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Structure and Expression of B-*myc*, a New Member of the *myc* Gene Family

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The *myc* family of genes contains five functional members. We describe the cloning of a new member of the *myc* family from rat genomic and cDNA libraries, designated B-*myc*. A fragment of cloned B-*myc* was used to map the corresponding rat locus by Southern blotting of DNA prepared from rat × mouse somatic cell hybrids. B-*myc* mapped to rat chromosome 3. We have previously mapped the c-*myc* to rat chromosome 7 (J. Sümegi, J. Spira, H. Bazin, J. Szpirer, G. Levan, and G. Klein, Nature [London] 306:497–498, 1983) and N-*myc* and L-*myc* to rat chromosomes 6 and 5, respectively (S. Ingvarsson, C. Asker, Z. Wirschubsky, J. Szpirer, G. Levan, G. Klein, and J. Sümegi, Somat. Cell Mol. Genet. 13:335–339, 1987). A partial sequence of B-*myc* had extensive sequence homology to the c-*myc* protein-coding region, and the detection of intron homology further indicated that these two genes are closely related. The DNA regions conserved among the *myc* family members, designated *myc* boxes, were highly conserved between c-*myc* and B-*myc*. A lower degree of homology was detected in other parts of the coding region in c-*myc* and B-*myc* not present in N-*myc* and L-*myc*. A 1.3-kilobase B-*myc*-specific mRNA was detected in most rat tissues, with the highest expression in the brain. This resembled the expression pattern of c-*myc*, although at different relative levels, and was in contrast to the more tissue-specific expression of N-*myc* and L-*myc*. B-*myc* was expressed at uniformly high levels in all fetal tissues and during subsequent postnatal development, in contrast to the stage-specific expression of c-*myc*.

The *myc* family contains five functional genes: c-*myc*, N-*myc*, L-*myc*, R-*myc*, and P-*myc*. It also contains one inactive pseudogene, L-*myc*-psi (6). The best-characterized member of the family, c-*myc*, was originally identified as the cellular homolog of v-*myc*, which is carried by avian leukemia virus MC29. It encodes a nuclear protein that is regularly expressed in a wide variety of proliferating cells. Illegitimate activation of c-*myc* is believed to contribute to the genesis or progression of many different tumors. Activation of retroviral insertion in the neighborhood of the gene represents an early step in the development of avian leukemia virus-induced lymphoid leukemia in the chicken (11). Juxtaposition to an immunoglobulin locus and subsequent constitutive expression is regarded as an essential step in the genesis of Burkitt lymphoma in humans, plasmacytoma in mice, and spontaneous immunocytoma in Louvain rats. Amplification of c-*myc* is believed to contribute to the progression of carcinomas (15), sarcomas (28), and leukemias (4, 38; C. Asker, C. Mareni, D. Coviello, S. Ingvarsson, M. Sessarego, P. Origone, G. Klein, and J. Sümegi, Leukemia Res., in press).

The N-*myc* and L-*myc* genes have been isolated on the basis of their frequent amplification in certain classes of human tumors. N-*myc* is amplified in a progression-related fashion in neuroblastomas (29), while the activation of L-*myc* has only been observed in a subset of small-cell lung cancer cells (3, 19). Expression of N-*myc* and L-*myc* is limited to certain developmental stages and tissue types (44). N-*myc*, L-*myc*, and c-*myc* have similar structures and encode similarly sized nuclear proteins (5, 6, 13, 32, 35, 41). N-*myc* and c-*myc* proteins bind DNA in an unspecific manner (23). It was thought that N-*myc* expression is limited to neuroectodermal tumors and is only activated by gene amplification, but recent findings indicate that it has a broader tissue specificity (17, 20, 30). R-*myc* and P-*myc* have been isolated on the basis of the homology of the L-*myc* third exon (6).

We isolated a new member of the *myc* family, designated B-*myc*, from a rat genomic library. B-*myc* was highly homologous to c-*myc*, but it localized on a different chromosome. B-*myc* was expressed in numerous rat tissues, with the highest expression in the brain.

MATERIALS AND METHODS

Hybridization probes. The following *myc* probes were used in this study: (i) pMc-*myc*54, the 1.9-kilobase (kb) internal *Hin*dIII fragment from the cDNA mouse c-*myc* containing the second exon and parts of the first and third exons (36); (ii) pRM44, a 4.4-kb *Eco*RI fragment containing the rat B-*myc* gene (Fig. 1); (iii) pRM05, a 0.5-kb *Pst*I fragment containing the part of the rat B-*myc* gene that shows homology to the c-*myc* first intron and second exon (Fig. 1); and (iv) pBC13, a 1.3-kb *Eco*RI fragment containing the complete B-*myc* cDNA clone (Fig. 1) and GAPDH, a 550-base-pair (bp) *Xba*I-*Hin*dIII fragment containing the human glyceraldehyde-3-phosphate dehydrogenase gene (42). The probes were ^{32}P labeled by nick translation (24) to a specific activity of 2×10^8 cpm/μg or by the oligonucleotide method (8) to a specific activity of 6×10^8 cpm/μg.

Cloning of rat B-*myc*. The rat genomic library was a gift from J. Bonner. It was prepared by partial *Hae*III restriction enzyme cleavage of individual male Sprague-Dawley rat liver DNA and by the addition of *Eco*RI linkers (26). The Charon4A vector was used. The library was screened with the pMc-*myc*54 probe by standard techniques (16). Both c-*myc* and B-*myc* were isolated on the basis of their hybridization with this probe under full stringency conditions. The rat brain cDNA library was prepared from entire adult rat Sprague-Dawley brain mRNA by the addition of *Eco*RI linkers (RL1002; Clonetech Laboratories, Inc., Palo Alto,

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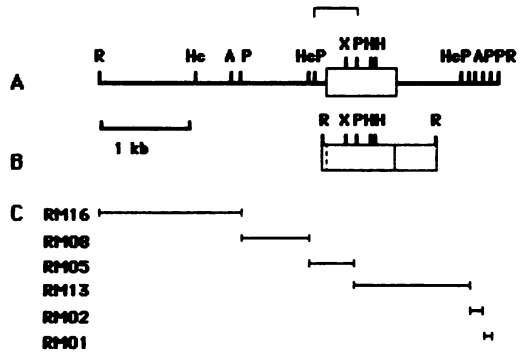

FIG. 1. Organization of the B-*myc* gene. (A) Partial restriction map of the 4.4-kb rat B-*myc* clone. Restriction endonuclease sites are abbreviated as follows: A, *Acc*I; H, *Hin*dIII; Hc, *Hin*cII; P, *Pst*I; R, *Eco*RI; X, *Xma*I. One of the *Eco*RI sites was created by linker addition during the cloning of the DNA. The bracket above the map of the clone indicates the sequenced region (see text for details). The boxes indicate the positions of putative exons. (B) Partial restriction map of the 1.3-kb rat B-*myc* cDNA clone. The *Eco*RI sites were created by linker addition during the cloning of the cDNA. (C) Fragments of the genomic clone that were isolated for use as oligonucleotide-labeled probes.

Calif.). The library was screened with the pRM05 probe by standard techniques (16). Fragments of interest were subcloned into the pUC9 vector.

Cell hybrids and Southern blotting. Somatic cell hybrids that segregate rat chromosomes were produced by fusing mouse hepatoma cells (BWTG3) with normal hepatocytes of the Sprague-Dawley rat strain (40). The chromosomal constitution of the hybrid clones is shown in Table 1. Fifteen micrograms of high-molecular-weight DNA was completely digested with *Eco*RI, *Bam*HI, or *Hin*dIII under the conditions recommended by the manufacturer (Amersham Corp., Arlington Heights, Ill.). The fragments were separated by electrophoresis in a 0.6% agarose gel and transferred to a nitrocellulose filter (33). *Hin*dIII-digested lambda phage DNA was run in parallel as a size marker. Hybridizations, washing, and autoradiography were carried out as described previously (39), except that the filters containing the human genomic DNA were washed at 60°C in 0.7× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

RNA analysis. Total cellular RNA was isolated from rat tissues by the guanidinium-isothiocyanate-cesium chloride method (9) and poly(A) selection. The RNA was glyoxylated, electrophoresed, and transferred to membranes (Hybond-N; Amersham). *Hin*dIII-digested lambda phage DNA was end labeled with ^{32}P and run in parallel as a size marker. Filters were prehybridized at 42°C overnight in a solution containing 6× SSC, 10× Denhardt solution, 1% sodium dodecyl sulfate (SDS), and 100 μg of boiled herring sperm DNA per ml. Hybridization reactions were incubated at 42°C for 18 h in a solution consisting of 6× SSC, 1% SDS, 50% formamide, 5% dextran sulfate, and 100 μg of herring sperm DNA per ml. The ^{32}P-labeled probe was added at a concentration of 2×10^6 cpm/ml. The membranes were washed twice at room temperature in 2× SSC–0.1% SDS for 30 min, 1 h at 65°C in 0.2× SSC–0.1% SDS, and 5 min at room temperature in 0.1× SSC. The filters were exposed to X-ray film (Fuji) with intensifying screens for 2 to 5 days. The radioactively labeled probe was removed by washing the filters 3 times for 10 min each in a boiling solution of 0.02× SSC–0.1% SDS. Filters were prehybridized immediately after stripping for 4 h and stored at 4°C in prehybridization solution. Densitometry was performed on a laser densitometer (Ultrascan XL; LKB Instruments, Inc., Rockville, Md.) using Gaussian fit analysis.

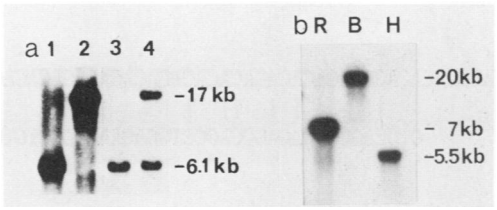

FIG. 2. (a) Southern blot analysis of mouse DNA (lane 1), rat DNA (lane 2), and two hybrid clones (lanes 3 and 4) cleaved with *Eco*RI and hybridized to the pRM44 probe (Fig. 1). The rat-specific germ line band is at 17 kb, and the mouse-specific band is at 6.1 kb. Lane 1, BWTG3; lane 2, Sprague-Dawley rat spleen; lane 3, LB251; lane 4, LB630. (b) Southern blot analysis of human placenta DNA cleaved with *Eco*RI (R), *Bam*HI (B), and *Hin*dIII (H) and hybridized to the pBC13 probe (Fig. 1). The *Eco*RI band is at 7 kb, the *Bam*HI band is at 20 kb, and the *Hin*dIII band is at 5.5 kb.

Nucleotide sequencing. DNA sequences were determined by the method of Sanger et al. (25) on fragments that were subcloned into M13 vectors (21). Sequences were analyzed by using the programs developed by Staden (34) and the University of Wisconsin Genetics Computer Group (7).

RESULTS

Cloning and characterization of a c-*myc*-related sequence. A rat gene, designated B-*myc*, was isolated when the rat genomic library was screened with the 1.9-kb *Hin*dIII fragment of the mouse cDNA c-*myc* gene (36). A weakly hybridizing clone, pRM44, was obtained, in addition to strongly hybridizing clones that contained the rat c-*myc* gene. A 1.3-kb B-*myc*-specific clone (pBC13) was isolated when the rat brain cDNA library was screened with the 0.5-kb *Pst*I fragment of the pRM44 clone (pRM05). A simplified map of pRM44 and pBC13 is shown in Fig. 1, indicating the region sequenced in this study. The 4.4-kb *Eco*RI fragment was subcloned and used as a probe for chromosome mapping. This fragment identifies single-copy rat, mouse, and human genes in genomic Southern experiments (Fig. 2). In Southern blot comigration studies with high-molecular-weight rat and mouse genomic DNA, pRM44 detected a fragment that was distinct from the c-*myc*-, N-*myc*-, and L-*myc*-specific bands. The respective sizes of the different *myc*-specific rat and mouse *Eco*RI fragments were 17 and 23 kb for c-*myc* (39), 7 and 7.7 kb for N-*myc*, and 18 and 14 kb for L-*myc*, respectively, while those for the B-*myc*-specific fragments were 6.1 and 17 kb, respectively (Fig. 2a).

Chromosome localization. The c-*myc*-related DNA fragment was mapped by screening an *Eco*RI- and *Bam*HI-digested rat × mouse hybrid panel with pRM44. The 17-kb *Eco*RI, 7.4-kb *Bam*HI, or 3-kb *Hin*dIII rat pRM44-specific bands were present in hybrid lines LB161, LB510, LB600, LB630, LB780, LB810, LB860, and LB1040 and were absent in 11 other hybrid lines: LB20, LB210B, LB210C, LB210D, LB210I, LB251, LB330TG2, LB330TG3, LB330TG6, LB1040TG3, and LB1040TG5. Examples of positive and negative hybrids are given in Fig. 2a. Comparison with the chromosomal segregation data (Table 1) indicated that only rat chromosome 3 gave a consistent pattern. We therefore concluded that the rat pRM44 locus is on chromosome 3.

Nucleotide and predicted amino acid sequence of the c-*myc*-related DNA sequence. We sequenced the 513-bp *Pst*I frag-

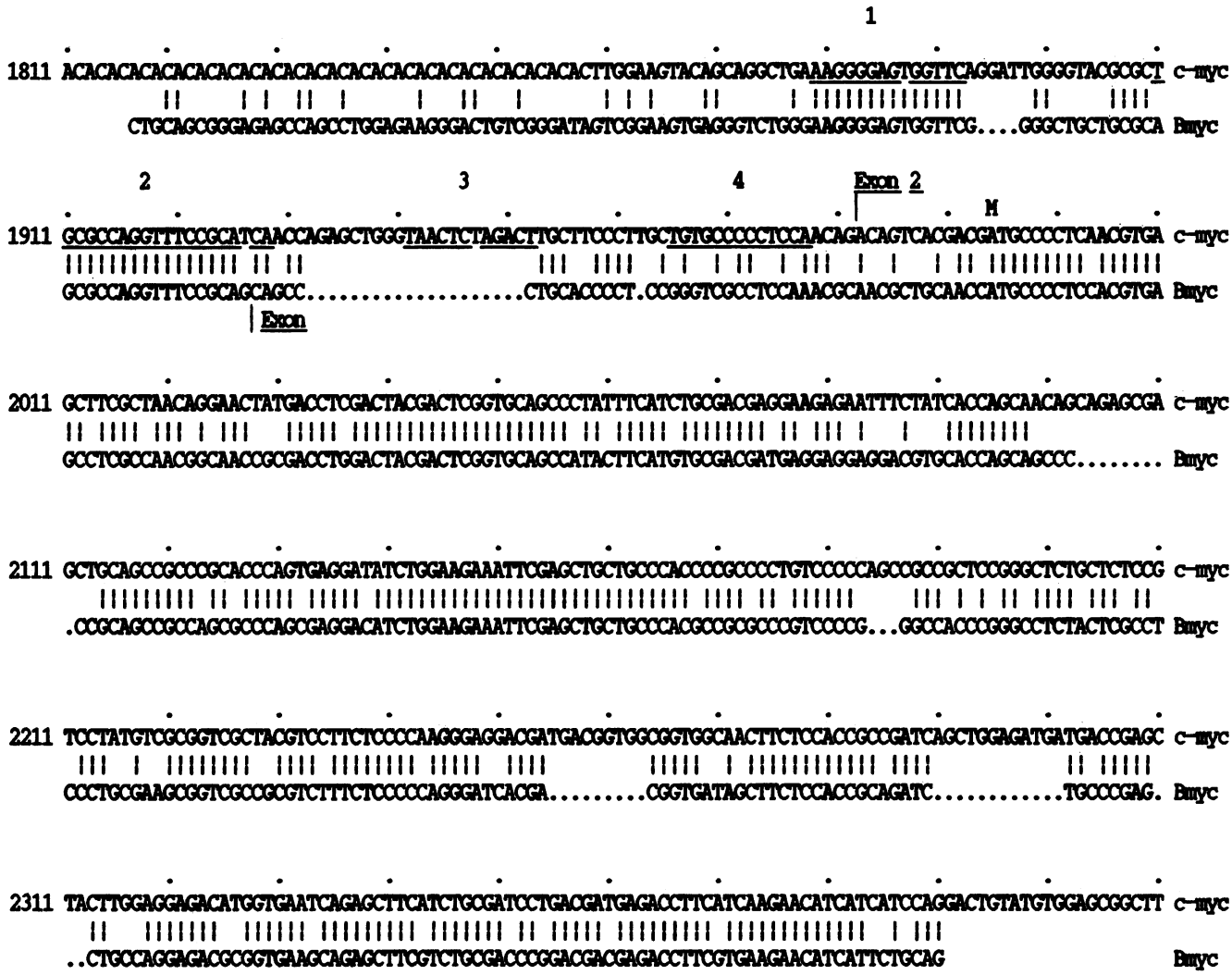

FIG. 3. Nucleotide comparison of the B-*myc* and c-*myc* (10) homologous regions. The c-*myc* sequence is from intron 1 and exon 2. Dots denote one-residue gaps that were added to maintain alignment. Sequence homologies are indicated by vertical lines. The underlined sequence of c-*myc* marked with numbers (1 through 4) shows regions of perfect homology among three species, human, mouse (Asker et al., in press), and rat (10). The M denotes the methionine initiation codon in c-*myc*.

ment of the rat plasmid pRM44. Comparison of pRM44 and rat c-*myc* (10) (Fig. 3) revealed that there is a high degree of homology. The sequenced part of pRM44 was homologous to the first intron of c-*myc* (79 bp showed 73% homology) and the 5′ part of the second exon (79% homology for 360 bp). Short stretches of the intron were also homologous with nonhomologous intervals. The underlined sequences in Fig. 3 represent the stretches of 100% homology among the c-*myc* sequences in mouse, human (2), and rat (10) genes. To obtain a consistent alignment between pRM44 and c-*myc*, the predicted amino acid sequences were compared (Fig. 4). Two *myc* boxes were evident, with 85 to 95% homology between pRM44 and c-*myc*. In the first box, which was conserved in c-*myc* and N-*myc*, but not in L-*myc*, there was almost perfect homology between c-*myc* and pRM44, with only two amino acid differences. The second box had perfect homology for 19 amino acid residues, but the homology dropped in the 3′ part of this box. There were some additional stretches of homology in other parts of the coding region. In comparison with c-*myc*, pRM44 had several deletions which occurred in nine regions and varied from 1 to 3 amino acids (14 amino acids in all). The only amino acid present in the sequenced region of pRM44 that was absent from c-*myc* was an aspartic acid at position 27.

Rat pRM44 is transcriptionally active. A single transcript of 1.3 kb was detected in total RNA or the poly(A)$^+$ fraction from all rat tissues examined and a mouse teratocarcinoma cell line. A B-*myc*-specific cDNA of equal size was isolated from an adult rat brain cDNA library (Fig. 1). A blot of total RNA from rat tissues hybridized to a 0.5-kb *Pst*I fragment of pRM44 that contained the DNA sequence homologous to the 3′ part of c-*myc* intron 1 and the 5′ part of exon 2 is shown in Fig. 5. The transcript was detected in brains, kidneys, hearts, intestines, lungs, livers, spleens, and thymuses from fetal, newborn, 12-day-old, and adult rats. Expression was highest in the brain. The relative expression in the other tissues is shown in Table 2. Comparison of pRM44 expression in fetal, newborn, 12-day-old, and adult rats did not show any major age-related differences in expression. Our results of c-*myc* expression in rat tissues were mostly in agreement with previously published results on the expression in mouse tissues (44), except that we detected higher

TABLE 1. Correlation of pRM44 with rat chromosomes in rat × mouse somatic cell hybrids

Hybrid	pRM44[a]	Correlation of pRM44 with the following rat chromosomes[b]:																				
		X	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
LB20	−	+	−	+	−	−	−	(−)	+	−	−	−	−	+	+	−	−	+	(+)	+	+	+
LB161	+	+	−	+	+	+	+	+	+	−	+	+	−	(−)	+	+	+	+	+	+	+	(+)
LB210B	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−
LB210C	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	−	−
LB210D	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	(−)
LB210I	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−
LB251	−	+	+	+	(−)	+	−	(−)	(+)	−	−	+	−	+	+	−	−	−	+	−	+	−
LB330TG2	−	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−
LB330TG3	−	−	−	−	−	+	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−
LB330TG6	−	−	−	+	−	+	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−
LB510	+	+	+	+	+	+	(−)	−	+	−	−	−	−	+	+	+	+	+	+	+	−	(−)
LB600	+	+	+	+	+	+	+	(+)	+	−	(−)	+	+	+	+	+	+	+	−	+	+	(−)
LB630	+	+	(−)	−	+	+	(+)	+	+	−	+	−	+	+	+	(+)	+	+	−	+	+	(−)
LB780	+	+	−	+	+	+	+	−	+	−	−	+	+	−	+	−	−	−	+	+	−	(+)
LB810	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	−	+	(+)
LB860	+	+	−	+	+	+	−	−	+	−	+	−	+	+	+	−	+	+	+	+	−	(+)
LB1040	+	+	−	−	+	+	(−)	+	+	−	−	+	+	+	−	−	+	+	−	+	−	+
LB1040TG3	−	−	−	−	−	+	−	+	+	−	−	(−)	+	−	−	−	+	+	−	+	−	−
LB1040TG5	−	−	−	−	−	+	−	+	(−)	−	−	−	+	−	−	−	+	+	−	+	−	−
No. of discordant clones		6	7	5	0	7	4	7	3	7	5	7	4	7	7	5	3	7	5	8	6	4

[a] pRM44 is the rat B*myc* probe (Fig. 1).
[b] Symbols and abbreviations: +, chromosome dosage factor (CDF) > 0.6; (+), CDF = 0.3 to 0.5; (−), CDF = 0.1 to 0.2; −, CDF = 0.

levels of c-*myc* mRNA in lung samples and lower levels in liver samples.

We analyzed the mRNA from different parts of the adult rat brain and found the highest expression in the cerebellum, striatum, and hippocampus but lower levels in other parts of the brain (data not shown).

DISCUSSION

The total size of the *myc* gene family is unknown, but it has been suggested that it is a dispersed family with members located on many different chromosomes (E. Legouy, R. A. DePinho, K. Zimmerman, P. Ferrier, R. Collum, and F. W. Alt, Nuclear Oncogene, in press). We isolated a lambda clone from a rat genomic library that contained sequences related to the central part of c-*myc*, namely, the first intron and the second exon. On the basis of its nucleotide sequence and pattern of expression, we concluded that the gene that was isolated is distinct from known members of the *myc* family. It was designated B-*myc*, since it is the first *myc* family member that is significantly expressed in the adult brain. Restriction enzyme mapping and comigration studies by Southern blotting showed that B-*myc* is not the rat homolog of c-*myc*, N-*myc*, or L-*myc*. Furthermore, the human genomic hybridization pattern of B-*myc*, P-*myc*, and R-*myc* revealed disparities; the *Eco*RI fragment sizes were 7 (Fig. 2b), 5.1, and 12 kb (6), respectively. Detection of this sequence in rat, mouse (Fig. 2a) and human (Fig. 2b) genomes demonstrated that the B-*myc* gene is conserved as a novel sequence in mammalian species, thereby implying a functional role. B-*myc* was expressed in all eight rat tissues tested. The transcriptional domain of the genes was estimated by hybridization of labeled DNA restriction fragments to rat brain RNA immobilized on nylon filters. The rat

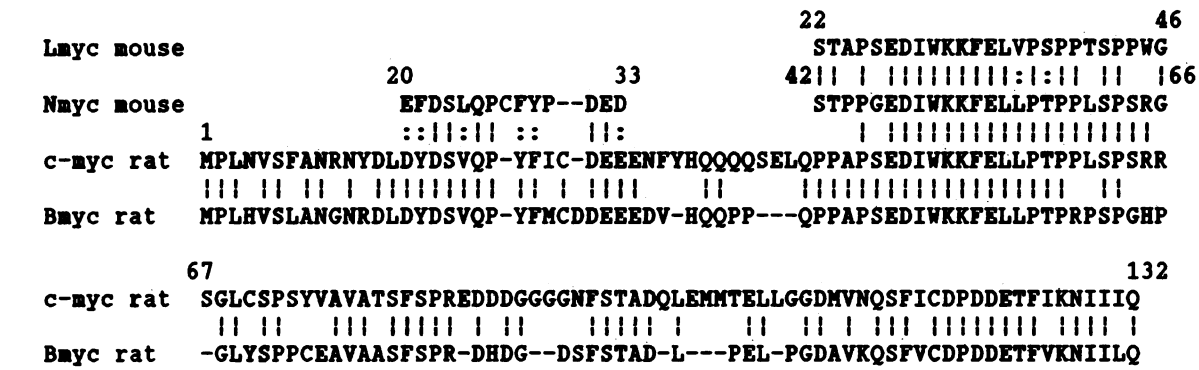

FIG. 4. Comparison of the rat c-*myc* (10), mouse N-*myc* (5), mouse L-*myc* (14a), and B-*myc* putative amino acid sequences. A solid line between amino acids indicates homology, and a double dot indicates a conserved substitution. The sequence of N-*myc* and L-*myc* is only indicated where high homology is detected among *myc* gene family members (*myc* boxes).

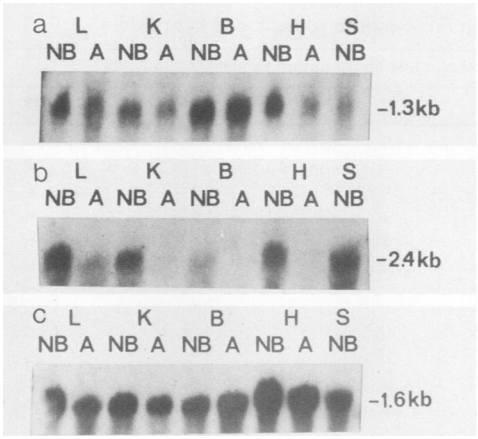

FIG. 5. Expression of B-*myc* and c-*myc* in several rat tissues by representative Northern blot analysis. The filters were sequentially hybridized to three different probes: pRM05 (a), pMc-*myc*54 (b), and GAPDH (for standardizing the RNA amount on the filters) (c). Abbreviations: L, lung; K, kidney; B, brain; H, heart; S, spleen; A, adult; NB, newborn.

genomic clone pRM44 probably contains a complete copy of the B-*myc* gene, as suggested by the detection of specific mRNA by using different *Pst*I fragments as probes. Three fragments (RM05, RM16, and RM13; Fig. 1) hybridized with the mRNA. The larger size of the DNA region covered by the probes compared with that of the mRNA and a comparison of the restriction enzyme maps of the genomic and cDNA clones suggested that the transcribed region is interrupted by an intron(s).

B-*myc* maps to rat chromosome 3. The c-*myc* gene has been assigned to rat chromosome 7 (39), while N-*myc* and L-*myc* have been mapped to chromosomes 6 and 5, respectively (12). We mapped B-*myc* to rat chromosome 3, which shows banding homology with mouse chromosome 2 (18, 43).

Nucleotide sequence of B-*myc*. The 513-bp fragment that we sequenced from the B-*myc* gene was homologous to the first intron and the 5′ part of the second exon of c-*myc* (Fig. 4). The pattern of c-*myc* intron homology was similar among species to that between B-*myc* and c-*myc*, suggesting that these DNA regions may be of functional importance. The *Cy*III actin gene subfamily is also known to have intron sequence homology among different gene members (1). The CA repeats found in rat and mouse c-*myc* intron 1, but not in the human c-*myc* gene (2, 10), were not present at the corresponding position in the rat B-*myc* gene. The splice acceptor site of c-*myc* was not conserved in B-*myc*, suggesting that the B-*myc* splice site is further upstream. B-*myc* should, therefore, have a longer noncoding 5′ exon compared with the second exon of c-*myc*.

The sequence around the initiation site for protein synthesis in c-*myc* was conserved in B-*myc*; this included the A at position −3 which is thought to be especially important for the initiation of protein synthesis (14). The predicted amino acid sequence of B-*myc* was highly homologous to that for c-*myc*. It was of interest that the threonine at position 58, the putative phosphorylation site that is mutated in the Burkitt lymphoma Raji (31), was conserved between the two genes, together with the surrounding region. The B-*myc* region that we sequenced was outside the *myc* family regions that are considered to be essential for transformation, nuclear localization, and DNA binding (27, 37). This region of c-*myc* tolerates insertions and partial deletions without losing transforming activity. Complete deletions disturb the *myc* activity in the rat embryo fibroblast cotransformation assay, however (27, 37).

The high homology of B-*myc* and c-*myc* suggests that these conserved sequences may play similar general roles in the B-*myc* and c-*myc* proteins. The conserved and divergent regions of the proteins should provide more restricted targets for future mutational analyses of overlapping and potentially divergent activities. The characteristic deletions of amino acids in B-*myc* relative to those in c-*myc* are in regions that have previously been noted to be subject to such structural variations among *myc*-related proteins (22), in agreement with the notion that these regions can tolerate changes in length.

Expression of B-*myc*. N-*myc* and L-*myc* are restricted in their tissue- and stage-specific expression, appearing to be involved in the early stages of differentiation, respectively, while c-*myc* expression is more generalized (44). B-*myc* expression was detected in all tissues examined, but it was detected at the highest levels in the brain (Fig. 5 and Table 2). While B-*myc* expression was similar to that of c-*myc*, the relative levels of expression differed in various tissues (Fig. 5). B-*myc* was expressed at similar levels in fetal and adult tissues and at intermediate stages (Fig. 6), whereas c-*myc* was downregulated in most adult tissues, particularly in the brain (44) (Table 2).

TABLE 2. Relative B-*myc* and c-*myc* RNA expression as a percentage of the highest expression

Tissue	Relative expression (%) of RNAs of [a]:							
	B-*myc*				c-*myc*			
	F	NB	12 days	A	F	NB	12 days	A
Brain	75 (4)	90 (8)	100 (4)	60 (8)	40 (5)	35 (8)	30 (4)	0 (8)
Lung	55 (2)	80 (7)	70 (1)	60 (3)	70 (2)	65 (7)	25 (1)	25 (3)
Kidney	40 (5)	40 (6)	30 (1)	35 (4)	25 (4)	25 (7)	10 (1)	10 (4)
Heart	35 (1)	25 (5)	40 (1)	15 (4)	20 (2)	25 (6)	ND	5 (4)
Intestine	ND	50 (2)	ND	60 (1)	ND	30 (2)	ND	5 (1)
Spleen	45 (1)	20 (4)	ND	ND	55 (1)	70 (4)	ND	10 (1)
Liver	ND	15 (2)	ND	15 (2)	30 (1)	40 (2)	ND	5 (2)
Thymus	25 (2)	15 (2)	ND	15 (2)	100 (2)	70 (2)	ND	30 (2)

[a] For each independent experiment, the intensity of a particular *myc* mRNA band in a given tissue was calculated as a percentage of the signal intensity generated with RNA from the most strongly expressing tissue (brain in the case of B-*myc* and thymus in the case of c-*myc*), which was arbitrarily set at 100%. Each value is an average (rounded off to the nearest multiple of 5) of 1 to 8 independent experiments (given in parentheses). Abbreviations: F, fetal; NB, newborn; 12 days, 12 days old; A, adult; ND, not determined.

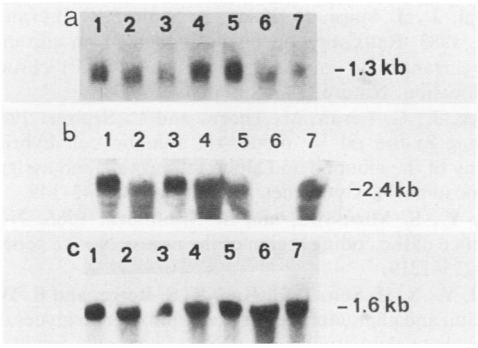

FIG. 6. Expression of B-*myc* and c-*myc* in rat brain (lanes 1 to 6) and kidney tissues (lanes 7) by representative Northern blot analysis. The filters were sequentially hybridized to the probes pRM05 (a), pMc-*myc*54 (b), and GAPDH (c). Lanes 1, brain, fetal; lanes 2, brain, newborn; lanes 3, brain, 3 days old; lanes 4, brain, 4 days old; lanes 5, brain, 12 days old; lanes 6, brain, adult; lanes 7, kidney, fetal.

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