## Lack of Correlation between N-*myc* and MAX Expression in Neuroblastoma Tumors and in Cell Lines: Implication for N-*myc*-MAX Complex Formation


Giuseppe Raschella, Antonino Romeo, Anna Negroni, et al.



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# Lack of Correlation between N-*myc* and MAX Expression in Neuroblastoma Tumors and in Cell Lines: Implication for N-*myc*-MAX Complex Formation[1]

**Giuseppe Raschella,[2] Antonino Romeo, Anna Negroni, Sabina Pucci, Carlo Dominici, Manuel A. Castello, Paola Bevilacqua, Armando Felsani, and Bruno Calabretta[2]**

*ENEA (Ente Nuove Tecnologie Energia e Ambiente) CRE Casaccia, Division of Molecular Biology, Via Anguillarese, 303, 00060 S. Maria di Galeria, Rome, Italy [G. R., A. R., A. N., S. P.]; Department of Pediatrics, University of Rome "La Sapienza," 00161 Rome, Italy [C. D., M. A. C.]; Istituto Regina Elena per lo Studio e la Cura dei Tumori, CRS, 00158 Rome, Italy [P. B., A. F.]; Istituto Tecnologie Biomediche, CNR, 00151 Rome, Italy [A. F.]; and Department of Microbiology, and Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, Pennsylvania 19107 [B. C.]*

## ABSTRACT

**Detectable levels of MAX messenger RNA were found in a set of human neuroblastoma tumors and established cell lines. MAX mRNA levels were independent of tumor stage and N-*myc* genomic amplification. By contrast, N-*myc* mRNA transcripts were detectable only in tumors with amplification of N-*myc* gene and in cell lines. Analysis by reverse transcriptase polymerase chain reaction and hybridization to specific oligodeoxynucleotide probes revealed approximately equal amounts of two MAX transcripts in all cases analyzed. Immunoprecipitations with a specific antibody to MAX detected two proteins of $M_r$ 21,000 and 22,000 in approximately equal amounts in all neuroblastoma lines regardless of N-*myc* amplification and/or expression. On the other hand, protein binding to the *myc* DNA consensus sequence correlated with N-*myc* expression in neuroblastoma cells. Thus, N-*myc* expression might be a limiting factor in the formation of the N-*myc*-MAX heterodimer in neuroblastomas.**

## INTRODUCTION

Neuroblastoma is the most frequent noncranial solid tumor of childhood with an occurrence of 10 per million children yearly (1). Deletion of the short arm of chromosome 1 (2) and the amplification and consequent overexpression of the N-*myc* oncogene (3) are common genetic features of this neoplasia. Clinical studies have demonstrated a correlation between N-*myc* amplification, advanced tumor stage, and poor outcome (3). Recently, we described an association between the expression of N-*myc* and that of the proliferation-associated genes histone H3, c-*myc* and *p53* (4). Treatment of a neuroblastoma cell line with antisense oligodeoxynucleotides resulted in inhibition of N-*myc* expression, reorganization of the cytoskeleton to a neuronal differentiated type, and decrease in the proliferation rate (5). In addition, N-*myc* antisense transcripts restricted the differentiative potential of primitive neuroectodermal cell lines (6). However, despite the wealth of data associating N-*myc* expression with differentiative (5, 7) and proliferative (8) events, the function of N-*myc* remains elusive. Like c-*myc*, N-*myc* localizes to the nucleus (9) and has been shown to bind to DNA *in vitro* (10). Recently, a protein was identified that is able to dimerize with c-*myc*, N-*myc* and L-*myc*. This protein, termed MAX in humans (10) and myn in mice (11), contains a helix-loop-helix and a leucine zipper domain which are essential for dimerization (10). At the 5′ of the helix-loop-helix, a basic region which is common to both MAX and *myc* genes allows the dimer to bind to DNA. Binding of MAX to *myc* proteins is highly specific; in fact, MAX fails to form a dimer with other proteins containing a helix-loop-helix or a leucine zipper domain or both (10). The *myc*-MAX complex has a higher affinity for the *myc* consensus core sequence CACGTG than does either MAX or *myc* alone (10). In mice, methylation of the recognition site inhibits DNA binding (11), suggesting that DNA methylation might regulate the *myc*-MAX complex *in vivo*. c-*myc*-MAX and N-*myc*-MAX association *in vivo* was demonstrated in human Burkitt's lymphoma (12) and in neuroblastoma cells (13), respectively. However, the questions of how MAX expression is regulated in the cell and whether there is a correlation with the regulation of its partner genes remain unanswered.

In this study, we compared the steady-state levels of MAX and N-*myc* mRNAs in a set of human neuroblastomas and cell lines. In addition, we detected comparable levels of MAX proteins in neuroblastoma lines and assessed the ability of whole cell extracts from two cell lines with opposite levels of N-*myc* expression to interact differentially with a *myc* binding consensus sequence. Our findings indicate that MAX expression is independent of N-*myc*, suggesting that the absence of simultaneous regulation of N-*myc* and MAX expression affects heterodimer formation in neuroblastoma cells.

## MATERIALS AND METHODS

**Tumors and Cell Lines.** Primary tumor and metastasis samples were obtained at diagnosis and staged according to the Evans system (14). Bioptic material used for molecular analyses was subjected to histological analysis to separate normal from tumor tissue. The IMR32 (15) and LAN-5 (5) cell lines were grown in RPMI 1640 (Sigma, St Louis, MO) supplemented by 15% fetal bovine serum (Sigma). The SK-N-SH cell line (16) was grown in minimal essential medium (GIBCO, Grand Island, NY) with 10% fetal bovine serum.

**RNA and DNA Analyses.** Total RNA was extracted as described (17). RNA was size-fractionated on agarose-formaldehyde denaturing gels and transferred to nylon membranes as described (4). Hybridization to molecular probes was carried out using high stringency conditions (4). Genomic DNA was extracted according to Blin and Stafford (18). Southern blot analysis was carried out as described (4). The N-*myc* probe was NB 19–21, kindly provided by Dr. F. Alt (Columbia University, New York, NY). The human c-*myc* probe was pMYC7.4 obtained by Dr. S. Ferrari (Modena University, Modena, Italy).

**Immunoprecipitations.** Cell lines were metabolically labeled for three hours with [$^{35}$S]methionine. Immunoprecipitations were carried out essentially asdescribed by Wenzel *et al.* (13) using a specific anti-MAX polyclonal antibody obtained by Dr. Edward B. Ziff (New York University, New York, NY). The antibody was raised against a synthetic peptide corresponding to a COOH-terminal sequence of MAX: LQTNYPSSDNSLYTNAKGGTIS. Immunoprecipitates were separated on a 15% polyacrylamide gel.

**RT-PCR[3] Analysis.** Unmodified oligodeoxynucleotides were synthesized and purified as described (19). The sequence of the 5′ MAX primer (MAX-1) was 5′-GGGCCGTAGGAAATGAGCGATAAC-3′. The 3′ primer (MAX-2) was 5′-CCGAGTGGCTTAGCTGGCCTCCAT-3′. The MAX-3 probe was

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[2] To whom requests for reprints should be addressed, at the Department of Microbiology, Thomas Jefferson University, 10th and Locust Streets, Philadelphia, PA 19107 (B. C.), or at the ENEA CRE Casaccia, Division of Molecular Biology, Via Anguillanese, 303, 00060 S. Maria di Galenà, Rome, Italy (G. R).

[3] The abbreviations used are: RT, reverse transcriptase; PCR, polymerase chain reaction; SSC, standard saline-citrate (1 × SSC is 0.15 M NaCl-0.015 M sodium citrate); SDS, sodium dodecyl sulfate; cDNA, complementary DNA.

5′-GAGCAACCGAGGTTTCAATC-3′. The MAX-4 probe was 5′-GCTCT-TCTGGAGCAGCAAGT-3′. Reverse transcription, PCR analysis, and hybridization to specific probes were carried out as described (19). Briefly, filters were prehybridized in 5 × SSC, 1% SDS for 1 h at 47°C. Hybridizations were carried out in 5 × SSC, 1% SDS, 100 μg/ml sonicated salmon sperm DNA, $10^7$ cpm of 5′ end labeled probe for 16 h at 47°C. Posthybridization washings were done in 2 × SSC, 0.5% SDS for 15 min at room temperature and in the same buffer for 15 min at 50°C. Filters were exposed in an X-ray cassette with an intensifier screen for 1–4 h.

**DNA-binding Assays.** Whole cell extracts were prepared by a rapid extraction method as described by Pagano *et al.* (20). Band shift assays were performed as follows: synthetic oligonucleotides corresponding to both strands of the *myc* consensus binding site ($E_{MS}$) oligonucleotide (5′-TCAG-ACCACGTGGTCGGGTGTTCCTGA-3′ and 5′-TCAGGAACACCCGAC-CACGTGGTCTGA-3′) (21) were used as probe. One strand was 5′-end-labeled with T4 polynucleotide kinase and [γ-$^{32}$P]ATP before annealing to the other strand. The annealed probe was purified by chromatography through a G-50 column. Probe DNA ($10^{-2}$ pmol) was incubated in a final volume of 20 μl with 40 mM KCl, 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.9), 1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol, 0.1 μg/μl polydeoxyinosinate-polydeoxycytidylate, 5 ng/μl of single-stranded unrelated oligonucleotide YY1 (5′-CCGAGCCCGCTTCAAAATGGAGACCCTC-3′), and 10 to 20 μg of whole cell extract for 15 min at room temperature in the presence or in the absence of an excess of specific unlabeled competitor ($E_{MS}$). In nonspecific competitions 1 pmol of unlabeled double stranded YY1 oligonucleotide was added. In experiments designed to compete out the DNA-protein interaction, 0.5 or 1 μg of a specific anti-N-myc monoclonal antibody (Oncogene Science, Uniondale, NY) or an anti-mouse H-2K$^d$ monoclonal antibody (Pharmingen, San Diego, CA) were added to the cell extracts at room temperature 10 min before the addition of the $^{32}$P-labeled probe. The mixtures were separated on a native polyacrylamide gel (5%), run on 0.5 × Tris- borate-EDTA (1 × is 0.090 M Tris borate-0.002 M EDTA) for 2 h, 30 min at 100 V at room temperature. The gel was dried and exposed to an X-ray film.

## RESULTS

**Construction of a MAX-specific Probe by RT-PCR.** A 5′-primer spanning the region from nucleotides −12 to 12 and a 3′-primer complementary to the sequence from nucleotides 442 to 465 of the MAX cDNA sequence reported by Blackwood and Eisenman (10) were synthesized. Total RNA from neuroblastoma cell line IMR32 was reverse-transcribed using the 3′ MAX primer, and the resulting cDNA was used as template for PCR. The PCR product was purified and restriction analysis was carried out for unambiguous identification. Digestion with the restriction enzyme *PstI* which recognizes a unique site in the reported cDNA sequence (10) at nucleotide 308 gave the two expected bands of 316 and 162 base pairs and a third band of 343 base pairs (not shown) suggesting the presence of two MAX mRNA transcripts in the IMR32 cells. The latter band was shown to be derived from the MAX mRNA transcript containing a 27-base insertion, 5′ of the basic region which was previously identified in Manca cells (10). The doublet obtained by RT-PCR was also identified as MAX by hybridization with two specific probes (MAX-3 and MAX-4) before being used in Northern and Southern analyses.

**Levels of MAX and N-*myc* Transcripts in Human Neuroblastomas.** Clinical stage and genomic features of tumors and cell lines used in this study are summarized in Table 1. Total RNA was extracted from 10 untreated primary neuroblastomas, 2 metastases, and the cell line IMR32 with a 25-fold N-*myc* amplification (22) and the cell line SK-N-SH lacking N-*myc* amplification (22) and analyzed by Northern blotting (Table 1, Fig. 1). Hybridization under stringent conditions to the MAX-specific probe revealed comparable amounts of 2.1-kilobase MAX transcripts in all samples independently from tumor stage and N-*myc* genomic amplification. Expression of MAX mRNA was readily detectable in each case analyzed. Hybridization of the same RNAs to an N-*myc* probe showed detectable amounts of transcripts in only the three primary tumors at stage IV and the metastasis (Fig. 1*B*, *Lanes 1, 4, 6,* and *7*) with amplified N-*myc* gene and in the N-*myc* amplified cell line IMR32 (Fig. 1*B*). Hybridization of the same RNAs to a human c-*myc* probe did not reveal any detectable signal (not shown).

Table 1 *N-myc amplification and clinical stage of the neuroblastomas analyzed*

| No. | Stage | N-*myc* copy no. |
|---|---|---|
| 1 | IV | 63 |
| 2 | II | <3 |
| 3 | IV (Metastasis) | <3 |
| 4 | IV | 20 |
| 5 | I | <3 |
| 6 | IV | 25 |
| 7 | IV (Metastasis of no. 6) | 29 |
| 8 | IV | <3 |
| 9 | IV-S | <3 |
| 10 | II | <3 |
| 11 | IV-S | <3 |
| 12 | III | <3 |
| Cell line | | |
| IMR 32 | | 25 (22) |
| LAN-5 | | 50 (22) |
| SK-N-SH | | <3 (22) |

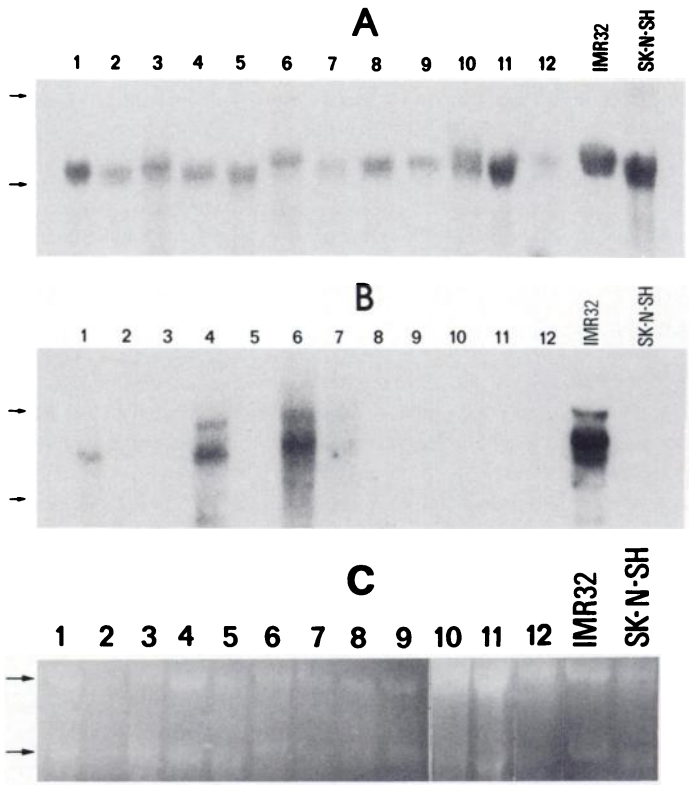


Fig. 1. Northern blot analysis of neuroblastomas RNAs. *A*, hybridization of 10 mg of total RNA to the MAX-specific probe (exposure time, 18 h). *B*, hybridization of the same filter to the N-*myc*-specific probe (exposure time, 18 h) after removal of the previous probe. *C*, ethidium bromide staining of the gel. *Arrows*, position of the 28S and 18S ribosomal RNAs. *Numbers 1* to *12* correspond to the neuroblastomas listed in Table 1.

**Detection of Two MAX Transcripts in Neuroblastoma.** To determine whether the heterogeneous MAX product detected by RT-PCR in IMR32 cells reflected the presence of more than one transcript, RT-PCR was carried out with the tumor RNAs from the Northern analysis with the primers used to construct the MAX-specific probe. RT-PCR products were transferred to a nylon membrane in duplicate and hybridized with two MAX probes: MAX-3 which is identical to 20 of the 27 nucleotides inserted 5′ of the basic region in several cDNAs of Manca cells mRNA (10) and able to

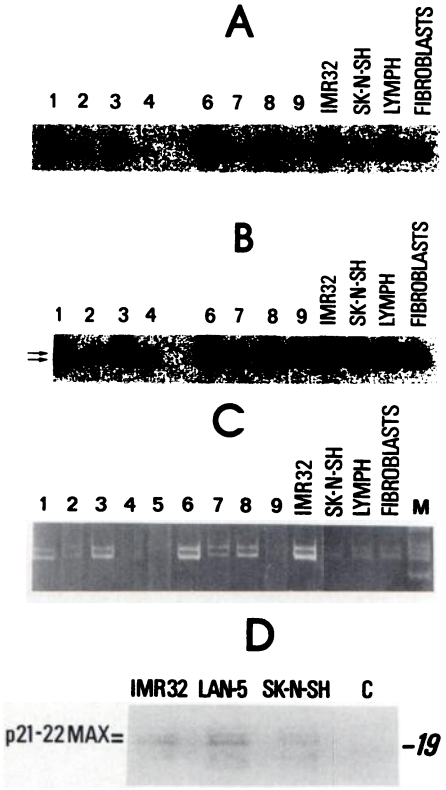


Fig. 2. Detection of MAX transcripts and proteins by RT-PCR and immunoprecipitation analyses. *A*, RT-PCR products were hybridized to probe MAX-3 after transfer to a nylon membrane (exposure time, 1 h). *B*, hybridization to probe MAX-4 (exposure time, 1 h). *C*, ethidium bromide staining of a 6% polyacrylamide gel of the RT-PCR products. *Numbers 1* to *12* are as in Fig. 1. Lymph and Fibroblasts are RNA from normal resting lymphocytes and normal proliferating fibroblasts respectively. *Arrows* in *B*, close doublet revealed by probe MAX-4. In *D*, MAX proteins were immunoprecipitated from [$^{35}$S]methionine-labeled IMR32, LAN-5, and SK-N-SH cells. As control, LAN-5 whole cell lysate was treated with preimmune serum (*Lane C*). *Left*, the position of p21–22 MAX doublet. *Right*, number indicates the position of a 19-kDa molecular mass marker.

detect only this form; and MAX-4 which is identical to nucleotides 250–269 of the MAX cDNA and able to detect both the inserted and noninserted MAX mRNA forms. The results of this experiment indicated the presence of both forms of MAX mRNA in all neuroblastomas tested (Fig. 2, *A* and *B*). The sample in Fig. 1, *Lane 5*, (Northern blot analysis) was not tested by RT-PCR because no RNA was still available for this analysis. Size fractionation of the RT-PCR products on a 6% polyacrylamide gel followed by ethidium bromide staining revealed two bands at 504 and 477 base pairs (Fig. 2*C*), in agreement with the hybridization results. Moreover, the relative abundance of the two MAX mRNA forms appeared to be equal in each sample. RT-PCR carried out with RNA of resting peripheral blood lymphocytes from a healthy donor and proliferating gengival fibroblasts from a primary culture also demonstrated transcription of the two MAX forms in both cases (Fig. 2, *A–C*) confirming that MAX expression is not limited to transformed and/or proliferating cells (12).

**Two MAX Proteins are Expressed in Neuroblastoma Cells.** To investigate if MAX protein(s) expression correlates with the presence of the MAX mRNAs in neuroblastomas, immunoprecipitations were carried out using a specific antibody to MAX on whole-cell lysates prepared from [$^{35}$S]methionine-labeled neuroblastoma cell lines LAN-5 which has a 50-fold N-*myc* amplification (22) and expresses N-*myc* mRNA high levels (5), IMR32 and SK-N-SH. A closely spaced doublet banding at $M_r$ 21,000–22,000 was detected in all samples (Fig. 2*D*) in agreement with the reported molecular weight for MAX proteins (12). Immunoprecipitation of the cell extract from LAN-5 cell line with preimmune serum did not detect any band in the $M_r$ 21,000–22,000 region (Fig. 2*D*).

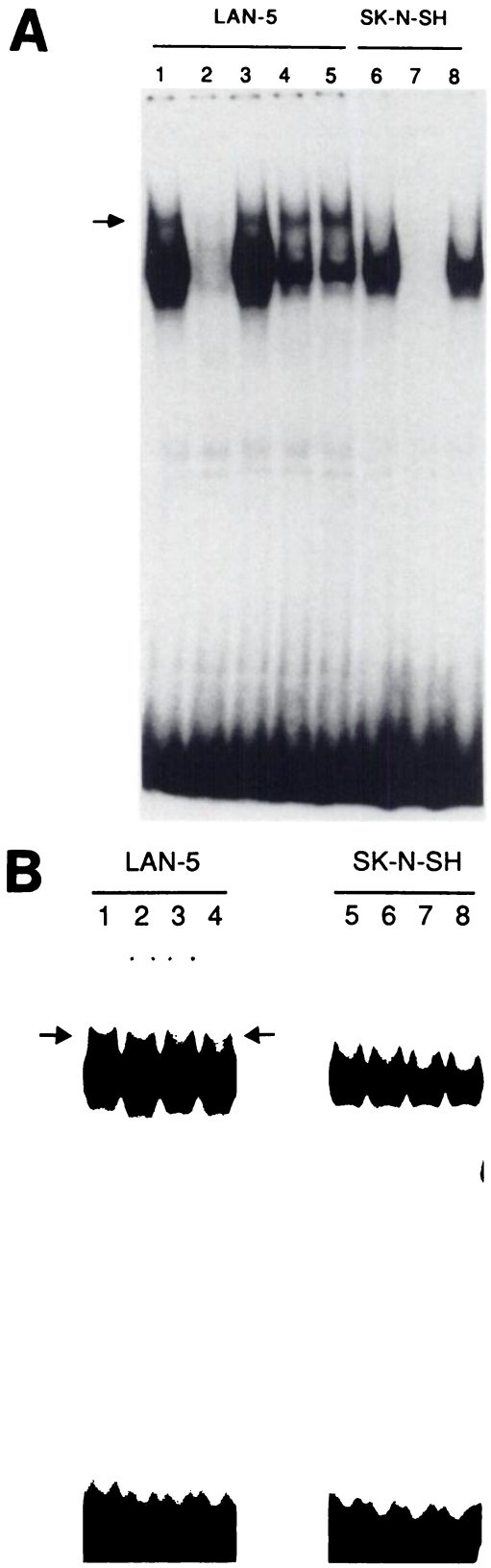


Fig. 3. In *A* gel mobility shift assays used whole extracts from LAN-5 (*Lanes 1–5*) or SK-N-SH (*Lanes 6–8*) and the $E_{MS}$ oligonucleotide as a probe. *Lanes 2* and *7*, specific competitions using 1 pmol of $E_{MS}$ double-stranded oligonucleotide. *Lanes 3* and *8*, nonspecific competitions with the YY1 oligonucleotide. *Lanes 4* and *5* the shifts were carried out using 10 μg of LAN-5 extracts; in all the other lanes 20 μg of extracts were used. *Arrow*, the distinctive band in LAN-5 shifts (see text for comments). Autoradiogram was exposed for 18 h. B, gel mobility shift assay in presence of anti a N-*myc* antibody. Whole extracts from LAN-5 (*Lanes 2* and *3*) and SK-N-SH cells were preincubated for 10 min at room temperature before electrophoresis mobility shift assay with 500 ng (*Lanes 2* and *6*) or 1 μg of a specific anti-N-myc antibody (*Lanes 3* and *7*). In *Lanes 4* and *8*, samples were preincubated with 1 μg of an unrelated monoclonal antibody. In *Lanes 1* and *5* samples were not preincubated. Exposure, 18 h.

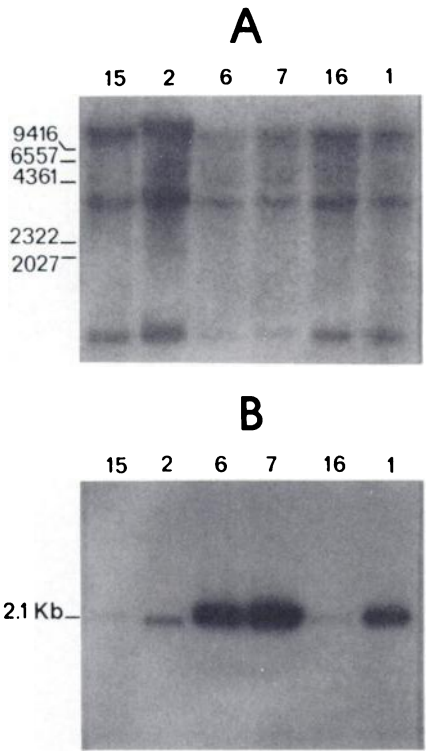


Fig. 4. Southern blot analysis of neuroblastoma genomic DNA. *Eco*RI digested genomic DNA was run on an agarose gel and transferred to a nylon membrane. *A*, hybridization to the MAX-specific probe (exposure time, 72 h). *B*, hybridization to the N-*myc*-specific probe (exposure time, 72 h). Samples 1, 2, 6 and 7 correspond to neuroblastomas listed in Table 1. Samples 15 and 16 are from two primary neuroblastomas for which no RNA was available for Northern and RT-PCR analyses.

**Binding Patterns to myc Recognition Sequences Differ in Neuroblastoma Cell Lines Expressing or Nonexpressing N-myc.** To determine whether the presence or the absence of the N-*myc* protein in neuroblastoma cells results in different binding patterns to the *myc* consensus sequence, we carried out electrophoresis mobility shift assay experiments using whole cell extracts from LAN-5 (high level of N-*myc* expression) and SK-N-SH (no detectable expression of N-*myc*). Whole cell extracts were prepared in high salt conditions to increase the solubility of myc proteins. Different band shift patterns were obtained using LAN-5 and SK-N-SH extracts (Fig. 3*A*). LAN-5 shifts showed an upper band (Fig. 3*A*, *arrow*) which was not present in SK-N-SH. Although also the ubiquitous transcription factor binds to the same DNA sequence recognized by *myc* proteins, it seems likely that the upper band seen in LAN-5 extracts derives from the binding of N-*myc*-MAX heterodimers to the oligonucleotide probe which does not occur in SK-N-SH extracts due to the lack of N-*myc* expression. To determine whether the upper band seen in Fig. 3*A* depended on N-*myc* expression in LAN-5 cells, total extracts from LAN-5 and SK-N-SH cells were preincubated with a monoclonal antibody to N-*myc* before electrophoresis mobility shift assay. Preincubation with the N-*myc* antibody reduced the density of the upper band seen with LAN-5 cell extracts (Fig. 3*B*, *Lanes 2* and *3*), whereas the pattern seen with the SK-N-SH extracts was not modified by the treatment. In control experiments, preincubation with an unrelated monoclonal antibody did not affect the intensity of the upper band seen with LAN-5 cell extracts (Fig. 3*B*, *Lane 4*).

**Southern Blot Analysis of Neuroblastoma Genomic DNA.** Hybridization of *Eco*RI-digested genomic DNA from five primary neuroblastomas and a metastasis of one of them to the MAX-specific probe revealed several bands ranging from more than 9 kilobases to less than 1 kilobase, a pattern that was invariant in all samples (Fig. 4*A*). Analysis of gene dosage, after normalization of the amount of DNA for each sample, revealed no genomic amplification of the MAX gene (not shown), suggesting that the transcription of MAX does not depend on amplification of the gene. Hybridization of the same filter to N-*myc*-specific probe, demonstrated amplification of N-*myc* in two tumors at stage IV and in one metastasis (Fig. 4*B*, *Lanes 1, 6*, and *7*).

## DISCUSSION

The association *in vivo* of N-myc oncoprotein with MAX phosphoprotein has been described in human neuroblastoma (13). This result suggests a role of the N-*myc*-MAX dimer in this neoplasia. Using a probe obtained by RT-PCR, we have detected MAX mRNA in 10 primary neuroblastomas, two metastases, and two cell lines, at comparable levels in all samples and independent of tumor stage or N-*myc* genomic amplification. Anti-MAX antibody immunoprecipitated two MAX proteins in all neuroblastoma cell lines tested. Thus, it seems unlikely that MAX expression is controlled in neuroblastoma by a posttranscriptional mechanism preventing translation of MAX mRNAs. By contrast, N-*myc* mRNA was present only in samples with amplified N-*myc* gene, suggesting that the latter is a limiting factor in the formation of the N-*myc*-MAX dimer in neuroblastoma. Unlike N-*myc*, the MAX levels of transcripts do not appear to depend on genomic amplification.

The detection of MAX transcripts in neuroblastomas that do not express N-*myc* raises the question of the function of *MAX* gene. It has been demonstrated in humans and mice that MAX can form an homodimer (10–12) the role of which in the cell might differ from that of the heterodimer. MAX protein(s) lacks the transactivation domain (22), yet MAX-MAX homodimers bind the same CACGTG sequence recognized by *myc* genes (10–12). Expression of MAX in excess over *myc* genes can shift the equilibrium in favor of homodimers, which in turn compete out the transactivating *myc*-MAX heterodimers (12), keeping the controlled genes in a state of transcriptional repression (23).

In neuroblastoma, the amplification and overexpression of N-*myc* is a marker of tumor aggressiveness (3). We can speculate that in tumors where only MAX expression is detectable, those genes under the transcriptional control of N-*myc* are silenced by MAX homodimers. The lack of expression of these genes could somehow decrease the tumor aggressiveness. The identification of neuroblastomas without N-*myc* amplification and overexpression, yet highly aggressive, might be explained with the activation of different pathways independent from those associated with N-*myc*-MAX expression. Alternatively, in tumors that do not express N-*myc*, the MAX protein could be in an inactive form. In light of the presence of a consensus signal for casein kinase II 3′ of the leucine zipper of MAX (10) it is possible that the activity of MAX protein is related to the phosphorylation state.

All of the neuroblastoma samples analyzed revealed the presence of two MAX transcripts, one of which derives from an in-frame insertion of 27 nucleotides in a region 5′ to the basic region. Since mutations outside the basic region helix-loop-helix-leucine zipper domain do not alter the ability of MAX to dimerize and bind to DNA (10, 11), it is conceivable that the presence of two MAX forms able to dimerize with N-*myc* serves to broaden the range of the gene activities controlled by the N-*myc*-MAX complex.

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## REFERENCES

1. Young, J. L. Jr., Gloeckler Ries, L., Silverberg, E., Horm, J. W., and Miller, R. W. Cancer incidence, survival, and mortality for children younger than age 15 years. Cancer (Phila.), *58:* 598–602, 1985.

2. Brodeur, G. M., Green, A. A., Hayes, F. A., Williams, K. J., Williams, D. L., and Tsiatis, A. A. Cytogenetic features of human neuroblastomas and cell lines. Cancer Res., *41:* 4678–4686, 1981.
3. Seeger, R. C., Brodeur, G. M., Sather, H., Dalton, A., Siegel, S. E., Wong, K. Y., and Hammond, D. Association of multiple copies of the N-*myc* oncogene with rapid progression of neuroblastomas. New Engl. J. Med., *313:* 1111–1116, 1985.
4. Raschellà, G., Negroni, A., Giubilei, C., Romeo, A., Ferrari, S., Castello, M. A., and Dominici, C. Transcription of N-*myc* and proliferation-related genes is linked in human neuroblastoma. Cancer Lett., *56:* 45–51, 1991.
5. Negroni, A., Scarpa, S., Romeo, A., Ferrari, S., Modesti, A., and Raschellà, G. Decrease of proliferation rate and induction of differentiation by MYCN antisense DNA oligomer in a human neuroblastoma cell line. Cell Growth & Differ., *2:* 511–518, 1991.
6. Whitesell, L., Rosolen, A., and Neckers, L. M. Episome-generated N-*myc* antisense RNA restricts the differentiation potential of primitive neuroectodermal cell lines. Mol. Cell Biol., *11:* 1360–1371, 1991.
7. Mugrauer, G., Alt, F. W., and Elkblom, P. V. N-*myc* protooncogene expression during organogenesis in the developing mouse as revealed by *in situ* hybridization. J. Cell Biol., *107:* 1325–1335, 1988.
8. Rosenbaum, H., Webb, E., Adams, J. M., Cory, S., and Harris, A. W. N-*myc* transgene promotes B-lymphoid proliferation, elicits lymphomas and reveals cross-regulation with c-*myc*. EMBO J., *8:* 749–755, 1989.
9. Ikegaki, N., Bukowski, J., and Kennett, R. H. Identification and characterization of the *NMYC* gene product in human neuroblastoma cells by monoclonal antibodies with defined specificities. Proc. Natl. Acad. Sci. USA, *83:* 5929–5933, 1986.
10. Blackwood, E. M., and Eisenman, R. N. MAX: A helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with MYC. Science (Washington DC), *251:* 1211–1217, 1991.
11. Prendergast, G. C., Lawe, D., and Ziff, E. B. Association of *myn*, the murine homolog of MAX, with c-*myc* stimulates methylation-sensitive DNA binding and *Ras* cotransformation. Cell, *65:* 395–407, 1991.
12. Blackwood, E. M., Luscher, B., and Eisenman, R. N. Myc and MAX associate *in vivo*. Genes & Develop., *6:* 71–80, 1992.
13. Wenzel, A., Cziepluch, C., Hamann, U., Schurmann, J., and Schwab, M. The N-*myc* oncoprotein is associated *in vivo* with the phosphoprotein MAX (p20/22) in human neuroblastoma cells. EMBO J., *10:* 3703–3712, 1991.
14. Evans, A. E., D'Angiò, G. J., and Randolph, J. A proposed staging for children with neuroblastoma. Cancer (Phila.), *27:* 374–378, 1971.
15. Schwab, M., Alitalo, K., Klempnauer, K-H., Varmus, H., Bishop, J. M., Gilbert, F., Brodeur, G., Goldstein, M., and Trent, J. Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. Nature (Lond.), *305:* 245–248, 1983.
16. Biedler, J. L., Helson, L., and Spengler, B. A. Morphology and growth tumourigenicity and cytogenetics of human neuroblastoma cells in continuous culture. Cancer Res., *33:* 2643–2649, 1973.
17. Chomczynski, P., and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., *162:* 156–159, 1987.
18. Blin, N., and Stafford, D. W. Isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res., *3:* 2303–2308, 1976.
19. Szczylik, C., Skorski, T., Nicolaides, N. C., Manzella, L., Malaguarnera, L., Venturelli, D., Gewirtz, A. M. and Calabretta, B. Selective inhibition of leukemia cell proliferation by BCR-ABL antisense oligodeoxynucleotides. Science (Washington DC), *253:* 562–565, 1991.
20. Pagano, M., Draetta, G., and Jansen-Durr, R. Association of cdk2 kinase with the transcription factor E2F during S phase. Science (Washington DC), *255:* 1144–1147, 1992.
21. Makela, T. P., Koskinen, P. J., Vastrik, I., and Alitalo, K. Alternative forms of MAX as enhancers or suppressors of *Myc-Ras* cotransformation. Science, *256:* 373–377, 1992.
22. Kohl, N. E., Gee, C. E., and Alt, F. W. Activated expression of N-*myc* gene in human neuroblastomas and related tumors. Science (Washington DC), *226:* 1335–1337, 1984.
23. Kretzner, L., Blackwood, E. M., and Eisenman, R. N. *Myc* and Max proteins possess distinct transcriptional activities. Nature (Lond.), *359:* 426–429, 1992.