Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5523–5527, June 1994
Cell Biology

MYCN is retained in single copy at chromosome 2 band p23–24 during amplification in human neuroblastoma cells

(oncogene/fluorescence *in situ* hybridization/tumorigenesis/comparative genomic hybridization)

RAFFAELLA CORVI, LUKAS C. AMLER, LARISSA SAVELYEVA, MANUELA GEHRING, AND MANFRED SCHWAB

Department of Cytogenetics, German Cancer Research Center, D-69120 Heidelberg, Germany

Communicated by David M. Prescott, February 22, 1994 (received for review December 29, 1993)

ABSTRACT **Amplification of the human N-*myc* protooncogene, *MYCN*, is frequently seen either in extrachromosomal double minutes or in homogeneously staining regions of aggressively growing neuroblastomas. *MYCN* maps to chromosome 2 band p23–24, but homogeneously staining regions have never been observed at this band, suggesting transposition of *MYCN* during amplification. We have employed fluorescence *in situ* hybridization to determine the status of *MYCN* at 2p23–24 in five human neuroblastoma cell lines. All five lines carried, in addition to amplified *MYCN* in homogeneously staining regions or double minutes, single-copy *MYCN* at the normal position. In one line there was coamplification of *MYCN* together with DNA of the host chromosome 12, to which *MYCN* had been transposed. Our results suggest a model of amplification where *MYCN* is retained at its original location. They further sustain the view that either the initial events of *MYCN* amplification or the further evolution of amplified *MYCN* copies follow mechanisms different from those leading to amplification of drug-resistance genes.**

Amplification is one of the mechanisms by which cellular oncogenes can be activated to express abnormally high levels of protein and to participate in tumorigenesis (1). The most consistent pattern is seen for human neuroblastoma, where the gene *MYCN* has been found amplified in both tumors and cell lines (2, 3). Amplification of *MYCN* is correlated with aggressive tumor growth (4) and is a predictor for clinical outcome of the patient (ref. 5; for review, see ref. 6).

MYCN maps to chromosome 2 band p23–24 (7). Amplified copies of *MYCN* localize to two types of chromosomal abnormalities, double minutes (DMs; ref. 3) and homogeneously staining regions (HSRs; refs. 7–10). Neither the resident site of *MYCN* nor other regions of the short (p) arm of chromosome 2 have been found to harbor amplified *MYCN*. This implies that the evolution of HSR involves the transposition of *MYCN* from its original location to distant chromosomal sites (3).

Although early events of *MYCN* amplification remain elusive, the question of whether *MYCN* is retained or deleted at its original location can be addressed. We have employed fluorescence *in situ* hybridization to determine the status of single-copy *MYCN* in five neuroblastoma cell lines. We found in all cases that *MYCN* was present on both apparently unrearranged chromosomes 2. The heterozygosity of microsatellite loci established that the two chromosomes 2 represented the parental homologs. Our data support a model of amplification in which *MYCN* is retained at its original site. Analyses of a HSR on chromosome 12 in one line revealed the coamplification of *MYCN* with chromosome 12 DNA, which reinforces our earlier suggestion (11) that the evolution of a HSR involves amplification subsequent to *MYCN* transposition into the host chromosome.

MATERIALS AND METHODS

Cell Lines and Preparation of Metaphases. Neuroblastoma line NGP was obtained from G. M. Brodeur (12), LA-N-5 from R. C. Seeger (13), and LS from R. Handgretinger (14). IMR-32 was from the American Type Culture Collection, and HD-MG-1 was established in our laboratory (unpublished work). For cytogenetic analyses the cells were treated with Colcemid, harvested, and fixed according to routine procedures.

DNA Probes and Labeling. As the probe for *MYCN* we employed cosmid pNb-101, isolated previously (unpublished work) on the basis of clone pNb-1 (2). The *MYCN* cosmid, a total chromosome 2 library (a gift of Joe Gray, University of California, San Francisco), and total DNA from LS and HD-MG-1 were labeled with biotin-16-dUTP (Boehringer Mannheim) according to ref. 15. The digoxygenin-labeled α-satellite probe for chromosome 2 was from Oncor.

Fluorescence *in Situ* Hybridization. Fluorescence *in situ* hybridization analysis was done as described (16, 17). Probe concentrations were 6 ng/μl for *MYCN*, 0.5 ng/μl for the chromosome 2 centromere probe, and 100 ng/μl for the chromosome 2 library. Repetitive sequences in pNb-101 or in the chromosome 2 library were suppressed with a 10-fold excess of *Cot-1* DNA (Bethesda Research Laboratories/Life Technologies). In reverse chromosome "painting" experiments (18) 1 μg of biotin-labeled tumor DNA from cell lines LS and HD-MG-1 was combined with 50 μg of *Cot-1* DNA in a final hybridization volume of 30 μl. The biotin-labeled probe was detected with avidin conjugated with fluorescein isothiocyanate, and the digoxigenin-labeled probe was detected with anti-digoxigenin antibody conjugated with rhodamine (Boehringer Mannheim) (for details, see ref. 16). Chromosomes were counterstained with either propidium iodide or 4′,6-diamidino-2-phenylindole dihydrochloride and embedded in antifade medium (Vectashield; Vector Laboratories). Equipment for evaluation of microscopic slides included an Axiophot microscope (Zeiss), an air-cooled charge-coupled-device camera (model CH250/a, Photometrics) with a KAF-1400-50 sensor chip (1348 × 1035 pixels; Kodak), and IPLabs-Spectrum software (version 2.1.1c; Signal Analytics, Vienna, VA) on a Macintosh IIfx computer, system 7.0 with a Formac ProOpt 650 optical disk drive. Images were displayed on a RasterOps 24 screen (1024 × 768 pixels).

Polymerase Chain Reaction (PCR) and Electrophoresis. PCR was performed as described (19), with the following modifications: 20 ng of DNA was used as a template for PCR; primer concentration was 0.5 μM in 50 mM KCl/10 mM Tris·HCl, pH 9.0/1% (vol/vol) Triton X-100 with 200 μM dGTP, 200 μM dCTP, 200 μM dTTP, 2 μM dATP, and 5 μCi

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: DMs, double minutes; HSR, homogeneously staining region.

(185 kBq) of [α-[35S]thio]dATP in a final reaction volume of 10 μl with an overlay of 30 μl of mineral oil. The samples were heated for 5 min at 95°C and 1 unit of DNA polymerase from *Thermus aquaticus (Taq* DNA polymerase) was added to initiate a "hot start." Reaction conditions were 95°C for 30 sec, 55°C for 75 sec, and 74°C for 30 sec for each cycle. The cycle was repeated 29 times with a final extension of 3 min at 74°C. Two microliters of the reaction mix was electrophoresed in a denaturing 5% polyacrylamide sequencing gel. The gel was dried and exposed for 12 hr to Kodak x-ray film. Sequences of primers were as described (20).

RESULTS

***MYCN* on Chromosome 2.** To determine the status of *MYCN* we have employed four long-term established lines—LA-N-5, NGP, IMR-32 and LS—and early passages of line HD-MG-1. All have *MYCN* amplification either in DMs (LA-N-5 and HD-MG-1) or in HSRs (NGP, IMR-32, and LS) and are characterized by a near-diploid karyotype with the presence of only few defined marker chromosomes, as determined by Giemsa staining (data not shown). Chromosome 2 appears unaltered as judged both by Giemsa staining (data not shown) and by fluorescence *in situ* hybridization with a total chromosome 2 library (Fig. 1), except for NGP (Fig. 1*A*), which contains a marker carrying chromosome 2 material, and for ≈30% of IMR-32 cells that have an additional HSR in 2q (data not shown).

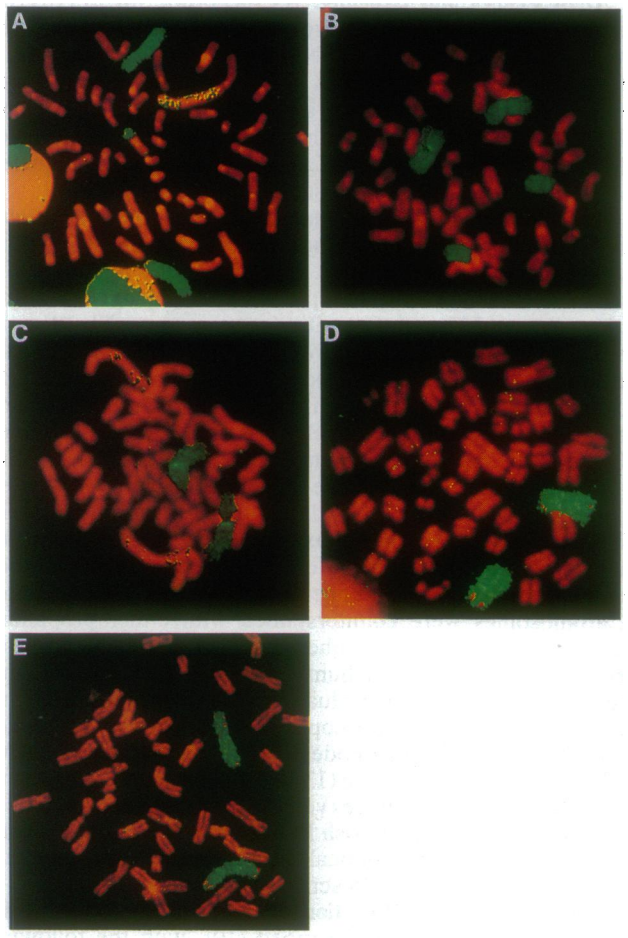

FIG. 1. Chromosome 2 status in neuroblastoma cell lines. NGP (*A*), IMR-32 (*B*), LS (*C*), LA-N-5 (*D*), and HD-MG-1 (*E*). A total chromosome 2 library labeled with biotin was hybridized to metaphases and detected with fluorescein-conjugated avidin (GIBCO/BRL) (green). The chromosomes were counterstained with propidium iodide (red). The signal is exclusively seen on the two copies of chromosome 2 and on the HSRs, showing that these cells do not contain markers with chromosome 2 material (except NGP).

The location of *MYCN* was determined in two-color analyses. *MYCN* cosmid pNb-101 was labeled with biotin, and the probe specific for the centromere of chromosome 2 was labeled with digoxygenin. Analyses of metaphases from a lymphocyte culture confirmed that the expected signals for *MYCN* were recorded (Fig. 2*A*, green). The centromere probe identified the two copies of chromosome 2 (Fig. 2 *B–F*, red).

Analyses of metaphases from the neuroblastoma cells revealed the presence of *MYCN* on the two copies of chromosome 2 in all five lines (Fig. 2 *B–F*). Additionally, amplified *MYCN* was detected in NGP in a large HSR on 4p and in LS in two HSRs on 12q. In IMR-32 the amplified *MYCN* was distributed in the majority of cells over two HSRs, both on 1p; ≈30% of the cells contained an additional HSR in 2q. In LA-N-5 and in HD-MG-1 the amplified *MYCN* mapped to DMs. Altogether, all five neuroblastoma lines showed two apparently unrearranged copies of chromosome 2 with *MYCN* present at the expected normal site.

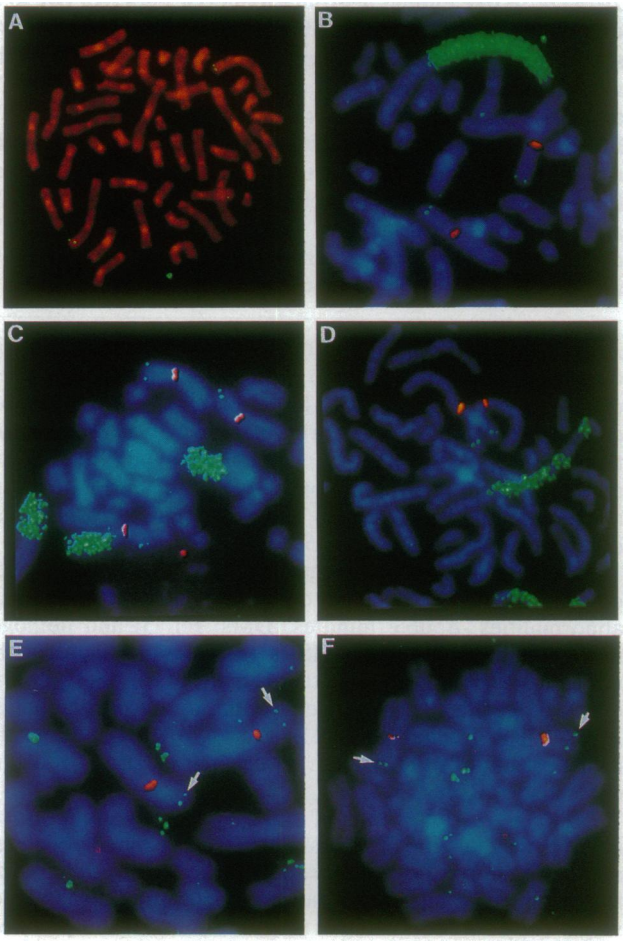

FIG. 2. Status of *MYCN* at 2p23–24. *MYCN* cosmid pNb-101 was labeled with biotin and chromosome 2-specific satellite DNA was labeled with digoxigenin, and the two probes were cohybridized to metaphase chromosomes. pNb-101 was detected with fluorescein-ated avidin (green), and satellite DNA with anti-digoxigenin-rhodamine Fab fragments (Boehringer Mannheim) (red). The chromosomes were counterstained either with propidium iodide (red) or with 4′,6-diamidino-2-phenylindole (blue). Each of the two copies of chromosome 2 carries two signals showing the presence of *MYCN* on the two chromatids. (*A*) Epstein–Barr virus-transformed normal human lymphocytes. (*B*) NGP. (*C*) IMR-32. (*D*) LS. (*E*) LA-N-5. (*F*) HD-MG-1. In cell lines with DMs (*E* and *F*) single-copy *MYCN* is indicated by arrows.

Presence of Two Homologous Chromosomes 2. To find out whether the two copies of chromosome 2 present in the neuroblastoma lines represented the parental homologous chromosomes, we analyzed five microsatellite loci consisting of $(CA)_n$ repeats (20) from the p arm, located on the distal and proximal side of *MYCN*, and from two loci from the q arm. To detect polymorphism we used PCR primers corresponding to sequences on either side of the $(CA)_n$ repeat (21). Line NGP was heterozygous for seven loci tested (Fig. 3). IMR-32 and LS were heterozygous for five loci and LAN-5 and HD-MG-1 for four loci (Table 1). These data demonstrate that the two copies of chromosome 2 are different and therefore represent the two parental homologs.

Coamplification of *MYCN* with Host Chromosomal DNA in a HSR. The two HSRs in LS are integrated in chromosome 12 (14). The roughly 100 copies of *MYCN* (14) are present within amplicons of relatively small size (11). To determine the composition of this HSR we employed fluorescence *in situ* hybridization using *MYCN* and a whole chromosome 12 library as probes. Cohybridization of the two probes revealed a scattered signal for *MYCN* over the HSR and for the chromosome 12 library both over the chromosome 12 and HSR sequences (Fig. 4*A*). With the chromosome 12 library as a probe, we again saw a signal both on the two complete derivative chromosomes 12 and on the intact chromosome 12 (Fig. 4*B*). These data demonstrate that chromosome 12 DNA is coamplified with *MYCN*.

To determine the complexity of the amplified chromosome 12 DNA, we labeled total DNA from LS and hybridized this probe to metaphase chromosomes of normal lymphocytes (Fig. 4*C*). A strong signal was seen on both copies of chromosome 12 around band q14. We did not detect a signal for *MYCN* on chromosome 2, presumably because of the low amount of DNA in the HSR derived from the *MYCN* locus. For comparison we used the same approach with DNA from line HD-MG-1, which contains DMs. A signal was detected on both copies of chromosome 2 at the site of the *MYCN* locus (Fig. 4*D*). In contrast to LS, we could not detect amplified sequences other than those derived from the *MYCN* locus.

DISCUSSION

Although amplification is a common route by which cellular oncogenes can become activated, little is known about the molecular mechanism involved. The inherent problem is that only the end point of the amplification process can be studied, from which it is impossible to draw firm conclusions about

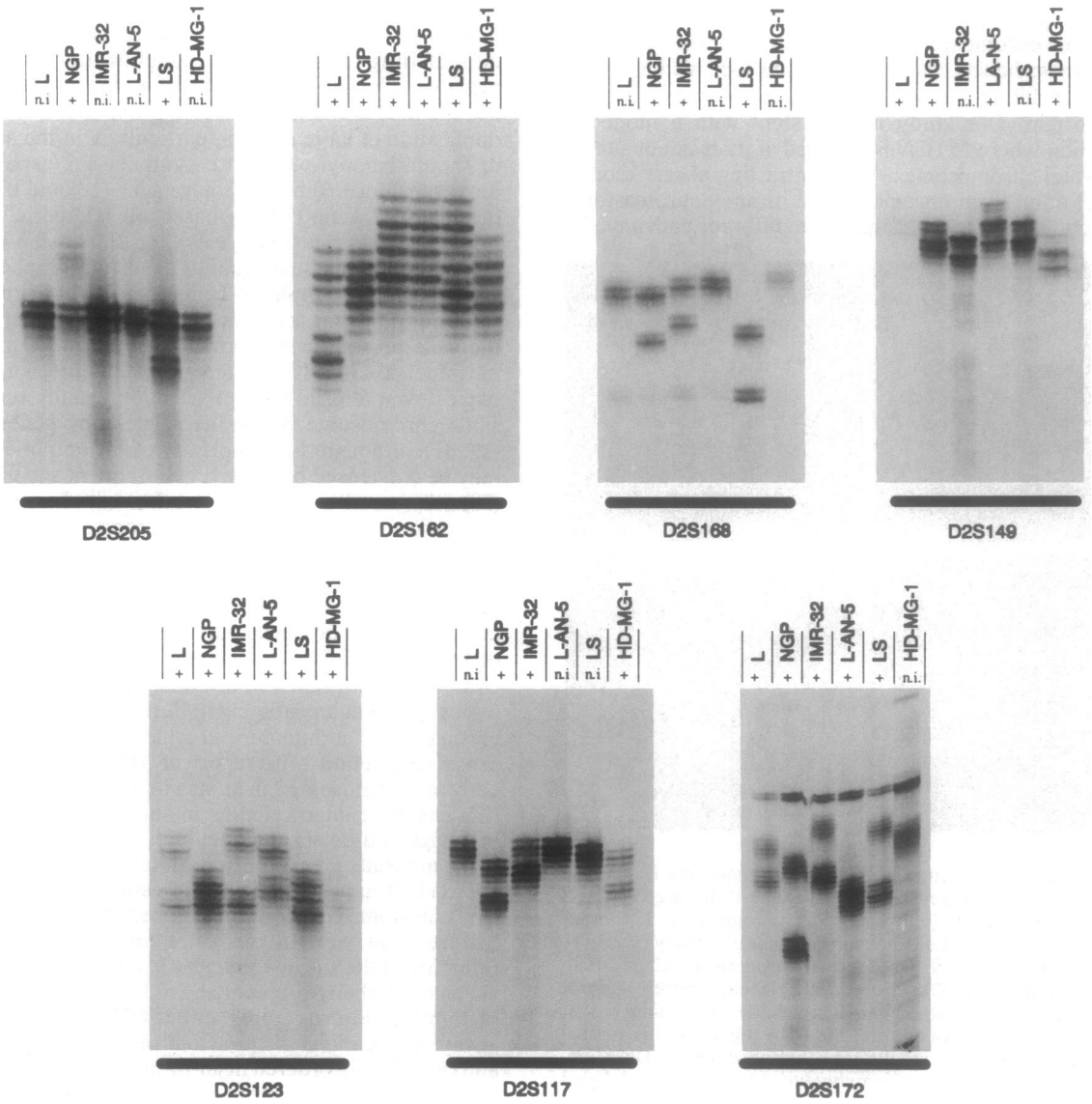

FIG. 3. Heterozygosity of chromosome 2 microsatellite loci. DNA from neuroblastoma lines and from unrelated lymphocytes (L) was amplified by PCR and the products were fractionated in a polyacrylamide sequencing gel. n.i., Not informative; +, heterozygous.

Table 1. Allelic status of microsatellite loci on chromosome 2 in neuroblastoma cell lines

Line	*D2S205*	*D2S162*	*D2S168*	*D2S149*	*D2S123*	*D2S117*	*D2S172*	Total no. of loci heterozygous
NGP	+	+	+	+	+	+	+	7
IMR-32	n.i.	+	+	n.i.	+	+	+	5
LA-N-5	n.i.	+	n.i.	+	+	n.i.	+	4
LS	+	+	+	n.i.	+	n.i.	+	5
HD-MG-1	n.i.	+	n.i.	+	+	+	n.i.	4

+, Heterozygous; n.i., not informative.

early amplification events. In this study we have addressed the question of whether the *MYCN* is retained at its resident site on 2p23–24 in neuroblastoma cell lines carrying amplification in HSRs or in DMs. Four long-term established neuroblastoma cell lines as well as early passages of a recently developed line unequivocally revealed retention of *MYCN* on both of the two copies of chromosome 2 present in each of the lines. This observation is in agreement with the previous mapping of anonymous DNA sequences isolated from the HSR of line IMR-32 to chromosome 2 (22, 23). Our conclusion that these represent the original two parental allelic *MYCN* copies is based on the finding that the two chromosomes 2 in all five lines are genetically different, as demonstrated by heterozygosity of microsatellite loci. Further, *in situ* hybridization with the total chromosome 2 library did not show chromosome 2 material to be present on other chromosomes; hence, the microsatellite loci we have analyzed in fact define the two homologous chromosomes 2 of parental origin. Our study is consistent with a model of amplification where *MYCN* is retained at its resident site on both parental chromosomes. The contrasting observation of *MYCN* deletion from chromosome 2 in one neuroblastoma line (24) could indicate that there are different pathways by which *MYCN* becomes amplified. Alternatively, it cannot be excluded that *MYCN* in this single case analyzed was deleted at some time during the somatic cell fusions used to demonstrate deletion. This latter possibility could be addressed through direct inspection of *MYCN* in the parental cell line by fluorescence *in situ* hybridization.

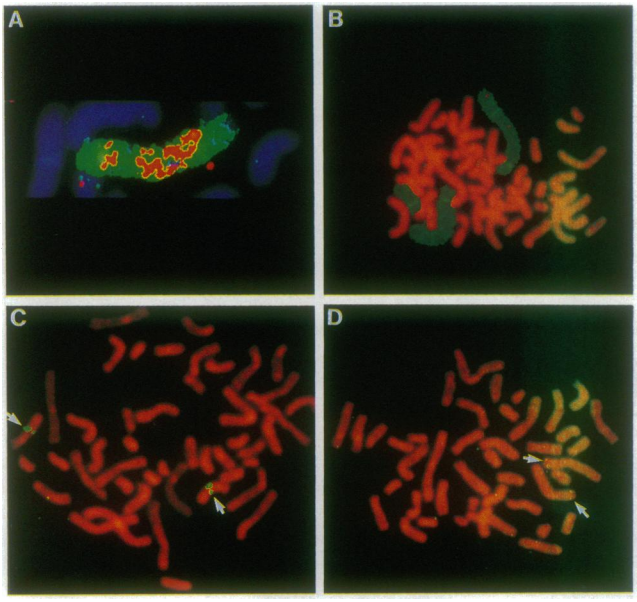

FIG. 4. Coamplification of *MYCN* and chromosome 12 DNA in a HSR of line LS. (*A*) Derivative chromosome 12 from cell line LS hybridized with *MYCN* (red) and total chromosome 12 library (green). (*B*) Hybridization of total chromosome 12 library (green) to a metaphase spread of LS. One normal chromosome 12 and both derivative chromosomes 12 each carrying a HSR are entirely stained. (*C*) Metaphase of normal human lymphocytes to which biotin-labeled DNA from LS was hybridized. The signal on the normal chromosomes 12 (arrows) results from hybridization of DNA amplified in LS. (*D*) Metaphase of lymphocytes to which biotin-labeled DNA from HD-MG-1 was hybridized. The signal on the normal chromosomes 2 (arrows) results from hybridization of DNA amplified in HD-MG-1.

Several mechanisms that have been proposed to account for unscheduled gene amplification, most of which are based on drug-resistance models. Early proposals suggested that repeated initiation of DNA replication at a single origin within a cell cycle leads to an "onionskin" structure (25), similar to that seen during developmentally regulated chorion gene amplification in *Drosophila* (26). This structure could be resolved through recombination into intra- or extrachromosomal DNA (25). Inspection of early events by chromosomal *in situ* hybridization, however, has revealed that initial products of drug-resistance gene amplification can be tens of megabases long (27–29) and therefore too large to fit extrareplication models. The initial event proposed to account for the duplication of large regions, particularly in the amplification of the carbamoyl-phosphate synthetase 2/aspartate carbamoyltransferase/dihydroorotase gene (28) and the adenylate deaminase 2 gene (30), is based on telomeric fusions and bridge–breakage–fusion cycles (28). Expansion of the initial duplication could subsequently lead to multiplication. As a key feature the amplified gene copies would reside on the same chromosome arm that carries the single-copy gene with the gene present in its normal position in the marker chromosome (27–30). It is interesting that the amplified gene copies even after long-term selection usually remain on the same chromosome where the single-copy gene is localized (29, 31), although the complexity of the amplified structures may be condensed by relatively infrequent secondary events (28). As an alternative model for initial events of drug-resistance, gene amplification chromosome breakage and deletion have been suggested to play a central role during amplification (32). Chromosome breakage is assumed to occur within a stalled replication bubble and could lead to a centric element observed during early events of amplification of the dihydrofolate reductance gene in a Chinese hamster ovary cell line (32). A particular feature of this proposed pathway is that an early event during amplification creates a chromosomal architecture in which the single-copy gene is prone to deletion. The report of *MYCN* deletion from one copy of chromosome 2 in human neuroblastoma line NO315L (24) has been interpreted as in support of the chromosome breakage-and-deletion model (32).

From results presented here and from previously published data (11) it appears as if amplification of *MYCN* in human neuroblastoma cells follows a reproducible pattern. Common features that we can recognize are predominantly (*i*) the retention of the single-copy gene in an apparently unarranged chromosomal environment at 2p23–24; (*ii*) the development of amplification structures, either DMs or HSRs, that reside always at locations distant from the original site of *MYCN*; and (*iii*) a highly ordered head-to-tail tandem repetition as the preferred common arrangement of *MYCN* amplicons in HSRs (11, 33). The latter is in contrast to the presence of inverted rearranged units found in stable cell lines carrying a

highly amplified adenylate deaminase 2 gene as the result of multiple selection steps (34) and the inverted repeats seen widely in other models (35–37). Even though we cannot determine directly the initial events during *MYCN* amplification, it seems intuitively unlikely that it follows pathways observed for the amplification of drug-resistance genes. Initial events by telomeric fusion and bridge–breakage–fusion cycles are not supported by the presence of the two apparently intact parental homologs of chromosome 2, at least there is no indication that large duplicated, or multiplicated, copies of the *MYCN* locus had ever developed at 2p. If they had developed initially, they would have to be excised in all cases with great precision to leave the original chromosomes 2 intact. Our analyses for microsatellite loci and the *in situ* hybridization with the *MYCN* probe did not reveal deletion or structural rearrangements at the very distal end of 2p that should accompany intrachromosomal multiplication followed by excision of the amplified sequences. Also, we have no reason to assume a selective force favoring elimination of amplified copies from 2p. The chromosome breakage–deletion mechanism appears also unlikely as an initial step toward amplification, because our data show the presence of *MYCN* on both of the homologous parental chromosomes.

The evolution of the HSR in line LS involves coamplification of *MYCN* together with host-chromosome DNA. We have yet to determine whether the HSRs in other cell lines result from coamplification of *MYCN* with the corresponding host-chromosome sequences. Detection of coamplification in line LS apparently was facilitated by the huge amount of host DNA amplified together with *MYCN*. The limited sensitivity of the reverse painting approach for detecting amplified DNA is illustrated by our inability to detect amplified *MYCN*. It is possible that HSRs in other lines also contain amplified host DNA, but the amount might be too low to allow detection by employing *in situ* hybridization with whole chromosomes libraries or by reverse painting approaches.

We conclude that it is presently impossible to delineate initial events of *MYCN* amplification. However, the results presented here enhance the idea that *MYCN* amplification follows a mechanism that is different from those leading to amplification of drug-resistance genes.

The technical assistance of Birgit Schneider and Kerstin Reumann and the secretarial skills of Ingrid Cederlund are greatly appreciated. This work was supported by grants from the Deutsche Forschungsgemeinschaft, the Dr. Mildred Scheel Stiftung, the Heidelberg–Mannheim Comprehensive Cancer Center, and the Israel–German Cancer Research Center Cooperation Program and is integrated into the frame of the European Communities Concerted Action Molecular Cytogenetics of Solid Tumors (BMH1-CT92-0156).

1. Alitalo, K. & Schwab, M. (1986) *Adv. Cancer Res.* **47**, 235–281.
2. Schwab, M., Alitalo, K., Klempnauer, K. H., Varmus, H. E., Bishop, J. M., Gilbert, F., Brodeur, G., Goldstein, M. & Trent, J. (1983) *Nature (London)* **305**, 245–248.
3. Kohl, N., Kanda, K., Schreck, P. R., Bruns, G., Latt, S., Gilbert, F. & Alt, F. (1983) *Cell* **35**, 359–367.
4. Brodeur, G., Seeger, R. C., Schwab, M., Varmus, H. E. & Bishop, J. M. (1984) *Science* **224**, 1121–1124.
5. Seeger, R. C., Brodeur, G. M., Sather, H., Dalton, A., Siegel, S. E., Wong, K. Y. & Hammond, D. (1985) *N. Engl. J. Med.* **313**, 1111–1116.
6. Schwab, M. & Amler, L. C. (1990) *Genes Chromosomes Cancer* **1**, 181–193.
7. Schwab, M., Varmus, H. E., Bishop, J. M., Grzeschik, K.-H., Naylor, S., Sakaguchi, A., Brodeur, G. M. & Trent, J. (1984) *Nature (London)* **308**, 288–291.
8. Cox, D., Yuncken, C. & Spriggs, A. I. (1965) *Lancet* **2**, 55–58.
9. Brodeur, G. M., Green, A. A., Hayes, F. A., Williams, K. J., Williams, D. L. & Tsiatis, A. A. (1981) *Cancer Res.* **41**, 4678–4686.
10. 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.
11. Amler, L. C. & Schwab, M. (1989) *Mol. Cell. Biol.* **9**, 4903–4913.
12. Brodeur, G. M., Sekhon, G. S. & Goldstein, M. N. (1977) *Cancer* **40**, 2256–2263.
13. Seeger, R. C., Danon, Y. L. & Hoover, R. (1982) *J. Immunol.* **128**, 983–989.
14. Rudolph, G., Schilbach-Stückle, K., Handgretinger, R., Kaiser, P. & Hameister, H. (1991) *Hum. Genet.* **86**, 562–566.
15. Langer, P. R., Waldrop, A. A. & Ward, D. C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6633–6637.
16. Barker, P. E. & Schwab, M. (1993) in *Methods in Molecular Genetics*, ed. Adolph, K. (Academic, Orlando, FL), Vol. 2, pp. 129–154.
17. Pinkel, D., Straume, T. & Gray, J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2934–2938.
18. Kallioniemi, A., Kallioniemi, O.-P., Sudar, D., Rutovitz, D., Gray, J. W., Waldman, F. & Pinkel, D. (1992) *Science* **258**, 818–821.
19. Saiki, R., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
20. Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morisette, J., Millasseau, P., Vaysseix, G. & Lathrop, M. (1992) *Nature (London)* **359**, 794–801.
21. Weber, J. L. & May, P. E. (1989) *Am. J. Hum. Genet.* **44**, 388–396.
22. Kanda, N., Schreck, R., Alt, F., Bruns, G., Baltimore, D. & Latt, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4069–4073.
23. Shiloh, Y., Shipley, J., Brodeur, G., Bruns, G., Korf, B., Donlon, T., Schreck, R. R., Seeger, T., Sakai, K. & Latt, S. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3761–3765.
24. Hunt, J. D., Valentine, M. & Tereba, A. (1990) *Mol. Cell. Biol.* **10**, 823–829.
25. Schimke, R. T. (1984) *Cell* **37**, 705–713.
26. Osheim, Y. N. & Miller, O. L., Jr. (1983) *Cell* **33**, 543–553.
27. Toledo, F., Smith, K. A., Buttin, G. & Debatisse, M. (1992) *Mutat. Res.* **276**, 261–273.
28. Smith, K. A., Gorman, P. A., Stark, M. B., Groves, R. P. & Stark, G. R. (1990) *Cell* **63**, 1219–1227.
29. Trask, B. J. & Hamlin, J. L. (1989) *Genes Dev.* **3**, 1913–1925.
30. Toledo, F., Le Roscouet, D., Buttin, G. & Debatisse, M. (1992) *EMBO J.* **11**, 2665–2673.
31. Wahl, G. M., Vitto, L., Padgett, R. A. & Stark, G. R. (1982) *Mol. Cell. Biol.* **2**, 308–319.
32. Windle, B., Draper, B. W., Yin, Y., O'Gorman, S. & Wahl, G. M. (1991) *Genes Dev.* **5**, 160–174.
33. Schneider, S. S., Hiemstra, J. L., Zehnbauer, B. A., Taillon-Miller, P., Le Paslier, D. L., Vogelstein, B. & Brodeur, G. M. (1992) *Mol. Cell. Biol.* **12**, 5563–5570.
34. Hyrien, O., Debatisse, M., Buttin, G. & Robert de Saint Vincent, B. (1988) *EMBO J.* **7**, 407–417.
35. Ford, M., Davies, B., Griffiths, M., Wilson, J. & Fried, M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3370–3374.
36. Stark, G. R., Debatisse, M., Giulotto, E. & Wahl, G. M. (1989) *Cell* **57**, 901–908.
37. Fried, M., Feo, S. & Heard, E. (1991) *Biochim. Biophys. Acta* **1090**, 143–145.