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Intrachromosomal Rearrangements Fusing L-*myc* and *rlf* in Small-Cell Lung Cancer

TOMI P. MÄKELÄ,[1] JUHA KERE,[2] ROBERT WINQVIST,[3] AND KARI ALITALO[1]*

Laboratory of Cancer Biology, Departments of Virology and Pathology,[1] and Department of Medical Genetics,[2] University of Helsinki, Haartmaninkatu 3, 00290 Helsinki, and Laboratory of Clinical Genetics, Oulu University Central Hospital, Kajaaanintie 52, 90220 Oulu,[3] Finland

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Chromosomal abnormalities affecting proto-oncogenes are frequently detected in human cancer. Oncogenes of the *myc* family are activated in several types of tumors as a result of gene amplification or chromosomal translocation. We have recently found the L-*myc* gene involved in a gene fusion in small-cell lung cancer (SCLC). This results in a chimeric protein with amino-terminal sequences from a novel gene named *rlf* joined to L-*myc*. Here we present a preliminary structural characterization of the *rlf*–L-*myc* fusion gene, which has been found only in cells with an amplified L-*myc* gene. In addition, we have used somatic cell hybrids to assign the normal *rlf* locus to the same chromosome (chromosome 1) on which L-*myc* resides. Finally, we have been able to establish a physical linkage between *rlf* and L-*myc* with pulsed-field gel electrophoresis. Our results demonstrate that normal *rlf* and L-*myc* genes are separated by less than 800 kb of DNA. Thus, the *rlf*–L-*myc* gene fusions are due to similar but not identical intrachromosomal rearrangements at 1p32. The presence of independent genetic lesions that cause the formation of identical chimeric *rlf*–L-*myc* proteins suggests a role for the fusion protein in the development of these tumors.

Specific chromosomal alterations have been associated with a large number of tumors (23), and a large body of evidence, especially from hematopoietic malignancies, indicates that these alterations are involved in the pathogenesis of their respective tumors (13). These abnormalities include translocations, inversions, deletions, and amplifications, and they have often been found to involve proto-oncogenes, which undergo deregulation or structural alterations due to the rearrangements (31). In a high percentage of Burkitt lymphomas, chromosomal translocations have been shown to involve the c-*myc* proto-oncogene on chromosome 8 and one of several immunoglobulin loci on chromosome 14, 2, or 22 (12). A consequence of these and analogous translocations involving c-*myc* (17, 20) is deregulation of c-*myc* transcription (45). The second general mechanism involves structural alterations of oncogene-encoded proteins due to the formation of a fusion gene and chimeric protein. Examples of the latter mechanism include the t(9;22) Philadelphia translocation of chronic myelogenous leukemia whereby a chimeric Bcr-Abl protein is formed (44), the t(1;19) E2A-Prl fusion in pre-B-cell leukemias (29, 38), and the t(15;17) Myl-retinoic acid receptor α fusion in acute promyelocytic leukemia (7, 15).

Several nonrandom somatic rearrangements that would not be detected in cytogenetic analysis have been recently discovered with molecular analysis of known oncogenes. For example, the *tal* (also called *scl* or TCL-5) gene was originally discovered because it was involved in the t(1;14)(p32;q11) translocation found only in a small subset of acute T-cell leukemias (4, 11, 18). Later it was found that the *tal* gene undergoes an intrachromosomal deletion at 1p32 in a high percentage of T-cell leukemias (9). Both types of rearrangement lead to a similar loss of coding potential of the gene. Other described cases include the tropomyosin-Trk from a colon carcinoma (34), the TCL-5 c-*myc* translocation in the HUT78 cell line (17), and the PTC oncogene found activated in the majority of papillary thyroid carcinomas (21). The PTC activation also appears to result from an intrachromosomal rearrangement, since the oncogene is formed as a fusion of two separate loci (H4 and *ret*), both of which map to human chromosome band 10q11.2 (21, 27).

In addition to c-*myc*, two other *myc* family genes are involved in human cancer. The N-*myc* gene is found amplified in a high percentage of neuroblastomas (8, 41). The L-*myc* gene was originally identified as an amplified, *myc*-related sequence from small-cell lung cancer (SCLC) (37). Like the other *myc* genes, it encodes a nuclear phosphoprotein of unknown function (40) and can transform cells both in vitro (6) and in vivo (36). Although gene amplification and high expression of L-*myc* are found frequently in SCLC (28, 47) and occasionally in other lung tumors (42), L-*myc* has not been implicated in other tumor types, possibly reflecting the restricted expression pattern of the L-*myc* proto-oncogene. The only described activation mechanism of L-*myc* is gene amplification, although c-*myc* has been found activated through several different types of DNA rearrangements (reviewed in reference 1).

We have recently characterized a fusion protein formed by L-*myc* and a novel gene named *rlf* from two unrelated SCLC cell lines (35). The protein is formed from a chimeric mRNA containing 5′ sequences from the *rlf* gene joined to the second and third exons of L-*myc*, joining 79 amino acids of *rlf* and 3 amino acids from the noncoding L-*myc* sequences to the amino terminus of the L-*myc* protein. Although the fusion protein is identical in both cases studied, the DNA rearrangements are dissimilar. They can be detected only with a *rlf* probe, indicating that the rearrangements have occurred some distance upstream of L-*myc*. The rearranged fusion genes have further been involved in DNA amplification in both of the cell lines. We were interested in studying the structural basis for the *rlf*–L-*myc* fusion mRNA, and here we present data on the normal *rlf* locus and on the structure of the rearrangement. Our results indicate that the

* Corresponding author.

fusion gene is formed through intrachromosomal rearrangements, which result in joining of 5′ regulatory and partial coding sequences of *rlf* upstream of the L-*myc* gene.

MATERIALS AND METHODS

Cell lines and normal DNAs. All cell lines were grown in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics. GLC28 cells were a kind gift from Charles Buys, CORL47 was from Gerard Evan, and NCI-H209 and NCI-H345 were from Adi Gazdar. Normal DNAs were from peripheral blood lymphocytes.

Somatic cell hybrids. A series of human × rodent (mouse or Syrian hamster) somatic cell hybrid clones was generated as described previously (10, 22). The fibroblast cell lines GM 3104 (Human Genetic Mutant Cell Repository), A9, and P3 served as human, mouse, and hamster controls, respectively. The human chromosomes contained in each hybrid cell line are shown in Fig. 6.

DNA probes. 119118 is a 250-bp polymerase chain reaction insert corresponding to the rearranged 5′ part of *rlf* obtained from the fusion cDNA clone G251 described by Mäkelä et al. (35). a8-0.9HE is a 0.9-kb *Hin*dIII-*Eco*RI insert from the a8 cDNA clone (35) representing more 3′ *rlf* sequences not participating in the rearrangement. The L-*myc* second-exon probe was a 400-bp *Eag*I-*Bss*HII insert from cDNA clone pU313, and the third-exon probe a 2.4-kb *Bam*HI-*Eco*RI insert from cDNA clone pU341. Both cDNA clones have been isolated from a U1690 cDNA library made from the U1690 SCLC cell line (5) as described previously (35) and represent the 3.8-kb mRNA (pU313; nucleotides 495 to 6800) and the 3.6-kb mRNA (pU341; nucleotides 367 to 6777) of L-*myc*. The EE-0.9 L-*myc* upstream probe was subcloned from a normal L-*myc* genomic clone isolated from this region (unpublished data). The c-*jun* probe was a 1.4-kb *Sal*I-*Eco*RI insert from the hcJ-1 plasmid (2; a kind gift from Peter Angel), and the *tal* probe was a 2.0-kb *Eco*RI insert from plasmid B2EE-2.0 (9; a kind gift from Richard Baer).

Southern blotting analysis. A 10-μg sample of high-molecular weight DNA was cleaved to completion with restriction endonucleases, separated in 0.8% agarose gels, and blotted onto nitrocellulose membranes. Hybridization of insert probes radiolabelled by random priming (16) and subsequent washes was done essentially as described previously (32). Before rehybridization, old signal was removed with 0.2 N NaOH for 15 min or by boiling in 0.1% sodium dodecyl sulfate for 10 min.

Pulsed-field gel electrophoresis (PFGE). Sample preparation from the GLC28 cell line and from normal leukocytes and electrophoresis were essentially as described previously (30). Briefly, cells were embedded in 0.5% low-melting-point agarose (Bethesda Research Laboratories) at 10^6 cells per ml, treated with 0.25 M EDTA, 1% sarcosyl, and 2 mg of proteinase K per ml at 50°C for 2 days, and washed and stored in 10 mM Tris–1 mM EDTA (pH 8.0). For restriction enzyme digestions, a 60-μl block was equilibrated in appropriate buffer. Digestions with 20 U of enzyme were done for 4 to 12 h. Electrophoresis was performed in 1% agarose gels at 125 to 170 V with pulse times of 30 to 180 s for 2 days in 0.25× Tris-borate-EDTA buffer. Blotting and hybridization were done as described above.

RESULTS

DNA rearrangements causing the formation of a *rlf*–L-*myc* fusion protein. The *rlf*–L-*myc* fusion protein detected in SCLC cell lines GLC28 and CORL47 was found to be encoded by a chimeric *rlf*–L-*myc* mRNA consisting of *rlf* sequences joined to the second exon of L-*myc* (35). The transcription of this mRNA is due to DNA rearrangements joining 5′ *rlf* sequences to L-*myc* as shown in Fig. 1. Hybridization of *Eco*RI-digested DNA with a *rlf* probe corresponding to the 5′ fusion cDNA showed rearranged and amplified bands of 16 and 19 kb in CORL47 and GLC28, respectively (Fig. 1A). The presence of a single major rearranged band suggested that the rearrangements have occurred prior to or at the onset of DNA amplification. Using the *rlf* probe, we detected the rearrangements in both cell lines with at least *Eco*RI, *Sac*I, and *Pvu*II restriction enzymes (the smallest rearranged bands were observed with *Pvu*II), but hybridization with L-*myc* cDNA probes failed to detect the rearrangements (Fig. 1B). This suggested that the breakpoints are closer to *rlf* than to L-*myc*; moreover, using the sizes of the rearranged restriction fragments and the restriction map of L-*myc*, we were able to estimate that both breakpoints are located less than 7 kb downstream of the *rlf* sequences used as a probe and more than 6 kb upstream of L-*myc*. Figure 1C shows partial restriction maps of normal

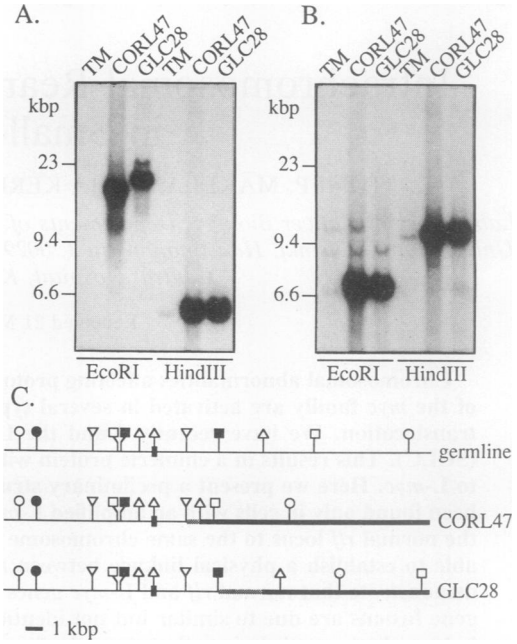

FIG. 1. *rlf*–L-*myc* rearrangement and amplification in two SCLC cell lines. (A) Southern analysis with the 119118 *rlf* probe representing sequences present in the fusion cDNA was carried out with DNA from the indicated sources. TM represents normal DNA. GLC28 and CORL47 DNAs contain rearranged, amplified bands of 19 and 16 kb with *Eco*RI, whereas *Hin*dIII digests show nonrearranged bands of 5.5 kb. (B) The same filter as in panel A was hybridized with an L-*myc* second-exon probe. The 10.0- and 6.6-kb bands in *Eco*RI digests represent two polymorphic alleles of L-*myc*; the 8.5-kb band represents the L-*myc* pseudogene (14). TM and CORL47 are heterozygous and GLC28 is homozygous for the 6.6-kb allele. Amplification of the 6.6-kb allele but no rearrangements are detected with the L-*myc* probe. (C) A partial restriction map of the region surrounding the 119118 probe (thick black box) from normal DNA and from GLC28 and CORL47 rearranged DNAs. Rearranged DNA is shown as a thick black line, and the shaded area represents the region where the rearrangements have occurred. Restriction sites: ○, *Eco*RI; ●, *Mlu*I; □, *Sac*I; ■, *Sca*I; ▽, *Hin*dIII; △, *Pvu*II.

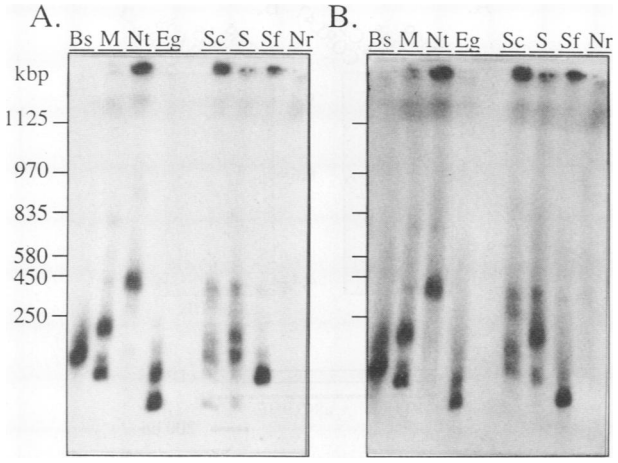

FIG. 2. PFGE analysis of GLC28 DNA hybridized with the 119118 *rlf* (A) or L-*myc* second-exon (B) probe. Note identical hybridization patterns for several enzymes. Several hybridizing bands in some lanes and weak bands in *Nru*I lanes are due to partial digestions repeatedly detected with certain enzymes. *Saccharomyces cerevisiae* chromosomes were used as size markers as described previously (30). Restriction sites: Bs, *Bss*HII; M, *Mlu*I; N, *Not*I; Eg, *Eag*I; Sc, *Sac*II; S, *Sal*I; Sf, *Sfi*I; Nr, *Nru*I.

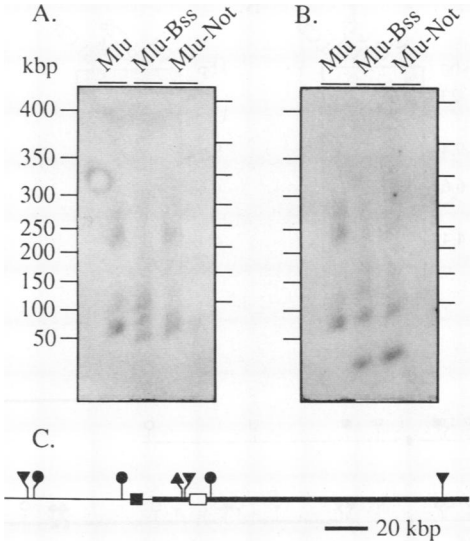

FIG. 3. PFGE analysis with indicated enzymes showing smallest common fragments of *rlf* (A) and L-*myc* (B) in GLC28 DNA with the same probes as in Fig. 2. Size markers are bacteriophage lambda concatemers. (C) Long-range restriction map of the rearrangement of L-*myc* and *rlf* in GLC28 cells. The thick line represents sequences from the normal *rlf* locus, and the thin line represents sequences from the normal L-*myc* locus. The breakpoint is less than 7 kb from the 119118 probe (white box) and over 15 kb upstream of L-*myc* (black box). Restriction sites: ▲, *Bss*HII; ●, *Mlu*I; ▼, *Not*I.

and rearranged DNAs surrounding the part of *rlf* participating in the rearrangements. The normal restriction map is based on a normal genomic clone from this region (data not shown).

PFGE analysis of the *rlf*-L-*myc* fusion gene in the GLC28 cell line. Because common fragments for *rlf* and L-*myc* were not detected in Southern hybridizations, we decided to use PFGE analysis with rare-cutting enzymes to construct a large-scale map of the region from the GLC28 cell line. Figure 2 shows PFGE analysis of GLC28 DNA hybridized with *rlf* (Fig. 2A) and L-*myc* (Fig. 2B) probes. The presence of a single amplified band with several digests suggested that the amplified region is larger than 500 kb. Common fragments for both probes were produced by at least *Mlu*I and probably by *Nru*I, although because of a reproducible partial digestion this fragment was very weak (Fig. 2). Although *Bss*HII and *Not*I digestions produced bands with similar sizes for both probes, double digestions indicated the presence of a single site for both enzymes between *rlf* and L-*myc* (Fig. 3A and B). On the basis of these digestions, together with the restriction map of the *rlf* region (Fig. 1C), we were able to construct a large-scale map of the rearranged region (Fig. 3C). The distance between the exons of *rlf* and L-*myc* in the rearranged gene of GLC28 was estimated to be 25 to 40 kb, and the smallest common fragment was the 45-kb *Mlu*I fragment.

Mapping of the breakpoint to a region 16 kb upstream of the L-*myc* gene in GLC28. To characterize the distance of the breakpoint from L-*myc* in more detail, a restriction map reaching about 30 kb upstream of L-*myc* was constructed by using normal genomic clones from this region. Comparison of the restriction sites of the rearranged DNA in GLC28 (shown as the thick line in Fig. 1C) showed a strong similarity with a region of the normal DNA upstream of L-*myc*. Common fragments detected in hybridization with the *rlf* probe (Fig. 4A) and with a probe from the L-*myc* upstream region (EE-0.9) confirmed this result (Fig. 4B). With use of the EE-0.9 probe, the breakpoint region was also narrowed to a 0.1-kb *Eco*RI-*Xba*I fragment shown as the gray area in Fig. 4C. Thus, the approximately 30-kb-long fusion gene in GLC28 contains 5′ regulatory domains, exon I (although we have not excluded the possibility of more than one exon), and 3.5 kb of the first intron from *rlf* joined to a region 16 kb upstream of L-*myc* (Fig. 4C). The EE-0.9 probe did not detect rearrangements in the CORL47 cell line, indicating that the rearrangements on the L-*myc* side varies considerably in the two cell lines. Thus, although the rearrangement in CORL47 is structurally similar to that of GLC28, the size of the first intron in CORL47 is over 33 kb (instead of 19.5 kb in GLC28; Fig. 1 and data not shown), and further mapping of the L-*myc* upstream region is needed for a detailed analysis of the rearrangement in CORL47.

The *rlf*-L-*myc* rearrangements have occurred in a large intron of *rlf*. The 5′ *rlf* sequences present in the fusion mRNA (0.3 kb) represent only a small part of the normal *rlf* message (7.0 kb; 35), and therefore we carried out hybridization analysis with a 3′ *rlf* probe obtained from a normal *rlf* cDNA (a8; 35), which was not present in the fusion mRNA. The 5′ 119118 probe detected amplified rearranged bands (Fig. 5A), whereas the 3′ a8-0.9HE probe detected only the normal pattern (Fig. 5B). Because the two probes, which cover only 1.2 kb of the 7-kb *rlf* mRNA, detect approximately 60 kb of genomic DNA divided in six bands, which all are larger than the probes, the normal *rlf* gene should contain at least six exons and probably represents a relatively large gene. The two probes did not detect common fragments with *Eco*RI or with several other enzymes (data not shown) in Southern analysis, indicating that the intron between the probes is longer than 15 kb (see restriction map in Fig. 4C). On the other hand, in PFGE analysis the whole 1.2-kb *rlf* cDNA clone was contained in a single 200-kb *Nar*I fragment (data not shown), which thus limits the maximum size of the

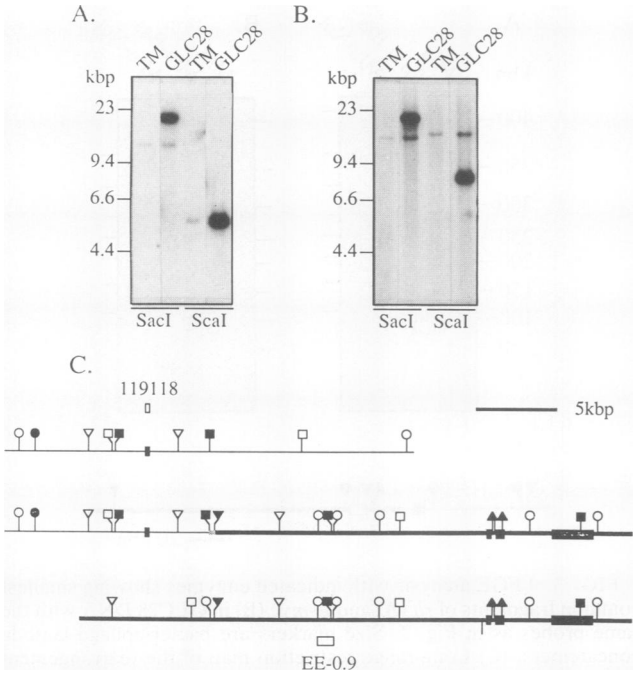

FIG. 4. Southern blotting analysis with the indicated enzymes with the *rlf* 119118 probe (A) and EE-0.9 L-*myc* probe (B) from normal (TM) and GLC28 DNA. Both probes hybridize to an identical 19-kb *Sac*I-digested rearranged band in GLC28. (C) Structure of the *rlf*–L-*myc* fusion gene in GLC28. A restriction map of the fusion gene (middle) is compared with the relevant regions of the normal *rlf* (top) and L-*myc* (bottom) genes. The white boxes represent the 119118 *rlf* probe (top) and EE-0.9 L-*myc* probe (bottom) used in construction of the map. Only the relevant sites surrounding the rearrangement are shown for *Hin*dIII, *Sac*I, and *Xba*I. Restriction sites: ○, *Eco*RI; ●, *Mlu*I; □, *Sac*I; ■, *Sca*I; ▽, *Hin*dIII; ▲, *Bss*HII; ▼, *Xba*I.

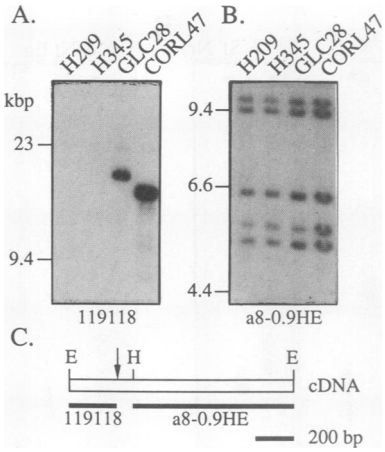

FIG. 5. Southern blotting analysis of *rlf*–L-*myc* rearrangements with 5′ and 3′ *rlf* probes. (A) *Eco*RI-digested DNAs were analyzed with the 119118 probe. Control DNAs from SCLC cell lines H209 and H345 have a single-copy band of 23 kb, whereas GLC28 and CORL47 also contain rearranged amplified bands. (B) The same filter was hybridized with the a8-0.9HE probe representing sequences more 3′ of those present in the fusion cDNA. All DNAs have a similar pattern of bands (10.2, 9.8, 6.6, 5.2, and 4.9 kb), and no rearrangements or amplifications are detectable with this probe. (C) Schematic map of a partial *rlf* cDNA clone (a8; 35) with relevant sites. Sequences present in the *rlf*–L-*myc* fusion cDNA are on the 5′ side of the breakpoint indicated by the arrow. The probes used in panels A and B are shown below.

intron. The intensities of the unrearranged *rlf* fragments seen in the rearranged cell lines were similar to those in control lanes, which suggested the presence of both normal alleles. However, the possibility of deletion of one allele is difficult to rule out without polymorphisms.

The normal *rlf* locus is on chromosome 1. In an earlier cytogenetic study including one of the rearranged cell lines (CORL47; 46), no abnormalities of chromosome 1 were detected. Because L-*myc* is located at 1p32, this suggests that the rearrangement is not a translocation or other cytogenetically visible recombination. We used human-rodent somatic cell hybrids to map the *rlf* gene. The results (summarized in Fig. 6) indicate 0% discordance with *rlf* and chromosome 1, whereas other chromosomes showed discordances of 20 to 87%. Sublocalization of *rlf* to 1pter-1q24 was possible with cell line RAG GM97 8–13, which has retained only this portion of chromosome 1. Identical localization from the same panel was also obtained with L-*myc* (data not shown). These results demonstrate that the rearrangements are intrachromosomal recombinations, which have not been detected with cytogenetic analysis.

Physical linkage of the unrearranged *rlf* and L-*myc* loci. In light of the somatic cell hybrid data, we decided to explore the possible physical linkage of *rlf* and L-*myc* with PFGE analysis of normal human DNA. Digestions with several rare-cutting enzymes hybridized with *rlf* and L-*myc* probes are presented in Fig. 7. Bands with identical mobilities were observed with both probes using *Mlu*I (780 kb) and *Nru*I (1050 kb) but not with several other enzymes yielding smaller fragments, establishing a physical linkage between *rlf* and L-*myc*. Double digestion with *Mlu*I and *Nru*I did not reduce the 780-kb *Mlu*I band (data not shown), which remained the smallest fragment containing both genes. The L-*myc* probe detected an additional 330-kb *Nru*I band, indicating that the *Nru*I digest was partial and that at least one *Nru*I site is located between the two probes. The physical linkage has subsequently been confirmed by coamplification of *rlf* and L-*myc* without rearrangements in several lung tumors (34a). Interestingly, *Mlu*I and *Nru*I were the two enzymes which gave common fragments in both the rearranged and normal configurations, and the size differences in the corresponding fragments are similar (730 kb for *Mlu*I and 630 kb for *Nru*I). Taking into account the low resolution of large fragments in PFGE, these findings would support the presence of a deletion of DNA between *rlf* and L-*myc*.

***tal* and c-*jun* are not associated with the *rlf*–L-*myc* rearrangements.** The linkage results indicated that both genes involved in the *rlf*–L-*myc* fusion gene are normally located at 1p32, a region implicated in several malignancies, including acute T-cell leukemia (9) and neuroblastoma (24). Therefore, we analyzed the rearranged cell lines with two oncogene probes from this region, *tal* and c-*jun*. Our results indicated that neither c-*jun* nor *tal* is located in the 780-kb *Mlu*I fragment containing *rlf* and L-*myc* and that they are not rearranged or amplified in the cell lines in which *rlf*–L-*myc* rearrangements were detected (data not shown).

DISCUSSION

We have recently described a fusion protein between *rlf* and L-*myc* found in two SCLC cell lines (35). In this study,

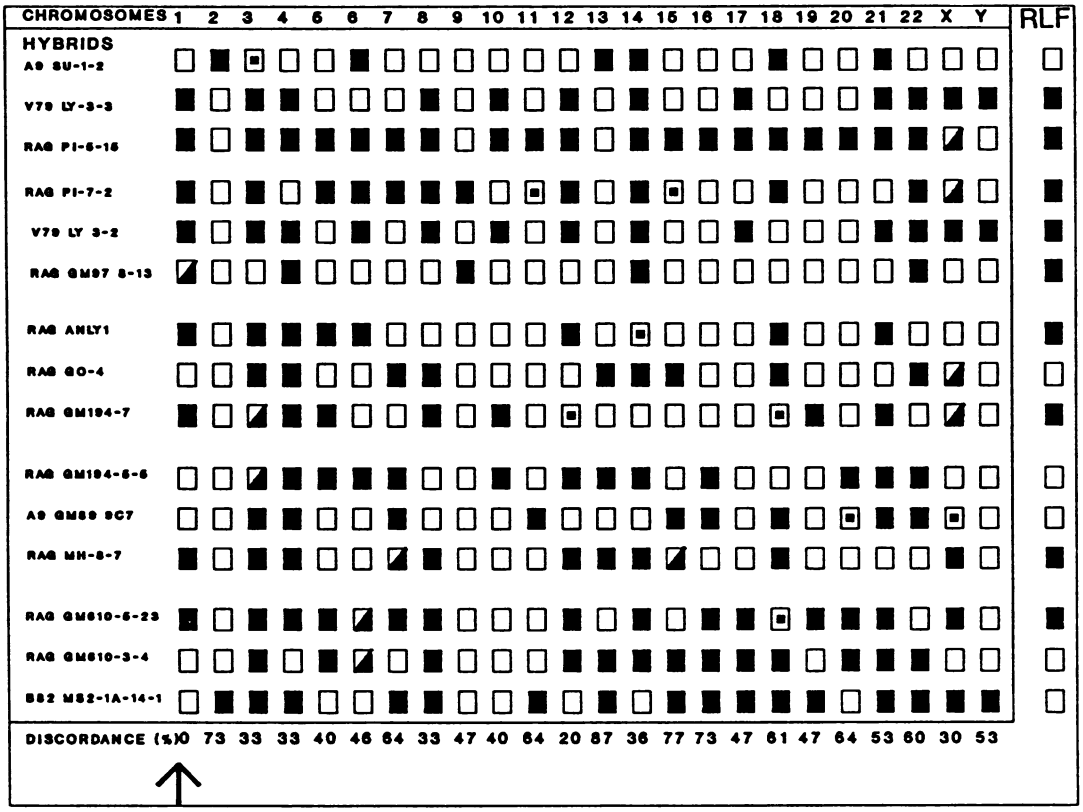

FIG. 6. Chromosomal localization of the *rlf* gene, determined by using a large panel of human × rodent somatic cell hybrids. The probe used for the hybridizations was 119118 (see Materials and Methods). The arrow at the bottom indicates the only human chromosome (chromosome 1) completely concordant with the hybridization result. Sublocalization of *rlf* to 1pter-q21 was also possible because cell line RAG GM97 8–13 has retained only this portion of chromosome 1. A filled box indicates the presence and an empty box indicates the absence of the indicated chromosome in a particular cell line. A half-filled box represents the presence of a well-characterized chromosome derivative, and a black dot in an open box represents a poorly characterized chromosome. Data from the latter two were not included in the mapping analysis. In the column labeled RLF, filled boxes indicate positive hybridization results and empty boxes indicate negative results.

we have analyzed the rearrangement leading to this fusion protein and found a physical linkage of the involved genes in normal DNA. Using somatic cell hybrids and large-scale restriction mapping with PFGE, we were able to demonstrate that *rlf* and L-*myc* reside within a 780-kb fragment on chromosome 1. This indicates that the *rlf*–L-*myc* fusion results from a small intrachromosomal rearrangement.

Although the chimeric *rlf*–L-*myc* mRNAs were found to be identical (35), the structures of the corresponding fusion genes are similar but not identical; the breakpoints in the two cases that we have analyzed differ on both the *rlf* and L-*myc* sides, suggesting that the mechanism of rearrangement is not sequence specific. PFGE analysis showed that the fusion gene in GLC28 contains regulatory sequences and (at least) one exon of *rlf* fused to a region 16 kb upstream of L-*myc*.

The intrachromosomal rearrangement leading to the fusion gene might occur in several ways. Because both cell lines with the rearrangement also show amplification of the fusion gene, the rearrangement may be associated with DNA amplification. However, because only a single rearranged band is seen in both cell lines with several digestions, the rearrangements must have occurred before DNA amplification. They could represent rearrangements associated with recombination of the initial amplification product, as shown in experimental amplification of the Chinese hamster adenylate deaminase gene (26). The rearrangements could also result from deletions of the germline counterparts of *rlf* or L-*myc*. Such amplification-associated deletions have been detected in experimental amplification of drug resistance genes (39) and also in amplifications of the N-*myc* gene in human neuroblastoma (25). If the *rlf*–L-*myc* rearrangements are associated with DNA amplification, they would resemble the amplification and rearrangement described for the c-*myc* and *pvt* genes in the COLO320DM cell line (43), in which rearrangement of *pvt* exon I 5′ of c-*myc* exon II has also apparently occurred prior to amplification.

The rearrangements may also have occurred as an earlier step in the pathogenesis of these SCLCs as the result of a deletion or an inversion between a *rlf* intron and a region upstream of L-*myc*. A deletion would require that *rlf* and L-*myc* normally reside in the same 5′-3′ orientation, and also that one allele of *rlf* sequences 3′ of the breakpoint be lost or integrated elsewhere. Our PFGE results showing similar reductions in the sizes of common fragments of *rlf* and L-*myc* in normal and rearranged DNA are in favor of this possibility. However, further studies are needed to unequivocally demonstrate the mechanism leading to these rearrangements.

Both the *rlf* and L-*myc* genes reside at 1p32, a chromosomal region implicated in several other malignancies. In addition to the t(1;14) translocation and *tal*^{d} deletion in T-cell acute leukemia (9), aberrations of the 1p32 region have also

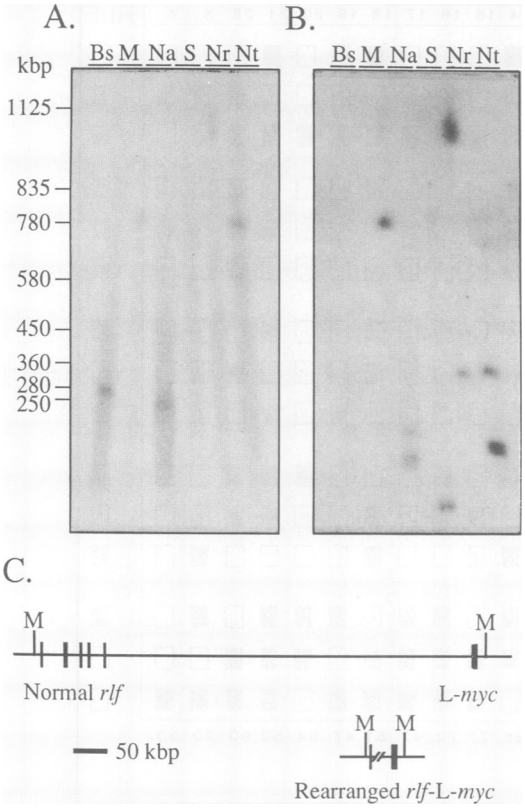

FIG. 7. PFGE analysis with indicated enzymes of normal DNA hybridized to the 119118 *rlf* probe (A) or to the L-*myc* second-exon probe (B). Size markers are as in Fig. 2. Bands common to both probes are seen in *Mlu*I (780 kb) and in *Nru*I (1,050 kb) digests. (C) Schematic diagram showing the structure of normal DNA and of the rearranged DNA of GLC28. The exact number of *rlf* exons and location of *rlf* and L-*myc* in normal DNA are not known and are only shown schematically. Bs, *Bss*HII; M, *Mlu*I; Na, *Nae*I; S, *Sal*I; Nr, *Nru*I; Nt, *Not*I.

been detected in human cutaneous malignant melanomas (3) and in neuroblastomas. A high percentage of neuroblastomas contain deletions of the short arm of chromosome 1 (19, 33), and molecular analysis has placed the majority of the breakpoints close to L-*myc* (24). It will be interesting to further explore the possible involvement of *rlf* or L-*myc* in these events.

The *rlf*–L-*myc* fusion gene in SCLC represents another DNA aberration detectable only with molecular analysis and thus resembles the *tal*^{d} deletion (9) and the rearrangement of the PTC oncogene in thyroid carcinomas (21). These findings suggest that intrachromosomal rearrangements may represent a large group of specific abnormalities previously undetected due to limitations of resolution of chromosomal analyses. We are currently investigating the incidence of the *rlf*–L-*myc* rearrangement from primary lung tumors and also from other malignancies with abnormalities in this region.

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