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Genetics

Cellular *myc* oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas

(B lymphocyte tumorigenesis/c-*myc* exon structure/immunoglobulin switch recombination region/altered c-*myc* transcription)

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ABSTRACT **Molecular cloning has recently established that the 15;12 chromosome translocations in murine plasmacytomas fuse DNA from chromosome 15 to the immunoglobulin heavy (H) chain locus, usually within the switch recombination region near the α constant region gene. We show here that the incoming DNA bears the cellular gene (c-*myc*) homologous to the oncogene (v-*myc*) of avian retrovirus MC29. In human Burkitt lymphomas bearing an 8;14 translocation, c-*myc* was also rearranged, apparently (in at least two cases) to an H chain switch recombination region (μ or α), and both products of a reciprocal chromosome exchange were detectable. Both the murine and human c-*myc* genes contain two exons homologous to v-*myc*, and additional 5′ and 3′ murine genomic segments (apparent exons) were defined by hybridization to c-*myc* mRNAs. In plasmacytomas, chromosome breakpoints fall near or within the 5′ exon and apparently disrupt the normal c-*myc* transcriptional unit, because plasmacytoma c-*myc* mRNAs differ from the mRNA in lines without c-*myc* rearrangement. The translocated gene presumably has lost its normal 5′ regulatory sequences and may well encode an altered *myc* polypeptide. We propose that altered expression of the c-*myc* gene, induced by translocation to an immunoglobulin locus, is a critical oncogenic event for these B lymphoid tumors. Two events may be required, because the plasmacytoma oncogene capable of transforming fibroblasts is not c-*myc*.**

Specific chromosome translocations may play a critical role in some malignancies. Those in certain tumors of B lymphocytes involve chromosomes carrying immunoglobulin loci (reviewed in refs. 1 and 2). In murine plasmacytomas, the distal region of chromosome 15 has translocated to the end of chromosome 12, where the heavy (H) chain locus resides, or, less frequently, to chromosome 6, on which the κ locus lies (3). Burkitt lymphomas of man display analogous translocations between chromosome 8 (band q24) and the bands of chromosomes 14, 2, or 22, on which the H chain, κ and λ loci reside (4–6). Such findings stimulated proposals (1, 2) that a specific gene on murine chromosome 15, and human chromosome 8, might promote lymphomagenesis when translocated near an immunoglobulin locus.

A candidate for the translocating murine DNA emerged with cloning (7–10) of nonimmunoglobulin DNA—termed LyR [lymphoid rearranging (8)], NIARD (9), or NIRD (10)—that has recombined near the α H chain constant region (C_α) gene in most plasmacytomas (7–10) and some B lymphomas (8). Recombination occurred within the "switch region" (S_α) 5′ to the C_α gene (7–10), which normally recombines with the μ switch region (S_μ) to bring an assembled H chain variable region (V_H) gene near C_α for α chain expression (reviewed in ref. 11). Because LyR DNA derives from chromosome 15 (10, 12, 13), the LyR-S_α clones span the 15;12 fusion point. Plasmacytomas with a translocation had one or more mRNAs smaller than the single [≈2.4 kilobase (kb)] species present in diverse other tumor lines, prompting us to suggest that the altered transcripts participated in plasmacytoma oncogenesis (8, 13).

To determine whether the transcripts derived from a known oncogene, we tested hybridization of several retroviral DNAs to clones of LyR DNA and homologous human DNA. We show here that both bear the cellular homologue (c-*myc*) of the oncogene (v-*myc*) carried by avian retrovirus MC29 (14) and that c-*myc* is rearranged in Burkitt lymphomas with the predominant 8;14 translocation. Thus both the human and mouse translocations involve the cellular gene activated in virally induced B lymphomas of chickens (15, 16). Significantly, the altered plasmacytoma c-*myc* mRNAs result from disruption of the normal c-*myc* transcriptional unit by the translocation. We suggest that translocation alters control of c-*myc* expression and may well generate an altered gene product. Since completion of this work, we have learned of studies assigning the human c-*myc* gene to the relevant region of chromosome 8 (6, 17) and showing that c-*myc* is rearranged and transcribed in plasmacytomas (18) and rearranged in other Burkitt lymphomas (6).

MATERIALS AND METHODS

The origin of mouse cell lines has been detailed (8). Human cell lines were kindly provided by D. Moss (Queensland Institute of Medical Research), who established lymphoblastoid (LCL) and Burkitt lymphoma (BL) lines from New Guinea patients W1 and W2, and by G. M. Lenoir (Lyon), who established IARC/BL36 and LCL174 from North African patient Lou. LY67 (19), BJAB (20), and Raji (21) are African lines, and Ramos (20) is American and J1 (19) is German. Southern blotting of cellular DNA was as described (22). Isolation of total cellular poly(A)$^+$ RNA and blot analysis were as in ref. 23 except that hybridization with v-*myc* probes was for 3 days at 37°C in 40% (vol/vol) formamide/5× NaCl/Cit/EDTA, with a 2-hr wash in 2× NaCl/Cit/EDTA at 50°C and then 30 min in 0.5× NaCl/Cit/EDTA at 50°C (NaCl/Cit/EDTA is 150 mM NaCl/15 mM sodium citrate/5 mM EDTA). Probes of LyR DNA (see Fig. 1) were nick-translated DNA fragments except for probes *c* and *d*, which were cloned in bacteriophage M13 and labeled by primer extension (24). The v-*myc* probes were the subcloned 1.5-kb *Pst* I fragment of MC29 DNA (14) and

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Abbreviations: H chain, immunoglobulin heavy chain; *C*, *V*, and *S*, constant, variable, and switch regions of immunoglobulin genes; LyR, lymphoid rearranging; kb, kilobase(s); bp, base pair(s); NaCl/Cit/EDTA, 150 mM NaCl/15 mM sodium citrate/5 mM EDTA.

two domain-specific fragments of it: the 5′ 590-base-pair (bp) *Pst/Sal* fragment (25) and the 3′ 900-bp *Cla/Pst* fragment (26). The *S* region probe was the 3′-terminal 4.7 kb of the germ-line C_α clone in figure 1*A* of ref. 8, which includes the entire S_α region and extends into the C_α gene.

NIH 3T3 mouse cells were transfected (27) with 5, 50, and 500 ng of cloned DNA per plate; monolayers were fed every second day and stored at 14–21 days.

RESULTS

Cloned Mouse and Human LyR DNAs Bear the c-*myc* Gene. The 20.5 kb of mouse chromosome 15 at the top of Fig. 1*A*, shown below to bear the c-*myc* gene, is defined by the three overlapping clones of rearranged LyR DNA. The J558 3′ clone, isolated as an aberrantly rearranged C_α gene in plasmacytoma J558 (8, 9), contains nearly all of the c-*myc* gene and its 3′ flanking region. The c-*myc* region in the clone from T lymphoma ST4 (8) extends 1.4 kb further upstream from that in J558 3′ and probes from it (*a* and *b* in Fig. 1*A*) allowed us to detect and clone a second J558 *Eco*RI fragment, termed J558 5′ (Fig. 1*A*), bearing the c-*myc* 5′ flanking sequence. Those sequences are linked to S_μ and represent the reciprocal translocation between chromosomes 15 and 12 (unpublished data). Two clones spanning 24 kb of the human germ-line c-*myc* locus (Fig. 1*B*) were isolated from a phage library (28) by using mouse LyR probes *f*, *g*, *h*, and *i*.

Evidence that the mouse and human clones bear the c-*myc* gene is, first, that v-*myc* probes (14, 25, 26) hybridized strongly to particular restriction fragments of the mouse and human clones (Fig. 2*A*). Second, LyR probes (*c* and *g* in Fig. 1) and a v-*myc* probe hybridized to the same sized poly(A)$^+$ RNA species in 15 murine tumors tested, as shown for four lines in Fig. 2*B*. Third, in genomic blots (not shown), mouse LyR probes hybridized to a human *Eco*RI fragment of the size (≈13 kb) reported for the c-*myc* gene (29) and weakly to the expected (25) chicken c-*myc* *Eco*RI, *Bam*HI, *Pvu* II, and *Sac* I fragments. Conversely, a v-*myc* probe labeled the ≈21-kb mouse and ≈13-kb human *Eco*RI fragments, but the weak signals indicated substantial c-*myc* divergence between birds and mammals. Finally, the map in Fig. 1*B* matches that recently reported (29) for a human c-*myc* clone, and sequences within the Lyr clones (unpublished results with Ora Bernard) correspond to those in v-*myc* (26).

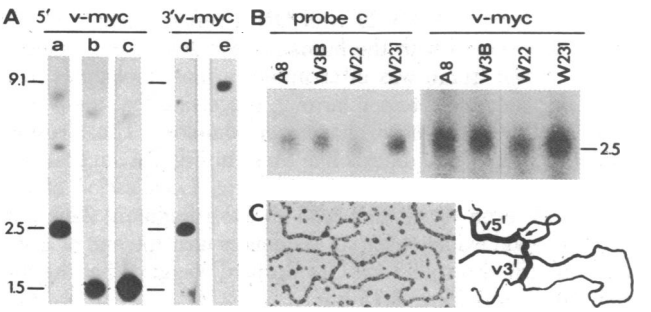

FIG. 2. Three types of evidence that LyR DNA bears the c-*myc* gene. (*A*) Hybridization of 5′ and 3′ v-*myc* probes to the mouse J558 3′ LyR DNA digested with *Xba* I + *Xho* I (lanes a and d) or with *Sac* I + *Eco*RI (b and e) or to human clone 1 digested with *Sac* I + *Eco*RI (lane c). Hybridization was in 6× NaCl/Cit/EDTA/0.1% NaDodSO$_4$ at 65°C. (*B*) Hybridization of LyR probe *c* and a v-*myc* probe (5′ + 3′) to total poly(A)$^+$ RNA from pre-B lymphoma ABLS-8, myeloid line WEHI 3B, T lymphoma WEHI 22, and B lymphoma WEHI 231, none of which have rearranged c-*myc* (8). (*C*) Heteroduplex of mouse c-*myc* with v-*myc*. The ST4 3′ c-*myc* clone in Fig. 1*A* (in pBR322) and pMC38 (ref. 15), which bears MC29 viral DNA, were linearized with *Cla* I (ST4) or *Hin*dIII (pMC38), and heteroduplexes were formed and spread at 35°C below the melting temperature. From seven measured molecules, duplex regions (mean ± SD) were 0.63 ± 0.11 kb for v5′ and 0.41 ± 0.12 kb for v3′. The single-stranded region between v5′ and v3′ was 1.2 ± 0.1 kb for c-*myc* and 0.12 ± 0.08 kb for v-*myc*.

c-*myc* Gene Includes Two Exons Homologous to v-*myc*. Like that in the chicken (25, 30), the c-*myc* gene in mouse and man (see also ref. 29) contains two exons homologous to 5′ and 3′ regions of v-*myc*, denoted v5′ and v3′ in Fig. 1. The heteroduplex in Fig. 2*C* shows that a mouse c-*myc* clone forms two

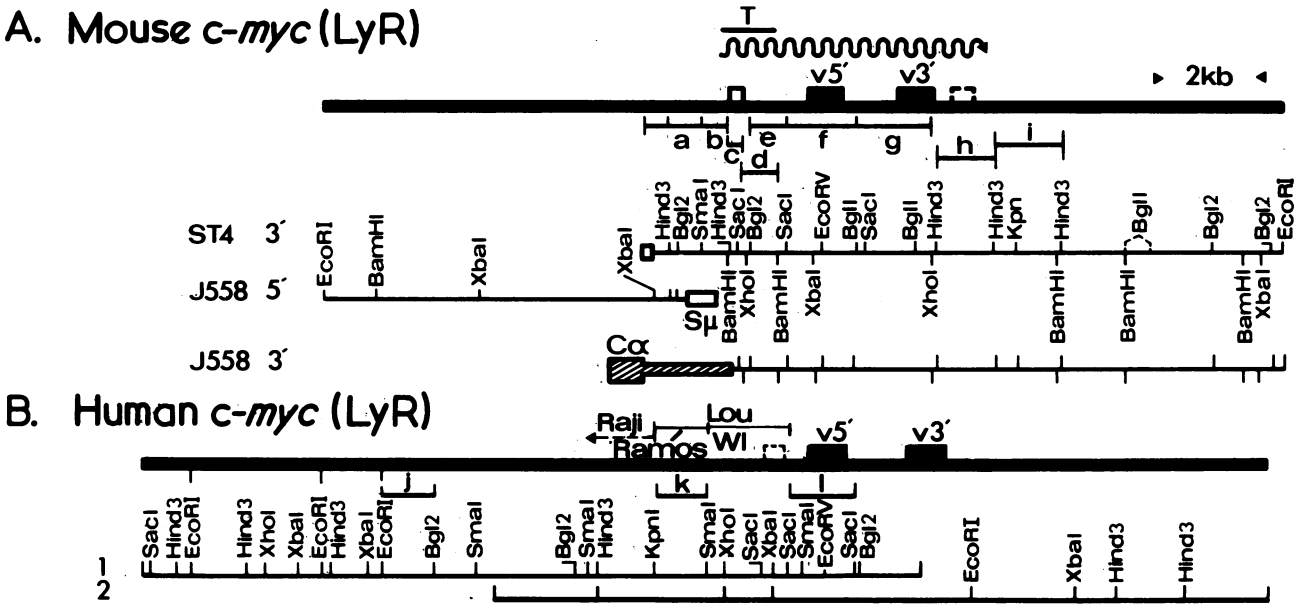

FIG. 1. Structure of the mouse (*A*) and human (*B*) germ-line c-*myc* (LyR) loci. Filled boxes v5′ and v3′ denote exons that hybridize with v-*myc*; nearby open boxes, presumptive exons of uncertain length; and the wavy line, the transcribed region so far defined (see text). T (top) denotes the translocation breakpoint region in 19 plasmacytomas and B lymphomas (refs. 8–11; unpublished data). Only indicated restriction endonuclease sites were mapped for the J558 5′ clone. Murine clones are *Eco*RI fragments in which c-*myc* has recombined with the C_α locus (hatched), with S_μ (the box in J558 5′), or an unknown region (boxed) in ST4 3′. Human c-*myc* clones are bounded by artificial *Eco*RI sites (28). Bars above the murine maps denote subclones and letters denote fragments used as probes. In *B*, apparent crossover regions in Burkitt lines are marked (see text) and a broken box denotes a region homologous to the plasmacytoma putative 5′ c-*myc* exon in murine region *e*.

duplex regions with MC29 viral DNA, separated by a 1.2-kb loop. As observed with the human c-*myc* gene (29) homology on the v-*myc* strand was interrupted in most molecules by a short single-stranded region (arrow), suggesting that the 5′ end of v3′ or the 3′ end of v5′ has lower homology. These results and analysis of many digests like those in Fig. 2*A* suggest that the murine intron is 1.0 ± 0.1 kb long. Localization of the human v5′ exon—e.g., to a 1.55-kb *Sac* I fragment (lane c in Fig. 2*A*)—and evidence for v3′ sequences near the end of clone 1 (unpublished results with Ora Bernard) suggest that the human intron is 1.25 ± 0.15 kb.

The heteroduplexes confirmed the orientation of c-*myc* determined from LyR (now c-*myc*) transcripts (8), as did hybridization (Fig. 2*A*): the 5′ v-*myc* probe labeled a 1.5-kb *Sac* I fragment (lane b) that maps to the left of the 9.1-kb *Sac* I/*Eco*RI fragment labeled by the 3′ probe (lane e). Significantly, the translocated c-*myc* and C_α genes are oriented oppositely: c-*myc* transcription (wavy line in Fig. 1*A*) proceeds rightward but that of C_α is leftward in α mRNA.

Murine c-*myc* Gene Has Additional 5′ and 3′ Segments. An ≈2.5-kb c-*myc* mRNA exists in thymocytes and splenocytes of mice (Fig. 3*A*) and chickens (31) and in every murine tumor line lacking a rearranged c-*myc* gene (Fig. 2*B* and ref. 8). Because v-*myc*-related sequences consist of only 1,580 bp (26) and the poly(A) tract only ≈100 bp, an extra ≈800 bp of c-*myc* sequences remain to be localized. The upper portion of Fig. 4 shows our current model for c-*myc* exon structure and the splices generating this "germ-line" c-*myc* mRNA. The exon 1.2 to 1.7 kb upstream from the v5′ exon was identified when we found that probe *c* labeled the 2.5-kb RNA in 10 lines lacking c-*myc* rearrangement (see, for example, Fig. 2*B*), whereas probe *e* did not, as is shown for pre-B lymphoma RAW 8 in Fig. 5 *B* and *C*. The germ-line c-*myc* mRNA also seems to include sequences downstream from v3′ (Fig. 4), because it hybridized to probe *h* (see RAW 8 in Fig. 5*D*), a region that did not hybridize detectably to v-*myc*, even at low stringency (not shown). A 3′ noncoding region highly divergent from that in v-*myc* (26) might explain these results, but there may also be an additional exon.

Presumptive Precursors of the Germ-Line c-*myc* mRNA. Total RNA from several lines, such as the two in Fig. 3*B*, contained poly(A)$^+$ c-*myc* species of ≈5.1, 4.3, and 3.7 kb, and a 3.3-kb species sometimes was resolved. The three largest were also labeled by the intron probe *e* (Fig. 3*C*). A similar spectrum of c-*myc* RNAs (6.5, 5.0, 4.4, 3.5, and 3.3 kb) exists in chicken nuclei (25). If such species reflect successive splices of introns, the murine germline c-*myc* gene contains at least three introns and four exons.

Translocation Disrupts the Germ-Line c-*myc* Transcriptional Unit. Fig. 4 shows that the chromosomal breakpoints occur near or within region *c*: the arrowed recombination points

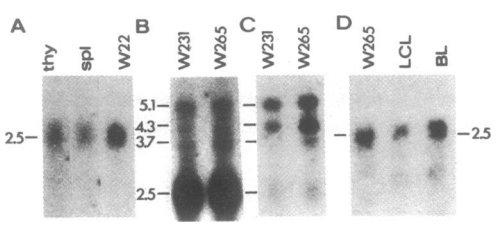

FIG. 3. Murine and human c-*myc* transcripts. (*A*) Detection of poly(A)$^+$ c-*myc* species in RNA from normal thymocytes (thy) and splenocytes (spl) the same size as in T lymphoma WEHI 22 by using probe *g*. (*B* and *C*) Detection of murine c-*myc* mRNA precursors with probe *g* (in *B*) and probe *e* (in *C*) in total poly(A)$^+$ RNA from B lymphoma WEHI 231 and myeloid line WEHI 265. (*D*) Human c-*myc* transcripts (≈2.6 kb) in Lou LCL174 and BL36 compared to the 2.5-kb species in WEHI 265, detected by using probe *g*.

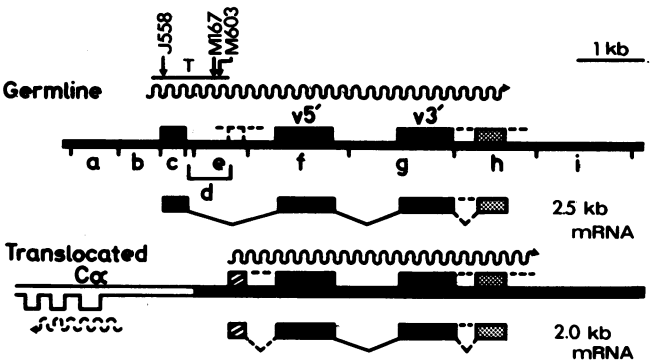

FIG. 4. Model for altered c-*myc* transcription resulting from the plasmacytoma translocation. A wavy line denotes a transcribed region, boxes exons, and T the predominant translocation region. Probe regions are lettered as in Fig. 1. The size and exact position of the apparent exons in regions *c*, *e*, and *h* are not yet known (see text); for example, *e* may be an extension of v5′, and *h* an extension of v3′ rather than separate exons. Splices that might account for the 2.5-kb germline transcript and the 2-kb plasmacytoma transcript are indicated.

for plasmacytomas M167 and M603 (10) lie to its right, that in J558 (8, 9) just to the left, and those in S107 (7) and 15 others fall within region T (unpublished results). Such lines contain a complex set of c-*myc* transcripts (8), typically 2.4, 2.0, 1.85, and 1.2–1.5 kb, as shown in Fig. 5*A* for four plasmacytomas and a B lymphoma. In contrast to germ-line c-*myc* mRNA, these transcripts lack sequences from the upstream exon defined by region *c* (Fig. 4); no mRNA from MOPC 104E, P3, BALTELM 1131, or MOPC 173 hybridized to probe *c* (Fig. 5*B*), nor did any from S107 or TEPC 1017 (not shown), and only a 2.5-kb species from SAMM 368 was detected. We conclude that translocation disrupts the 5′ exon or removes 5′ regulatory sequences. Moreover, because these lines, like most others (8–11), retain a germ-line c-*myc* allele, absence of the 2.5-kb germ-line transcript (defined by hybridization to probe *c*) in all, except perhaps SAMM 368, suggests that this allele is turned off in most plasmacytomas.

A Germ-Line c-*myc* Intron Is Expressed in Plasmacytoma RNAs. As shown previously (probe *f* in figure 4 of ref. 8) and documented further in Fig. 5*C*, "germ-line intron" probe *e* (Fig. 4) hybridizes to multiple poly(A)$^+$ RNAs in lines bearing a rearranged c-*myc* gene. These appear to include certain c-*myc* mRNAs, because an ≈2.0-kb and often ≈1.85-, 1.5-, and 1.2-kb species were labeled in 10 lines examined. The new "exon" probably lies near the 3′ end of region *e* (Fig. 4), because its 5′ segment (probe *d*) hybridized only weakly. The lower part of Fig. 4 depicts possible splices for the 2-kb c-*myc* RNA. Comparison of Fig. 5 *A* and *C* indicates that some species labeled by probe *e*, such as the 2.7-kb RNA in P3, do *not* bear a v-*myc* exon. Thus translocation may activate expression of more than one class of transcript. Expression of previous intron sequences might reflect triggering of a cryptic promoter within region *e* or altered splicing.

Variable 3′ Regions on Plasmacytoma c-*myc* RNAs. Plasmacytoma 2.0- and 1.2- to 1.5-kb species in most lines were labeled by 3′ probe *h*, but arrows in Fig. 5*D* indicate that the ≈1.85-kb species present in some lines (see Fig. 5*A*) was not labeled. Thus certain plasmacytoma species may be spliced differently in their 3′ regions.

Burkitt Lymphomas with t(8;14) Contain a Rearranged c-*myc* Gene. In Burkitt lines W1 BL, Lou BL (IARC/BL36), Ramos, and Raji, which bear the common 8;14 translocation (refs. 19, 20, 21; G. Lenoir and M. Garson, unpublished results), and in W2 BL, c-*myc* rearrangement was evident from the new fragments (arrow) in *Eco*RI digests (Fig. 6*A*) and *Bam*HI and *Hin*dIII digests (not shown). This does not merely

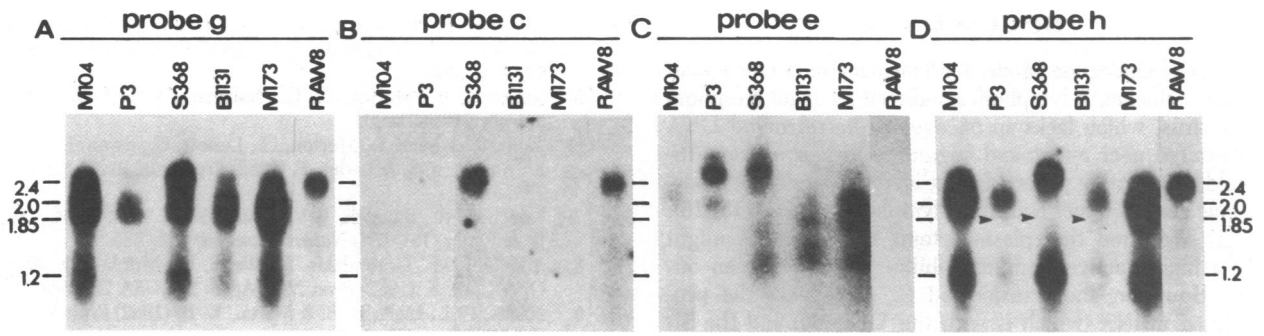

FIG. 5. Hybridization of four c-*myc* probes to total poly(A)$^+$ RNA in plasmacytomas MOPC 104E, P3, SAMM 368, MOPC 173, B lymphoma BALTELM 1131, and pre-B lymphoma RAW 8. One c-*myc* allele has recombined with S_α in SAMM 368, MOPC 173, and BALTELM 1131 and with unknown DNA (not S_α) in MOPC 104E and P3, but c-*myc* is not rearranged in RAW 8 (8). Hybridization with the different probes was either sequentially to the same filter or to a duplicate filter from the same gel.

reflect genetic polymorphism, because lymphoblastoid (nontumorigenic) cell lines (LCL in Fig. 6) from patients W1, W2, and Lou displayed only the normal ≈13-kb *Eco*RI fragment, as did leukocytes from 14 leukemic individuals: four chronic and two acute lymphocytic and five chronic and three acute myeloid (not shown). BJAB, an unusual Burkitt lymphoma line that lacks a translocated chromosome 8 (20), had no new fragment in *Eco*RI (Fig. 6) or *Hin*dIII digests. None was seen in *Eco*RI digests of LY67 or J1 (not shown), which respectively bear 8;22 and 8;2 translocations (19) involving the same band of chromosome 8 as in a t(8;14). Thus a second gene in that region may be implicated.

If t(8;14) is reciprocal (33), a breakpoint upstream from c-*myc* would place the gene and its 5′ flanking region on different restriction fragments, as in J558 (Fig. 1*A*). Fig. 6*B* shows that probes either 2.3 or 8.1 kb upstream from c-*myc* (*k* or *j* in Fig. 1*B*) labeled fragments in W1 BL, Lou BL, and Raji distinct from those labeled by the gene probe in Fig. 6*A*. Thus both expected products of reciprocal exchange are detectable. By determining which product was labeled by probes *j*, *k*, and *l*, we localized the recombination points in four lines to the regions indicated in Fig. 1*B*. For instance, in Lou BL and W1 BL, because probe *k* (and *j*) detects only the "reciprocal rearrangement product," which was not labeled by *l*, the recombination point must lie 1–2 kb 5′ of the v5′ exon, between regions *k* and *l*. In contrast, the Raji breakpoint appears to lie 3–9 kb 5′ of v5′ (Fig. 1*B*). Of eight rearrangements detected by Taub *et al.* (6), three appeared to lie outside the 12.5-kb *Eco*RI fragment.

Analogy with plasmacytomas led us to search for C_H switch regions associated with these rearrangements. A murine S_α probe, homologous to human S_μ and S_α (34), hybridized to the same size *Eco*RI fragments of W1 and W2 BL and Ramos DNA as c-*myc* probes (compare arrowed fragments in Fig. 6 *C* and *A*). For W1 and W2, this was confirmed with *Bam*HI digests. Hence it appears that, in at least these two lines, c-*myc* has recombined near S_μ or S_α. S_μ is more likely because the full complement of S_α sequences appears to remain in germ-line context (Fig. 6*C*). Translocation near S_μ has been reported for two other Burkitt lines (6), but it has been inferred to occur within the V_H locus for another line (35).

Fig. 3*D* shows that, unlike the mouse c-*myc* transcripts, c-*myc* mRNAs in a Burkitt line and a lymphoblastoid line were the same size and the level was increased no more than 2-fold, in accord with other data (36).

Translocated c-*myc* Gene Does Not Transform Fibroblasts. Plasmacytoma DNA can transform NIH 3T3 fibroblasts (37), so we tested the c-*myc*-bearing J558 3′ clone in four experiments and the J558 5′ clone in two. No transformed foci were found, although Harvey sarcoma virus DNA gave numerous foci (≈10^3 per μg), and the number of foci was not depressed by added J558 3′ DNA. These results suggest that the plasmacytoma oncogene scored on fibroblasts (37) is *not* c-*myc*. In accord with that conclusion, clones from three NIH 3T3 foci transfected with S107 plasmacytoma DNA did not contain the rearranged S107 c-*myc* gene or the reciprocal rearrangement. Moreover, the transforming activity is spared by digestion with *Hin*dIII (37), whereas the c-*myc* gene is cut (Fig. 1*A*).

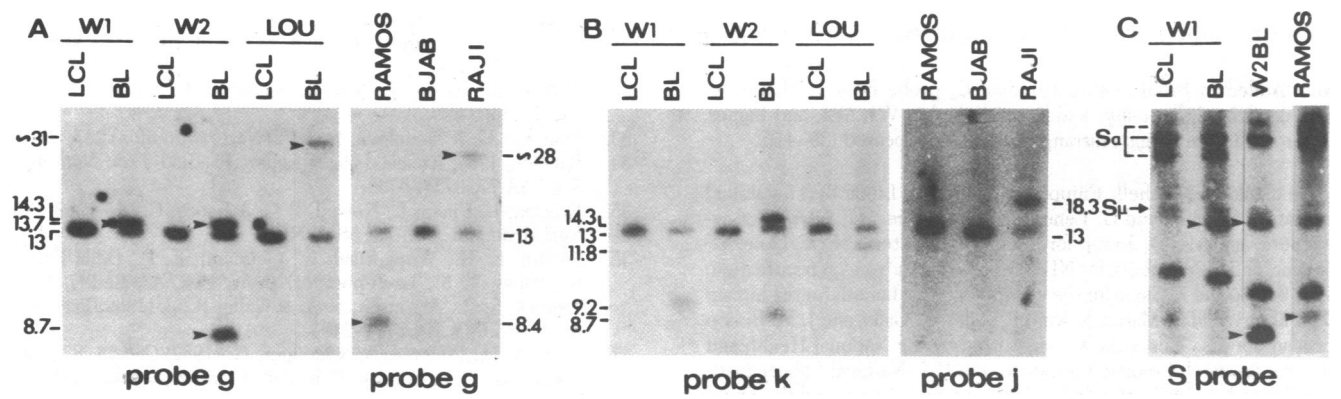

FIG. 6. Southern blot analysis revealing c-*myc* gene rearrangement in Burkitt lymphomas. *Eco*RI-digested DNA from the indicated Burkitt lymphomas (BL) and lymphoblastoid cell lines (LCL) were scored in *A* with c-*myc* gene probe *g* (identical results were obtained with *l*); in *B*, with probes *k* and *j* from the c-*myc* 5′ flanking region; in *C*, with an *S* region probe, using the same filters as in *A*. Bands labeled S_α in *C* denote ≈25- and 30-kb *Eco*RI fragments characterized for germ-line C_α genes (32) and a larger fragment that replaces the 25-kb fragment in certain individuals. S_μ denotes a fragment ascribed to germ-line S_μ, because it is present in nonlymphoid lines but is replaced, as expected, by rearranged fragments in most B lymphoid lines (e.g., W1 LCL in *C*).

DISCUSSION

An Altered Oncogene Model for Plasmacytoma Oncogenesis. In most chicken B lymphomas induced by avian lymphoid leukosis virus, which lacks an oncogene, the retroviral DNA has integrated near c-*myc* and augmented its expression 15- to 50-fold, stimulating proposals that increased levels of a normal c-*myc* gene product feature in lymphomagenesis (15, 16). Klein (1) suggested that plasmacytoma translocations might couple a highly active immunoglobulin promoter to an oncogene. However, the translocated C_α gene lacks the promoter for α mRNA (which is with the V_H gene) and the 5′-to-5′ orientation of c-*myc* and C_α rules out any joint promoter. Moreover, plasmacytomas do not have a high level of c-*myc* mRNAs: 14 lines with a translocated c-*myc* gene averaged ≈20 copies per cell, about 5-fold higher than the mean for 18 pre-B and T lymphomas lacking rearrangement, some of which had higher levels than some plasmacytomas (8). Normal thymocytes and splenocytes (Fig. 3A) have on the order of 1 or 2 copies per cell.

Because chromosome breakpoints in plasmacytomas fall within a region (T in Fig. 4) spanning a 5′ c-*myc* exon, translocation must separate the c-*myc* gene from its normal promoter and 5′ regulatory machinery and place it under some lymphoid regulatory element for immunoglobulin genes. Conceivably, the S_α–C_α region contains an element that acts bidirectionally, such as an enhancer, that could stimulate a cryptic promoter within region *e* (Fig. 4), or S_α, to generate the specific plasmacytoma mRNAs. Our proposal (8, 13) that these mRNAs have a role in the malignancy implies that they encode an altered *myc* polypeptide oncogenic for mature B cells. Because the 408-bp sequence (8) across region *c* in Fig. 4 ends with a 346-bp open reading frame in the c-*myc* orientation, the exon in that region may well encode part of the germ-line *myc* polypeptide. If so, absence of region *c* from the plasmacytoma mRNAs (Fig. 5*B*) means that any polypeptides they encode must differ at least at their NH_2 termini.

More data on the Burkitt lines are needed to determine whether the altered oncogene model will hold for them. Perhaps the altered context of the human c-*myc* gene induces changes in the mRNA structure not yet discernible.

Activation of c-*myc* Probably Is Not the Only Step in Plasmacytoma Induction. Our finding that c-*myc* is not the plasmacytoma gene that can transform fibroblasts parallels results with chicken lymphomas, in which c-*myc* is activated (15, 16) but a separate gene is scored on fibroblasts (38). Plasmacytoma oncogenesis thus appears to involve both translocation of c-*myc* and activation of another oncogene. One event might trigger the other, or the events might occur independently.

Note Added in Proof. Using a human C_μ probe from T. Honjo, we have confirmed that *c-myc* and S_μ are linked in W1, W2, and Ramos. Other studies on c-*myc* rearrangement have appeared (39–41).

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