Differential Regulation of the N-myc Gene in Transfected Cells and Transgenic Mice

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The N-myc gene is expressed specifically in the early developmental stages of numerous cell lineages. To assay for sequences that could potentially regulate N-myc expression, we transfected constructs that contained murine N-myc genomic sequences linked to a reporter gene and genomic clones that contained the complete human or murine N-myc genes into cell lines that either express or do not express the endogenous N-myc gene. Following either transient or stable transfection, the introduced N-myc sequences were expressed regardless of the expression status of the endogenous gene. In contrast, when the clones containing the complete human N-myc gene were introduced into the germline of transgenic mice, expression in some transgenic lines paralleled the tissue- and stage-specific expression of the endogenous murine gene. These findings demonstrate differences in the regulation of N-myc genes in recipient cells following in vitro versus in vivo introduction, suggesting that early developmental events may play a role in the regulation of N-myc expression.

The myc family of nuclear oncogenes consists of three well-defined, structurally similar members, c-myc, N-myc, and L-myc; these genes encode related but distinct nuclear proteins (for a review, see reference 3). The three myc-family genes have been implicated in various types of malignancies and, when deregulated, have been shown to have similar transforming activities in vivo and in vitro (1, 4, 11, 13, 28, 42, 43, 58). The normal function of myc-family proteins has not been elucidated. However, changes in myc expression have been correlated with the response of cultured cells to differentiation agents or mitogenic stimuli (14, 19, 20, 22, 23, 48). In addition, the three myc proteins share regions of homology with known transcriptional regulatory and cell lineage determination proteins (6, 9, 29, 34, 49, 50, 53). These findings, coupled with the expression patterns of myc genes during normal development (see below), suggest that a function of myc proteins may be to regulate gene expression during cellular growth and differentiation.

Despite encoding proteins with highly conserved regions and shared transforming activities, the c-myc, N-myc, and L-myc genes are conserved as distinct sequences in many vertebrate species, suggesting unique functional roles. This possibility is supported by the unique activation pattern of each gene in spontaneously arising tumors and the differential developmental stage- and tissue-specific expression of each gene in normal cells (5, 24, 25, 30, 35–37, 41, 51). During normal development, c-myc expression occurs in a diverse set of tissues, where it is often correlated with sites of cell proliferation; in contrast, high-level expression of N-myc and L-myc appears restricted to specific tissues (40, 60). Detailed studies suggest that N-myc expression is often a feature of early differentiation stages, regardless of cell proliferative state (15, 22, 60); for example, postmitotic but not yet differentiated neuroblasts in the brain express high N-myc RNA levels (33). In general, results with differentiating tissues and cell lineages support the notion that differential or perhaps combinatorial expression of myc-family genes may be important in normal development.

Molecular mechanisms that regulate expression of the c-myc gene in cultured cells have been examined in detail (for a review, see reference 32), but few studies have addressed regulation of the N-myc and L-myc genes (27, 47). Furthermore, the mechanisms that regulate the differential tissue- and stage-specific expression of myc genes during in vivo development have not been defined in any system. The expression pattern of the N-myc gene is distinct from that of other well-characterized, developmentally expressed genes. In particular, many genes that have been studied in cell transfection and transgenic mouse systems encode proteins expressed in the later developmental stages of restricted cell lineages (for reviews, see references 31 and 38). In contrast, N-myc expression occurs in immature rather than mature cells and is not restricted to a single, or even a few, cell lineages. We now describe a series of cell transfection and transgenic mouse studies that begin to define molecular aspects of the developmental control of N-myc gene expression.

MATERIALS AND METHODS

Construction of N-myc:CAT fusion genes. Fragments of the murine N-myc gene were subcloned into the pGAT-C vector, a derivative of pGEM-4 that has a bacterial chloramphenicol acetyltransferase gene and a simian virus 40 polyadenylation sequence subcloned at the HindIII site of the polylinker (Olivier Brison, personal communication). The 600-base-pair (bp) SacI-BamHI murine N-myc fragment was subcloned directly into the corresponding sites of the pGAT-C polylinker (construct 3, Fig. 1A). The 1.9-kilobase (kb) EcoRI-BamHI and the 2.8-kb EcoRI-BglII fragments of murine N-myc were subcloned into the SmaI-BamHI sites of pGAT-C following Klenow-directed fill in of the EcoRI site (constructs 4 and 5, Fig. 1A). The 1-kb

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FIG. 1. Murine N-myc flanking sequences drive nonspecific expression of a heterologous CAT gene. (A) A schematic of the murine N-myc gene is shown (12). Fragments 3 through 7 were subcloned upstream of the bacterial chloramphenicol acetyl transferase gene as described in Materials and Methods. (B) Analysis of CAT activity in transiently transfected HeLa cells. Lane 1, RSV-CAT (16); lane 2, pGCAT-C vector alone; lanes 3 through 7, corresponding N-myc:CAT constructs diagrammed in 1A. Unacylated (CM) and monoacetylated (1 and 3) forms of chloramphenicol are indicated; representative results are shown.

HincII-EcoRI fragment of the murine N-myc gene was treated with Klenow to fill in the EcoRI site and was subcloned in a 5' to 3' orientation into the Smal site of pGCAT-C (construct 6, Fig. 1A). The same HincII-EcoRI fragment was inserted in a 5' to 3' orientation upstream of the N-myc EcoRI-BamHI fragment by subcloning into the Klenow-treated EcoRI site of the polylinker of construct 4 (construct 7, Fig. 1A).

Construction of murine N-myc transgene. To distinguish a murine N-myc transgene from the endogenous N-myc gene, an Xbal linker was inserted at a unique HincII site in the 3' untranslated region of the murine N-myc gene. The plasmid pN7.7, consisting of the 7.4-kb EcoRI genomic N-myc fragment subcloned in PUC18 (12), was digested with HincII, resulting in restriction at the HincII site contained in the murine N-myc gene and the HincII site of the polylinker. Xbal linkers were ligated to the resulting blunt ends. Following digestion with Xbal to remove excess linkers, the resulting Xbal fragments (a 3.8-kb fragment that contained the 3' end of the N-myc gene and the PUC18 plasmid and a 6.5-kb fragment that contained the remainder of the N-myc gene) were separated by electrophoresis. The 3.8-kb plasmid-containing fragment was then treated with calf intestinal phosphatase and religated to the 6.5-kb fragment in the correct orientation to yield plasmid pN7.7-X. We determined that the altered N-myc gene could cooperate with an activated Ha-ras oncogene to transform primary rat embryo fibroblasts with an efficiency similar to that of the wild-type gene; therefore, none of the manipulations interfered with the assayable expression of the N-myc gene (unpublished results).

Cell culture, DNA transfections, and CAT assays. The 38B9 and 70Z cell lines were cultured in RPMI 1640 supplemented with 10% fetal calf serum. All other cell lines were cultured in Dulbecco modified Eagle medium supplemented with 10% newborn calf serum. DNA transfection into the 38B9, 70Z, and 104E cell lines was performed by using electroporation techniques (57). Other stable and transient DNA transfections were performed by the calcium phosphate precipitation technique (18). Approximately 5 x 106 HeLa and LAN-5 cells were transfected with 15 ng of the N-myc:CAT constructs shown. Cells were harvested after 48 h, and extracts were prepared and assayed for CAT activity by standard methods (17). Approximately 5 x 107 L-cells and HeLa cells were transfected with 10 ng of the human N-myc-containing plasmid DNA (pNG-2 [26]) and 30 ng of high-molecular-weight carrier DNA. The transfected populations were harvested after 48 h, and RNA was prepared. Approximately 106 3T3 cells were stably transfected with 10 ng of the human N-myc containing plasmid pNG-2, 2 ng of LTRneo, and 30 ng of high-molecular-weight carrier DNA. Approximately 107 38B9, 70Z, and 104E cells were stably transfected with 50 ng of pNG-2 and 10 ng of LTRneo. Independent stable transfectants were selected in media supplemented with 1 or 2 mg of (38B9) G418 per ml and expanded for RNA and DNA preparation.

RNA and DNA preparation, blotting analysis, and S1 nuclease analysis. RNA and DNA were prepared, electrophoresed, blotted, and probed with nick-translated fragments as previously described (24, 25). The human cDNA fragment, p11-1, was used to detect the human N-myc gene (26), and the murine cDNA fragment, M2.1, was used to detect the murine N-myc gene (60).

S1 nuclease analyses were performed on 10 ng of total RNA with uniformly labeled single-stranded DNA probes and analyzed by electrophoresis on 5% acrylamide-urea gels as previously described (26). A previously described genomic 800-bp Xhol-PstI fragment containing the third exon of the murine N-myc gene was used to detect endogenous murine N-myc expression (see Fig. 3 [12]). A previously described 250-bp AvaI fragment spanning the exon 2 to 3 junction of the human N-myc gene was used to detect specific expression of an introduced human gene (see Fig. 3 [26]). To specifically detect expression of the murine N-myc transgene, the 540-bp PstI-ScaI fragment from the third exon of the transgene was used. Since the transgene has an Xbal linker inserted at the HincII site, transcripts from the transgene are fully protected, while endogenous transcripts are digested at the linker sequence, yielding protected fragments of 120 and 420 bp.

Microinjection and identification of transgenic mice. The plasmid pNG-2 (26) was digested with HindIII, and the 13-kb genomic human N-myc fragment was isolated by agarose gel electrophoresis. The pN7.7-X plasmid was digested with NdeI (an NdeI site is located 80 bp downstream of the 5' EcoRI site of N-myc) and 260 bp downstream of the poly-
linker), and a 7.6-kb fragment that contains the murine N-myc gene and a limited amount of plasmid sequence was isolated by gel electrophoresis. Both fragments were purified by electrodialysis, followed by centrifugation through CsCl gradients and dialysis against a solution of 7.5 mM Tris (pH 7.5)-0.1 mM EDTA (pH 8.0) (21). Fragments were diluted to a final concentration of 1 µg/ml and injected into the pronucleus of (C57BL × CBA)F2 or (C57BL × DBA)F2-fertilized eggs (21). Injected eggs were transferred on the same or the following day to 0.5-day pseudopregnant F1 foster mothers. Offspring were analyzed 2 weeks after birth for the presence of the injected fragment by Southern blot analysis of tail DNA. Transgenic lines were established by mating to either (C57BL × CBA)F1 male or (C57BL × CBA)F1 or (C57BL × DBA)F1 female mice.

RESULTS

Expression of transiently transfected N-myc constructs. To assay for potential transcriptional control elements, we constructed fusions between the 5' and 3' flanking regions as well as the untranslated regions of the murine N-myc gene and the bacterial chloramphenicol acetyltransferase gene (HeLa) and a neuroblastoma cell line that expresses the enzymatic CAT activity in extracts prepared from the transiently transfected cells. Similar results were obtained with both cell lines; only representative results with the HeLa cell line are shown. The N-myc3:CAT fusion construct contained only 600 bp of 5' N-myc sequence but generated efficient expression of the heterologous CAT gene (Fig. 1, compare lanes 2 and 3), indicating that elements contained within this region provide transcriptional promoter activity. The expression of the N-myc3:CAT construct in HeLa cells suggested that additional sequences might be required for down regulation of the N-myc promoter in these cells. Therefore, additional first exon, first intron, 5' flanking, and 3' untranslated sequences of the murine N-myc gene were fused upstream of the CAT gene (Fig. 1A, fragments 4 through 7). All constructs yielded similar efficient expression of CAT activity in HeLa cells (Fig. 1B, lanes 4 through 7).

To further assay for potential regulatory sequences capable of driving specific expression of the N-myc gene, the entire murine N-myc clone (Fig. 1A) was introduced by transient transfection procedures into LAN-5 and HeLa cells, as well as into two murine cell lines that do not express the endogenous gene (3T3 and L-cells [60]). Cells were harvested 48 h after transfection, and RNA was analyzed for ability to protect a single-stranded DNA probe specific for the murine N-myc gene from digestion with S1 nuclease. The transfected murine N-myc gene was expressed in all cell lines tested regardless of the expression status of the endogenous gene (data not shown).

The murine N-myc clone transfected into cell lines contained 1.5 kb of 5' flanking sequence and 0.2 kb of 3' flanking sequence (12). To test for potential regulatory elements in additional flanking sequence, the human N-myc gene, for which 3.5 kb of upstream and 3 kb of downstream flanking sequence were readily available (26; Fig. 2A), was transfected into the murine L-cell and human HeLa cell lines, both of which do not express the endogenous N-myc gene. Cells were harvested 48 h after transfection, and RNA was assayed for expression of the transfected gene by S1 nuclease analysis with a probe specific for the human N-myc gene. The transfected human N-myc sequence again was expressed in both cell lines despite lack of expression of the endogenous N-myc gene (Fig. 2B). S1 nuclease analysis confirmed that transcriptional initiation occurred at the normal sites for the human N-myc gene (see below).

Expression of stably transfected human N-myc sequences. A high copy number of largely extrachromosomal sequences is introduced following transient transfection, raising the possibility that inappropriate expression of the N-myc gene in the transient transfection experiments resulted from a limiting quantity of a negative regulatory factor or a dependence on chromosomal integration. To test these possibilities, the human N-myc gene assayed transiently was introduced by stable transfection into a murine cell line that does not express the endogenous N-myc gene (3T3 [60]). The N-myc gene was introduced by cotransfection with an LTRneo vector, and DNA from G418-resistant clones was screened by Southern blotting analysis for integrated, cotransfected human N-myc sequences. The blotting analysis revealed that the clones varied in copy number of the transfected sequence from 1 to 2 copies to greater than 10 copies per cell (Fig. 2C). All transfected 3T3 clones produced substantial levels of steady-state N-myc mRNA, with some relatively low-copy-number clones producing levels that approached those found in a human neuroblastoma that produces 50-fold baseline N-myc levels as a result of gene amplification (Fig. 2C). However, the relative expression per copy of introduced sequence was quite variable, suggesting integration site effects on levels of transcription. With few exceptions (Fig. 1C, lanes 4 and 5), the size of the introduced human
N-myc transcript indicated that the normal transcriptional start site and polyadenylation signal were used. To confirm that expression of the transfected human N-myc gene in 3T3 cells initiated from the associated N-myc promoter, we performed S1 nuclease analyses, as previously described (26), on RNA from five of these clones to map the initiation site of the transcripts. These studies confirmed that the transfected N-myc genes initiated from the normal set of transcriptional initiation sites with an essentially identical utilization pattern to that observed for the endogenous N-myc gene in human neuroblastoma cell lines (data not shown).

To examine the regulation of an introduced N-myc gene in additional cell lines, the human gene was introduced by cotransfection with the LTRneo vector into cell lines representing the pre-B (38B9), advanced pre-B (70Z), and plasma (104E) cell stages of B-lineage development. Expression of the endogenous murine N-myc gene is down regulated during B-cell differentiation and, therefore, is detectable in the pre-B cell line (38B9) but not in the more advanced pre-B cell line (70Z) or in the mature myeloma cell line (104E) (60). Expression of the introduced human N-myc gene was assayed by S1 nuclease analysis with a probe specific for the human N-myc gene (Fig. 3, lower panel). In contrast to the expression of the endogenous gene in the pre-B cell line, expression of the introduced human N-myc sequence again was detectable in transfected clones derived from all three cell lines (Table 1).

In summary, murine and human N-myc gene sequences introduced by transfection into numerous cell lines generally are expressed regardless of the expression status of the endogenous N-myc locus. Expression of introduced N-myc sequences occurs in both transient and stable transfection assays, in assays of heterologous gene expression, and in human and murine cell lines (Table 1).

Expression of N-myc gene sequences in transgenic mice. The lack of down regulation of the N-myc gene following transfection into cell lines that do not express the endogenous gene might result from a missing regulatory element in the transfected sequence. However, another possibility is that sequence elements necessary for regulated expression are present but the cell does not act appropriately on an introduced N-myc gene. We used transgenic mouse technology to test the importance of early developmental events in correct regulation of N-myc expression. Both the murine and human N-myc sequences used in transfection experiments (Fig. 1A and 2A) were introduced into the mouse germline by injection into fertilized eggs. Five transgenic founders resulted from injection of the murine sequences (of which four transmitted the gene to progeny), and six transgenic founders resulted from injection of the human N-myc sequences (of which three transmitted the gene to progeny) (Table 2). The copy number of murine and human transgene sequences varied from 1 to 2 to greater than 10 copies arranged in a head-to-tail configuration at a single integration site (Table 2; other data not shown).

Transgenic expression in the hN1 transgenic line. To determine whether transgene expression was regulated in these animals, we assayed RNA from newborn and adult tissues of transgenic animals for transgenic versus endogenous N-myc expression. For this purpose, we used an S1 nuclease assay with probes specific for either the transgene or endogenous gene (Fig. 3; lower panel). These analyses were performed on RNA from pooled tissues of a number of transgenic siblings and on individual animals with similar results. Expression of the human transgene was detectable in all three transgenic lines (Table 2) and was generated, at least predominantly, from the normal human N-myc transcriptional initiation sites (data not shown). The hN1 line expressed the transgene in a manner that paralleled endogenous gene expression (Fig. 3). Like the endogenous N-myc gene, the transgene was expressed at highest levels in the newborn brain and kidney, with levels of expression declining in these tissues in the adult. Expression of the endogenous N-myc gene in other newborn and adult tissues, although detectable, was much lower than expression in the

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### Table 1. Summary of transfection results

<table>
<thead>
<tr>
<th>Cell line</th>
<th>N-myc gene introduced</th>
<th>No. of clones testeda</th>
<th>Expressionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LAN-5</td>
<td>Murine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HeLa</td>
<td>Murine and human</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Murine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-cell</td>
<td>Murine and human</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>3T3</td>
<td>Murine</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Stable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3T3</td>
<td>Human</td>
<td>8</td>
<td>+ (8/8)</td>
</tr>
<tr>
<td>38B9</td>
<td>Human</td>
<td>3</td>
<td>− + (3/3)</td>
</tr>
<tr>
<td>70Z</td>
<td>Human</td>
<td>2</td>
<td>− + (2/2)</td>
</tr>
<tr>
<td>104E</td>
<td>Human</td>
<td>4</td>
<td>− + (4/4)</td>
</tr>
</tbody>
</table>

* a. Clones determined by Southern blotting analyses to contain transfected N-myc sequences.

* b. Expression detectable; −, no expression detectable. Numbers in parentheses indicate number with detectable expression per total number tested.
newborn brain and kidney. Low-level transgene expression was also faintly detectable in the newborn intestine, lung, thymus, and heart; expression in other tissues was beyond our detection limits (Fig. 3). Therefore, within the limits of our assay, the overall pattern of transgene expression in the hN1 line paralleled endogenous expression.

Transgene expression during postnatal development of brain and kidney. Expression of the endogenous N-myc gene declines during the postnatal development of the brain and kidney (60; see Fig. 5). To examine the regulation of transgene expression in more detail, we determined the developmental time course of transgenic N-myc expression in these organs. For this purpose, RNA was prepared from the brains and kidneys of hN1 transgenic mice at a number of postnatal developmental stages and assayed for transgene and endogenous N-myc expression. Expression of the transgene in the hN1 line paralleled that of the endogenous gene in both the developing brain and kidney (Fig. 4), indicating that the transgene is regulated in parallel to the endogenous gene in these tissues. The regulated in vivo expression of the human N-myc gene in the hN1 transgenic line demonstrates that sequences capable of directing correct expression of the N-myc gene are contained within the limited sequence introduced into the mouse germline. Furthermore, the human N-myc transgene is regulated in transgenic mice, suggesting that the specific sequence elements and factors that control N-myc expression are conserved between these species.

**Human N-myc transgene expression in other transgenic lines.** The transgenic line hN2 expressed the N-myc transgene at high levels in the newborn brain, with a decline in expression in the adult brain (Fig. 5A). However, the transgene was not expressed in the newborn kidney but, instead, was expressed in the adult kidney. Furthermore, the transgene was expressed at significant levels in the adult lung. In the transgenic line hN3, the transgene was expressed in the newborn brain but expression did not decline in the adult

![FIG. 4. Transgenic and endogenous N-myc expression in the hN1 transgenic line during postnatal development of the brain and kidney. RNA was prepared from the brain and kidney of hN3 transgenic mice at the indicated postnatal time points and assayed for human N-myc expression as described for Fig. 3.](image-url)

![FIG. 5. Expression of the N-myc transgene in the hN2 and hN3 transgenic lines. Expression of the N-myc transgene was monitored in the hN2 (A) and hN3 (B) transgenic lines as described in the legend to Fig. 3. Endogenous N-myc expression in these transgenic lines was identical to that of nontransgenic siblings (data not shown).](image-url)
brain. No expression was detected in the newborn kidney (Fig. 5B). Expression of the endogenous gene in both transgenic lines was identical to that in nontransgenic siblings (data not shown). The cause of the aberrant transgene expression in these mice is unclear. One possibility is that this phenomenon is related to the higher copy number of the N-myc transgene in these two lines than in the hN1 line, although the difference is not greater than fivefold (Table 1). The most likely possibility for the partial regulation in the hN2 and hN3 lines is that the differential expression of the transgene results from influences exerted by the locus of integration (see Discussion). Although the regulation in these lines is incomplete, we note that transgene expression is still restricted.

Expression of the murine N-myc transgene. Expression of the murine transgene, as determined by S1 nuclease protection assays (see Material and Methods), was detectable in three of four transgenic lines (Table 2). The murine transgene was reproducibly expressed in the newborn brain and, in one line, expression was also detectable in the newborn kidney. However, transgene expression levels were substantially lower than endogenous levels, making detection of the transgene difficult and preventing more extensive analyses of expression in the various tissues. This low level of transgenic expression was observed even in a mouse line containing greater than 10 copies of the transgene.

**DISCUSSION**

In vitro versus in vivo regulation of an introduced N-myc gene. We have found apparently normal expression of a genomic clone containing the complete N-myc gene after introduction into transgenic mice but were unable to reproduce endogenous regulation patterns following transfection into cell lines. It is unlikely that expression of the stably transfected N-myc sequences resulted from the proximal integration of the long terminal repeat enhancer element of the cotransfected neo vector, since similar levels of N-myc expression were observed when an enhancerless thymidine kinase-neo construct was substituted (K. Zimmerman and F. Alt, unpublished results). Furthermore, the consistent expression of the human N-myc gene in numerous independent stable and transient transfectants indicates that expression of the introduced sequence in stable transfectants does not simply result from random truncations associated with integration. The nature of the mechanisms that provide correct expression of the transgenic N-myc gene is unclear. However, our inability to readily reproduce N-myc regulation in vitro suggests the possibility that factors mediating this regulation are only transiently present within cells and that their appearance is an early developmental event; such a transient mechanism was proposed for the formation and maintenance of secondary structures in chromatin (54). A similar expression scenario to that which we report here for the N-myc gene was recently reported for al-acid glycoprotein and K14 keratin genes following introduction into transgenic mice or transient transfection into cell lines (10, 52).

**Low-level expression of the murine N-myc transgene.** The murine N-myc transgene appeared to be appropriately expressed in some animals, but its expression level was substantially lower than that of the human transgenes or the endogenous murine N-myc gene. Decreased expression levels of the murine transgene might result from an inserted linker sequence in the 3' untranslated region, but the similar transforming efficiencies of the gene with and without the inserted linker sequence makes this possibility unlikely (see Materials and Methods). Alternatively, low-level expression of the murine transgene may indicate that the murine N-myc clone used, as opposed to the human N-myc clone used, lacks elements that confer high-level transgene expression (44, 46). In this regard, recent transfection studies indicate that the murine N-myc sequence used in our construct is inefficient at promoting expression of a linked neo gene (8).

**Regulation of N-myc transgene expression.** The occurrence of apparently normally regulated expression of the human N-myc transgene in the hN1 transgenic line suggests that the anomalous expression patterns of introduced N-myc sequences in other transgenic lines results from position effects rather than the absence of necessary target regulatory elements within the clone used. Such position-dependent effects have been seen with a number of transgenes (2, 7, 45). The absence of strong, position-independent N-myc promoter elements might reflect the fact that expression of N-myc is not restricted to a cell line or a few cell lineages. Instead, the N-myc gene is expressed in a wide range of lineages at early developmental times, with expression becoming gradually restricted at later stages (15, 33, 60). Transgenic studies of two other genes with similar developmentally regulated expression patterns, the homeobox containing Hox-1.3 and Hox-1.4 genes, also found differences between transgene and endogenous gene expression (55, 56, 59). In the case of the Hox-1.4 gene, expression occurred in the same tissues as the endogenous gene but differed quantitatively (59); we have not eliminated this possibility for the aberrant expression patterns observed for some of the N-myc transgenes.

The regulated expression of an N-myc transgene in mice may provide a system for defining elements involved in regulating normal N-myc expression. Currently, studies indicate that the necessary regulatory elements are contained within a discrete region surrounding and including the N-myc gene. Finally, transgenic lines that express an N-myc transgene in all or some sites of endogenous N-myc expression should provide a means of complementing mice harboring potentially lethal gene disruption mutations of the endogenous N-myc loci (8).

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2102 ZIMMERMAN ET AL.


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