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Transcriptionally active c-*myc* oncogene is contained within NIARD, a DNA sequence associated with chromosome translocations in B-cell neoplasia

[NIARD (non-immunoglobulin-associated rearranging DNA)/transcription activation by chromosome translocation]

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ABSTRACT **NIARD (non-immunoglobulin-associated rearranging DNA) is located on mouse chromosome 15 at the break point of a commonly observed translocation event involving chromosomes 15 and 12 in murine plasmacytomas. The human cellular analogue of the v-*myc* oncogene of avian myelocytomatosis virus, strain MC-29, is known to reside on the distal end of human chromosome 8 and has been observed to translocate to chromosome 14 in Burkitt lymphomas. Using a cDNA clone specific for the transcript of the human c-*myc* gene (H c-*myc*), we show that the mouse c-*myc* (M c-*myc*) gene is contained within NIARD. NIARD-associated chromosome translocations occurred 1.3–2 kilobases (kb) 5′ of the mouse c-*myc* gene where NIARD recombines with the switch region of the C_α immunoglobulin gene in various murine plasmacytomas. The mouse c-*myc* encoding region within NIARD spanned <2.4 kb of DNA and expressed a low level of a 2.3-kb polyadenylylated RNA in BALB/c spleen. Increased (10- to 20-fold) levels of rearranged mouse c-*myc* transcripts (i.e., ≈1.8–2.1 kb) were observed in plasmacytomas that have NIARD-associated chromosome translocations. Human c-*myc* and NIARD probes detected DNA rearrangements of human c-*myc* in four of seven Burkitt lymphomas. DNA sequences adjacent to the human c-*myc* gene recombined with the C_μ immunoglobulin gene locus on chromosome 14 in several Burkitt lymphomas. The activation of the c-*myc* oncogene by chromosome translocation implicates its involvement in B-cell oncogenesis.**

Non-immunoglobulin-associated rearranging DNA (NIARD) is a unique DNA sequence in the mouse genome that has recently been shown to generate aberrantly rearranged C_α immunoglobulin genes in various BALB/c plasmacytomas (1–3). The NIARD sequence recombines with the switch (S) region of the C_α gene but does not have any striking sequence homology with it (refs. 1–3; unpublished data). NZB plasmacytomas appear to prefer rearranging NIARD outside of the C_H gene locus (1). NIARD rearrangements were not observed in the normal B-lymphocyte genome (1–3) and appear to be rare events in T-cell lymphomas and other neoplastic cells not of B-lymphocyte origin (refs. 1–3; unpublished data). NIARD has been localized to chromosome 15 (2, 4) and is associated with a specific chromosome translocation [*T* (12; 15)] (1, 4) which is frequently observed in plasmacytomas (5–7). A similar type of translocation event is associated with Burkitt lymphomas (8, 9) wherein the distal end of chromosome 8 translocates to the heavy chain locus residing on chromosome 14 (10, 11). Interestingly, the human cellular analogue of the v-*myc* oncogene of the avian MC-29 retrovirus has recently been localized to the distal end of human chromosome 8 (12).

In this report, we demonstrate that (*i*) NIARD contains the mouse analogue of the MC-29 oncogene (c-*myc*); (*ii*) increased amounts of structurally different mouse c-*myc* (i.e., NIARD) transcripts are produced in plasmacytomas compared to normal mouse spleen; (*iii*) human c-*myc* and NIARD probes detect human c-*myc* DNA rearrangements in Burkitt lymphomas and some of these appear to result from chromosome 8 translocations to the C_μ immunoglobulin locus on chromosome 14 (12).

MATERIALS AND METHODS

RNA Isolation. Total poly(A)$^+$RNA and membrane bound polysomal poly(A)$^+$RNA were isolated from plasmacytomas and cultured myeloma cells essentially as described (13, 14). BALB/c spleen and liver total poly(A)$^+$RNAs were prepared by extraction in guanidinium thiocyanate and phenol at 65°C as described (15).

Hybridizations. Filters for Southern hybridizations were prepared as described by Southern (16). For genomic DNA blot hybridizations, filters were prehybridized in 5× standard saline citrate (NaCl/Cit; 1× is 0.15 M NaCl/0.015 M Na citrate)/5× Denhardt solution (0.1% polyvinylpyrrolidone/0.1% Ficoll/0.1% bovine serum albumin)/50% deionized formamide/5 mM EDTA/0.2% $NaDodSO_4$ at 42°C for 4 hr. Hybridizations were performed in 5× NaCl/Cit containing, 10% dextran sulfate, 1× Denhardt solution, 25 mM Na P_i (pH 6.5), 0.2% $NaDodSO_4$, 40% deionized formamide, 5 mM EDTA, salmon sperm DNA at 50 μg/ml, poly(rA) at 10 μg/ml, and label equivalent to 1–2 × 10^6 cpm/ml for 16–24 hr at 40–44°C. Filters were washed twice at 55–58°C for 1 hr in 0.2 × NaCl/Cit containing 0.2% $NaDodSO_4$ except where indicated and exposed to x-ray film with an intensifier screen.

RNAs were denatured at 65°C for 10 min in 50% formamide/2 mM Mops [(3-(*N*-morpholino)propanesulfonic acid]/50 mM NaOAc/10 mM EDTA, pH 7.0 (Mops buffer) and applied to a 1% agarose gel prepared in 6% formaldehyde/Mops buffer; they were transferred to nitrocellulose essentially as described by Thomas (17). Prehybridization and hybridization procedures were performed essentially as described above for genomic Southern blots. Single-stranded DNA hybridization probes were prepared in M13 phage vectors mp8 and mp9 essentially as described by Hu and Messing (18).

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Abbreviation: NIARD, non-immunoglobulin-associated rearranging DNA; S region, switch region; NaCl/Cit, standard saline citrate; bp, base pair(s); kb, kilobase(s).

RESULTS

Genetic Rearrangement Sites Within the NIARD Sequence. NIARD was molecularly cloned by virtue of its fusion to an aberrantly rearranged C_α immunoglobulin heavy chain gene isolated from a partial *Eco*RI library of J558 myeloma DNA (1). The entire NIARD sequence 5′ of the S_α region in J558 α4 originates from chromosome 15 and appears to recombine directly with S_α via a chromosome translocation event (refs. 1, 2, and 4; unpublished data). The recombination sites within NIARD for J558 (1, 3) and two other IgA-producing myelomas (M167 and M603) (2) are shown in Fig. 1*A*. Studies on a larger panel of IgA- and non-IgA-producing plasmacytomas indicated that all retain the NIARD *Bam*HI site immediately 5′ of the S_α sequence in J558 α4 (Fig. 1*A*) but several lose the NIARD *Sst* I site ≈700 base pairs (bp) 3′ of this *Bam*HI site (ref. 1; unpublished data). The 700-bp *Bam*HI–*Sst* I fragment in J558 α4 is identical to its germ-line counterpart on mouse chromosome 15 (1, 4). Molecular clones of the NIARD rearrangement present in MPC-11 cells (which is not associated with C_α or any other C_H gene) also retain this *Bam*HI site and contain all the 5′ flanking NIARD DNA observed in the J558 α4 clone (unpublished data). Therefore, most of the NIARD information 3′ of the *Bam*HI site is dispensable for any NIARD functions that are required subsequent to the rearrangement event.

Mouse Homologue of Human c-*myc* Resides in the NIARD Sequence. Single and double restriction endonuclease digests of J558 α4 DNA were hybridized to a cDNA clone (pRyc-7.4) of the human c-*myc* gene transcript (Fig. 2). These and other mapping studies located the pRyc-7.4 hybridizing sequence within NIARD (Fig. 1*A*). The NIARD plasmid pα25BH3.4 (1)

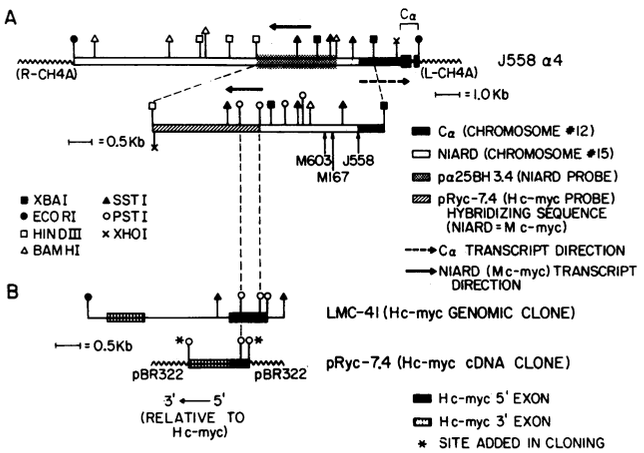

FIG. 1. (A) Location and direction of transcription of mouse c-*myc* gene within the NIARD sequence of genomic clone J558 α4. ■, C_α immunoglobulin gene and flanking sequences on chromosome 12; □, NIARD sequence originating from chromosome 15; ▨, and ▨, NIARD plasmid pα25BH3.4 and mouse c-*myc* gene (as defined by its homology to human c-*myc* cDNA clone, pRyc-7.4), respectively, contained within the NIARD sequence. pRyc-7.4 was isolated from a cDNA library prepared from K562 cells (unpublished data) by hybridization with the 3′ exon of a human c-*myc* genomic clone (19). pRyc-7.4 contains a complete 3′ c-*myc* exon and 60% of the 5′ c-*myc* exon. The cDNA was cloned into the *Pst* I site of pBR322 by poly(dC)·poly(dG)tailing. The recombination sites within NIARD for the J558 (1, 3), M603 (2), and M167 (2) plasmacytomas are indicated as are the direction of NIARD (i.e., mouse c-*myc*) and C_α transcripts. The C_α gene is not transcribed in the aberrantly rearranged J558 α4 gene but would be transcribed from the opposite strand as NIARD (as so indicated) if an appropriate immunoglobulin variable region (V_H) promoter were present instead of NIARD. (*B*) Location of exons in human c-*myc* genomic clone LMC-41 (19) and human c-*myc* cDNA clone pRyc-7.4. The positions of conserved *Pst* I sites present in both NIARD (mouse c-*myc*) and human c-*myc* are indicated by dashed lines.

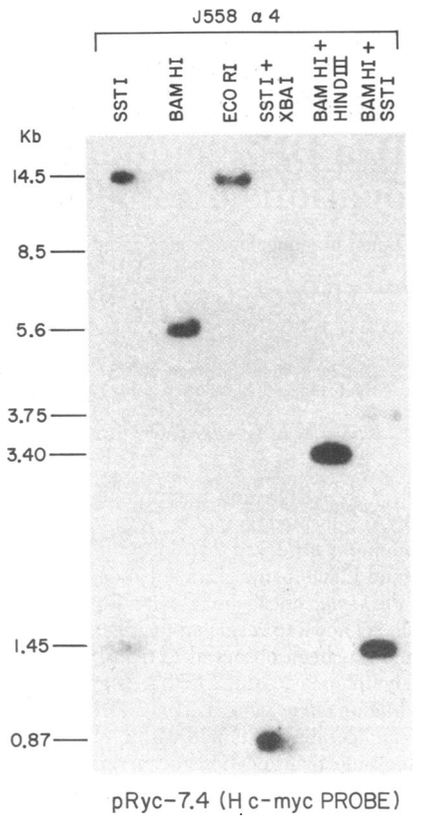

FIG. 2. Southern blot of restriction endonuclease digests of J558 α4 hybridized to human c-*myc* cDNA clone pRyc-7.4; 0.25 μg of digested J558 α4 was applied to each lane. Filter was pretreated for 2 hr at 65°C in 3× NaCl/Cit containing 5× Denhardt solution, 5 mM EDTA, and 0.1% $NaDodSO_4$. For ≈16 hr at 65°C hybridization was performed in 3× NaCl/Cit containing 1× Denhardt solution, 5 mM EDTA, salmon sperm DNA at 50 μg/ml, poly(rA) at 10 μg/ml, and 0.1% $NaDodSO_4$ with ≈1 × 10^5 cpm of nick-translated pRyc-7.4 per ml (≈1.5 × 10^8 cpm/ml). The filter was subsequently washed for 1 hr in two changes of 0.1× NaCl/Cit containing 0.1% $NaDodSO_4$ at 60°C, blotted dry, and autoradiographed for 36 hr with an intensifier screen.

appeared to contain the entire mouse c-*myc* hybridizing sequence. Cleavage with *Sst* I separated the pRyc-7.4 hybridizing region into two bands of different intensities (Figs. 1 and 2). *Pst* I restriction sites appeared to be conserved at the 5′ ends of both the mouse and human c-*myc* genes (Fig. 1).

The c-*myc* gene is not perfectly conserved in mouse and man. The intensity of hybridization of pRyc-7.4 to NIARD decreased with filter washes at higher stringency (i.e., 50–55°C to 60–65°C) (data not shown). The human c-*myc* gene [≈3.3 kilobases (kb)] has been shown to possess two similar sized exons (≈750 bp each) separated by a ≈1.8-kb intron (19). The mouse c-*myc* gene within our NIARD sequence appeared to be no more than 2.4 kb long. This conclusion is based in part on the complete absence of hybridization of the pRyc-7.4 probe beyond the *Xho* I site at the 3′ end of pα25BH3.4. This would define the end of the 3′ exon as being no further than this *Xho* I site. By comparison to a human c-*myc* genomic clone (19), the 5′ end of the mouse c-*myc* gene within NIARD is tentatively localized to two *Pst* I restriction sites which appear to be conserved at the 5′ end of both mouse and human c-*myc* genes (Fig. 1). The assignment of the mouse c-*myc* 5′ end is also in agreement with blotting experiments performed with plasmacytoma poly(A)$^+$RNAs (see below). The mouse c-*myc* gene, residing in the translocated NIARD sequence may have a smaller intron than the human c-*myc* gene but proof of this idea requires more confirmation.

Activation of NIARD Transcripts in Plasmacytomas. RNA blots were performed to identify NIARD- (i.e., mouse c-*myc*)

and human c-*myc*-related transcripts in normal mouse tissue (i.e., BALB/c spleen and liver) and mouse plasmacytomas and in HeLa cells, respectively. Four representative plasmacytomas were chosen for these experiments: J558 (α), H2020 (α), MPC-11 (γ2b), and PC3741 (μ). Both J558 and H2020 have NIARD rearrangements and both involve the S_α region on chromosome 12 (1, 4). The NIARD rearrangement in MPC-11 does not involve any sequences within the C_H gene locus (ref. 1; unpublished data). PC3741 (an NZB μ-producing plasmacytoma) does not appear to have any rearrangement within ≈10 kb 5′ and ≈7 kb 3′ of the NIARD mouse c-*myc* gene (1).

Total cell poly(A)$^+$ and membrane-bound polysome poly(A)$^+$ RNAs were isolated from these four plasmacytomas and total poly(A)$^+$RNA was prepared from BALB/c spleen and liver. RNA blotting results with total poly(A)$^+$RNA from plasmacytomas were identical to data obtained with poly(A)$^+$RNAs isolated from polyribosomes. The polysomal poly(A)$^+$RNAs were ≈3-fold purified relative to total cell poly(A)$^+$RNAs (13, 14). Hybridization results obtained with nitrocellulose filter blots of formaldehyde/agarose gel-fractionated, formamide-denatured poly(A)$^+$RNAs to pα25BH3.4 (NIARD probe) and pRyc-7.4 (human c-*myc* probe) are shown in Fig. 3. NIARD (mouse c-*myc*) and human c-*myc* probes detected identical-sized poly(A)$^+$RNA species; low levels of a 2.3-kb mouse c-*myc* (i.e., NIARD) transcript were present in spleen and liver. The HeLa cell c-*myc* transcript was slightly smaller than the normal mouse NIARD transcript (i.e., ≈2.15 kb compared to ≈2.3 kb). Increased amounts (10- to 20-fold) of NIARD transcripts (i.e., mouse c-*myc*) appeared in all four plasmacytomas tested. Plasmacytomas that had a NIARD rearrangement (with or without S_α) contained new transcripts 1.8–2.1 kb long. PC 3741 (which displays no obvious NIARD rearrangement) contained a 2.3-kb NIARD transcript and a ≈2.15-kb transcript that migrated with the HeLa cell c-*myc* transcript.

NIARD and C_α Are Transcribed from Opposite DNA Strands. As indicated in Fig. 1, the mouse c-*myc* gene within NIARD is oriented opposite to the direction of the C_α coding region. In order to confirm this result and to estimate the limits of the NIARD (mouse c-*myc*) transcripts, we prepared single-stranded DNA probes in M13 phage cloning vehicles for RNA blot hybridizations. The entire upper strand of the 3.4-kb *Hin*dIII–*Bam*HI fragment in pα25BH3.4 (Fig. 4) was inserted into M13 mp9. The upper strand of a 230-bp *Sst* I–*Bam*HI fragment at the 3′ end of the pα25BH3.4 insert and a 200-bp *Hpa* II fragment centrally located within the 3′ adjacent 700-bp *Bam* HI–*Sst* I fragment in J558 α4 were independently cloned by treatment with Klenow DNA polymerase followed by blunt end ligation into the *Hin*cII site of M13 mp8. Only the 3.4-kb *Hin*dIII–*Bam*HI probe detected the NIARD (mouse c-*myc*) and human c-*myc* [HeLa cell poly(A)$^+$RNA] transcripts. The 230-base *Sst*I–*Bam*HI probe only hybridized to a 1.45-kb species of poly(A)$^+$RNA not observed with the human c-*myc* probe. The 200-base *Hpa* II fragment probe gave a pattern identical to that of the 230-base *Sst*I–*Bam*HI probe (data not shown).

The simplest interpretation of the experiment presented in Fig. 4 is that the rearranged mouse c-*myc* transcripts initiate within the 3.4-kb *Hin*dIII–*Bam*HI NIARD fragment. The lower molecular weight transcripts detected by the *Bam*HI–*Sst* I and *Hpa* II fragment probes may originate elsewhere in the mouse genome. Interestingly, an unrelated 200-bp *Hpa* II fragment located 350 bp 3′ of the mp8 SS *Hpa* II probe described in Fig. 4 detected a small family of homologous sequences in the mouse genome (unpublished data). In the case of the rearranged mouse c-*myc* RNAs, it would seem unlikely that other mouse c-*myc* exons are located near the J558 α4 recombination site and that these would be attached to other mouse c-*myc* exons located within the pα25BH3.4 sequence (Fig. 1) by differential splicing because of the following. (*i*) Several *Pst* I sites within the 3.4-kb *Bam*HI–*Hin*dIII fragment also were observed at the 5′ end of the 5′ human c-*myc* exon (19) (Fig. 1), suggesting that the entire mouse c-*myc* encoding region is within this 3.4-kb DNA fragment. (*iii*) The NIARD recombination sites in M167 and M603 are considerably 5′ of the J558 rearrangement site (2) (Fig. 1). Therefore, if additional exons exist near the J558 α4 recombination site these would be absent in the NIARD rearrangements observed in M167 and M603. (*iii*) The NIARD rearrangement in MPC-11 generates the same size class of poly(A)$^+$RNA observed in J558 and H2020 even though NIARD does not recombine with S_α in MPC-11 (1), suggesting that C_α flanking sequences do not contain any mouse c-*myc* exons.

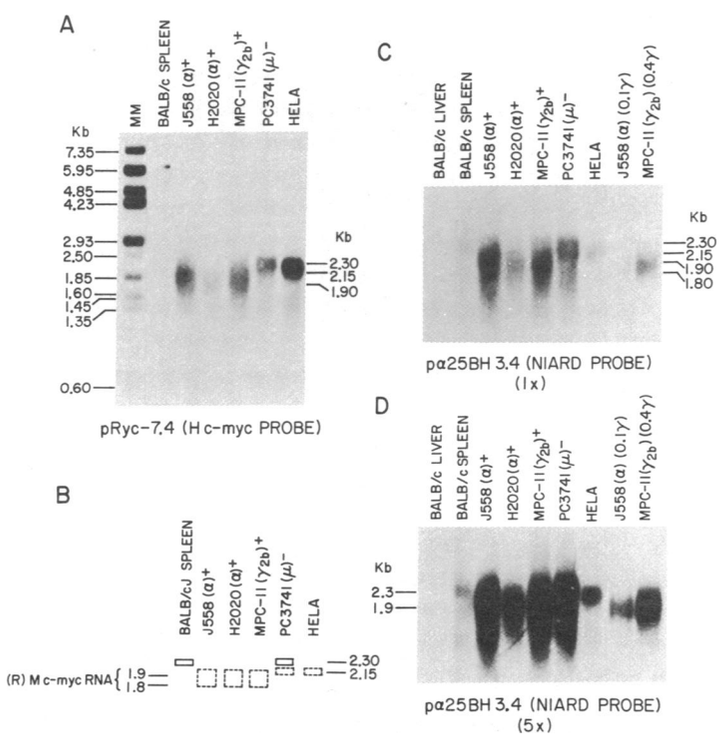

FIG. 3. Hybridization of RNA blots of various poly(A)$^+$RNAs to either pRyc-7.4 (human c-*myc*) or pα25BH3.4 (mouse c-*myc*). Lane MM in *A* represents DNA molecular weight markers prepared by digestion of pα25BH3.4 with various restriction endonucleases. DNA markers were treated in the same manner as poly(A)$^+$RNAs. The internal DNA markers were observed to migrate in a manner identical to that of rRNA species in the denaturing formaldehyde/agarose gel system. +, existence of a NIARD rearrangement; −, absence of an obvious NIARD rearrangement (i.e., in the case of PC 3741). (*B*) Schematic representation of the data *A*, *C*, and *D*. (R) M c-*myc* RNA in *B*, rearranged mouse c-*myc* RNA sizes. (*D*) Five-times-longer exposure of *C*. Except where indicated, 5 μg of BALB/c liver, spleen, and HeLa cells and 2 μg of J558, H2020, MPC-11, and PC 3741 membrane-bound polysome poly(A)$^+$RNAs were added in all cases.

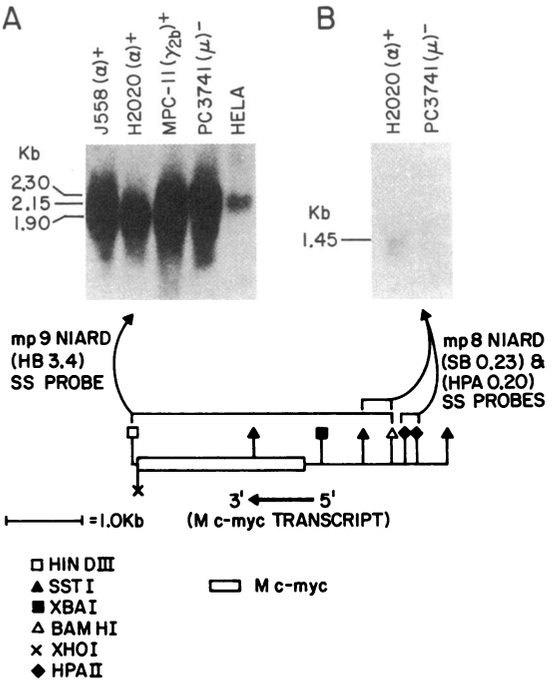

FIG. 4. Hybridization of RNA blots of poly(A)$^+$RNAs to single-stranded DNA probes prepared in M13 phages mp8 and mp9. HB 3.4, *Hin*dIII–*Bam*HI 3.4-kb upper strand, SB 0.23, *Sst* I–*Bam*HI 230-base upper strand; HPA 0.20, *Hpa* II 200-base upper strand. Results are presented for HB 3.4 and SB 0.23 single-stranded probes. Data with HPA 0.20 single-stranded probe were identical to those with SB 0.23 probe. The single-stranded DNA probes were at $\approx 2 \times 10^6$ cpm/ml ($\approx 4 \times 10^8$ cpm/μg) in both *A* and *B*.

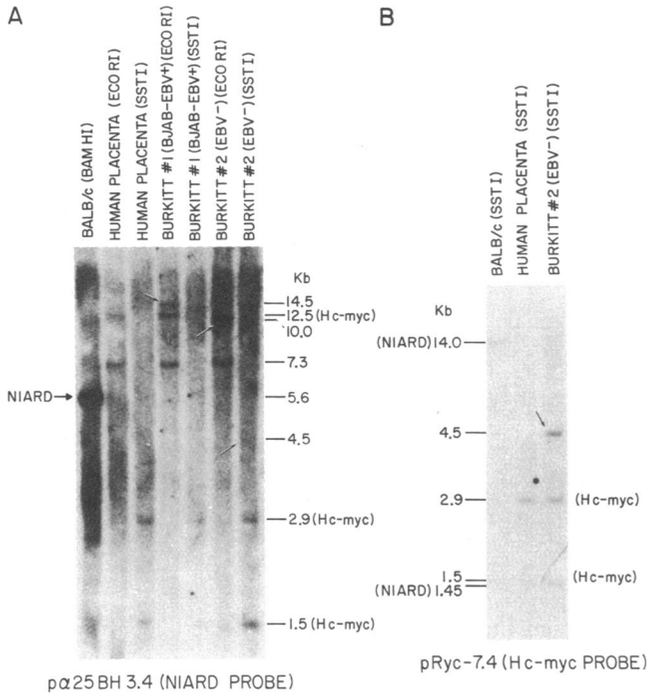

FIG. 5. Southern blots of restriction endonuclease digests of BALB/c liver, human placenta, and two Burkitt DNA samples hybridized to either pα25BH3.4 or pRyc-7-4 probes. Burkitt #1, BJAB (GC) [American Burkitt lymphoma superinfected with Epstein–Barr virus (EBV)]; Burkitt #2, EBV$^-$ Loucks Burkitt sample of African origin. Human placenta DNAs in *A* and *B* are unrelated samples. Arrows indicate the presence of bands unique to the Burkitt DNA sample detected by either human c-*myc* or mouse c-*myc* probes. Bands corresponding to the human c-*myc* gene (19) and NIARD (1) are so indicated.

Context of the Human c-*myc* Gene in Burkitt Lymphoma. The human c-*myc* gene has been observed to translocate along with the distal end of chromosome 8 to the human heavy chain immunoglobulin gene locus on chromosome 14 (12). Therefore, we investigated whether DNA rearrangement events occur in close proximity to the human c-*myc* gene in a panel of Burkitt lymphomas. Fig. 5 displays two Southern blots of BALB/c liver, human placenta, and two Burkitt lymphoma cell lines hybridized to pα25BH3.4 (NIARD probe) and pRyc-7.4 (human c-*myc* probe). These results are summarized as follows. (*i*) NIARD and human c-*myc* uniquely detect each other in their corresponding genomes. (*ii*) The human c-*myc* gene displays an altered context in both Burkitt DNA samples. Because human contextual differences also could be due to genetic polymorphisms, we analyzed a larger panel of Burkitt cell lines. We could detect c-*myc* rearrangements in only two (CA46 and JD38IV) of the five additional Burkitt lymphoma cell lines (20); in both of these, a new 22-kb *Bam*HI band was present (Fig. 6*B*). Since *Bam*HI cuts outside of the human c-*myc* gene and outside of the unrearranged human C_μ gene (21), we also rehybridized the same filter with a 1.2-kb *Eco*RI genomic probe of the C_μ gene that detects the first two exons and part of the third exon of C_μ. The two 22-kb bands hybridized with the C_μ probe (Fig. 6A, lanes 4 and 7). Cleavage with *Hin*dIII and *Eco*RI, confirmed that, in cell lines CA46 and JD38II, the human c-*myc* gene translocated in close proximity to the human C_μ gene (data not shown).

DISCUSSION

We have shown that the C-*myc* oncogene is adjacent to the site of a chromosome translocation that is commonly observed in mouse and human B-cell neoplasia. These translocations appear to be mediated by a DNA sequence originally defined as NIARD (1, 4). Mouse plasmacytomas display 10- to 20-fold increased levels of mouse c-*myc* (or NIARD) transcripts compared to normal mouse spleen cells. The actual hybridization intensity differences presented in Figs. 3 and 4 represent a 20- to 40-fold increase in NIARD transcripts. However, most plasmacytomas containing NIARD rearrangements display a slightly increased copy number of rearranged NIARD sequences compared to germ-line controls (1) (i.e., to 2- to 3-fold higher). We presume

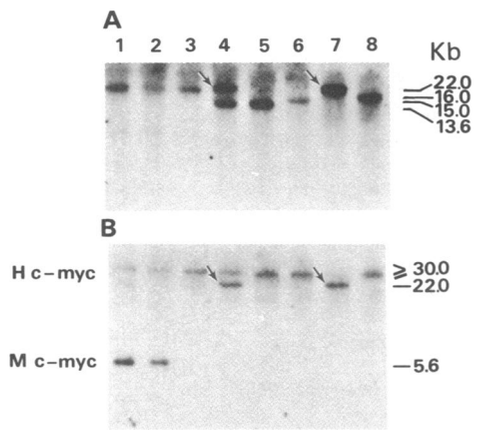

FIG. 6. Southern blotting analysis of *Bam*HI-digested DNA derived from different Burkitt lymphoma cell lines. Lanes: 1 and 2, two independent somatic cell hybrids between mouse myeloma cells (21) and P3 HR-1 Burkitt lymphoma cells (12); 3, P3HR-1 Burkitt cell line; 4–7, Burkitt lymphomas CA46, AD876, EW36, and JD38-IV cell lines, respectively (20); 8, PAF, simian virus 40-transformed human fibroblastic cell line. (*A*) Hybridization with a C_μ probe. The C_μ probe was washed off and the filter was rehybridized to the pRyc-7.4 probe (*B*). Arrows in *A* and *B* indicate the identical-sized band detected by both the pRyc-7.4 and the C_μ probes.

that this is due to the hypotetraploidy of these tumors and, therefore, a higher cell copy number of the translocated chromosome. Consequently, we choose to estimate our RNA blot data as indicating a 10- to 20-fold increase of NIARD transcription in plasmacytomas. A more heterogeneous profile of smaller transcripts (i.e., 1.8–2.1 kb compared to ≈2.3 kb for spleen poly(A)$^+$RNA] is observed in plasmacytomas harboring a NIARD rearrangement. In addition, mouse spleen appears to contain a higher level (≈5-fold) of NIARD transcripts than mouse liver (Fig. 3*D*). This result may suggest that NIARD is preferentially transcribed in lymphocytes. In addition, the level of c-*myc* transcripts (compared to the BALB/c spleen control) appears to be higher in mouse plasmacytomas than in HeLa cells (Fig. 3 *A*, *C*, and *D*). High levels of c-*myc* transcripts may be a characteristic of neoplastic B cells. However, more work will be required to assess the general significance of our results.

Our findings strongly suggest that the c-*myc* gene in mouse and man is involved in B-cell oncogenesis. We have also observed rearrangements of the human c-*myc* gene in four of seven Burkitt lymphoma cell lines and demonstrated that in at least two of these cell lines the rearrangement involves the C_μ gene locus on human chromosome 14. Chromosome translocation of the c-*myc* gene to the chromosome harboring the heavy chain immunoglobulin gene family in mouse and man results in significantly higher transcriptional activity of the c-*myc* gene. The locations of DNA rearrangement sites within NIARD and RNA transcripts detected by single-stranded DNA probes would collectively argue that a promoter site is somehow newly activated or more efficiently utilized subsequent to a NIARD translocation event. This promoter may exist in a repressed state in its former context on chromosome 15 and this repression may be lifted by either the translocation event or some other structural DNA modification such as mutation. Alternatively, this secondary promoter may be enhanced by sequences on chromosome 12. If enhancement is occurring, these enhancer elements may reside within the immediate boundaries of the C_α gene. However, this sequence environment does not appear to be obligatory for enhanced transcription of new mouse c-*myc* transcripts because (*i*) enhanced transcription of NIARD occurs in the PC 3741 plasmacytoma which displays no obvious NIARD rearrangement (1) and (*ii*) NIARD rearrangements need not be associated with the C_H gene locus because increased levels of new transcripts are evident in MPC-11. Clearly, more refined structural work is needed to completely characterize these new transcripts and their potential role in B cell neoplasia.

In bursal lymphomas, the avian c-*myc* gene is activated by the integration of an avian leukosis virus promoter (22–24). The v-*myc* gene of avian myelocytomatosis virus (strain MC-29) has been found to generate foci of transformed cells when transfected into NIH 3T3 cells (25) whereas the avian c-*myc* gene, activated by the integration of an avian leukosis virus promoter (22–24), does not transform NIH 3T3 cells (26).

Preliminary results indicate that the J558 α4 genomic clone does not yield foci upon transfection into NIH 3T3 cells compared to a high level of foci observed with the cloned T24 human bladder carcinoma oncogene (ref. 27; unpublished data). Therefore, the human c-*myc* and mouse c-*myc* genes may represent a class of oncogenes that are incapable of rapid transformation of NIH-3T3 cells via DNA transfection. Further studies are obviously required to confirm and support this important observation. The increased expression or production of structurally altered c-*myc* gene transcripts could represent a step of a complex pathway resulting in the activation of dominant acting c-*onc* genes eventually yielding neoplasia. We propose that DNA sequences with properties analogous to those of NIARD exist on chromosomes other than human chromosome 8 and mouse chromosome 15. The activation of cellular oncogenes by a NIARD-like chromosome translocation may constitute a general mechanism of cellular neoplastic transformation.

Note Added in Proof. Our recent data now show that mouse c-*myc* contains at least three exons. The 5′-most exon is broken off by NIARD translocation.

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