain of the retroviral gene. A 350-base-pair (bp) region of this 2.0-kb *Eco*RI fragment has been sequenced and found to contain two separate blocks of nucleotides totaling 133 bp that exhibit a 78% homology with exon 2 of the human c-*myc* gene (32). This homology has led to the prediction, therefore, that a c-*myc*-related gene, termed N-*myc*, exists in the human genome. To investigate further the potential relationship between the human c-*myc* gene and N-*myc* and other possibly related cellular genes that may be amplified in human neuroblastoma cells, we performed a series of Southern transfers of *Eco*RI-digested human tumor DNAs and hybridized them under low stringency with probes derived from regions of the human c-*myc* gene within which each of its three exons are located. The results show that human neuroblastoma cells and the retinoblastoma cell line Y79T amplify sequences partially homologous to the third exon of c-*myc* in addition to those partially homologous to its second exon, but they do not amplify DNA with comparable homology to the first exon of this human oncogene. We also show that the amplified second and third c-*myc* exon-related sequences lie on adjacent *Eco*RI fragments in human DNA and that BE(2)-C-59 is encoded by the same amplified genomic fragment that contains the information related to the third exon of c-*myc*.

MATERIALS AND METHODS

Cell lines and culture. The origin and maintenance of the human neuroblastoma cell lines used here have been de-

* Corresponding author.

scribed briefly (27) and in detail (5) and include the HSR-containing lines BE(2)-C and NAP(H), the DM-containing lines SK-N-BE(1), NAP(D), CHP-234, SMS-KAN, SMS-KCN, and SMS-MSN, the ABR-containing line SMS-KANR, and the non-HSR–DM-containing line SH-SY5Y. Nonneuroblastoma cell lines include the fibroblast line WI-38 (16), the retinoblastoma line Y79T (13), the neuroendocrine colon tumor line COLO 321 (29), the small cell lung carcinoma line NCI-H82 (39), the cortical adenocarcinoma and colon adenocarcinoma lines SW-13 (12) and SW-48 (9, 12), respectively, and the bladder carcinoma line SW-800 (12). Normal control tissues include peripheral blood lymphocytes, placenta, and liver. Cell lines were supplied by June L. Biedler, Memorial Sloan-Kettering Cancer Center.

Preparation of partial cDNA libraries. The isolation of polysomal poly(A)$^+$ RNA from neuroblastoma cells, the synthesis of cDNA and double-stranded cDNA, and the cloning of double-stranded cDNA into the *Pst*I site of pBR322 via G-C tailing were all carried out as recently described (24), except that second-strand synthesis was performed by incubating cDNA with the Klenow fragment of polymerase I for 20 h at 15°C. Conditions for the annealing of C-tailed double-stranded cDNA and G-tailed *Pst*I-digested pBR322 and for the transformation of *Escherichia coli* DH-1 have also recently been reported (24). Transformants were scored by their ability to grow on L-agar plates containing 10 μg of tetracycline per ml. Transformation frequencies were routinely on the order of 5×10^5/μg of plasmid DNA.

Probe preparation and library screening. C_0t 10-300 DNA from neuroblastoma cell line BE(2)-C and from human placenta was prepared by standard hydroxyapatite chromatography as described previously (27). Samples of this DNA were then nick translated (30) with ^{32}P-labeled precursors to a specific activity of 0.5×10^8 to 2.0×10^8 cpm/μg and, after exhaustive hybridization against cellulose-bound placental DNA to remove highly repeated sequences (8), were used directly to screen partial cDNA libraries. Screening procedures were those of Grunstein-Hogness (14) with hybridizations carried out at 65°C in 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–8 mM EDTA–300 μg of denatured salmon sperm DNA for 24 to 48 h. After initial hybridization with placental probe and exposure to X-ray film, filters were washed in 0.1 N NaOH and 2× SSC at room temperature for 30 min followed by five washes in 2× SSC, exposed to X-ray film to ensure that all radioactive signal had been removed, and then rehybridized with neuroblastoma probe. Clones scoring as positive in this assay, i.e., those that hybridized more intensely with the neuroblastoma probe than with the placental probe, were picked, gridded onto new tetracycline-containing L-agar plates, and screened a second time in the same manner. Only those clones scoring as positives in both screens were studied further.

Preparation and analysis of DNA and RNA. Preparation of high-molecular-weight DNA and poly(A)$^+$ RNA from cells and tissues was carried out as reported previously (21, 25–27). DNA and RNA transfer experiments were performed as described by Southern (34) and Thomas (35), respectively, with modifications (21). Low-stringency hybridization conditions included prehybridization of the baked DNA-containing nitrocellulose filters in 1× Denhardt solution, 4× SSC, and 50 μg of denatured salmon sperm DNA per ml at 42°C for 6 h, followed by hybridization with ^{32}P-nick-translated probes in 40% formamide, 10× Denhardt solution, 5× SSC, and 50 μg of sheared salmon sperm DNA per ml at 42°C for 42 h, and finally washing the hybridized filters twice for 45 min at 44°C in 0.4× SSC–0.2% sodium dodecyl sulfate and once for 45 min at 50°C in the same buffer.

Plasmid preparation. All plasmids were prepared with a modification of the method of Norgard (28) as described by Melera et al. (24), except that the chloramphenicol step necessary to obtain sufficient amounts of ColE1 plasmids (10) was omitted when using the vector pUC 8 (36).

Cloning of genomic DNA. Genomic DNA was digested with *Eco*RI under conditions described by the vendors and size fractionated on low-melting agarose gels. The size range of interest was identified by comparison with an ethidium bromide-stained well containing markers, and the DNA within that size range was eluted from the gel (31) and cloned by direct ligation into the *Eco*RI sites of pBR322 or pUC 8. The clones of interest were identified via the Grunstein-Hogness colony screening technique with appropriate ^{32}P-labeled probes.

RESULTS

We have recently shown (27) that the C_0t 10-300 fraction of human neuroblastoma DNA contains a large number of sequences that (i) cross-hybridize with high intensity to a variety of restriction endonuclease-digested neuroblastoma DNAs; (ii) do not cross-hybridize with similar digests from a number of other cell types, including those displaying either malignant or nonmalignant phenotypes; and (iii) are not present in the C_0t 10-300 fraction of placental or WI-38 DNA. Hence, they represent a family of amplified neuroblastoma-specific DNA sequences.

The hybridization specificity of these C_0t 10-300 sequences suggested that they might serve as useful probes with which to screen cDNA libraries in an attempt to identify

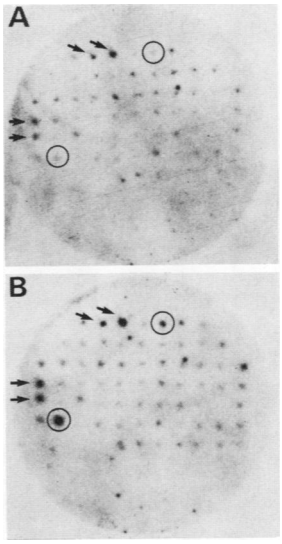

FIG. 1. Use of C_0t 10-300 genomic DNA to screen a partial cDNA library prepared from BE(2)-C cells. The partial cDNA library was gridded onto nitrocellulose filters, one of which is shown here. The filters were first hybridized with ^{32}P-labeled C_0t 10-300 DNA from human placenta (A) and then, after washing to remove the radioactivity, hybridized again with ^{32}P-labeled C_0t 10-300 DNA from BE(2)-C cells (B). A total of 5×10^3 colonies were screened in this manner. Arrows indicate those colonies that hybridized with equal intensity to both probes, and circles show those that hybridized with greater intensity to the tumor cell probe. The circle on the lower left in each panel marks the colony designated DH1/BE(2)-C-59.

mRNAs encoded by amplified DNA. Therefore, ^{32}P-labeled probes were prepared from the C_0t 10-300 genomic DNA fraction of both neuroblastoma cell line BE(2)-C and human placenta and were used to screen 5×10^3 colonies from a partial BE(2)-C cDNA library. An example of such a screen is shown in Fig. 1. The left circle indicates a clone, DH1/BE(2)-C-59, that hybridizes more intensely with neuroblastoma probe than with placental probe, whereas the arrows indicate clones that hybridized to equal intensity with both probes. From the 5×10^3 colonies initially screened, 32 appeared to hybridize with greater intensity to the BE(2)-C C_0t 10-300 probe. Of these, five were shown by Southern blots to be homologous to amplified neuroblastoma DNA. The results of a typical blotting experiment are shown in Fig. 2B. *Eco*RI digests of SK-N-BE(1), BE(2)-C, NAP(H), and placental DNA were transferred to nitrocellulose and hybridized with nick-translated pBE(2)-C-59. As shown, hybridization to high intensity with a 3.8-kb *Eco*RI fragment was apparent in SK-N-BE(1), BE(2)-C, and NAP(H) but not in placental DNA. The remaining four plasmids each hybridized with a 3.8-kb *Eco*RI fragment in neuroblastoma DNA similar in size to that hybridized by pBE(2)-C-59. When these four plasmids, ranging in length from 450 to 550 base pairs, and pBE(2)-C-59, now known from DNA sequence data to be 559 base pairs in length, were hybridized with one another, their inserts all cross-reacted (data not shown), suggesting that each had been derived from the same or closely related mRNAs. As a control for these observations, a clone that did not show differential hybridization during screening was also used to probe *Eco*RI digests of BE(2)-C, NAP(H), and placental DNA. pBE(2)-C-32 hybridized equally well with an 8.6-kb fragment in both neuroblastoma and placental DNA (Fig. 2A). Since the amount of DNA and the conditions used for these blots would not readily allow visualization of single-copy DNA, this 8.6-kb *Eco*RI fragment must represent a sequence normally present in repetitive copy in human DNA, whereas the 3.8-kb fragment hybridized by pBE(2)-C-59 must represent a sequence normally present in low or single copy but amplified in the SK-N-BE(1), BE(2)-C, and NAP(H) genomes.

That the 3.8-kb *Eco*RI fragment can be found among the amplified DNA sequences of other neuroblastoma cells is shown in Fig. 3. DNAs obtained from various cell lines derived from different patients and containing HSRs, DMs, abnormally banding regions (ABRs), or no cytological evidence for amplification were digested with *Eco*RI and analyzed by Southern blots with pBE(2)-C-59 as probe. In every case in which cytological evidence for amplification has been shown to exist (Fig. 3, lanes b, d, f–j, n, and o), the 3.8-kb *Eco*RI fragment is amplified. The variations in hybridization intensities generally follow those previously seen with C_0t 10-300 sequences obtained from individual cell lines to probe a similar panel of digests (27) and suggest that different levels of amplification of the 3.8-kb fragment occur from line to line. In neuroblastoma cell line SH-SY5Y, in which the evidence for amplification at both the cytological (5) and DNA sequence level (27) is questionable, the level of hybridization of the 3.8-kb fragment with pBE(2)-C-59 (Fig. 3, lane s) approximates that observed with placental DNA (Fig. 3, lane q). A series of hybridization experiments with dilutions of BE(2)-C DNA indicates that relative to placenta, BE(2)-C-59 sequences are amplified ca. 150-fold in the BE(2)-C genome (data not shown).

To determine whether BE(2)-C-59 sequences were amplified in other human tumors, including some containing amplified DNA, i.e., HA-L, NCI-H82, and Y79T, *Eco*RI restriction digests of a variety of nonneuroblastoma tumor DNAs were analyzed on the same gel (Fig. 3, lanes c, k, l, m, p, and r). As shown, BE(2)-C-59 was found to cross-hybridize with the DNA from the retinoblastoma cell line Y79T (lane l), but it cross-hybridized to none of the other cell lines tested. The size of the *Eco*RI fragment hybridized in

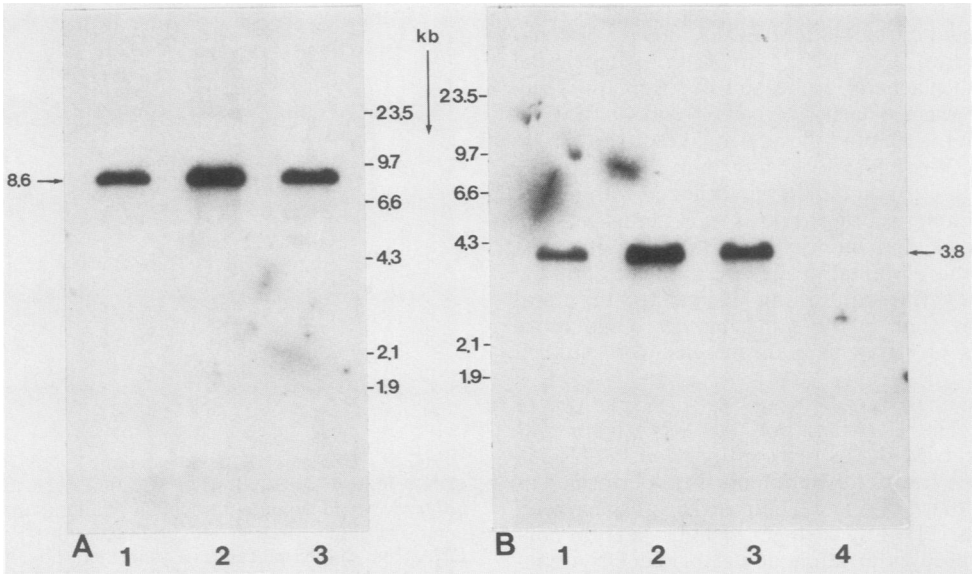

FIG. 2. Hybridization of pBE(2)-C-59 and pBE(2)-C-32 with genomic DNA. *Eco*RI digests of neuroblastoma DNA were electrophoresed, transferred to nitrocellulose paper, and hybridized with nick-translated pBE(2)-C-59 or pBE(2)-C-32 as the probe; specific activity, 10^8 cpm/μg. Molecular weight markers were *Hin*dIII-digested lambda, visualized by ethidium bromide staining. (A) 2.5 μg of DNA from BE(2)-C (lane 1), NAP(H) (lane 2), and placenta (lane 3) hybridized with pBE(2)-C-32. (B) 2.5 μg of DNA from SK-N-BE(1) (lane 1), BE(2)-C (lane 2), NAP(H) (lane 3), and placenta (lane 4) hybridized with pBE(2)-C-59. Overexposure of this radioautograph allows visualization of the 3.8-kb band in single-copy amounts in lane 4.

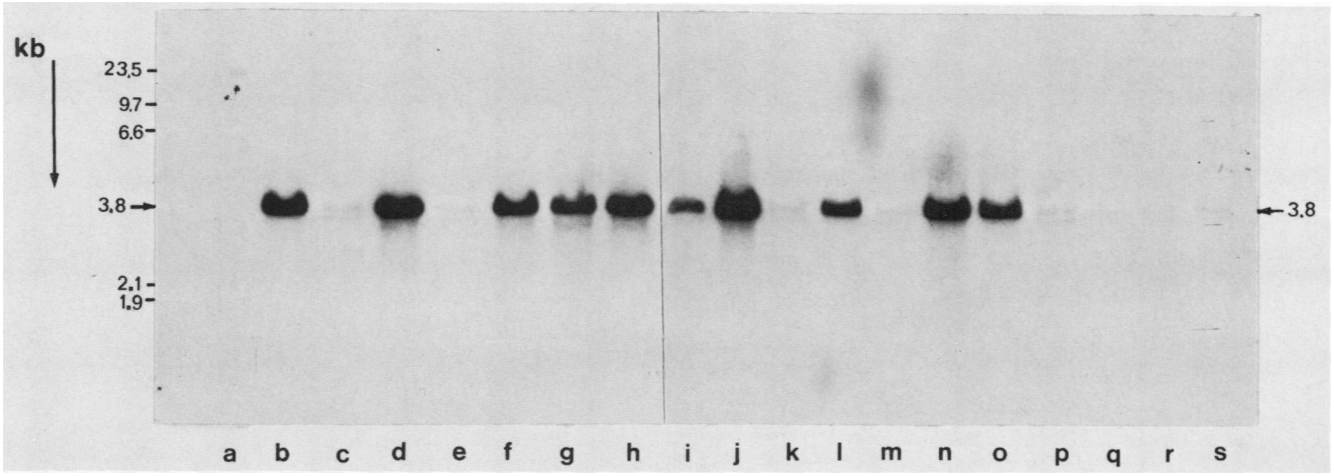

FIG. 3. Hybridization of genomic DNA from a variety of human tumor cell lines with pBE(2)-C-59. *Eco*RI digests of DNA were electrophoresed, transferred to nitrocellulose, and hybridized with pBE(2)-C-59 nick translated to a specific activity of 1.2×10^8 cpm/μg. Molecular weight markers were as described in the legend to Fig. 2. DNA (15 μg) was used in each lane. Lanes: a, lambda DNA; b, SMS-MSN; c, SW-800; d, SMS-KCN; e, WI-38; f, NAP(D); g, NAP(H); h, CHP-234; i, BE(1)-N; j, BE(2)-C; k, HA-L; l, Y79T; m, SW-13; n, SMS-KAN; o, SMS-KANR; p, SW-48; q, placenta; r, NCI-H82; and s, SH-SY5Y.

retinoblastoma DNA, 3.8 kb, suggests that it is the same sequence hybridized by BE(2)-C-59 in neuroblastoma cells. The level of amplification of BE(2)-C-59 in the Y79T genome is estimated by comparison with BE(2)-C DNA to be ca. 30-fold.

Since BE(2)-C-59 represents a cDNA sequence, it was used as a probe in a Northern blot analysis of BE(2)-C poly(A)$^+$ RNA to identify the template from which it had been synthesized. The results of these experiments (Fig. 4) indicate that BE(2)-C-59 shares homology with two prominent BE(2)-C RNAs of 3,000 and 1,500 bases in length. These two poly(A)$^+$ RNAs are present in varying amounts in other neuroblastoma cell lines containing different levels of amplified copies of BE(2)-C-59 genes but are not readily detectable in cells that do not contain elevated copies of this gene, including the neuroblastoma line SH-SY5Y in which we have identified only the 3-kb transcript (data not shown). Although we have not accurately quantitated the level of expression of the BE(2)-C-59 gene with the level of its amplification, it is clear from the data presented that the steady-state level of BE(2)-C-59 mRNA in the different cell lines varies directly with the gene copy number.

Our initial efforts to identify the BE(2)-C-59 sequence as a transcript of a known human gene was influenced by the knowledge that elevated levels of oncogene expression had been demonstrated in various human and murine tumor types to be gene amplification mediated (1, 11, 22, 33). We, therefore, initially screened neuroblastoma DNA with a number of different known oncogene probes, including H-*ras*, K-*ras*, c-*myc*, v-*rel*, v-*abl*, v-*sis*, and c-mos but obtained

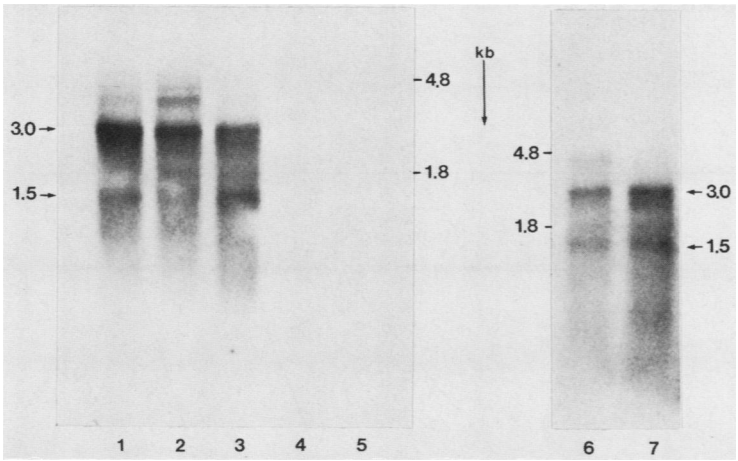

FIG. 4. Hybridization of poly(A)$^+$ RNA from neuroblastoma cells with pBE(2)-C-59. Poly(A)$^+$ RNA from a number of neuroblastoma cell lines was electrophoresed on formamide-formaldehyde gels, transferred to nitrocellulose, and hybridized with pBE(2)-C-59 nick translated to a specific activity of 10^8 cpm/μg. Molecular size markers were 28S and 18S rRNAs taken to be 4,810 and 1,800 nucleotides in length, respectively. Visualization of the markers was by methylene blue staining of the nitrocellulose filter. Lanes: 1, BE(2)-C (5 μg); 2, NAP(H) (5 μg); 3, CHP-234 (5 μg); 4, placental poly(A)$^+$ RNA (5 μg); 5, BE(2)-C poly(A)$^-$ RNA (5 μg); 6, SMS-KAN (2.5 μg); and 7, SMS-KANR (2.5 μg).

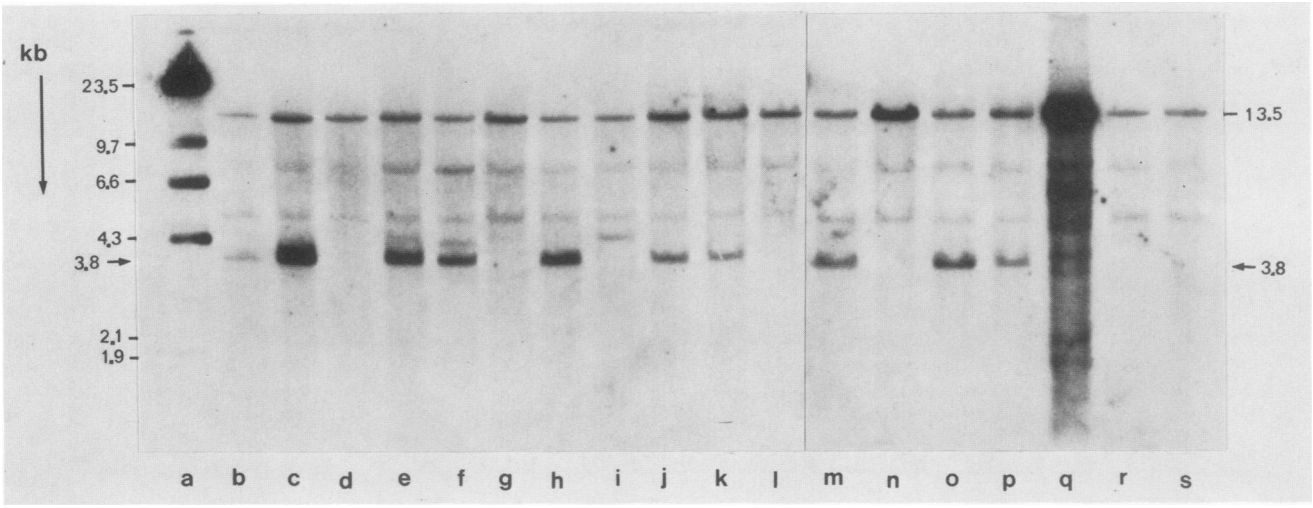

FIG. 5. Hybridization of genomic DNA from a variety of human tumor cell lines with the third exon region of human c-*myc*. *Eco*RI digests of DNA from various human tumor cell lines were electrophoresed, transferred to nitrocellulose, and hybridized under reduced stringency with a probe, pMC41 3RC, representing the 3′ exon region of the human c-*myc* gene and kindly supplied by Robert C. Gallo. Molecular weight marker used was *Hin*dIII-digested lambda DNA, visualized by hybridization with nick-translated lambda DNA that had been added to the probe. Specific activity of the probe was 5×10^7. DNA (15 μg) was used in each lane. Lanes: a, lambda DNA; b, SK-N-BE(1); c, BE(2)-C; d, SW-13; e, NAP(D); f, NAP(H); g, HA-L; h, SMS-KCN; i, WI-38; j, SMS-MSN; k, Y79T; l, SW-48; m, CHP-234; n, SW-800; o, SMS-KAN; p, SMS-KANR; q, NCI-H82; r, placenta; and s, SH-SY5Y.

negative results until the stringency with which the hybridizations were performed was lowered to allow annealing of more distantly related sequences. Under these conditions and using a probe, pMC41 3RC, containing the third exon region of the human c-*myc* gene, we were able to detect a prominent 3.8-kb *Eco*RI fragment in DNAs from those neuroblastoma cell lines and a retinoblastoma cell line known to contain amplified DNA (lanes b, c, e, f, h, j, k, m, o, and p) in addition to the 13.5-kb *Eco*RI fragment that is known to contain the human c-*myc* gene (Fig. 5). This 3.8-kb restriction fragment was not observed in DNA from the small cell lung carcinoma cell line NCI-H82 (lane q) which contains amplified c-*myc* genes (22), nor was it detected in other human tumor cells containing amplified DNA or in normal controls lacking amplified domains.

To determine whether sequences distantly related to the remainder of the human c-*myc* gene were also amplified in neuroblastoma cells, hybridization probes from regions containing its first and second exons were prepared as indicated in Fig. 6. When the second exon region was used as a probe (Fig. 7), a prominent 2.1-kb *Eco*RI fragment was observed in the same cell lines in which the 3.8-kb c-*myc* third exon-related fragment had been seen. This 2.1-kb fragment was not observed in NCI-H82 (lane n) DNA or in the neuroendocrine tumor cell line COLO 321 (lane l) which also contains amplified c-*myc* genes (1). When hybridized with a probe containing the first exon of the c-*myc* gene (Fig. 8), no hybridization, except for that expected with the c-*myc* gene itself, was detected in any of the cellular DNAs analyzed, including those previously shown to contain amplified se-

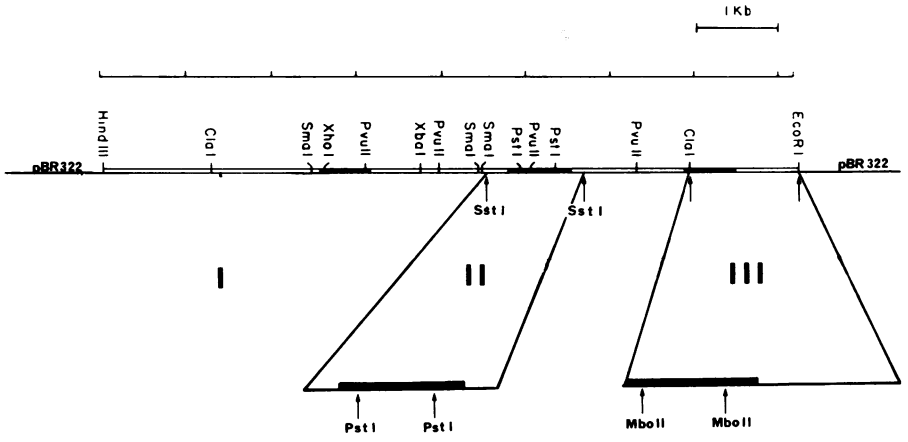

FIG. 6. Generalized restriction map of the cloned human c-*myc* gene, MC-41, provided by Michael Cole. The map is taken from Watt et al. (38). The numerals I, II, and III indicate those fragments referred to as exon region probes. The *Pst*I fragment from exon II and the *Mbo*II fragment from exon III actually lie within the exons per se, which are indicated by the solid black lines.

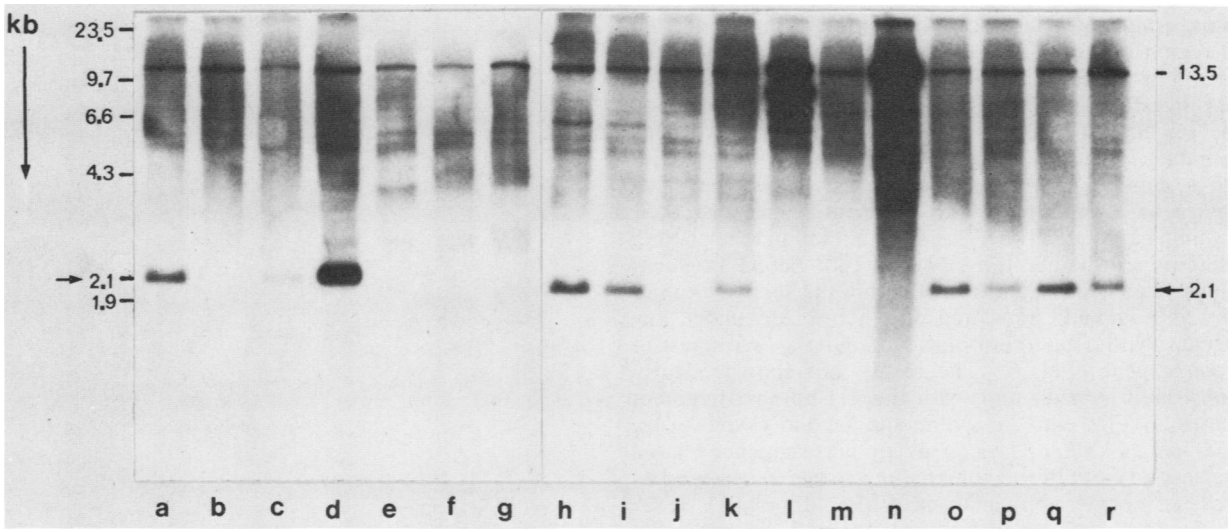

FIG. 7. Hybridization of genomic DNA from a variety of human tumor cell lines with the second exon region of human c-*myc*. *Eco*RI digests of DNA from a number of different human tumor cell lines were electrophoresed, transferred to nitrocellulose, and hybridized under reduced stringency with the second exon region probe of c-*myc* shown in Fig. 6 that had been isolated as an *Sst*I fragment from a low-melting agarose gel and nick translated to a specific activity of 7×10^7 cpm/μg. Markers used were the same as in Fig. 5, but are not shown. A 15-μg amount of DNA was used in each lane. Lanes: a, CHP-234; b, SW-48; c, SK-N-BE(1); d, BE(2)-C; e, HA-L; f, placenta; g, SW-800; h, NAP(D); i, NAP(H); j, SH-SY5Y; k, Y79T; l, COLO 321; m, WI-38; n, NCI-H82; o, SMS-KAN; p, SMS-KANR; q, SMS-KCN; r, SMS-MSN.

quences related to the second and third c-*myc* exon regions. Hence, although sequences sharing partial homology with both the second and third exon regions of the human c-*myc* gene are present and amplified in neuroblastoma cells and in a retinoblastoma cell line as well, no such sequence sharing a similar degree of homology with the first exon region of the c-*myc* gene is amplified in these cells. The inability of the second and third c-*myc* exon region probes to detect their respective 2.1- and 3.8-kb *Eco*RI fragments in cells which do not carry them in amplified amounts is presumably due to the low degree of homology between them. Furthermore, because of this low degree of homology, we cannot rule out the existence of a nonamplified c-*myc* exon 1-related sequence in the human genome.

Since pBE(2)-C-59 and pMC41 3RC both hybridized with an amplified 3.8-kb *Eco*RI fragment in neuroblastoma cells, we cloned that fragment from BE(2)-C cells and used it as a probe to demonstrate its homology to both sequences. The cloned 3.8-kb fragment hybridizes well with pBE(2)-C-59 under normal stringency and, as expected, requires lowered stringency to hybridize with pMC41 3RC (Fig. 9). To show that sequences related to those within the third exon of c-*myc* are located in the 3.8-kb fragment, we purified the 465-bp *Mbo*II fragment which lies within the third exon of the c-*myc* gene (Fig. 6), nick translated it, and hybridized it under low stringency with the cloned 3.8-kb fragment and obtained positive results (data not shown). Hence, both BE(2)-C-59 and sequence related to the third exon of the human c-*myc* gene reside within the 3.8-kb *Eco*RI fragment. However, since pBE(2)-C-59 and pMC41 3RC do not cross-hybridize with each other, even under low stringency (data not shown), they apparently do not overlap to any appreciable extent. Recently obtained DNA sequence data to be presented elsewhere (R. W. Michitsch and P. W. Melera, manuscript in preparation) show that BE(2)-C-59 does not contain an open reading frame but does contain a 3′ polyadenylate tail 24 residues in length preceded 12 bases in the 5′-ward direction by the polyadenylation signal AAUAAA. Coupled with the fact that BE(2)-C-59 was synthesized by reverse transcription of oligodeoxythymidylate-primed poly(A)$^+$ mRNA, it is reasonable to suggest that it represents the 3′-untranslated region of an mRNA molecule, at least part of which is encoded by the 3.8-kb *Eco*RI fragment.

We next cloned the amplified 2.1-kb *Eco*RI BE(2)-C

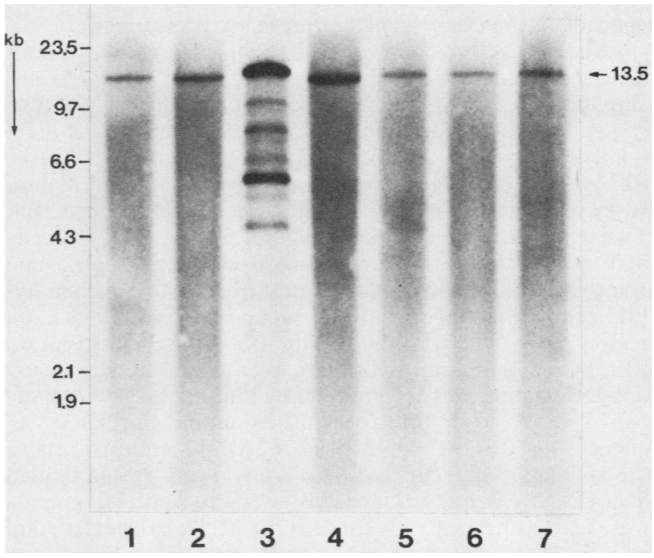

FIG. 8. Hybridization of DNA from human tumor cell lines with the first exon region of c-*myc*. *Eco*RI digests of DNA from several human tumor cell lines were electrophoresed, transferred to nitrocellulose, and hybridized under reduced stringency with the nick-translated first exon region of c-*myc* shown in Fig. 6 that had been isolated as a ca. 7-kb fragment on low-melting agarose after double digestion of the clone with *Sst*I and *Eco*RI. Probe specific activity was 4×10^7 cpm/μg. A 15-μg amount of DNA was used in each lane. Molecular weight markers are not shown but were the same as those in Fig. 5. Lanes: 1, CHP-234; 2, SW-13; 3, COLO 321; 4, SW-48; 5, BE(2)-C; 6, placenta; 7, NAP(H).

genomic fragment that shares homology with the second exon region of c-*myc* (Fig. 9C). This clone and the 3.8-kb fragment were then used as probes and hybridized to partial *Eco*RI digests of BE(2)-C DNA to determine whether they reside on adjacent *Eco*RI fragments. The results of this experiment (Fig. 10) show that although both probes hybridize to a number of fragments of various sizes, they also hybridize to a common 5.9-kb fragment, suggesting their direct linkage (i.e., 3.8 kb + 2.1 kb = 5.9 kb) in the genome. To determine whether the cloned 2.1-kb *Eco*RI fragment contains sequence related to that within the second exon of c-*myc*, it was nick translated and hybridized under low stringency with a Southern blot containing a *Pst*I digest of the c-*myc* gene (Fig. 6). The result, not shown, clearly demonstrated hybridization with the 411-bp *Pst*I fragment indicated in Fig. 6 to lie within the second c-*myc* exon. Hence, the 2.1-kb *Eco*RI fragment contains sequence related to the second exon of the human c-*myc* gene. When used as probes in a Northern blot analysis of BE(2)-C poly(A)$^+$ RNA (Fig. 11), both the 2.1- and 3.8-kb fragments hybridize to the same size BE(2)-C poly(A)$^+$ RNAs originally shown in a similar analysis with pBE(2)-C-59 as probe (Fig. 4) to be overproduced in neuroblastoma cell lines containing amplified DNA. Since BE(2)-C-59 is contained within the 3.8-kb *Eco*RI fragment and, from its nucleotide sequence, represents the 3′ terminus of either the 1.5- or the 3.0-kb transcript or both, either one or both of these mature transcripts must end within the 3.8-kb fragment. Since, in the genome, the 2.1-kb fragment lies adjacent to the 3.8-kb fragment (Fig. 10) and hybridizes to both the 1.5- and 3.0-kb RNAs (Fig. 11), it cannot lie downstream of the 3.8-kb fragment and must, therefore, lie upstream. Hence, the direction of transcription of these two amplified *Eco*RI fragments must be from the 2.1-kb fragment into and perhaps through the 3.8-kb fragment, the same direction in which their related sequences in the c-*myc* gene are normally transcribed.

Although we have shown that sequences related to both the second and third exons of the human c-*myc* gene are amplified in human neuroblastoma cells, and have also shown that the genomic fragments that harbor these c-*myc*-related sequences hybridize with cytoplasmic poly(A)$^+$ RNAs, our attempts to show that c-*myc*-related information is present in these mature RNAs have not been successful. Twenty micrograms of poly(A)$^+$ RNA from BE(2)-C cells and human liver were denatured, electrophoresed, and transferred to nitrocellulose as described above. When hybridized with either ^{32}P-labeled second or third c-*myc* exon region probes, or with the 465-bp *Mbo*II fragment (Fig. 6), BE(2)-C RNA did not hybridize, even when the stringency of hybridization was lowered below that necessary to allow c-*myc* DNA to hybridize with its amplified-related sequences in BE(2)-C DNA (Fig. 12A). In contrast, these probes readily detected a 2.6-kb c-*myc* transcript in human liver RNA. When the cloned 2.1- or 3.8-kb *Eco*RI fragments were used as probes, only the BE(2)-C RNA hybridized, and no signal was observed in the track containing liver RNA (Fig. 12B). This is a somewhat surprising result as far as the 2.1-kb fragment is concerned, since it is most likely the same *Eco*RI fragment cloned, partially sequenced, and shown by Schwab et al. (32) to contain two regions of c-*myc* exon 2 homology (see below). Hence, under the conditions of hybridization used here, the amount of c-*myc*-related sequence present in the 2.1-kb genomic fragment, i.e., two separate blocks of nucleotides totaling 133 bp with 78% homology to exon 2 of the human c-*myc* gene, is apparently insufficient to detect c-*myc* transcripts at the level of expression found in human liver. That the 2.1-kb *Eco*RI fragment is, itself, readily detectable by hybridization under reduced stringency with a c-*myc* exon 2 probe (Fig. 6) suggests that either additional homology outside the 411-bp *Pst*I fragment may exist between these two DNA fragments or our RNA transfer and hybridization protocols are not as efficient as our DNA protocols in detecting distant sequence homologies. Although it is clear, therefore, that the results in Fig. 12A do not allow us to conclude that all c-*myc* information is excluded from the mature transcripts of the BE(2)-C-59 gene, they do suggest, given that these transcripts are overproduced in BE(2)-C cells and may account for as much as 0.1% of the total poly(A)$^+$ RNA, that the amount of c-*myc*-related information present in them is small or widely diverged.

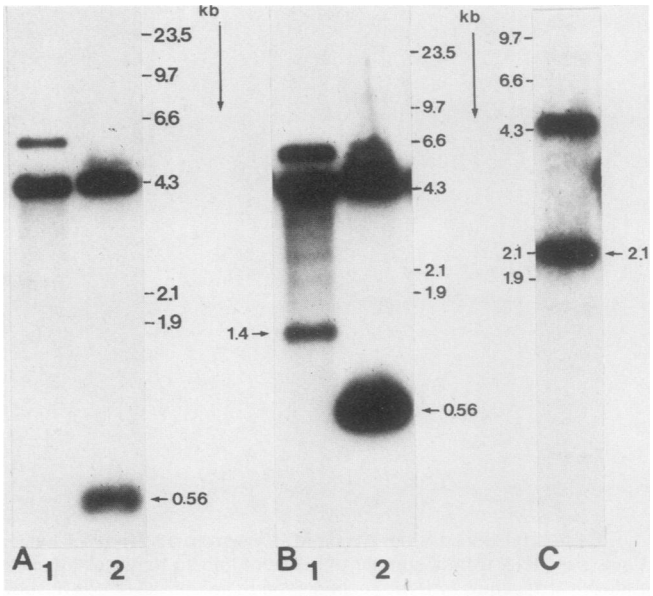

FIG. 9. Cloning of the amplified 3.8- and 2.1-kb *Eco*RI fragments from BE(2)-C DNA. *Eco*RI digests of BE(2)-C DNA were electrophoresed on low-melting agarose gels. Bands corresponding to molecular weights of 2.1 and 3.8 kb were cut out, and the DNA extracted. The resulting DNA fragments were cloned by direct ligation into the *Eco*RI site of pBR322. Transformants were selected by growth on ampicillin, and recombinants were identified by screening with either pBE(2)-C-59 (for the 3.8-kb fragment) or the second exon region of c-*myc* (for the 2.1-kb fragment). In the latter case hybridization was done at reduced stringency. (A) Hybridization of pMC41 3RC and pBE(2)-C-59 with the cloned 3.8-kb *Eco*RI fragment used as probe. pMC41 3RC was double-digested with *Eco*RI and *Hin*dIII to release its 1.4-kb insert, and pBE(2)-C-59 was digested with *Pst*I to release its 559-bp insert. After electrophoresis and transfer to nitrocellulose, hybridization was carried out under normal stringency. A 1-μg amount of DNA was used in each lane. Lanes: 1, pMC41 3RC; 2, pBE(2)-C-59. (B) The same experiment as in (A) but hybridization was carried out under reduced stringency. Lanes: 1, pMC41 3RC; 2, pBE(2)-C-59. (C) Hybridization of the cloned 2.1-kb *Eco*RI fragment with the second exon region of c-*myc*. The plasmid presumably containing the 2.1-kb *Eco*RI fragment was digested with *Eco*RI and, after electrophoresis and transfer to nitrocellulose, was hybridized under reduced stringency with the second exon-region probe of c-*myc*, prepared as described in the legend to Fig. 7.

DISCUSSION

We have previously shown that a large number of independently established human neuroblastoma cell lines derived

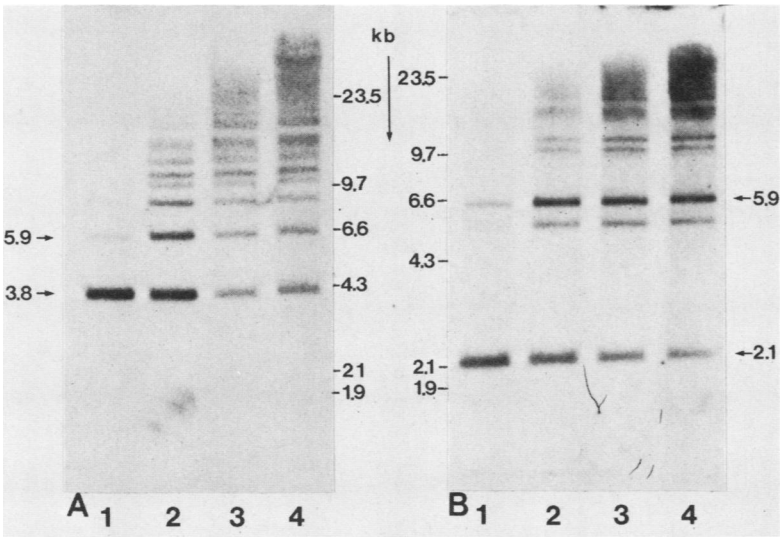

FIG. 10. Hybridization of the cloned 3.8- and 2.1-kb *Eco*RI fragments with partial *Eco*RI digests of BE(2)-C DNA. Different amounts of DNA from BE(2)-C cells was digested with 1 U of *Eco*RI for 1 h at 37°C and then electrophoresed, transferred to nitrocellulose, and hybridized with either the 3.8- or 2.1-kb *Eco*RI fragments. (A) Hybridization with the cloned 3.8-kb *Eco*RI fragment as probe. Specific activity of the probe was 8×10^7 cpm/μg. Lanes: 1, 1 μg of BE(2)-C DNA; 2, 2 μg; 3, 4 μg; 4, 8 μg. (B) Hybridization with the cloned 2.1-kb *Eco*RI fragment as probe. Specific activity of the probe 5×10^7 cpm/μg. Lanes: 1, 1 μg of BE(2)-C DNA; 2, 2 μg; 3, 4 μg; 4, 8 μg.

from various patients amplify a common array of *Eco*RI DNA fragments in addition to some that may be cell line specific (27). Hybridization in situ has indicated that prevalent HSRs, DMs, and ABRs harbor these fragments and, most importantly, that many of the same fragments are present in HSRs and ABRs located at widely different chromosomal locations and in extrachromosomal DMs as well. The data reported here show that among this array of amplified DNA fragments is the transcriptionally active gene ultimately responsible for the production of the cDNA clone BE(2)-C-59. We have cloned two exon-containing *Eco*RI fragments from this gene and have used them as probes to show that the gene is amplified in all of the neuroblastoma cell lines that contain HSRs, DMs, or prominent ABRs and in a retinoblastoma cell line that contains an HSR (Fig. 3). Moreover, recent in situ hybridization studies with pBE(2)-C-59 as the probe have verified the location of this gene to be within these chromosomal structures (J. L. Biedler and P. W. Melera, unpublished data).

Concomitantly, we have shown that many neuroblastoma cell lines amplify DNA fragments containing partial sequence homology to both the second and third exon, but not the first exon, of the human c-*myc* oncogene. Although there is no amplified exon 1-related fragment, the fact that fragments containing information related to exons 2 and 3 of c-*myc* are linked together in the human genome, are transcriptionally active, and are apparently transcribed in the same direction, i.e., from the fragment containing exon 2-related sequences toward the fragment containing exon 3-related sequences, suggests that a gene containing c-*myc*-related sequence exists and is both amplified and expressed in these human tumor cell lines. These results confirm and extend those of Schwab et al. (32) and Kohl et al. (20), both of whom reported the finding of an amplified 2.0-kb *Eco*RI fragment in neuroblastoma cells, identified by virtue of its partial homology with the 5′ domain of the v-*myc* gene. Interestingly, neither of these authors reported any homology between the 3′ domain of v-*myc* and amplified neuroblastoma DNA, and

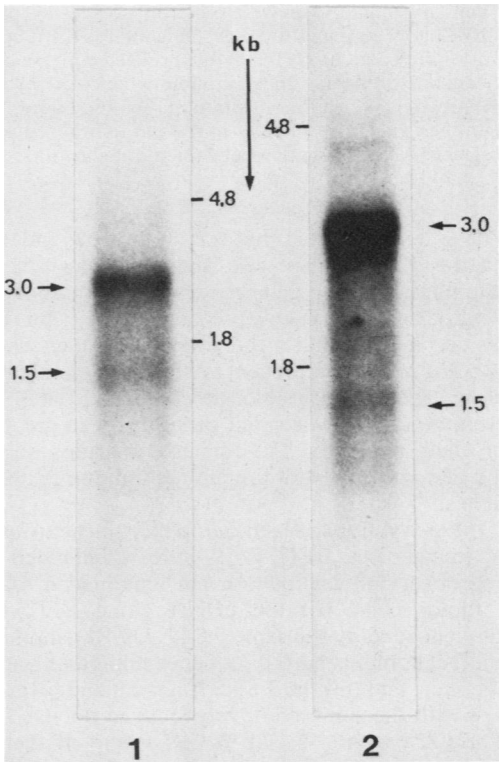

FIG. 11. Hybridization of BE(2)-C poly(A)$^+$ RNA with the cloned 3.8- and 2.1-kb *Eco*RI fragments. Poly(A)$^+$ RNA from BE(2)-C cells was electrophoresed through a formamide-formaldehyde gel, transferred to nitrocellulose, and hybridized with either the 3.8- or 2.1-kb *Eco*RI fragments. Specific activity of both probes was 10^8 cpm/μg. Markers used were 28S and 18S rRNA as discussed in the legend to Fig. 4. Note that lanes 1 and 2 were run on different gels for different times. Lane 1, 2 μg of RNA. Hybridization with the 3.8-kb *Eco*RI fragment. Lane 2, 15 μg of RNA. Hybridization with the 2.1-kb *Eco*RI fragment.

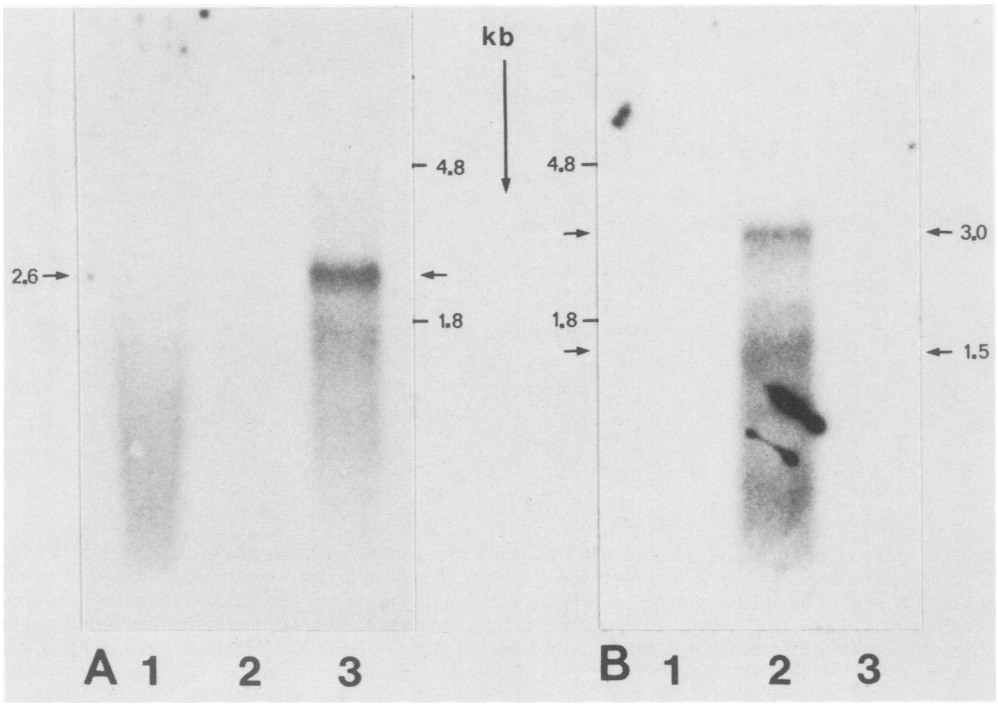

FIG. 12. Hybridization of poly(A)$^+$ RNA from BE(2)-C cells and human liver with pMC41 3RC. A 20-μg amount of poly(A)$^+$ RNA from BE(2)-C cells or human liver were electrophoresed on formamide-formaldehyde gels, transferred to nitrocellulose, and hybridized with pMC41 3RC, nick translated to a specific activity of 10^7 cpm/μg. After exposure to X-ray film, the same filter was washed to remove the majority of its radioactivity and then rehybridized with the cloned 3.8-kb *Eco*RI fragment nick translated to a specific activity of 2×10^7 cpm/μg. Reducing the formamide concentration in the c-*myc* hybridizations from 50 to 30% did not increase the hybridization between c-*myc* and BE(2)-C RNA. (A) Hybridization with pMC41 3RC. Lanes: 1, 20 μg of BE(2)-C poly(A)$^-$ RNA; 2, poly(A)$^+$ RNA from BE(2)-C; 3, poly(A)$^+$ RNA from liver. (B) Same filter as in (A) but hybridized with the 3.8-kb *Eco*RI fragment. Hybridization of a similar filter with the 2.1-kb *Eco*RI fragment gave the same results (data not shown).

yet we have demonstrated that homology does indeed exist between the 3′ exon of c-*myc* and neuroblastoma DNA. Since the human c-*myc* gene represents a mammalian cell equivalent of the avian c-*myc* protooncogene from which v-*myc* was derived (37) and if the amplified sequences related to c-*myc* do, in fact, represent a structurally related gene (see below), then the degree of divergence of the 3′ domain of v-*myc* and the c-*myc*-related gene has been greater than that for their respective 5′ domains, perhaps suggesting retention of some ancestral function within the 5′ domain of these genes.

That the c-*myc*-related gene amplified in neuroblastoma cells is, in fact, the BE(2)-C-59 gene is indicated by the following observations: (i) both are amplified in the same human tumor cells; (ii) the cDNA clone BE(2)-C-59 is ultimately encoded by the same 3.8-kb *Eco*RI genomic DNA fragment that contains partial sequence homology with the 3′ exon of c-*myc*; and (iii) the 3.8-kb fragment and pBE(2)-C-59 hybridize with the same two overproduced poly(A)$^+$ RNAs also hybridized by the 2.1-kb *Eco*RI fragment that shows homology to exon 2 of c-*myc*. Hence, the overproduced RNAs transcribed from the two genomic fragments containing c-*myc*-related sequences are the same size, i.e., 3,000 and 1,500 bases, as those hybridized by pBE(2)-C-59. Since BE(2)-C-59 represents the 3′ end of a poly(A)$^+$ RNA molecule, the simplest explanation for these results is that the BE(2)-C-59 gene encodes two transcripts, both of which extend from the 2.1-kb *Eco*RI fragment into the 3.8-kb fragment, at which point at least one, and perhaps both, terminate. Whether any c-*myc*-related information is found in the mature transcripts of the BE(2)-C-59 gene, however, remains to be determined, as does any possible functional relatedness between the products of the c-*myc* and BE(2)-C-59 genes.

Since the amplified 2.1-kb *Eco*RI fragment found by us to share partial sequence homology with the second exon of c-*myc* is most likely the same amplified *Eco*RI fragment reported by Schwab et al. (32) and Kohl et al. (20) to be 2.0 kb in size and to share partial sequence homology with the 5′ domain of v-*myc*, the BE(2)-C-59 gene is probably the so-called N-*myc* gene (20). A discrepancy does exist, however, in the number of transcripts reported to be encoded by this gene. Whereas Kohl et al. (20) had reported the presence of a 3.2-kb transcript in IMR-32 cells, we have consistently identified both a 3.0-kb transcript and a 1.5-kb transcript in five different neuroblastoma cell lines. Although differences in the sizes of c-*myc* transcripts have been reported and shown to be due to rearrangements of the gene or differential utilization of 5′ start sites (2, 4, 15), no such information is yet available for N-*myc*. The formal possibility exists, therefore, that the BE(2)-C-59 gene and the N-*myc* gene are closely related but not identical.

The ability to prepare amplification-unit-specific probes with which to screen cDNA libraries permits a rapid means of identifying transcripts of amplified genes. Brison et al. (8) have indicated that amplification levels of 10-fold can be detected when using this duplicate screen technique for genomic libraries, and the same level of detection ought to hold for cDNA libraries as well. The success of this approach depends upon both the level of amplification and the

level of expression of the target gene. In general, the representation of a given sequence in a cDNA library varies with the abundance of its template in the starting RNA population. Amplification of a transcriptionally active gene, then, not only increases the number of its copies in the kinetically defined probe but also increases the representation of its transcripts in the cDNA library. It has been estimated that to recover low-abundance transcripts (i.e., <14 copies per cell) from cDNA libraries, screening of ca. 1.7×10^5 colonies is required (23). Since an amplification of only 10-fold decreases that number to a more easily manageable 1.7×10^4, it is clear that this method, although not as elaborate as others (18), offers a rapid and convenient way in which to obtain cloned transcripts of amplified genes.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health to the Sloan-Kettering Institute for Cancer Research and from the Special Projects Committee of the Society of the Memorial Sloan-Kettering Cancer Center, the Milton Schamach Foundation, and the Jeanette Warren Davison Fund for Cancer Research to P.W.M.

We thank June L. Biedler for continued support and interest in this research.

ADDENDUM IN PROOF

We have determined the nucleotide sequence of the 3′ exon of the human N-*myc* gene and have shown that its protein-encoding region shares significant but dispersed homology with the analogous protein-encoding region of the human c-*myc* gene (R. W. Michitsch and P. W. Melera, submitted for publication). Hence, the failure of 3′ exon c-*myc* probes to detect N-*myc* transcripts in Northern blots, as reported here, does not result from an absence of homology between these two sequences.

LITERATURE CITED

1. **Alitalo, K., M. Schwab, C. C. Lin, H. E. Varmus, and J. M. Bishop.** 1983. Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (c-*myc*) in malignant neuroendocrine cells from a human colon carcinoma. Proc. Natl. Acad. Sci. U.S.A. **30**:1707–1711.
2. **ar-Rishdi, A., K. Nishikura, J. Erikson, R. Watt, G. Rovesa, and C. M. Croce.** 1983. Differential expression of the translocated and the untranslocated c-*myc* oncogene in Burkitt lymphoma. Science **222**:390–393.
3. **Balaban-Malenbaum, G., and F. Gilbert.** 1977. Double-minute chromosomes and the homogeneously staining regions in chromosomes of human neuroblastoma cell lines. Science **198**:739–741.
4. **Bernard, O., S. Cory, S. Gerondakis, E. Webb, and J. M. Adams.** 1983. Sequence of the murine and cellular myc oncogenes and two modes of myc transcription resulting from chromosome translocation in three lymphoid tumours. Eur. Mol. Biol. Organ. J. **2**:2375–2383.
5. **Biedler, J. L., M. B. Meyers, and B. A. Spengler.** 1983. Homogeneously staining regions and double minute chromosomes: prevalent cytogenetic abnormalities of human neuroblastoma cells. Adv. Cell. Neurobiol. **4**:267–307.
6. **Biedler, J. L., and B. A. Spengler.** 1976. Metaphase chromosome anomaly: association with drug resistance and cell-specific products. Science **191**:185–187.
7. **Bostock, C. J., E. M. Clark, N. G. L. Harding, P. M. Mounts, C. Tyler-Smith, V. van Heyningen, and P. M. B. Walker.** 1979. The development of resistance to methotrexate in a mouse melanoma cell line. I. Characterisation of the dihydrofolate reductases and chromosomes in sensitive and resistant cells. Chromosoma **74**:153–177.
8. **Brison, O., F. Ardeshir, and G. R. Stark.** 1982. General method for cloning amplified DNA by differential screening with genomic probes. Mol. Cell. Biol. **2**:578–587.
9. **Chen, T. R., R. J. Hay, and M. L. Macy.** 1982. Karyotype consistency in human colorectal carcinoma cell lines established *in vitro*. Cancer Genet. Cytogenet. **6**:93–117.
10. **Clewell, D. B.** 1972. Nature of Col E_1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
11. **Collins, S., and M. Groudine.** 1982. Amplification of endogenous *myc*-related DNA sequences in a human myeloid leukemia cell line. Nature (London) **298**:679–681.
12. **Fogh, J., W. Wright, and J. D. Loveless.** 1977. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J. Natl. Cancer Inst. **58**:209–214.
13. **Gilbert, F., G. Balaban, W. R. Breg, B. Gallie, T. Reid, and W. Nichols.** 1981. Homogeneously staining region in a retinoblastoma cell line: relevance to tumor initiation and progression. J. Natl. Cancer Inst. **67**:301–306.
14. **Grunstein, M., and D. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. U.S.A. **72**:3961–3965.
15. **Hamlyn, P. H., and T. H. Rabbitts.** 1983. Translocation joins c-*myc* and immunoglobulin γ1 genes in a Burkitt lymphoma revealing a third exon in the c-*myc* oncogene. Nature (London) **304**:135–139.
16. **Hay, R., M. Macy, and J. Shannon (ed.).** 1981. American tissue type collection, catalogue of strains II, p. 73. American Type Culture Collection, Rockville, Md.
17. **Kanalas, J. J., and D. Parker Suttle.** 1984. Amplification of the UMP synthase gene and enzyme overproduction in pyrazofurin-resistant rat hepatoma cells. J. Biol. Chem. **259**:1848–1853.
18. **Kanda, N., R. Schreck, F. Alt, G. Bruns, D. Baltimore, and S. Latt.** 1983. Isolation of amplified DNA sequences from IMR-32 human neuroblastoma cells: facilitation by fluorescence-activated flow sorting of metaphase chromosomes. Proc. Natl. Acad. Sci. U.S.A. **80**:4069–4073.
19. **Kempe, T. D., E. A. Swyryd, M. Bruist, and G. R. Stark.** 1976. Stable mutants of mammalian cells that overproduce the first three enzymes of pyrimidine nucleotide biosynthesis. Cell **9**:541–550.
20. **Kohl, N. E., N. Kanda, R. R. Schreck, G. Bruns, S. A. Latt, F. Gilbert, and F. W. Alt.** 1983. Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell **35**:359–367.
21. **Lewis, J. A., J. P. Davide, and P. W. Melera.** 1982. Selective amplification of polymorphic DHFR gene loci in Chinese hamster lung cells. Proc. Natl. Acad. Sci. U.S.A. **79**:6961–6965.
22. **Little, C. D., M. M. Nau, D. H. Carney, A. F. Gazdar, and J. D. Minna.** 1983. Amplification and expression of the c-myc oncogene in human lung cancer cell lines. Nature (London) **306**:194–196.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Melera, P. W., J. P. Davide, C. A. Hession, and K. W. Scotto.** 1984. Phenotypic expression in *Escherichia coli* and nucleotide sequence of two Chinese hamster lung cell cDNAs encoding different dihydrofolate reductases. Molec. Cell. Biol. **4**:38–48.
25. **Melera, P. W., C. A. Hession, J. P. Davide, K. W. Scotto, J. L. Biedler, M. B. Meyers, and S. Shanske.** 1982. Antifolate-resistant Chinese hamster cells: mRNA directed overproduction of multiple DHFRs from a series of independently derived sublines containing amplified DHFR genes. J. Biol. Chem. **257**:12939–12949.
26. **Melera, P. W., D. Wolgemuth, J. L. Biedler, and C. Hession.** 1980. Antifolate-resistant Chinese hamster cells: evidence from independently derived sublines for the overproduction of two DHFRs encoded by different mRNAs. J. Biol. Chem. **255**:319–322.
27. **Montgomery, K. T., J. L. Biedler, B. A. Spengler, and P. W. Melera.** 1983. Specific DNA sequence amplification in human neuroblastoma cells. Proc. Natl. Acad. Sci. U.S.A. **80**:5724–

5728.
28. **Norgard, M. V.** 1981. Rapid and simple removal of contaminating RNA from plasmid DNA without the use of RNase. Anal. Biochem. **113**:34–42.
29. **Quinn, L. A., G. E. Moore, R. T. Morgan, and L. K. Woods.** 1979. Cell lines from human colon carcinoma with unusual cell products, double minutes, and homogeneously staining regions. Cancer Res. **39**:4914–4924.
30. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
31. **Rozek, C. E., and N. Davidson.** 1983. *Drosophilia* has one myosin heavy-chain gene with three developmentally regulated transcripts. Cell **82**:23–34.
32. **Schwab, M., K. Alitalo, K.-H. Klempnauer, H. E. Varmus, J. M. Bishop, F. Gilbert, G. Brodeur, M. Goldstein, and J. Trent.** 1983. Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumor. Nature (London) **305**:245–248.
33. **Schwab, M., K. Alitalo, H. E. Varmus, J. M. Bishop, and D. George.** 1983. A cellular oncogene (c-ki-*ras*) is amplified, overexpressed, and located within karyotypic abnormalities in mouse adrenocortical tumour cells. Nature (London) **303**:497–501.
34. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
35. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
36. **Veira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
37. **Vennstrom, B., D. Sheiness, J. Zabielski, and J. M. Bishop.** 1982. Isolation and characterization of c-*myc*, a cellular homolog of the oncogene (v-*myc*) of avian myelocytomatosis virus strain 29. J. Virol. **42**:773–779.
38. **Watt, R., K. Nishikura, J. Sorrentino, A. ar-Rushdi, C. M. Croce, and G. Rovera.** 1983. The structure and nucleotide sequence of the 5′ end of the human c-*myc* oncogene. Proc. Natl. Acad. Sci. U.S.A. **80**:6307–6311.
39. **Whang-Peng, J., P. A. Bunn, C. S. Kao-Shan, E. C. Lee, D. N. Carney, A. Gazdar, and J. D. Minna.** 1982. A nonrandom chromosomal abnormality, deletion 3p(14–23), in human small cell lung cancer (SCLC). Cancer Genet. Cytogenet. **6**:119–134.