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Increased expression of *myc*-related oncogene mRNA characterizes most BALB/c plasmacytomas induced by pristane or Abelson murine leukemia virus

(carcinogenesis/Southern blots)

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ABSTRACT **RNA blots of poly(A)-containing RNA from normal livers and spleens and from a number of transplantable hematopoietic and lymphoid BALB/c tumors, including early and late generation plasmacytomas, were hybridized with probes for four *onc* genes. *abl* RNA was abundant only in those tumors producing Abelson virus, *bas* RNA was found in approximately equal amounts in normal tissues and plasmacytomas, and *myb* RNA was absent in normal liver and plasmacytomas. Normal liver and spleen RNA showed faint traces of *myc* hybridization, but *myc* RNA was increased in most plasmacytomas. In one plasmacytoma, TEPC 1165, a particularly abundant amount of *myc* RNA was found, principally as a 3.5-kilobase band. In the other plasmacytomas, bands of 2.4- or 1.8-kilobase *myc* RNA were found. Southern blots of DNA from tumors that contained 2.4-kilobase or larger *myc* RNA showed *myc* hybridization to an *Eco*RI fragment of about 21 kilobase pairs, similar to the *myc* band in normal DNA. *Eco*RI digests of DNA from two tumors that expressed *myc* RNA of 1.8 kilobases showed an additional smaller *myc* band, suggesting that the *myc* gene is rearranged in these plasmacytomas. The basis for increased *myc* gene transcription in plasmacytomas is not understood, but the evidence suggests that different mechanisms may be operating in different plasmacytomas. Apparently, neither *myc* gene amplification nor *myc* gene rearrangement is required for increased *myc* transcription.**

Retroviruses that rapidly induce tumors in their hosts contain, in their genomes, certain sequences (*onc* genes) that appear to be responsible for induction or maintenance of cellular transformation. DNA sequences homologous to these viral *onc* genes can be found in the genomic DNA of normal cells not only of the host for each particular virus but in most mammalian cells. Expression of low levels of RNA from some of these *onc* genes can be detected in normal cells, but abnormally large amounts of these RNA transcripts have been observed only in tumors. These transcripts or, more likely, the proteins encoded by the RNAs are thought to be important in many types of carcinogenesis not just in tumors of viral origin. For recent reviews of these topics, see refs. 1–3.

To determine whether increased expression of any of the known *onc* genes plays a role in the development of mouse lymphoid tumors, several *onc* genes were used to probe messenger-enriched RNA [poly(A)$^+$RNA] from a variety of pristane-induced BALB/c plasmacytomas (4), from normal tissues, from T- and B-cell lymphomas, and from other tumors. Three *onc* genes associated with lymphoid tumors were used as hybridization probes: c-*myc* from normal chicken DNA (5) and v-*myc* from avian myelocytomatosis virus MC 29 (6), v-*myb* from avian myeloblastosis virus (7), and v-*abl* from Abelson murine leukemia virus (A-MuLV) (8). In addition, v-*bas* from BALB/c murine sarcoma virus (9) was included as an example of *onc* genes associated with soft tissue sarcomas rather than lymphoid tumors. Only *myc* RNA was consistently elevated in plasmacytomas. Because chromosomal rearrangements are consistently found in pristane-induced plasmacytomas (10) and the non-immunoglobulin DNA sequences called NIARD (11), NIRD (12), and LyR (13) have been shown to be rearranged frequently in lymphoid tumors, studies were done to look for changes in *myc* gene loci at the DNA level. We observed rearrangements in *myc* DNA in certain plasmacytomas, and these are strikingly similar to those reported for NIARD and LyR.

MATERIALS AND METHODS

Preparation and Hybridization of RNA and DNA Blots. All normal tissues and tumors (see Table 1) were of BALB/c origin. Total RNA was prepared by extraction of pulverized frozen tissues in 6 M urea/3 M LiCl (16) followed by phenol extraction and ethanol precipitation. Five micrograms of RNA that had been enriched for poly(A)$^+$ sequences by two cycles of absorption to and elution from oligo(dT)-cellulose was denatured in 14 mM methylmercury hydroxide and subjected to electrophoresis in 1% agarose/5 mM methylmercury hydroxide gels (17). Several duplicate sets of identical RNAs were subjected to electrophoresis and blotted (18) onto *o*-diazophenyl thioether paper (19). The duplicate blots were prehybridized and hybridized (20) with different DNA probes that had been labeled with ^{32}P by nick-translation (21) to a specific activity of 1.4–2.7 × 10^8 cpm/μg. Diluted samples of these RNAs were also applied directly to diazophenyl thioether paper in 100 μl of 0.4 M NaOAc (pH 5) by suction using a 96-well manifold. These dot blots were prehybridized and hybridized as above.

Total DNA was extracted (22) from pulverized frozen tissue, digested to completion by restriction endonucleases, electrophoresed on 0.7% agarose gels, stained with ethidium bromide, transferred to nitrocellulose membranes (23), and hybridized with nick-translated DNA probes.

With DNA probes of mouse origin (*abl* and *bas*), stringent conditions (20) were used for prehybridization, hybridization, and washing of RNA and DNA blots. For avian probes (v-*myc*, c-*myc*, and v-*myb*), relaxed conditions were used as described by Eva *et al.* (24).

DNA Probes. The v-*bas* probe was a 0.6-kilobase-pair (kbp) *Hin*dIII/*Bam*HI fragment of the cloned genome of BALB/c murine sarcoma virus that had been subcloned in pBR322 (9) and was provided by P. Andersen and S. Tronick. The v-*abl*

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Abbreviations: A-MuLV, Abelson murine leukemia virus; kbp, kilobase pair(s); kb, kilobase(s).

probe was a 1.2-kbp *Bgl* II fragment isolated from cloned A-MuLV (8) and subcloned using *Eco*RI linkers in pBR322. The v-*myc* probe was a 2.8-kbp *Bam*HI fragment of the cloned genome of avian myelocytomatosis virus MC 29 (6) provided by T. Papas. The c-*myc* probe was a 0.85-kbp *Cla* I/*Eco*RI fragment derived from and including virtually all of the second exon of c-*myc*. It had been isolated from a normal chicken genomic library (5) and subcloned in pBR322 and was furnished by T. Papas. The v-*myb* probe was a 1.3-kbp *Kpn* I/*Xba* I fragment isolated from cloned avian myeloblastosis virus (7) and subcloned in pBR322 and was provided by T. Papas and M. Baluda.

RESULTS

***bas* RNA.** Fig. 1*A* shows that the normal tissues and the tumors contain two RNAs, 1.0 and 1.2 kilobases (kb), that hybridized with the v-*bas* probe under stringent conditions. Approximately the same intensity of hybridization is seen in the blots of 5 μg of poly(A)$^+$RNA from each tissue tested.

***myb* RNA.** None of the plasmacytomas, induced with or without Abelson virus, nor normal liver contained RNAs that hybridized with v-*myb*. RNA from normal spleen and A-MuLV lymphosarcomas did hybridize with v-*myb* (data not shown). These studies will be described in detail elsewhere.

***abl* RNA.** Fig. 1 *B* and *C* shows no detectable RNA hybridization with the v-*abl* probe except in ABPC 4, ABPC 33, ABLS 8, and ABLS 19 RNA. These four A-MuLV-induced tumors contain abundant 6.7-kb *abl* RNA. This RNA represents the A-MuLV genomic RNA, because it also hybridizes with a probe for the *gag* gene (27) of Moloney murine leukemia virus (data not shown). A 5.4-kb *abl* RNA is present in P 1798 and ABPC 33. This probably represents a subgenomic RNA fragment that may encode the Abelson protein (28, 29).

***myc* RNA.** Blots such as those presented in Fig. 2 *A* and *B* show only a faint smear of v-*myc* hybridization with 5 μg of poly(A)$^+$RNA from normal liver and spleen, B-lymphocytic neoplasms, and Histio 212. A faint but discrete band of 2.4-kb *myc* RNA is seen in BALABMC 20 and Meth A. Intense bands of hybridization characterize 11 of the 14 plasmacytomas studied to date. TEPC 1017, XRPC 24, MOPC 104E, and SP 2/0 (data not shown) show 1.8-kb *myc* bands that are broad and may consist of two or more incompletely resolved bands of hybridizing RNA. A 2.4-kb *myc* RNA band is present in ABPC 33, ABPC 4, TEPC 1194, TEPC 1033, TEPC 1119, MOPC 104E, and ABPC 18 (data not shown). This broad band could also be made up of two or more RNA species. Note that MOPC 104E (and possibly XRPC 24) contains both 1.8- and 2.4-kb *myc* RNAs. TEPC 1165 contains by far the greatest amount of *myc* RNA of all the tissues surveyed to date, and it uniquely contains prominent bands at 3.5 and 2.8 kb. Three plasmacytomas, TEPC 1173 (Fig. 2*A*) and TEPC 1196 and ABPC 22 (data not shown), contained only faint 2.4-kb bands of *myc* RNA. The only T lymphoma so far examined, P 1798, was the only non-plasma-cell tumor to contain an amount of *myc* RNA comparable with that seen in most plasmacytomas. These results are summarized in Table 1. Identical results (not shown) were obtained when a duplicate blot of Fig. 2*A* was hybridized with the c-*myc* probe.

Intensities of v-*myc* hybridization of diluted RNAs in dot blots such as those shown in Fig. 2*C* and other experiments not shown suggest that TEPC 1165 has >350 times the amount of *myc* RNA in its poly(A)$^+$RNA as normal liver. MOPC 104E, XRPC 24, and TEPC 1194 contain ≈15 times and TEPC 1017, TEPC 1033, ABPC 4, and ABPC 33 contain ≈4 times the amount of *myc* RNA present in normal liver.

***myc* DNA.** Southern blots of *Eco*RI digests of genomic DNAs that have been hybridized with c-*myc* are shown in Fig. 3. Normal liver and most tumors exhibit one band of *myc* hybridization at about 21 kbp. TEPC 1165, TEPC 1173, XRPC 24, and TEPC 1017, exhibit *myc* hybridization with an additional *Eco*RI fragment while TEPC 1033 showed hybridization to an additional 27-kbp fragment.

DISCUSSION

Most (11 out of 14) of the mouse plasmacytomas examined here and the T lymphoma P 1798 contain abundant discrete RNA transcripts that hybridized with the avian v-*myc* probe. This contrasts with the results obtained with the other *onc* gene probes: v-*bas*, which detects two small RNAs, 1.0 and 1.2 kb, in approximately equal abundance in normal tissues and tumors; v-*myb*, which shows no hybridization with plasmacytoma RNA; and v-*abl*, which, P1798 excepted, hybridizes only to 6.7-kb

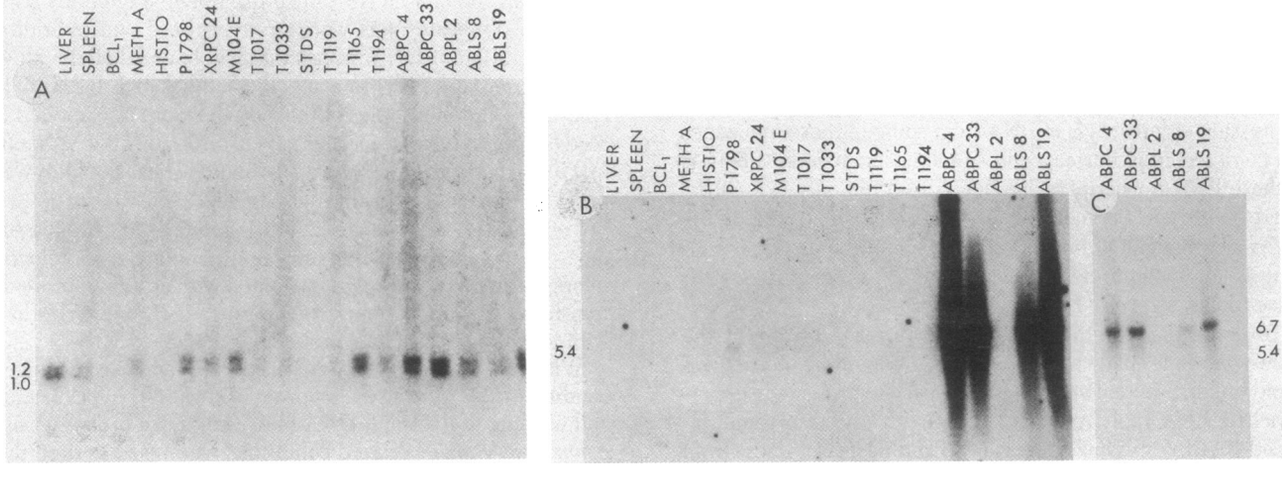

FIG. 1. (*A*) *bas* hybridization of RNA blots of normal and tumor poly(A)$^+$RNAs (5 μg each) after separation on 1% agarose/methylmercury hydroxide gels. Sources of RNA are indicated at the tops of the lanes; M = MOPC and T = TEPC as designated in Table 1 and the text. Sizes of hybridizing bands (in kb) are indicated on the left. Sizes were determined from ethidium bromide-stained plant virus RNA standards [tobacco mosaic virus, 6.34 kb (25), brome mosaic virus, 0.87, 2.3, 3.1, and 3.4 kb (26)] (lanes STDS) and from endogenous 18S and 28S rRNA (2.0 and 4.7 kb). The 0.6-kb v-*bas* fragment was nick-translated to 1.4×10^8 cpm/μg and used in stringent hybridization. The hybridized washed blot was exposed to Kodak XAR-5 film for 2 days at −70°C with a Dupont Lightning Plus intensifying screen. (*B*) *abl* hybridization of RNAs as in *A*. The 1.2-kb v-*abl* fragment was nick-translated to 2.7×10^8 cpm/μg. The blot was exposed for 3 days at −70°C with an intensifying screen. (*C*) The five right-most bands in *B* after exposure for only 6 hr at −70°C with screen.

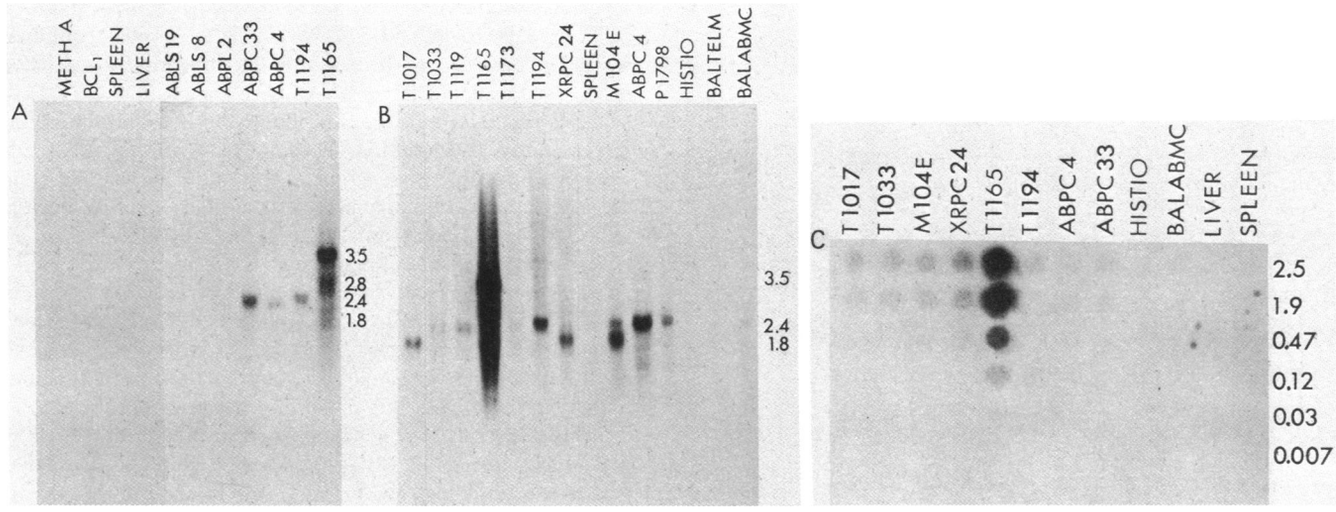

FIG. 2. (*A* and *B*) *myc* hybridization of RNA blots as in Fig. 1. The 2.8-kb v-*myc* and 0.7-kb c-*myc* fragments were nick-translated to 2.3 and 1.8×10^8 cpm/μg, respectively. Exposures were for 2 days at −70°C with intensifying screens. A blot identical to that shown in *A* was hybridized with c-*myc*. The hybridization results using v-*myc* and c-*myc* were identical, so only the v-*myc* pattern is shown. (*C*) Dot blot hybridization of dilutions of poly(A)$^+$RNA from the tissues and tumors indicated at the tops of the lanes. The amount of poly(A)$^+$RNA in each dilution is indicated at the right of each row of dots. Hybridization was done with the v-*myc* probe as in *A* and *B*, and blots were exposed to film for 6 days at −70°C with an intensifying screen.

RNAs that also encode viral *gag* sequences and appear to be full-length A-MuLV genomic RNA. One A-MuLV-induced tumor, ABPL 2, contains no detectable *abl* mRNA, suggesting that this tumor no longer produces Abelson virus, similar to other cases recently described (30).

As shown in Fig. 2 *A* and *B*, the *myc* RNA in the mouse tumors surveyed here usually takes the form of a 2.4-kb species, although some tumors contain *myc* RNAs of other sizes. The possibility that the unusually large (3.5-kb) *myc* RNA found in great abundance in TEPC 1165 represents a defective *myc*-containing virus is unlikely because this RNA species did not hybridize with any of the retrovirus probes for *gag*, *pol*, or *env*

Table 1. Tissues examined and summary of hybridization results

Tissue	Characteristics	*myc* RNA	*myc* DNA
BALB/c liver	Normal	—	21
BALB/c spleen	Normal	—	ND
BCL_1 spleen	B-cell leukemia (ref. 14)	—	ND
TEPC 1119	PCT, Gen 3 (IgA)	2.4	21
TEPC 1165	PCT, Gen 3 & 7 (IgA,κ)	3.5, 2.8,(2.4, 1.8)	21
TEPC 1173	PCT, Gen 3 (untyped)	(2.4)	21
TEPC 1194	PCT, Gen 2 (IgA)	2.4	21
TEPC 1196	PCT, Gen 3 (untyped)	[(2.4)]	ND
TEPC 1017	PCT, Gen 13 (IgD,κ)	1.8	21, 14
TEPC 1033	PCT, Gen 21 (IgD,κ)	2.4	27, 21
XRPC 24	PCT, Gen 42 (IgA,κ)	(2.4), 1.8	21, 14
MOPC 104E	PCT, Gen 87 (IgM,λ)	2.4, 1.8	ND
SP 2/0	PCT, cultured cells (none)	[1.8]	ND
ABLS 5	ALS, Gen 10 (none)	[(3.5)]	ND
ABLS 8	ALS, Gen 13 (none)	—	21
ABLS 19	ALS, Gen 11 (none)	—	21
ABPL 2	APL, Gen 7 (none)	—	21
ABPC 4	APCT, Gen 14 (IgA,κ)	2.4	21
ABPC 18	APCT, Gen 3 (IgG2b,κ)	[2.4]	21
ABPC 22	APCT, Gen 7 & 8 (IgM,κ)	[(2.4)]	ND
ABPC 33	APCT, Gen 24 (IgA,κ)	2.4	ND
BALABMC 20	A-MuLV-induced mast cell tumor, Gen 11	(2.4)	21
BALTELM 1131	B-cell lymphoma, Gen 12	—	ND
P 1798	T-cell lymphoma, Gen 206	2.4	ND
Histio 212	Histiocytoma, Gen 5	—	ND
Meth A	Methylcholanthrene-induced fibrosarcoma (ref. 15)	(2.4)	ND

Sizes of hybridizing RNA band(s) are in kb; dash indicates no more than trace hybridization with no definite band; parentheses indicate faint bands and brackets indicate bands not shown in Fig. 2. Sizes of hybridizing DNA (*Eco*RI) fragments are in kbp. PCT, pristane-induced plasmacytoma; Gen x (. . .), number of transplant generations (type of myeloma protein secreted); ALS, Abelson leukemia virus/pristane-induced B-cell lymphosarcoma; APL, Abelson leukemia virus/pristane-induced plasmacytoid lymphosarcoma; APCT, Abelson leukemia virus/pristane-induced plasmacytoma; ND, not done.

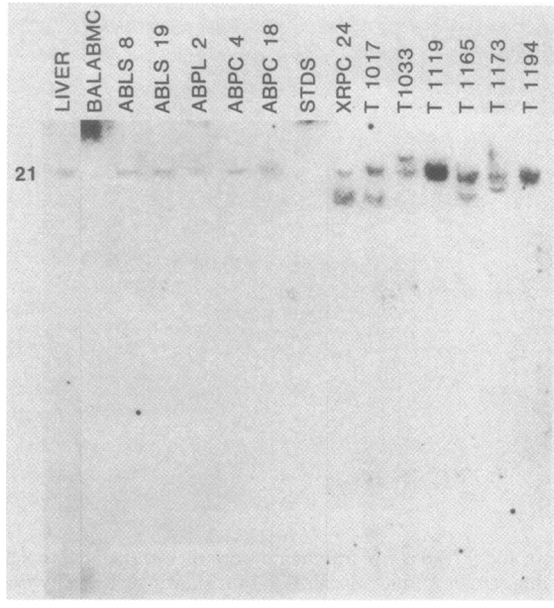

FIG. 3. (*A*) *myc* hybridization of *Eco*RI fragments of genomic DNAs from tissues and tumors after electrophoretic separation on 0.7% agarose and blotting onto nitrocellulose. DNA sources are indicated at the tops of the lanes. The c-*myc* 0.85-kb fragment was nick-translated to 1.8×10^8 cpm/μg and used for hybridization. Size (in kbp) of the germ line *myc*-hybridizing fragment is indicated on the left [determined from ethidium bromide-stained standards of *Hin*dIII-digested phage λ DNA run in parallel lanes (not shown)]. The sizes of the rearranged *myc* fragments are given in Table 1.

genes (data not shown). Other possible explanations for this large *myc* RNA (e.g., defective RNA processing) should be investigated. The 1.8-kb *myc* RNA seen in four tumors is unusual in that such a small *myc* RNA has not been described before in any species (31–35).

The *myc* gene in normal cells, and most plasmacytomas, is on an *Eco*RI fragment of about 21 kbp (Fig. 3). TEPC 1165, TEPC 1033, TEPC 1173, XRPC 24, and TEPC 1017 contain *myc* on a second *Eco*RI fragment in addition to the 21-kbp *Eco*RI fragment. This suggests that at least one, but not all, of the *myc*-bearing chromosomes in these tumors underwent a DNA rearrangement in the vicinity of c-*myc*. Such rearrangement could affect the size of the RNA transcript or the rate of transcription of *myc* RNA, as in avian systems where promoter insertion (32) and upstream (33) and downstream promotion (36) of c-*myc* transcription has been described. The plasmacytomas with *myc* on *Eco*RI fragments of 21- and 14-kbp produce abundant *myc* RNA of 1.8 kb, while all the other plasmacytomas produce only a 2.4-kb *myc* RNA, except TEPC 1165, which has larger *myc* RNAs. These results suggest that increased *myc* transcription occurs from both rearranged and unrearranged *myc* genes. Thus, large-scale DNA rearrangements near the c-*myc* locus are not essential for increased expression of *myc* RNA.

It may be significant that the four tumors that have 1.8-kb *myc* RNA, of which at least two have *myc* DNA rearrangements, have been carried in transplant for several years. Of the early-generation plasmacytomas (cf. Table 1), only TEPC 1165 contains 1.8-kb *myc* RNA and shows *myc* DNA rearrangements. Thus, it is possible that *myc* DNA rearrangements that result in transcription of 1.8-kb *myc* RNA occur late in the life of a transplanted plasmacytoma and are not connected with early transformation events.

Increased *myc* transcription in plasmacytomas could be due to amplification of c-*myc* genes at the DNA level, as has been reported in some human tumors (37, 38). Although some plasmacytoma *myc* DNA bands in Fig. 3 are darker than those of other plasmacytomas and of normal liver, the intensity of DNA hybridization does not correlate with the expression of *myc* RNA as assessed by intensity of RNA hybridization in Fig. 2. This suggests that *myc* gene amplification is not a requirement for increased *myc* transcription.

Another possible explanation for the abundant *myc* RNAs in plasmacytomas is that *myc* protein is a differentiation-specific molecule, and plasmacytomas may represent cells arrested at a developmental stage in which *myc* is abundantly expressed. Nothing more than a faint smear of *myc* hybridization was seen with RNA from liver and spleen. Spleen cells contain very few plasma cells, so until a more appropriate source of control normal RNA is found, we cannot rule out this possibility. It is important to investigate this possibility because recent data indicate that several *onc* genes appear to be expressed at relatively high levels only during certain stages of pre- and postnatal development in the mouse (39).

The reciprocal chromosome translocation commonly seen in plasmacytomas (10) involving chromosome 15 and chromosomes that contain Ig structural genes may be responsible for some *myc* DNA rearrangements. This may result in an unusual form of activation of the *myc* locus in the translocated or untranslocated arm of chromosome 15 (40). More karyotypic characterizations of plasmacytomas that have increased *myc* gene expression are needed to understand whether a correlation exists. The data so far suggest that chromosomal translocations involving the *myc*-containing chromosome do not always result in an altered restriction fragment. Possibly the chromosomal translocations characteristic of plasmacytomas happen early in the life of the tumor, but the translocation may occur at a site too distant from the *myc* locus to affect the size of *myc* restriction fragments. Later in the life of the tumor, subsequent DNA rearrangements such as deletions could bring DNA from the translocated chromosome—e.g., Cα genes—closer to the *myc* gene. This could yield altered *myc* restriction fragments and perhaps also cause altered *myc* RNA transcripts. It is not yet known whether increased *myc* RNA levels can be found in the absence of chromosome translocation, but increased levels of 2.4-kb *myc* RNA can be found in the absence of altered *myc* DNA restriction fragments. Thus, there may be several mechanisms that can result in increased *myc* expression in mouse plasmacytomas.

A gene referred to as NIARD (11) or NIRD (12) (for non-immunoglobulin rearranging DNA) or LyR [for lymphoid rearranging (13)] has a combination of restriction fragments and mRNA transcripts in plasmacytomas that is strikingly similar to those reported here for *myc*. Thus, it is likely that NIARD/LyR DNA has sequences in common with the mouse homologue of avian c-*myc*. This identification has also been made elsewhere (41).

Note Added in Proof. Marcu *et al.* (42), using a mouse probe homologous to the avian *myc* gene, have made observations similar to those presented here: (*i*) elevation of *myc* RNA levels in most mouse plasmacytomas, (*ii*) altered restriction fragments in *myc* genes of some plasmacytomas, and (*iii*) smaller *myc* RNAs expressed in those plasmacytomas that contain smaller *myc* DNA restriction fragments.

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