Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 5724–5728, September 1983
Medical Sciences

Specific DNA sequence amplification in human neuroblastoma cells

(gene amplification/chromosome abnormalities/neuronal malignancy)

KATE T. MONTGOMERY*, JUNE L. BIEDLER†, BARBARA A. SPENGLER†, AND PETER W. MELERA*‡

*Laboratory of RNA Synthesis and Regulation and †Laboratory of Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University, 1275 York Avenue, New York, New York 10021

Communicated by Paul A. Marks, May 26, 1983

ABSTRACT Southern blot analysis of a number of *Eco*RI-digested human neuroblastoma DNAs has revealed the presence of a family of discrete restriction fragments, the majority of which are amplified in most, but not all, of the neuroblastoma cell lines tested. None of these sequences is abundantly present in DNA from other human tumors of different tissue origins, including several either known or presumed to contain amplified DNA. Hence, these sequences appear to be specifically amplified by neuroblastoma cells. Hybridization with metaphase chromosomes *in situ* has localized these sequences to either the homogeneously staining regions or double-minute chromosomes of different neuroblastoma cell lines, indicating that these chromosomal structures, although present in cell lines established from different patients, share many sequences and may have a common, but as yet unknown, function.

Gene amplification is one of several mechanisms whereby cells can alter phenotypic expression when increased amounts of specific proteins are required, for example, during development (1–4) and in the face of environmental challenge when overproduction of specific proteins can impart resistance to otherwise lethal concentrations of cytotoxic agents (5–9). At the chromosomal level, gene amplification has been shown in a number of different experimental systems to be indicated by the presence of structural abnormalities called HSRs (homogeneously staining regions) and DMs (double-minute chromosomes). Originally reported by Biedler and Spengler (10) to be present in antifolate-resistant Chinese hamster lung cells overproducing dihydrofolate reductase, as well as in cells of two different neuroblastoma lines, SK-N-BE(2) and IMR-32, HSRs have since been demonstrated by hybridization *in situ* to contain amplified copies of (*i*) the DHFR gene in antifolate-resistant mouse and Chinese hamster cells (11, 12); (*ii*) DNA sequences that encode mRNA species whose function is not yet known in the mouse adrenocortoid tumor cell line Y-1 (13); and (*iii*) the c-*myc* gene in the neuroendocrine tumor cell line COLO 320 (14). Similarly, a large body of evidence now exists which suggests that DMs contain amplified DNA (13–18), although direct demonstration by hybridization *in situ* has not been possible due to their small size and random nonchromosomal location. Indeed, it is most likely that HSRs and DMs are alternate cytological manifestations of the same phenomenon—i.e., the somatic cell amplification of genomic DNA.

On the basis of these observations we have undertaken a study of the putatively amplified DNA sequences of human neuroblastoma cells. Initially shown by Biedler and Spengler (10) and Balaban-Malenbaum and Gilbert (19) to contain HSRs, the great majority of cell lines derived from these highly malignant and clinically refractory childhood tumors are now known to display either HSRs or DMS [see review by Biedler *et al.* (20)]. However, the functional significance of these structures in these cells is not understood, and attempts to equate their presence with the overproduction of specific proteins, readily demonstrated in some of the amplification systems mentioned above, or with the expression of neurotransmitter-synthesizing enzymes, have so far been unsuccessful (21). Therefore, we have begun this study by relying on the kinetic properties of DNA and, via a simple C_0t fractionation technique, have prepared genomic DNA probes containing amplified sequences. Using these probes we have been able to (*i*) directly demonstrate the amplification of DNA sequences in human neuroblastoma cells; (*ii*) provide evidence that common as well as different sequences are amplified in different neuroblastoma cell lines; (*iii*) show that, except for one possible exception, neuroblastoma cells amplify sets of DNA sequences not amplified in other HSR- or DM-containing tumor cells; and (*iv*) localize these amplified sequences to the HSRs and, in one case, the DMs of these human tumor cells.

MATERIALS AND METHODS

Cell Lines and Culture. The human neuroblastoma cell lines used in this work have recently been discussed (20) and include the HSR-containing lines NAP(H) and BE(2)-C [a clone of the SK-N-BE(2) line] and the DM-containing lines NAP(D), CHP-234, SMS-KAN, and SMS-KCNR. Two additional lines, SH-SY5Y (a clone of the SK-N-SH line) and SMS-KANR, neither of which contains evident HSRs or DMs but instead displays possible abnormally banding regions (ABRs), were also analyzed. Nonneuroblastoma human tumor cell lines studied were COLO 321, SK-Mel 28, SW-13, SW-48, SW-800, NCI-H-82, HA-L, and Y79T. Control DNA was prepared from both WI-38, a diploid human fibroblast line, and human placenta. Refer to Table 1 for further details.

Preparation of High Molecular Weight DNA and Southern Blot Analysis. High molecular weight DNA was obtained from cultured cells as reported previously (33). Normal human placental DNA was prepared essentially as described by Blin and Stafford (34). *Eco*RI digestions were carried out in the buffers recommended by the suppliers, at 37°C for 5–7 hr or overnight, using 4–7 units of enzyme per μg of DNA. Digestion products were separated by size on 0.8% nondenaturing agarose gels and transferred to nitrocellulose filter paper by the method of Southern (35). Filters were prehybridized and then hybridized with ^{32}P-labeled nick-translated probes and washed, as described (36). They were exposed to Kodak X-Omat XAR5 film with intensifying screens for 12–48 hr at −80°C.

DNA-Cellulose Preparation. High molecular weight human placental DNA (2 mg of DNA per 100 mg of ABM cellulose)

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.

Abbreviations: HSR, homogeneously staining region; DM, double-minute chromosome; NaCl/Cit, 0.15 M sodium chloride/0.015 M sodium citrate, pH 7; kb, kilobase(s); ABR, abnormally banding region.
‡To whom reprint requests should be addressed.

Table 1. Description of cell lines analyzed

Cells	Tissue type	Chromosome abnormality	Refs.
SH-SY5Y	Neuroblastoma*	†	20, 22, 23
SK-N-BE(1)	Neuroblastoma	DMs	20, 21, 23
BE(2)-C	Neuroblastoma*	HSRs	23
NAP(D)	Neuroblastoma	DMs	20, 21, 23
NAP(H)	Neuroblastoma	HSRs	20, 21
CHP-234	Neuroblastoma	DMs	20, 24
SMS-KAN	Neuroblastoma	DMs	20
SMS-KANR	Neuroblastoma	‡	20, 22
SMS-KCNR	Neuroblastoma	DMs	20
WI-38 (CCL 75)	Fibroblasts	None	25
Placenta	Normal tissue	None	This work
COLO 321	Neuroendocrine (colon)	HSRs	26
SW-13	Cortical adenocarcinoma		27
SW-48	Colon adenocarcinoma	None	27, 28
SW-800	Bladder carcinoma		27
SK-Mel-28	Melanoma	None	29
HA-L	Melanoma	HSRs	30
NCI-H82	Small cell lung carcinoma	HSRs	31
Y79T	Retinoblastoma	HSR	32

* Clone.
† One possible ABR.
‡ Five possible ABRs.

was bound to reprecipitated ABM-cellulose powder by the method described by Brison *et al.* (37). The DNA-cellulose powder was regenerated after each use by washing with 0.1 M NaOH/0.01 M EDTA, followed by repeated washings in TE (0.01 M Tris, pH 7.0/0.001 M EDTA) and stored at 4°C in TE.

C_0t Fractionation of Genomic DNAs and Probe Preparation. Genomic DNA was sheared to an average size of 425 base pairs, heat denatured at 105°C for 7 min, and allowed to reanneal in 0.12 M phosphate buffer at 65°C to C_0t 10. Double-stranded DNA was removed by hydroxylapatite chromatography and the remaining single-stranded DNA was dialyzed, concentrated, denatured, and allowed to reanneal to C_0t 300. The double-stranded DNA isolated by hydroxylapatite chromatography after the second reannealing was taken to represent the C_0t 10–300 fraction. One-microgram aliquots of this DNA were nick-translated (38) with ^{32}P-labeled precursors to a specific activity of 0.5–2.0 × 10^8 cpm/μg or with ^{3}H-labeled precursors to 1 × 10^8 dpm/μg. Highly repeated sequences were then removed by exhaustive hybridization of the probes with the cellulose-bound placental DNA. Between 30% and 50% of the nick-translated C_0t 10–300 DNA was routinely removed by this procedure.

Chromosome Preparation and *in Situ* Hybridization. Hybridization *in situ* was performed essentially as described by Chandler and Yunis (39). Metaphase plates, prepared as outlined previously (33), were digested with ribonuclease A and denatured for 2 min at 70°C in 50% formamide/0.3 M NaCl/0.03 M sodium citrate, pH 7 (2× NaCl/Cit). ^{3}H-Labeled nick-translated neuroblastoma probes were suspended at a final concentration of 100 ng/ml in 50% formamide/2× NaCl/Cit/10% dextran sulfate 500/Denhardt's reagent and 100 μg of salmon sperm DNA per ml. Following denaturation at 70°C for 15 min, the probe was applied to the denatured chromosomal DNA and hybridization was carried out for 3 or 18 hr at 37°C. Slides were rinsed briefly in 2× NaCl/Cit, washed in three 10-min changes of 50% formamide/2× NaCl/Cit at 42°C, rinsed again in 2× NaCl/Cit, then dehydrated, coated with emulsion, stored in light-tight boxes in the cold, and stained as described (33). Exposure time at 4°C ranged from 3 to 11 days.

RESULTS

Human Neuroblastoma Cells Contain Amplified DNA Sequences. Southern transfers of *Eco*RI-digested genomic DNA from both normal human and neuroblastoma cells were hybridized with a C_0t 10–300 probe obtained from the DM-containing neuroblastoma cell line CHP-234. The results of this experiment (Fig. 1*A*) indicate that though the probe contains sequences that hybridize with low intensity to a small number of *Eco*RI restriction fragments in the digests of WI-38 and placental DNA, it has many sequences that hybridize with great intensity to a large array of *Eco*RI restriction fragments found in both the CHP-234 and BE(2)-C neuroblastoma DNA digests. When a C_0t 10–300 probe prepared from WI-38 DNA was hybridized to Southern transfers of *Eco*RI digests of the same four DNAs (Fig. 1*B*), no such intense hybridization was observed. Rather, the intensity and patterns of hybridization in all cases are similar to that seen when WI-38 and placental DNA are hybridized with the C_0t 10–300 CHP-234 probe (Fig. 1*A*, lanes a and d).

The pattern of hybridization seen in Fig. 1*B* is consistently observed when probes obtained from normal DNA are hybridized with either normal or neuroblastoma DNA and when neuroblastoma tumor probes are hybridized with normal DNA or with DNA obtained from nonneuroblastoma tumors that do not contain HSRs or DMs (Fig. 1*C*). This control pattern always includes a doublet of approximately 8.8 kb and 7.8 kb in addition to several other bands, depending on the exposure time and the amount of DNA used in the digest. Presumably these bands represent sequences normally found in the C_0t 10–300 fraction of human genomic DNA. Therefore, they are present in the normal probe as well as in the tumor probe and in nearly equal amounts in all genomic digests. The control pattern is obscured when a C_0t 10–300 neuroblastoma probe is hybridized with neuroblastoma DNA because of the intensity of hybridization caused by the presence of amplified sequences in both. Taken together the data presented in Fig. 1 demonstrate the presence of amplified DNA sequences in the CHP-234 and BE(2)-C genomes and show that these sequences are not present in amplified amounts either in nonneuroblastoma tumor DNAs or in the normal human DNAs tested.

Variation in Amplification Pattern Among Different Neuroblastoma Cell Lines. In addition to establishing the presence of amplified DNA sequences within the human neuroblastoma genome, the data in Fig. 1 also indicate that, whereas several *Eco*RI fragments are amplified in both CHP-234 and BE(2)-C cells, others appear only in the CHP-234 digests (namely, 10.2, 3.4, and 2.6 kb). When a C_0t 10–300 probe was prepared from cell line BE(2)-C and hybridized with *Eco*RI-digested BE(2)-C and CHP-234 DNA (Fig. 2*A*, lanes a and b), the 10.2-, 3.4- and 2.6-kb fragments did not hybridize, indicating that these sequences are not contained in the BE(2)-C probe and that they are not amplified in BE(2)-C cells.

Other examples of different *Eco*RI fragments amplified by different neuroblastoma cell lines and reactive with the BE(2)-C and CHP-234 C_0t 10–300 probes are shown in Fig. 2 *A* and *B*, and examples of specific hybridization pattern differences between neuroblastoma cell lines with the use of other C_0t 10–300 probes have also been found (unpublished data). Although the extent of these differences varies from small [namely, SMS-KAN versus NAP(H), Fig. 2*B*, lanes a and b] to large (namely, SMS-KCNR, Fig. 2*B*, lane d, versus any of the other DNAs in

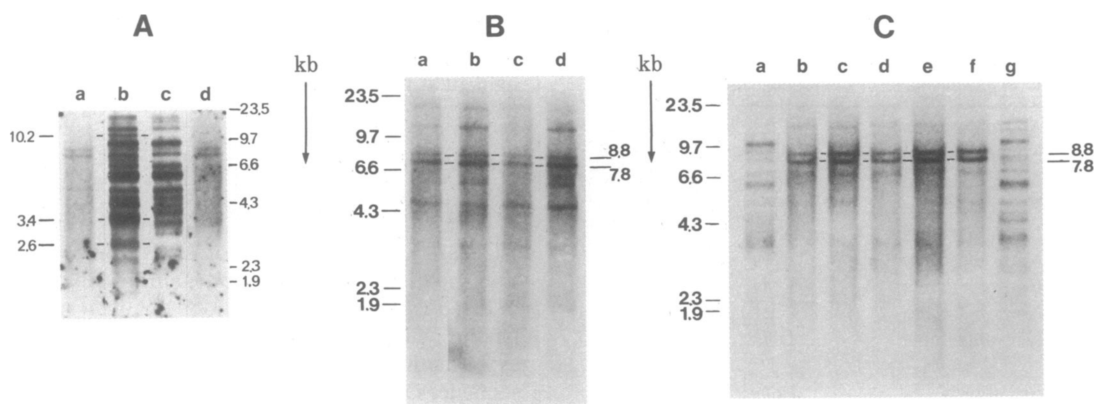

FIG. 1. Southern blot analysis of human neuroblastoma and other human tumor DNAs. High molecular weight DNA was digested with *Eco*RI at an enzyme-to-DNA ratio of 4–7 units/μg overnight at 37°C. Samples were then adjusted to 40 mM Tris·HCl, pH 7.9/7 mM sodium acetate/1 mM EDTA/2% Ficoll/0.1% bromophenol blue and electrophoresed for 16–20 hr in 0.8% agarose at 50 V. The molecular weight marker used was *Hin*dIII-cut λ DNA. Transfer of DNA to nitrocellulose paper and hybridization with ^{32}P-labeled C_0t 10–300 probes was as described. To assay for partial digestion the enzyme-to-DNA ratios were varied from 35:1 to 4:1 and incubations run from 18 hr to 4 hr, respectively. No differences in Southern patterns were observed between these two extremes; hence, partial digestion was ruled out as a cause for the variable banding patterns observed among neuroblastoma cell lines. (*A*) Hybridization with a C_0t 10–300 probe from CHP-234 DNA. Lane a, 5 μg of WI-38; lane b, 5 μg of CHP-234; lane c, 5 μg of BE(2)-C; lane d, 5 μg of placenta. (*B*) Hybridization with a C_0t 10–300 probe from WI-38 DNA. Lane a, 5 μg of WI-38; lane b, 5 μg of CHP-234; lane c, 5 μg of BE(2)-C; lane d, 5 μg of placenta. (*C*) Hybridization with a C_0t 10–300 probe from CHP-234 DNA. Lane a, 2.5 μg of NAP(H); lane b, 10 μg of SK-Mel-28; lane c, 10 μg of SW-13; lane d, 10 μg of placenta; lane e, 10 μg of SW-48; lane f, 10 μg of SW-800; lane g, 2.5 μg of CHP-234. kb, Kilobases.

Fig. 2), it is clear that a great deal of cross-hybridization occurs between the amplified DNAs of human neuroblastoma cells, suggesting that the same or similar DNA sequences are amplified in all of these tumor cell lines. However, the restriction pattern differences cannot be simply interpreted due to the heterogeneity of the probes used and therefore may be due either to restriction site polymorphisms or to the amplification of different sequences by different lines. Additionally, not all neuroblastoma cell lines can be shown to contain amplified DNA sequences at the levels of detection being considered here. When hybridized with a C_0t 10–300 probe from CHP-234, the pattern of hybridization displayed by the SH-SY5Y cell line appears normal (Fig. 2*B*, lane e). Hence, DNA sequence amplification, although prevalent among neuroblastoma cell lines, may not be required for maintenance of the neuroblastoma phenotype.

Amplified Sequences Are Associated with Specific Cytologic Markers. A C_0t 10–300 probe prepared from the HSR-containing NAP line was used for hybridization *in situ* with metaphase chromosome preparations from NAP(H) and the DM-containing line CHP-234. The intensity of hybridization over the HSR on NAP(H) chromosome 7p clearly indicates that amplified DNA sequences reside within that region (Fig. 3*b*). Hybridization of the NAP probe with CHP-234 also yielded positive results and showed specific signal over this cell's palely stained DMs (Fig. 3*d*). This represents direct demonstration by hybridization *in situ* of amplified DNA sequences within DMs and most likely results from the fact that the CHP-234 DMs are readily visualizable and that the probe used contains many of the DNA sequences contained within them.

Sequences Amplified and Maintained by Neuroblastoma Cells Are Not Amplified and Maintained by Other HSR- or DM-Containing Human Tumors. To rule out the possibility that the amplified sequences present in human neuroblastoma cells are amplified in all HSR- or DM-containing tumor cells, whatever their tissue of origin, we hybridized a C_0t 10–300 probe from the BE(2)-C line with a panel of *Eco*RI-digested DNAs from four nonneuroblastoma human tumor lines known to contain HSRs or DMs, one of which, the neuroendocrine tumor, COLO 320/321, was recently shown to contain amplified DNA (14, 40). The result of this analysis is shown in Fig. 4 and clearly

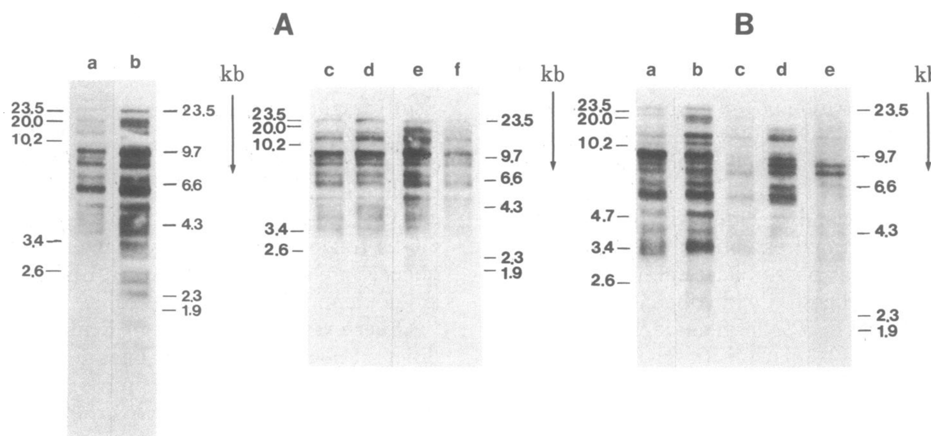

FIG. 2. Southern blot analysis of different neuroblastoma DNAs. Experimental conditions are as described in the legend to Fig. 1. (*A*) Hybridization with a C_0t 10–300 probe from BE(2)-C DNA. Lane a, 2 μg of CHP-234; lane b, 4 μg of BE(2)-C; lane c, 2.5 μg of NAP(H); lane d, 2.5 μg of NAP(D); lane e, 1 μg of SMS-KAN; lane f, 2.5 μg of SMS-KANR. (*B*) Hybridization with a C_0t 10–300 probe from CHP-234 DNA. Lane a, 5 μg of NAP(H); lane b, 5 μg of SMS-KAN; lane c, 5 μg of SMS-KANR; lane d, 5 μg of SMS-KCNR; lane e, 10 μg of SH-SY5Y.

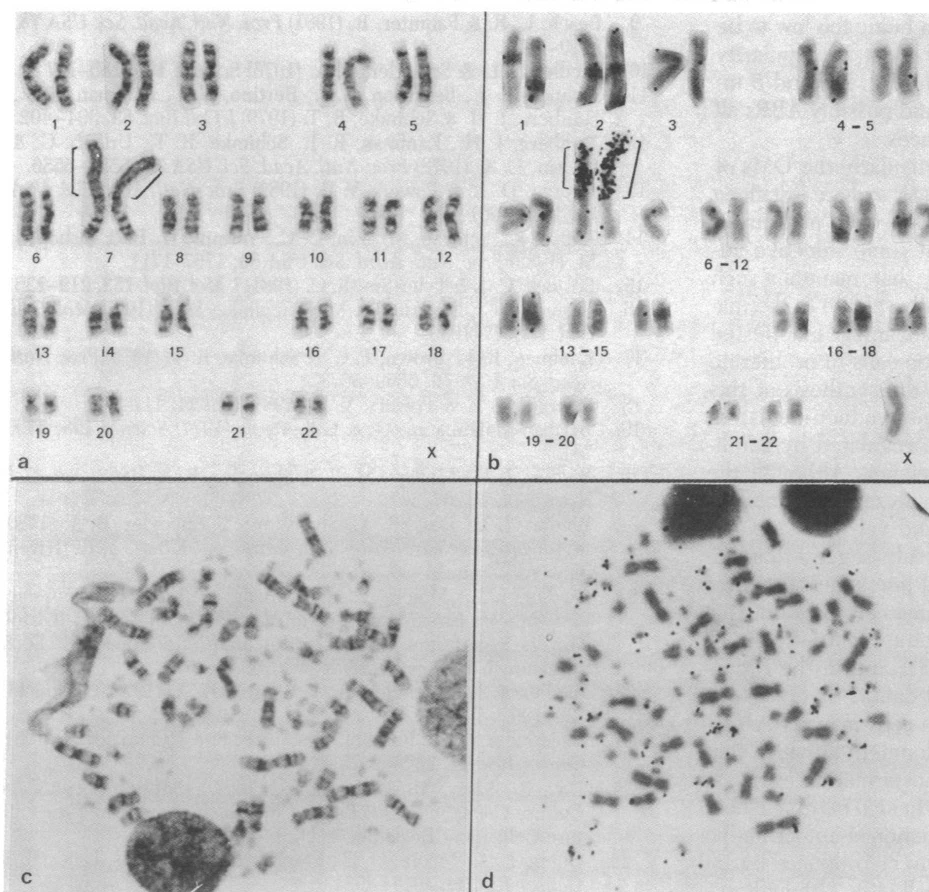

FIG. 3. Human neuroblastoma cells hybridized *in situ* with a C_0t 10–300 probe from NAP(H) DNA. (*a*) Trypsin–Giemsa banded karyotype of the NAP(H) cell line. Brackets indicate the two long HSRs on both chromosome 7 homologs (at 7p22). (*b*) Karyotype of NAP(H) cell hybridized *in situ* with 100 ng of tritiated NAP(H) DNA probe per ml. Brackets indicate HSRs. Cells were hybridized for 18 hr, as described, and exposed for 7 days at 4°C. Approximately 70% of the 130 silver grains are over the two HSRs. Only 3% of grains are between chromosomes. (*c*) Trypsin–Giemsa banded metaphase cell of the CHP-234 cell line. Numerous, nonbanded, palely staining DMs are visible. (*d*) Metaphase CHP-234 cell hybridized *in situ* with the NAP(H) DNA probe for 3 hr as described. Slides were exposed for 11 days at 4°C. Approximately 75% of the 130 silver grains overlie some 60 discernable DMs, whereas only 7% overlie chromosomes.

indicates that the majority of sequences amplified and maintained by neuroblastoma cells are not amplified and maintained by other human tumor cells. In only one case, that of the retinoblastoma line Y79T, was there any indication of a banding pattern or hybridization intensity approaching that of neuroblastoma. However, because the embryological identity of retinoblastomas is sometimes questionable, there is a possibility that the Y79T line is ontogenetically related to neuroblastoma. Indeed, the Y79T line has been shown to express neuronal-specific enzymes (24).

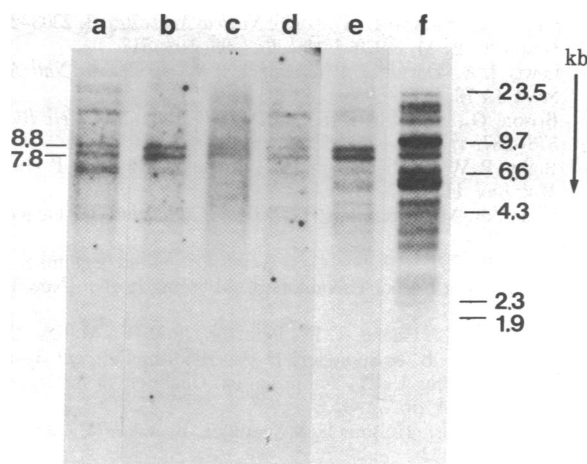

FIG. 4. Southern blot analysis of other HSR- and DM-containing human tumor DNAs. Conditions for analysis were the same as described in the legend to Fig. 1. Hybridization was performed with a C_0t 10–300 probe from BE(2)-C DNA. Lane a, 5 μg of placenta; lane b, 5 μg of NCI-N82; lane c, 5 μg of COLO 321; lane d, 5 μg of HA-L; lane e, 5 μg of Y79T; lane f, 1 μg of BE(2)-C.

DISCUSSION

The work presented here demonstrates the presence of amplified DNA sequences in the HSRs and DMs of human neuroblastoma cells and shows that, while some cells amplify sequences that others do not, there is a great deal of cross-hybridization between the amplified sequences isolated from cells established in culture from different patients. Though it is likely that the amplified DNA detected here represents, in other cells, a mixture of unique and low copy number sequences, the significance of its amplification remains unclear.

Whereas the results show that some qualitative differences exist between the DNA amplified by different neuroblastoma cell lines, hybridization intensity differences such as those between SMS-KAN and SMS-KANR (Fig. 2*A*, lanes e and f, and Fig. 2*B*, lanes b and c) suggest that overall differences in the degree of amplification may also occur. The SMS-KAN line was established from a primary tumor explant; all cells contained DMs with a mean number per cell of approximately 150. The SMS-KANR line was established 8 months later from the bone marrow of the same patient in clinical relapse. The presence of shared, structurally abnormal marker chromosomes indicates that the SMS-KAN and SMS-KANR lines were derived from a common cell precursor (20); however, DMs were not detected in SMS-KANR cells. Interestingly, SMS-KANR cells display five ABRs (22) known to be sites of low level DHFR gene amplification in antifolate-resistant Chinese hamster lung cells (33). That ABRs may also represent sites of amplified genes in neuroblastoma cells is indicated by the Southern blots in Fig. 2, which show that DNA fragments amplified by DM-containing SMS-KAN cells are also amplified, albeit to a lower level, in the ABR-containing SMS-KANR cell line. Our failure to observe amplified sequences in the SH-SY5Y line (Fig. 2*B*, lane e), even though it may contain a single, small ABR, is most eas-

ily explained by its degree of amplification being too low to be detected by the methods used here. In any event, the similarity of Southern patterns shown in Fig. 1*A* and Fig. 2 *A* and *B* indicates that neuroblastoma HSRs, DMs, and possibly ABRs all share many of the same amplified sequences.

Because it is known that HSRs and particularly the DMs of drug-resistant cells are unstable structures (41) and are lost along with their amplified copies of DNA when selection pressure is removed, it is reasonable to assume that some selective advantage is afforded neuroblastoma cells that maintain such structures. That advantage could be causally related to the malignant phenotype by bestowing a growth advantage manifested by the ability of these normally nonmitotic cells to proliferate both *in vivo* and in culture. Presumably the synthesis of this growth-promoting substance would be gene product-mediated; hence, its increased production could be regulated by amplification or enhanced transcriptional efficiency. Although the great majority of neuroblastoma cells analyzed contains amplified DNA, one, SH-SY5Y, may not and yet maintains the malignant phenotype (42). Preliminary results suggest that neither Harvey-*ras*- nor Kirsten-*ras*-related *onc* gene sequences are amplified by any of our neuroblastoma cell lines but that a sequence related to the human c-*myc* gene is amplified in all neuroblastoma cell lines, except SH-SY5Y, and in the retinoblastoma line Y79T as well (unpublished data).

Alternatively, different neuroblastoma cells may reflect different stages of normal neuroblast development at which they have been arrested due to the initial transformation event. In this case the initial amplification of DNA *in vivo* may have been in normal response to the temporal production of autocrine-like regulator substances, which are thought to be produced by and have regulatory effects upon the same cell (43). Although presumably transient during development, the production of such factors by neuroblastoma cells may, as a concomitant of malignant transformation, become constitutive, thereby stabilizing the presence of the amplified DNA *in vivo* and *in vitro*. The various Southern banding patterns we have observed could reflect cells transformed at different developmental stages, and the SH-SY5Y line may simply have been transformed at a time when no such regulatory factors and hence no amplified DNA was present. Interestingly, the transient amplification of actin genes during development of chicken muscle has been reported (44) and the possibility that gene amplification may be among those regulatory mechanisms that mediate the progression of cells through specific developmental pathways remains a viable subject for further study.

This work was supported in part by grants from the National Institutes of Health (to the Sloan-Kettering Institute and to J.L.B.) and from the Special Projects Committee of the Society of the Memorial Sloan-Kettering Cancer Center (to J.L.B. and P.W.M.). K.T.M. is a predoctoral Research Fellow supported in part by the Westchester Women for Cancer Research.

1. Brown, D. D. & Dawid, J. B. (1968) *Science* **160**, 272–280.
2. Gall, J. G. (1968) *Proc. Natl. Acad. Sci. USA* **60**, 553–560.
3. Spradling, A. C. & Mahowald, A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1096–1100.
4. Glover, D. M., Zaha, A., Stocker, A. J., Santelli, R. V., Pueyo, M. T., deToledo, S. M. & Lara, F. J. S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2947–2951.
5. Alt, F. W., Kellems, R. E., Bertino, J. R. & Schimke, R. T. (1978) *J. Biol. Chem.* **253**, 1357–1370.
6. Wahl, G., Padgett, R. A. & Stark, G. R. (1979) *J. Biol. Chem.* **254**, 8679–8689.
7. Bostock, C. J., Clark, E. M., Harding, N. G. L., Mounts, P. M., Tyler-Smith, C., van Heyningen, V. & Walker, P. M. B. (1979) *Chromosoma* **74**, 153–177.
8. Melera, P. W., Lewis, J. A., Biedler, J. L. & Hession, C. (1980) *J. Biol. Chem.* **255**, 7024–7028.
9. Beach, L. R. & Palmiter, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2110–2114.
10. Biedler, J. L. & Spengler, B. A. (1976) *Science* **191**, 185–187.
11. Dolnick, B. J., Berenson, R. J., Bertino, J. R., Kaufman, R. J., Nunberg, J. H. & Schimke, R. T. (1979) *J. Cell Biol.* **83**, 394–402.
12. Nunberg, J. H., Kaufman, R. J., Schimke, R. T., Urlaub, G. & Chasin, L. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5553–5556.
13. George, D. L. & Powers, V. E. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1597–1601.
14. Alitalo, K., Schwab, M., Lin, C. C., Varmus, H. E. & Bishop, J. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1707–1711.
15. Bostock, C. J. & Tyler-Smith, C. (1981) *J. Mol. Biol.* **153**, 219–236.
16. Brown, P. C., Beverley, S. M. & Schimke, R. T. (1981) *Mol. Cell. Biol.* **1**, 1077–1083.
17. Kaufman, R. J., Brown, P. C. & Schimke, R. T. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5669–5673.
18. George, D. L. & Powers, V. E. (1981) *Cell* **24**, 117–123.
19. Balaban-Malenbaum, G. & Gilbert, F. (1977) *Science* **198**, 739–741.
20. Biedler, J. L., Meyers, M. B. & Spengler, B. A. (1983) *Adv. Cell Neurobiol.* **4**, 267–307.
21. Biedler, J. L., Ross, R. A., Shanske, S. & Spengler, B. A. (1980) in *Advances in Neuroblastoma Research*, ed. Evans, A. E. (Raven, New York), pp. 81–96.
22. Biedler, J. L., Melera, P. W. & Spengler, B. A., *Chromosomes and Cancer: From Molecules to Man*, The 5th Annual Bristol-Meyers Symposium on Cancer Research, eds. Rowley, J. D. & Ultmann, J. E. (Academic, New York), in press.
23. Biedler, J. L., Spengler, B. A. & Ross, R. A. (1979) *Gaslini* **11**, 128–139.
24. Schlesinger, H. R., Rorke, L., Jamieson, R. & Hummeler, K. (1981) *Cancer Res.* **41**, 2573–2575.
25. Hay, R., Macy, M. & Shannon, J., eds. (1981) *American Type Culture Collection, Catalogue of Strains II* (American Type Culture Collection, Rockville, MD), p. 73.
26. Quinn, L. A., Moore, G. E., Morgan, R. T. & Woods, L. K. (1979) *Cancer Res.* **39**, 4914–4924.
27. Fogh, J., Wright, W. C. & Loveless, J. D. (1977) *J. Natl. Cancer Inst.* **58**, 209–214.
28. Chen, T. R., Hay, R. J. & Macy, M. L. (1982) *Cancer Genet. Cytogenet.* **6**, 93–117.
29. Carey, T. E., Takahashi, T., Resnick, L. A., Oettgen, H. F. & Old, L. J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3278–3282.
30. Trent, J. M., Thompson, F. H. & Ludwig, C. (1983) *Cancer Res.* **43**, 4733–4737.
31. Whang-Peng, J., Bunn, P. A., Jr., Kao-Shan, C. S., Lee, E. C., Carney, D. N., Gazdar, A. & Minna, J. D. (1982) *Cancer Genet. Cytogenet.* **6**, 119–134.
32. Gilbert, F., Balaban, G., Berg, W. R., Gallie, B., Reid, T. & Nichols, W. (1981) *J. Natl. Cancer Inst.* **67**, 301–306.
33. Lewis, J. A., Biedler, J. L. & Melera, P. W. (1982) *J. Cell Biol.* **94**, 418–424.
34. Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3**, 2303–2308.
35. Southern, E. M. (1976) *J. Mol. Biol.* **98**, 503–517.
36. Lewis, J. A., Davide, J. P. & Melera, P. W. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6961–6965.
37. Brison, O., Ardeshir, F. & Stark, G. R. (1982) *Mol. Cell. Biol.* **2**, 578–587.
38. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
39. Chandler, M. E. & Yunis, J. J. (1978) *Cytogenet. Cell Genet.* **22**, 352–356.
40. Hubbell, H. R. (1982) in *Gene Amplification*, ed. Schimke, R. T. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), pp. 193–198.
41. Biedler, J. L., Chang, T. D., Peterson, R. H. F., Melera, P. W., Meyers, M. B. & Spengler, B. A. (1983) in *Rational Basis for Chemotherapy*, UCLA Symposia, ed. Chabner, B. A. (Liss, New York), Vol. 4, pp. 71–92.
42. Biedler, J. L., Helson, L. & Spengler, B. A. (1973) *Cancer Res.* **33**, 2643–2652.
43. Sporn, M. B. & Todaro, G. J. (1980) *N. Engl. J. Med.* **303**, 878–880.
44. Zimmer, W. E. & Schwartz, R. J. (1982) in *Gene Amplification*, ed. Schimke, R. T. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), pp. 137–146.