Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 3761–3765, June 1985
Genetics

Differential amplification, assembly, and relocation of multiple DNA sequences in human neuroblastomas and neuroblastoma cell lines

(N-*myc*/gene mapping/human chromosome 2/tumor progression)

YOSEF SHILOH*†, JANET SHIPLEY*†, GARRETT M. BRODEUR‡, GAIL BRUNS*†, BRUCE KORF*†, TIM DONLON*†, RHONA R. SCHRECK*†, ROBERT SEEGER§¶, KAZUO SAKAI*†, AND SAMUEL A. LATT*†||**

*Mental Retardation Center and Genetics Division, Children's Hospital, Boston; Departments of †Pediatrics and ||Genetics, Harvard Medical School, Boston, MA 02115; ‡Department of Pediatrics, Washington University School of Medicine, St. Louis, MO 63110; and the §Children's Cancer Study Group and ¶University of California at Los Angeles, Los Angeles, CA 90024

Communicated by J. Herbert Taylor, February 4, 1985

ABSTRACT **DNA amplification, manifested by homogeneously staining regions in chromosomes and by extrachromosomal, double minute bodies, is characteristic of many neuroblastoma cell lines. Sequences recruited from a specific domain on the short arm of chromosome 2 (2p) are amplified in advanced-stage primary neuroblastomas, whereas sequences from distinctly different regions of 2p are amplified in the neuroblastoma cell line IMR-32. Five different DNA segments, which include the oncogene N-*myc*, three other fragments derived from the homogeneously staining region of the neuroblastoma cell line IMR-32, and a fifth fragment, derived from the neuroblastoma cell line NB-9, showed differential and variable amplification in 24 advanced-stage neuroblastoma tumors out of 112 tested specimens. All five fragments were mapped within the chromosomal region 2p23-2p25 by three different approaches. However, eight other fragments cloned from the homogeneously staining region of IMR-32 cells, which were not amplified in the tumor tissues examined, were mapped to two more proximal domains of 2p, thousands of kilobases apart from each other and from the chromosomal domain that is amplified in the tumors. These results establish the amplification, to different degrees, of a variable-sized segment of one domain near the terminus of 2p in advanced neuroblastomas. These tumors might ultimately be distinguished according to the pattern of amplification of DNA segments within this domain. The data presented also indicate the existence of a new and complex amplification mechanism in at least one neuroblastoma cell line (IMR-32), which involves not only relocation of DNA from specific genomic domains but also the formation of novel units by splicing together very distant DNA segments.**

There is now firm evidence for the association of DNA amplification, particularly that of several oncogenes, with a number of malignancies, such as leukemia, neuroblastoma, and retinoblastoma (1–3). DNA amplification is evident cytologically either as homogeneously staining regions (HSRs) or double minute bodies (DMs) in both primary tumor cells and cell lines established from the tumors (1, 2, 4). Molecular analysis of the structure and function of the amplified sequences and elucidation of the mechanism of their amplification should prove useful in understanding the role of specific genes in tumorigenesis.

Isolation and cloning of DNA from a HSR of one neuroblastoma cell line, IMR-32, has provided one approach for studying DNA amplification. Eleven DNA segments, which were cloned from the HSR found in chromosome 1 in these cells, were mapped to the short arm of the normal chromosome 2 (2p), thereby indicating a complex amplification–transposition process (5). One of those 11 sequences, probe 8 (the eighth largest), was found to be amplified in several other neuroblastoma and retinoblastoma cell lines (6, 7), in which the recently described putative oncogene N-*myc* is amplified as well (6–10). Molecular probes for N-*myc* have now been isolated by three approaches (6, 8, 11). In two studies, N-*myc* has been mapped to 2p (6, 12), more precisely to the distal region p23-p24 (12). Hence, amplification of this domain on 2p may play a role in the progression of neuroblastoma and retinoblastoma.

The biological significance of DNA amplification in neuroblastoma cell lines was reinforced by the observation that the DNA sequence detected by the N-*myc* probe pNb-1 (8) was amplified in a number of advanced-stage primary neuroblastomas (13). We have now confirmed and extended these studies by using another N-*myc* probe, NB-19-21 (6), on a larger series of DNA samples. In addition, we have further subdivided the tumors that amplify this sequence into nested subsets amplifying increasing numbers of additional sequences initially isolated from two neuroblastoma cell lines. Detailed regional mapping of these sequences in the normal genome indeed shows that differential and variable amplification of a specific region on the distal part of 2p occurs in neuroblastoma tissues. However, additional, distant DNA sequences have joined the process in the formation of the HSR in IMR-32 cells.

MATERIALS AND METHODS

DNA from Tumor Tissues. One-hundred twelve primary tumors were tested, most of which were obtained from the Children's Cancer Study Group (CCSG), with the rest obtained at the coauthors' institutions [55 of these tumors were included in a previous study done with the N-*myc* probe pNb-1 (13)]. The tumors had been classified according to the clinical staging system of Evans *et al.* (14). DNA was prepared from the tissues as described (7, 13) and was quantified by fluorometry (15).

Probes. The 11 HSR-specific probes were previously isolated from a Charon 21A library constructed from *Hin*dIII-digested DNA of the HSR-bearing chromosome 1 of IMR-32 cells (5). They were numbered 1–11, the numbers referring to decreasing size [ranging from 6.7 to 0.46 kilobase (kb)]. NB-9-A is a 1.65-kb *Hin*dIII–*Eco*RV fragment subcloned from a 3.2-kb *Hin*dIII segment isolated from cells of the neuroblastoma line NB-9, in which it is involved in a DNA rearrangement also involving part of probe 8 (6, 7). Segment NB-9-A is

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: 2p, short arm of human chromosome 2; HSR, homogeneously staining region; DM, double minute body; kb, kilobase(s).

**To whom correspondence and reprint requests should be addressed at: Children's Hospital, Genetics Division, 300 Longwood Avenue, Boston, MA 02115.

amplified in NB-9 cells but not in IMR-32. The two independently derived N-*myc* probes NB-19-21 [a 2.1-kb *Eco*RI fragment cloned from NB-19 cells (6)] and pNb-1 [a 1.0-kb *Bam*HI–*Eco*RI fragment cloned from the cell line Kelly (8)] were used in parallel, in Boston and St. Louis, respectively, to probe DNA from the same tumors. The restriction maps for these probes (7, 8) are similar but not entirely identical, perhaps reflecting polymorphic variation. H2-26, a single-copy 3.8-kb *Hin*dIII fragment derived from chromosome 13 (generously contributed by M. Lalande), served as an internal single-copy control in the former studies, permitting quantitative analysis (16) of DNA amplification. A 2.7-kb subclone of probe 1 (5) was used, rather than the entire probe 1, which detects repeated sequences.

Somatic Cell Hybrids. The somatic cell hybrids G24A4, G35A5, G35D3, and RRP-5-3 were derived from fusions of either mouse cells (RAG) or Chinese hamster cells (E36) with human cells and were characterized for human chromosome complements by analysis of human isozyme markers and by cytogenetic techniques (17, 18). In the course of their maintenance in culture, spontaneous rearrangements of human chromosome 2 occurred in these hybrids, and therefore they contain different portions of this chromosome. These hybrid lines were again characterized by analysis of human chromosome 2 markers malate dehydrogenase 1, which has been mapped to band 2p23 (19), and isocitrate dehydrogenase 1, mapped to 2q32-2qter (19), and by testing of their DNA for hybridization with a fragment of the immunoglobulin κ gene, kindly provided by Philip Leder, which has been mapped to human 2p12 (20). DNA from the two human–mouse hybrids A9 Call 1-9-3 and A9 Call 1-13, which contain the chromosome 2 regions qter → p23 and p23 → pter, respectively, and which were described earlier (8, 21), was a generous gift from K.-H. Grzeschik (Munster, Federal Republic of Germany).

Molecular Techniques. Digestion by restriction enzymes of genomic DNA, gel electrophoresis, Southern blotting, hybridization, and scanning densitometry were performed as described (5–7, 13, 16). *In situ* hybridization was done according to Harper and Saunders (23).

RESULTS

Amplified Sequences in Neuroblastoma Tissues. Amplification of the various DNA fragments used in this study in 112 primary neuroblastomas was examined by using them as probes against Southern blots of tumor DNAs digested to completion with either *Hin*dIII or *Eco*RI. DNA samples from three negative controls (normal blood, placenta, and a normal lymphoblastoid line) and one positive control (IMR-32) were run on each gel, and each blot was also hybridized with the control probe H2-26 (22). The copy number of every probe in each sample was estimated by scanning densitometry readings (16), which were normalized for experimental variation against the standard probe H2-26. The results (Figs. 1 and 2, Table 1) showed differential and variable amplification of at least 10-fold of 5 of the 13 probes used in 24 of the 112 tumors. The pattern of this amplification enabled the division of these tumors into nested sets—according to the probes that were amplified in their DNA (Fig. 1).

In all 24 tumors, both N-*myc* probes that were used independently in two laboratories showed similar degrees of amplification of from 10- to 270-fold (Table 1). This result is an extension of a previous study done with the N-*myc* probe pNb-1 on 11 of these tumors (13). All 24 tumors had been clinically classified as advanced-stage tumors (stage III or stage IV), and thus the correlation between N-*myc* amplification and advanced tumor progression (with poor prognosis) (13) is further substantiated.

Four DNA segments (the IMR-32 probes 8, 3, and 1 as well as probe NB-9-A) were amplified in smaller subsets of these tumors (Fig. 1), with copy numbers ranging between 20

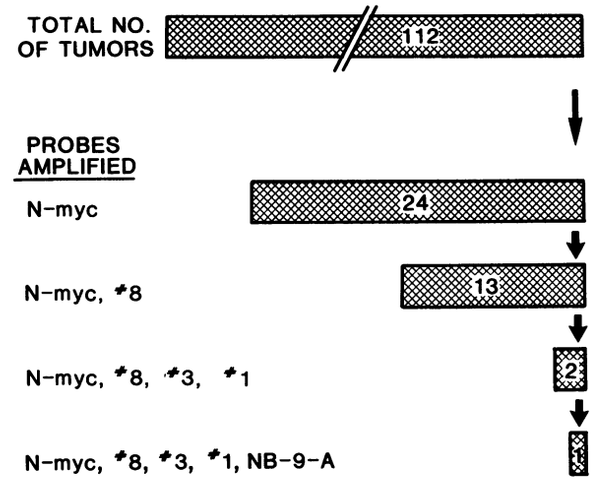

FIG. 1. Partition of 112 primary neuroblastomas into nested subsets characterized by specific amplification patterns. On the right is the number of tumors in which probes, listed on the left, are amplified. Each descending group is a subset of the one above.

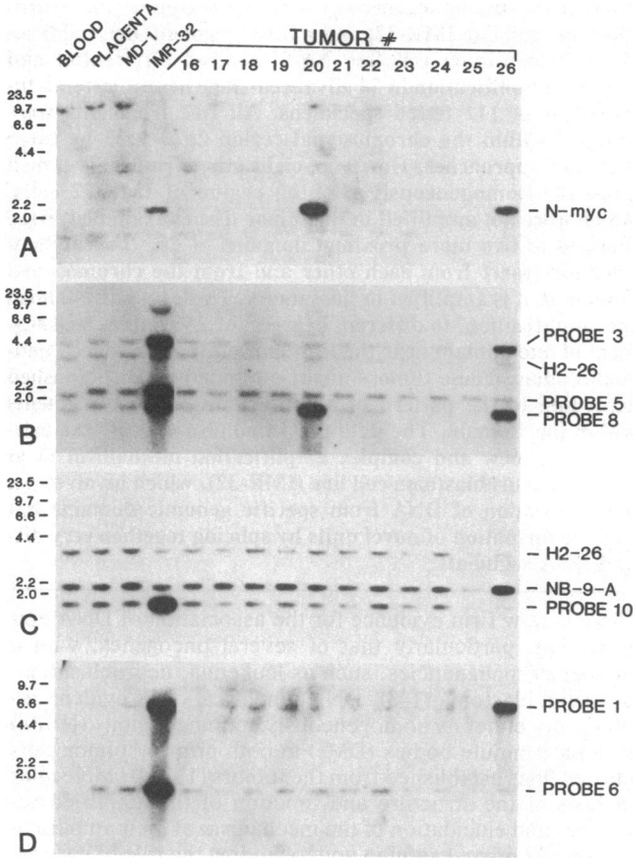

FIG. 2. Southern blots showing differential amplification of five probes (N-*myc*, probes 1, 3, 8, and NB-9-A) in several primary neuroblastomas (numbered 16–26). Two micrograms of DNA digested with either *Eco*RI (*A*) or *Hin*dIII (*B–D*) was run in each lane. The blots were hybridized with probes that had been end-labeled using T4 DNA polymerase. Note tumor 26, the only one that showed amplification of all five probes, four of which are amplified in other tumors, in different, smaller combinations. Probes 5, 6, and 10 were not amplified in any tumor. H2-26 is a single-copy control probe that served to normalize the densitometry readings. Normal human DNA from blood, placenta, or lymphoblasts (MD-1) was included, as was IMR-32 DNA.

Table 1. Amplification of different DNA segments in 24 primary neuroblastomas

Tumor no.	Copy number per haploid genome				
	Probe N-*myc**	Probe 8	Probe 3	Probe 1	Probe NB-9-A
26	220	90	50	60	20
8	130	120	40	260	1
20, 30, 43, 45, 53, 64, 66, 73, 81, 84, 86	10–270	30–240	1	1	1
4, 13, 33, 34, 51, 60, 63, 78, 83, 89, 92	10–230	1	1	1	1

The extent of amplification was estimated by using scanning densitometry of autoradiograms from Southern blots.
*Based on a value of 25 for the amplification of N-*myc* probe NB-19-21 in IMR-32 DNA, published previously (6) and confirmed independently in our laboratory.

and 260 (Table 1). In no case was amplification of any probe observed without N-*myc* also being amplified in the same tumor.

Mapping of the Amplified Sequences in Normal Cells. Mapping of the amplified sequences in the normal genome, and especially their localization relative to one another, is an early step in elucidating the structure and the origin of the amplified unit. All of the 11 HSR probes had been mapped previously to 2p (5, 6), and N-*myc* (pNb-1) has been localized recently to the region 2p23-2p24 (12). We have used three different approaches to document mapping of these and related probes to different regions on 2p: (*i*) *in situ* hybridization to metaphase chromosomes, (*ii*) segregation of sequences homologous to these probes in somatic cell hybrids with different human chromosome constitution, and (*iii*) quantitative hybridization of some of these probes to DNA from cells with unbalanced karyotype with regard to different portions of 2p. Fig. 3 shows the autoradiographic grain distribution over the genome following *in situ* hybridization of one of those probes, whereas Fig. 4 shows the distribution of six different probes over chromosome 2. It is evident from these results that the four probes tested thus far that are highly amplified in tumor tissues are located in the distal region of the short arm of chromosome 2 (2p23-2p25) (Fig. 4 *A–D*), whereas two of the IMR-32-derived probes, which are

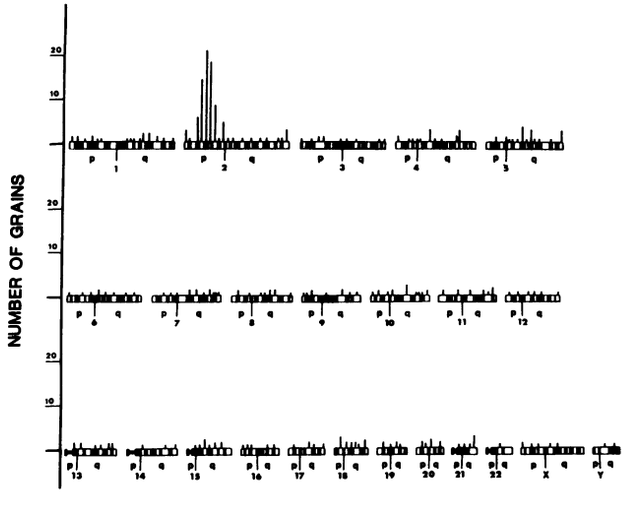

FIG. 3. Diagram showing silver grain distribution over the chromosomes of normal lymphocytes following *in situ* hybridization of probe 6. Three-hundred eleven grains were scored in two different experiments, of which 67 (22%) were on 2p.

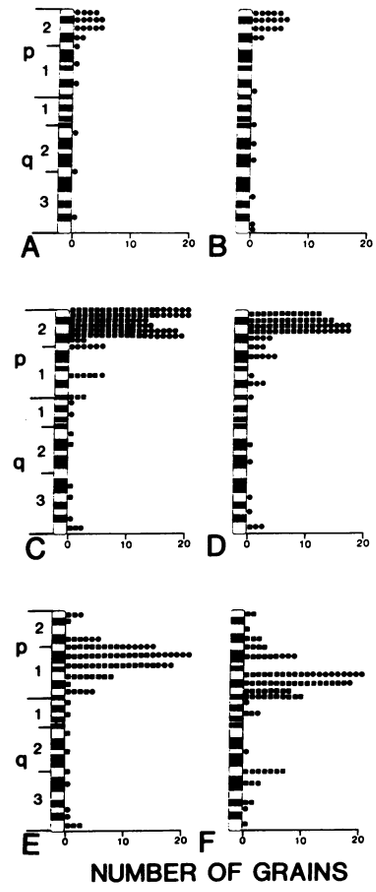

FIG. 4. Regional localization on 2p of six probes by *in situ* hybridization to normal lymphocyte chromosomes. The first group [*A*, probe NB-19-21A (N-*myc*); *B*, probe 8; *C*, probe 3; *D*, probe 1] is all amplified in tumors as is probe NB-9-A (data not shown), which localizes to the same region. The second group (*E*, probe 6, and *F*, probe 10) localizes more proximally on 2p and is amplified in IMR-32 but not in any tumors studied so far. Probe 11, which is not amplified in the tumors, maps to the same domain as probe 10 (data not shown). Between 75 and 207 cells (97–334 grains) were scored for the probes shown. Different symbols refer to grains scored in different experiments. On the average, 15–25% of the grains were on 2p. For these experiments, intact *Hin*dIII segments, subcloned in pBR322, were used for all probes, except for the following: probe 8, a 0.85-kb *Bam*HI–*Eco*RI fragment; NB-19-21A, a 1.0-kb *Eco*RI–*Bam*HI fragment; probe 1, a 2.7-kb *Hin*dIII–*Eco*RI fragment.

not amplified in the tumors, reside in more proximal parts of 2p: the peaks of grain distributions for probes 6 and 10 are over 2p15-2p16 and 2p13, respectively (Fig. 4 *E* and *F*).

The results of the *in situ* hybridization experiments are supported by the segregation pattern of the same probes in somatic cell hybrids containing various portions of 2p (Fig. 5). All five DNA segments, which by *in situ* hybridization map to 2p23-2p25, are missing from the DNA of hybrid G35D3, which contains a rearranged human chromosome 2 with a terminal deletion of 2p23-2pter. Part of band 2p23 still exists in the rearranged chromosome, as indicated by the presence of human malate dehydrogenase 1 in this hybrid. All of the other probes, except probes 4 and 6, clearly hybridized to their corresponding human fragment in the DNA of this hybrid. The absence of signal with probes 4 and 6 in G35D3 suggests the existence of a previously unsuspected interstitial deletion in 2p in this hybrid. The segregation of the 13 probes in the two A9 Call hybrids, which separate the region between band 2p23 and the distal end of 2p (2pter) from the rest of chromosome 2, further shows that the five distal sequences define a distinct chromosomal domain that

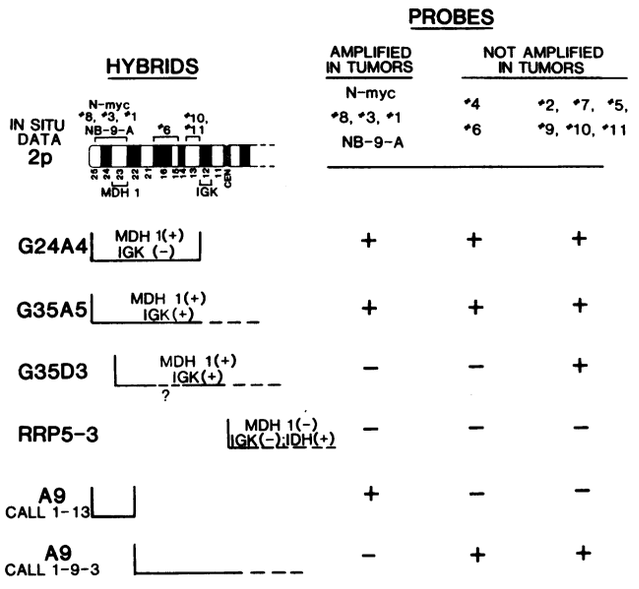

FIG. 5. Segregation of human chromosome 2-derived sequences in somatic cell hybrids with various rearrangements of this chromosome. The different portions of 2p contained in each hybrid are indicated. Ten micrograms of digested hybrid DNA was electrophoresed in a 0.7% agarose gel, transferred to nitrocellulose, and probed with end-labeled fragments. MDH 1, malate dehydrogenase 1; IDH, isocitrate dehydrogenase 1; IGK, immunoglobulin κ gene.

is separate from the location of the other DNA segments discussed.

Probes that map to different domains by *in situ* hybridization and hybrid cell analysis also hybridize with different relative intensities to DNAs from cells with chromosomal imbalance affecting different parts of 2p (Fig. 6). Monosomy of the region between either the middle of band 2p23 or band 2p24 and the terminus of 2p results in an ≈50% decrease in the intensity of the signal obtained with N-*myc* and probes 8, 3, and 1, whereas trisomy of these regions is associated with an ≈50% increase of the intensity relative to control DNA samples from blood and placenta. In contrast, monosomy of a smaller segment, distal to the middle of band 2p25, does not affect the intensity of the signal due to hybridization of these probes. These quantitative DNA blotting experiments thus further narrow the chromosomal domain that is amplified in the tumors to a segment distal to the middle of band 2p24 but proximal to the middle of band 2p25. This observation is consistent with previous data (12) indicating that the pNb-1 probe for N-*myc* maps to 2p23-2p24, with 2p24 now considered to be the more probable location of this domain. In contrast to the results with probes N-*myc*, 8, 3, and 1, probes 6, 4, and 10 hybridize to an equal extent with DNA from all of the fibroblast strains employed (Fig. 6).

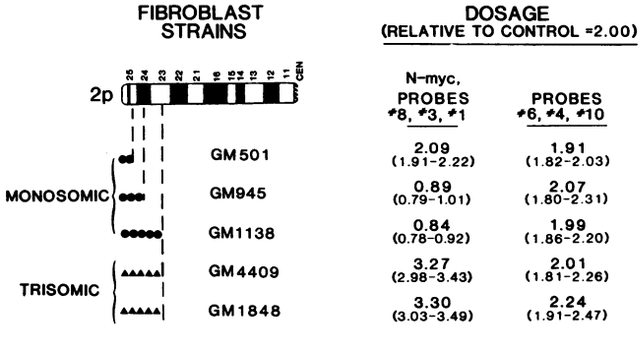

FIG. 6. Dosage of different 2p-derived probes in DNA from fibroblast strains with unbalanced rearrangements of 2p. The probes were hybridized to Southern blots of fibroblast DNA digests, and the signal obtained was quantitated by scanning densitometry and normalized against the reading of the standard probe H2-26. The average ratio for each probe was derived from two to six independent readings. Shown for each cluster of probes is the mean and the range of each set of average ratios.

DISCUSSION

Until recently, DNA amplification, primarily of various oncogenes, has been documented mainly in tumor-derived cell lines (2, 3). Now there is evidence linking this phenomenon to actual *in vivo* tumor stage advancement (13). Early studies have shown that amplification of a specific oncogene, N-*myc*, is a common phenomenon in primary neuroblastomas and retinoblastomas (3, 8, 10, 13, 24).

In this report, we show that a specific region of chromosome 2 is coamplified in several primary neuroblastomas along with N-*myc* in a differential and variable pattern. We have been able to subdivide the tumors with N-*myc* amplification into nested subsets in which additional probes are amplified. This finding may lead to a molecular classification of advanced-stage neuroblastomas, the clinical correlates of which will have to be determined. It is likely that many of the probes utilized represent various parts of an amplification unit (amplicon), which, in different tumors, extends to different lengths. Our data strongly suggest that N-*myc* may be at or near the core of this unit.

The possibility that a sequence other than N-*myc* is the functional and structural core of the neuroblastoma amplicon cannot be absolutely excluded at this time. However, there is circumstantial evidence for a functional role of N-*myc* in the progression of neuroblastoma as well as retinoblastoma. Increased expression of this gene accompanies its amplification in cell lines derived from both types of tumors (10, 11, 24), and, in neuroblastoma tissue, N-*myc* expression is most pronounced in the undifferentiated neuroblasts (24). Furthermore, increased expression of N-*myc*, without amplification of the genomic N-*myc* sequence, has been observed in retinoblastoma cells (10).

The actual size of the amplification unit in the tumors summarized in Table 1 has not yet been determined. Thousands of kilobase units are probably the norm when the amplification of certain genes is favored in cultured cells (2, 25, 26). In IMR-32 HSRs, the amplicon length has been estimated to be in the 3000-kb range (5). Other functional genes may reside within a region this large. An example of an expressed sequence that might be such a gene, whose function is still unknown, has been documented recently by Kohl *et al.* (27).

Three independent approaches have been utilized to establish that DNA segments incorporated into the 3000-kb repeat unit of the HSR of the human neuroblastoma cell line IMR-32 are derived from three domains of the short arm of human chromosome 2 that are separated from each other by several thousands of kilobases. Since each segment appears, by *in situ* hybridization, to be evenly distributed over the HSR (ref. 5 and other data not shown), at an approximately identical copy number (50–75 per haploid genome), it appears that the unit forming this HSR is assembled as a single event. Moreover, in most cases, the size of the amplified fragments is identical to that of the single copy fragment present in normal cells. Hence, the relocation/amplification event probably does not proceed via a spliced RNA species that then serves as a template for reverse transcription, as has been hypothesized and shown with *Drosophila* for *copia* elements (28) and for some pseudogenes (29).

Our data thus are compatible with the existence of a novel splicing and amplification event (Fig. 7) that occurs at the level of DNA, perhaps involving an extrachromosomal intermediate that, in principle, might be detectable only during

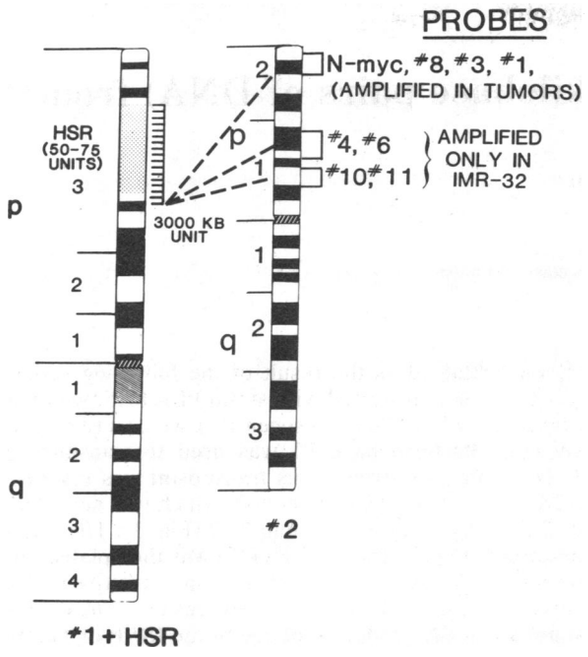

FIG. 7. A schematic showing the three different domains on 2p as defined by different probes and the proposed assembly of sequences from the different domains into one amplification unit in IMR-32 cells. Probe NB-9-A, which is not amplified in IMR-32 cells but is amplified in one of the primary tumors tested (Table 1), is also located in the most distal domain of 2p. Based on the data of Fig. 5, probes 2, 5, 7, and 9 are also assumed to map in the same domain as do probes 10 and 11, but *in situ* hybridization data to confirm this speculation have not yet been obtained.

the event itself. Although evidence of a similar phenomenon has not yet been described for neuroblastoma cell lines other than IMR-32, it may become apparent upon studying these lines in more detail. Evidence for somatic recombination between less distant sequences (probes 8 and NB-9-A) has already been obtained for formation of the DMs of the neuroblastoma cell line NB-9 (6, 7), and it would be surprising, were such a DNA splicing mechanism (Fig. 7) to exist, that it would not have more widespread occurrence.

The nature of the sequences in 2p, especially those in the domain centered around 2p24 (2p23-2p25), which relocate, amplify, and, at times, rearrange in tumors of neuroectodermal origin, and the mechanism of the apparently novel DNA relocation–rejoining process documented so far for IMR-32 are both interesting biological and medically relevant questions. Isolation of additional sequences from this region by using recombinant libraries enriched for chromosomes 1 and 2 has been initiated in our laboratory. The identification of candidate sequences for the junctions of the amplified regions provides one excellent point from which such studies can be initiated.

The interaction with Dr. Naotoshi Kanda, Dr. Fred Alt, and Ms. Nancy Kohl, with whom the initial work on DNA amplification in neuroblastomas cells was done, continues to be appreciated. We are also grateful for the assistance of Drs. Stephen Sallan and Cynthia Kretschmar in obtaining tissue samples and to Dr. Karl Heinz Grzeschik for his gift of hybrid DNA samples. This research was supported by grants from the American Cancer Society (CD-36, S.A.L. and associates; IN-36, G.M.B.) and the National Institutes of Health (HD18658). Y.S. is a Chaim Weizmann Postdoctoral Fellow. G.M.B. is a Junior Faculty Clinical Fellow of the American Cancer Society (650) and was supported in part by the Fern-Waldman Memorial Research Fund. R.S. acknowledges support from National Institutes of Health Grants CA22794, CA27678, CA36011, and CA16042. The Children's Cancer Center Study Group (CCSG) provided DNA from 106 tumors. Principal investigators of CCSG, in addition to R.S., who participated and their National Institutes of Health grant support are as follows: D. Hammond, H. Sather, J. Weiner, Group Operations Office, Los Angeles, CA13539; R. Heyn, Ann Arbor, CA02971; A. Ablin, San Francisco, CA17829; R. Chard, Seattle, CA10382; P. Coccia, Cleveland, CA20320; S. Leiken, Washington, D.C., CA03888; J. Ortega, Los Angeles, CA02649; H. Maurer, Chicago, CA07431; F. Ruymann, Columbus, CA03750; S. Piomelli, New York, CA03526; V. Albo, Pittsburgh, CA07349; J. Lukens, Nashville, CA26270; R. Neerhout, Portland, CA26044; P. Zeltzer, San Antonio, A. Evans, Philadelphia, CA11796; D. Miller, New York, CA14557; R. Baehner, Indianapolis, CA13809; H. Cohen, Rochester, CA11174; B. Lampkin, Cincinnati, CA26126; S. Feig, Los Angeles, CA27678; N. Shahidi, Madison, CA05436; R. Tannous, Iowa City, CA29314; and D. Tubergen, Denver, CA28851. Four additional tumors were provided by J. Casper, Milwaukee.

1. Cowell, J. K. (1982) *Annu. Rev. Genet.* **16**, 21–59.
2. Stark, G. R. & Wahl, G. M. (1984) *Annu. Rev. Biochem.* **53**, 447–491.
3. Schwab, M., Alitalo, K., Varmus, H. E. & Bishop, J. M. (1984) in *Cancer Cells: Oncogenes and Viral Genes*, eds. Van de Woude, G. F., Levine, J. A., Topp, W. C. & Watson, J. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 215–220.
4. Biedler, J. L., Melera, P. W. & Spengler, B. A. (1983) in *Chromosomes and Cancer*, eds. Rowley, J. D. & Ultmann, J. E. (Academic, New York), pp. 117–138.
5. Kanda, N., Schreck, R., Alt, F., Bruns, G., Baltimore, D. & Latt, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4069–4073.
6. Kohl, N., Kanda, N., Schreck, R., Bruns, G., Latt, S. A., Gilbert, F. & Alt, F. W. (1983) *Cell* **35**, 359–367.
7. Sakai, K., Kanda, N., Shiloh, Y., Donlon, T., Schreck, R., Shipley, J., Dryja, T., Chaum, E., Chaganti, R. S. K. & Latt, S. A. (1985) *Cancer Genet. Cytogenet.*, in press.
8. Schwab, M., Alitalo, K., Klempnauer, K.-H., Varmus, H., Bishop, J. M., Gilbert, F., Brodeur, G., Goldstein, M. & Trent, J. (1983) *Nature (London)* **305**, 245–248.
9. Michitsch, R. W., Montgomery, K. T. & Melera, P. W. (1984) *Mol. Cell. Biol.* **4**, 2370–2380.
10. Lee, W.-H., Murphree, A. L. & Benedict, W. (1984) *Nature (London)* **309**, 458–460.
11. Montgomery, K. T., Biedler, J. L., Spengler, B. A. & Melera, P. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5724–5728.
12. Schwab, M., Varmus, H. E., Bishop, J. M., Grzeschik, K.-H., Naylor, S. L., Sakaguchi, A. Y., Brodeur, G. & Trent, J. (1984) *Nature (London)* **308**, 288–291.
13. Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E. & Bishop, J. M. (1984) *Science* **224**, 1121–1124.
14. Evans, E. A., D'Angio, G. J. & Randolph, J. (1971) *Cancer* **27**, 374–381.
15. Brunk, C. F., Jones, K. C. & James, T. W. (1979) *Anal. Biochem.* **92**, 497–500.
16. Tantravahi, U., Kirschner, D. A., Beauregard, L., Page, L., Kunkel, L. & Latt, S. A. (1983) *Hum. Genet.* **64**, 33–38.
17. Bruns, G. A. P., Leary, A. C., Eisenman, R. E., Bazinet, C. W., Regina, V. M. & Gerald, P. S. (1978) *Cytogenet. Cell Genet.* **22**, 172–176.
18. Bruns, G. A. P., Mintz, B. J., Leary, A. C., Regina, V. M. & Gerald, P. S. (1979) *Biochem. Genet.* **17**, 1031–1059.
19. Bootsma, D. & Kidd, K. K. (1984) *Cytogenet. Cell Genet.* **37**, 22–46.
20. Rabbitts, T. H. (1983) *Biochem. Soc. Trans.* **11**, 119–126.
21. Grzeschik, K.-K., Meyerova, A., Bender, K. & Johannsman, R. (1978) *Cytogenet. Cell Genet.* **22**, 412–414.
22. Lalande, M., Dryja, T. P., Schreck, R. R., Shipley, J., Flint, A. & Latt, S. A. (1984) *Cancer Genet. Cytogenet.* **13**, 283–295.
23. Harper, M. E. & Saunders, G. F. (1981) *Chromosoma* **83**, 431–439.
24. Schwab, M., Ellison, J., Busch, M., Rosenau, W., Varmus, H. E. & Bishop, J. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4940–4944.
25. Schimke, R. T. (1984) *Cell* **37**, 705–713.
26. Federspiel, N. A., Beverley, S. M., Schilling, J. W. & Schimke, R. T. (1984) *J. Biol. Chem.* **259**, 9127–9240.
27. Kohl, N. E., Gee, K. E. & Alt, F. W. (1984) *Science* **226**, 1335–1337.
28. Flavell, A. J. (1984) *Nature (London)* **310**, 514–516.
29. Hollis, G. F., Hieter, P. A., McBride, O. W., Swan, D. & Leder, P. (1982) *Nature (London)* **296**, 321–325.