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Molecular cloning of translocations involving chromosome 15 and the immunoglobulin C_α gene from chromosome 12 in two murine plasmacytomas

(class switching/oncogenesis)

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ABSTRACT **Expression of IgA by plasmacytomas occurs as a result of a DNA rearrangement that brings the variable region gene, V_H, a few kilobases 5′ to the constant region gene, C_α. In this study, we show that the allelic nonexpressed C_α gene also is rearranged in most plasmacytomas. Cloning, restriction mapping, heteroduplex analyses, and sequence analyses of the nonproductively rearranged C_α genes from two plasmacytomas, M603 and M167, have demonstrated that the nonproductive rearrangement occurs within the α switching region, S_α. In each case, the same DNA sequence has been joined to the 5′ side of C_α and we have termed this DNA "NIRD" (for nonimmunoglobulin rearranged DNA). Southern blotting analyses of genomic DNAs from various IgG-, IgM-, or IgA-producing plasmacytomas suggest that NIRD is rearranged in almost all plasmacytomas. However, NIRD rearranges to the S_α region only in IgA-producing cells, not in IgM or IgG producers. Cytogenetic evidence has shown that T(12;15) translocations are common in murine plasmacytomas. Immunoglobulin heavy chain genes are located on chromosome 12, and the translocation breakpoint in plasmacytomas occurs near the immunoglobulin genes. NIRD has been mapped to chromosome 15 by Southern blotting analysis of mouse–hamster cell lines, suggesting that the nonproductively rearranged C_α clones represent the T(12;15) translocations identified cytogenetically. Therefore, we have identified a region of DNA on chromosome 15 that is commonly rearranged in transformed mouse lymphocytes. We speculate on the significance of NIRD in neoplastic transformation of mouse lymphocytes.**

Murine plasmacytomas (also referred to as myelomas) have been observed to have specific chromosomal translocations in which the distal portion of chromosome 15 is translocated to either chromosome 12 or chromosome 6 (1). The immunoglobulin heavy chain and κ light chain gene families are located on chromosomes 12 and 6, respectively, and the translocational breakpoint on chromosome 12 occurs at the same region of the chromosome as do the immunoglobulin genes. Trisomy of chromosome 15 occurs in most murine T-cell and some B-cell leukemias. Based on these observations, it has been suggested that a cellular oncogene located on the distal portion of chromosome 15 may become abnormally activated, through translocation or triplication, and result in the neoplastic transformation of murine lymphoid cells (1).

One explanation for the involvement of the immunoglobulin loci in the chromosomal translocations is that immunoglobulin genes undergo DNA rearrangements in B cells. These rearrangements may predispose the immunoglobulin genes to undergo chromosomal translocation. Conceivably, translocation of immunoglobulin genes, which are actively transcribed in B cells, could increase the rate of transcription of genes residing near the recombinational breakpoint. There are two types of immunoglobulin gene rearrangements (reviewed in ref. 2). First, separate variable (V), diversity (D), and joining (J) gene segments are rearranged to create a functional V gene. These V-D-J or V-J joining events also are denoted V gene formations for the heavy and light chains, respectively. The second type of rearrangement, heavy chain or C_H switching, occurs among the closely linked C_H genes of which there are eight in the mouse (5′C_μ-C_δ-$C_{\gamma 3}$-$C_{\gamma 1}$-$C_{\gamma 2b}$-$C_{\gamma 2a}$-C_ε-C_α3′). The C_H genes determine the class or isotype of the antibody—e.g., C_μ makes IgM, C_α makes IgA, etc. All C_H genes except for C_δ have repetitive elements to their 5′ sides that are denoted switch (S) regions. For example, if the B cell is to switch from its initial expression of IgM to the synthesis of IgA, then the S_μ and S_α regions are joined, deleting the intervening DNA and placing V_H near C_α.

Immunoglobulin gene expression in B cells exhibits allelic exclusion; that is, in an individual B cell, only one heavy chain allele and one light chain allele are expressed. The chromosome containing the expressed immunoglobulin gene undergoes a productive DNA rearrangement. Often, the chromosome carrying the unexpressed gene also is rearranged and these are denoted nonproductive rearrangements.

In the study reported here, we analyzed the nature of nonproductive C_α rearrangements in murine plasmacytomas. Our results show that most IgA-producing plasmacytomas have a nonproductively rearranged C_α on a 13-kilobase (kb) *Eco*RI fragment. Analysis of nonproductively rearranged C_αs from two plasmacytomas, M603 and M167, demonstrated that the nonproductive rearrangements occurred within the S_α region in both cases and that the same 5′ sequence had been joined to C_α. We have denoted this sequence "nonimmunoglobulin rearranging DNA" or NIRD. Southern blotting analyses indicated that, whereas most IgA-expressing tumors have a NIRD-S_α rearrangement, IgG- or IgM-expressing tumors have different NIRD rearrangements. Thus, in IgA-producing plasmacytomas, both S_α regions are rearranged whereas in IgG-producing plasmacytomas, neither S_α region is rearranged.

Most significantly, we also have demonstrated that the NIRD sequence is located on mouse chromosome 15. Because the C_α gene originates from chromosome 12, these results demonstrate that the nonproductively rearranged C_α clones from M603 and M167 represent the junction of a translocation involving chro-

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Abbreviations: V, variable; D, diversity; J, joining; S, switch; kb, kilobase(s); NIRD, nonimmunoglobulin rearranging DNA; bp, base pair(s).

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mosome 15 and chromosome 12. Furthermore, the results of the Southern blotting analyses mentioned above define a small region on chromosome 15 that is almost always rearranged in murine plasmacytomas.

METHODS

Construction and Isolation of the CHM603α30 and CHM167α7 Clones. CHM603α30 was isolated from an *Eco*RI partial library of plasmacytoma M603 DNA in Charon 4A by screening with a C_α cDNA probe (3). CHM167α7 was isolated using a 5′ C_α genomic clone probe to screen a *Hae*III/*Alu* I-digested *Eco*RI linker library of plasmacytoma M167 DNA in Charon 4A (4).

DNA Blots. Ten micrograms of total genomic DNA was digested to completion with restriction endonuclease, size-separated on a 1% (wt/vol) agarose gel, and transferred to nitrocellulose filters (5). Filters were prehybridized, hybridized, and washed by using the conditions of Wahl *et al.* (6). Washed filters were exposed to preflashed Kodak XAR-5 film at −70°C with a Dupont Cronex Lightning Plus intensifying screen for 1–3 days.

Somatic Cell Hybrids. Somatic cell hybrids between Chinese hamster and mouse spleen cells (BALB/c) were generated and maintained as described (7). The mouse chromosome content of the hybrid clones was determined enzymatically and cytogenetically. Each hybrid clone was analyzed for the expression of 25 enzymes representing linkage group assigned to 16 of the 19 autosomes and the X chromosome as described (8, 9). Enzymatic, cytogenetic, and Southern blotting analyses were carried out on parallel cultures of each hybrid clone.

Preparation of NIRD Probe. The 440-base-pair (bp) *Msp* I fragment from α30 (see Fig. 2) was gel purified and subcloned into the *Cla* I site of pBR322.

Other Analyses. Heteroduplex analysis was carried out as described by Davis *et al.* (10). DNA sequence analysis was performed by the partial cleavage method of Maxam and Gilbert (11).

RESULTS

Most IgA-Producing Plasmacytomas Have Similar Nonproductive C_α Gene Rearrangements. In preliminary experiments, we analyzed genomic Southern blots of DNA from eight independently arising IgA-producing plasmacytomas with a 5′ C_α probe (unpublished data). As expected, sperm DNA digested with *Eco*RI revealed a germ-line 5′ C_α band at 9.5 kilobases (kb) whereas the different plasmacytomas gave variable-size fragments which corresponded to the productively rearranged C_α gene. Unexpectedly, however, a 13-kb *Eco*RI fragment was present in seven plasmacytomas (H8, M511, M167, M603, S63, T15, and Y5236) but was absent from one (W3207). This finding suggested that similar or possibly identical nonproductive C_α gene rearrangements occurred repeatedly and prompted us to clone and analyze examples of such nonproductively rearranged C_α genes.

The construction and screening of genomic libraries from the DNA of the plasmacytomas M603 and M167 have been described elsewhere (3, 4). Clones representative of the 13-kb *Eco*RI fragment from M603 (CHM603α30) and M167 (CHM167α7) are shown in Fig. 1.

Nonproductive C_α Clones α7 and α30 Are Identical by Heteroduplex Analysis. Heteroduplex analyses of the M603 nonproductively rearranged C_α clone α30 with the germ-line C_α clone α29 indicated that the two clones are homologous throughout the C_α gene region and for about 2.6 kb 5′ to the C_α gene (Fig. 1). Thus, there has been a rearrangement 2.6 kb

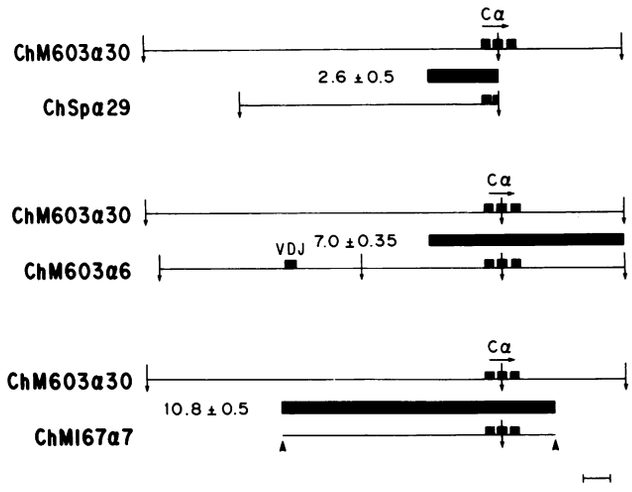

FIG. 1. Comparison of CHM603α30 to other C_α clones by heteroduplex analysis. Homology between α30 and CHSpα29 (a germ-line C_α clone), CHM603α6 (containing the expressed C_α gene from plasmacytoma M603), and CHM167α7 is shown by solid bars (sizes shown in kb). Natural *Eco*RI sites are indicated by arrows; linker *Eco*RI sites are indicated by triangles. CHM167α7 contains 3.6 kb of additional DNA 3′ to the C_α gene which is not shown in the figure because our blotting studies indicate that it is a cloning artifact and it does not occur 3′ to the C_α gene in the M167 genome (data not shown). Scale marker = 1 kb.

5′ to the C_α gene in α30 and the rearrangement occurs within the S_α region. Heteroduplex analyses comparing the α30 clone to the productively rearranged M603 C_α clone α6 (3) revealed that the 5′ half of α30 is not homologous to the 5′ portion of α6 and thus demonstrate that in α30 the sequences upstream from the S_α sequences are neither J_H sequences nor M603 V_H sequences. However, when the two nonproductively rearranged C_α clones, α30 and α7, were compared to one another by heteroduplex analysis, homology was observed not only throughout the C_α genes but also in their 5′ flanking sequences for at least 7.5 kb. These results therefore demonstrate that the nonproductively rearranged C_α genes in the M603 and M167 plasmacytomas have identical or similar DNA sequences joined to the C_α gene. This sequence, of unknown origin, is denoted NIRD.

NIRD Sequences Are Joined to the S_α Region. In order to characterize the sites at which NIRD sequences and the germ-line C_α flanking sequences were joined, we determined the DNA sequences at the boundaries between these two DNAs. Fine structure restriction mapping of the two clones in a 1.5-kb region surrounding the S sites (Fig. 2) confirmed the homology observed by heteroduplexing and led to the sequence-determination strategies shown. The sequences illustrated in Fig. 3 demonstrate that the S_α recombination point in the two clones differs by 94 bp. In addition, clone α7 contains approximately 70 bp more NIRD sequences than does clone α30. Thus, although clones α7 and α30 are homologous by heteroduplex analyses, they join NIRD and germ-line C_α flanking sequences at slightly different points.

Because clones α7 and α30 both have recombinational breakpoints within the S_α region, it is likely that the rearrangement was due to a nonproductive C_H switching event. Indeed, the S_α recombination breakpoint from α30 lies only 2 bp away from the S_α site used in generating the expressed α gene in the plasmacytoma T15 (12).

Most IgA-Producing Plasmacytomas Have Nonproductive α Genes That Contain NIRD. The identity of the NIRD sequence in the M167 and M603 nonproductively rearranged C_α

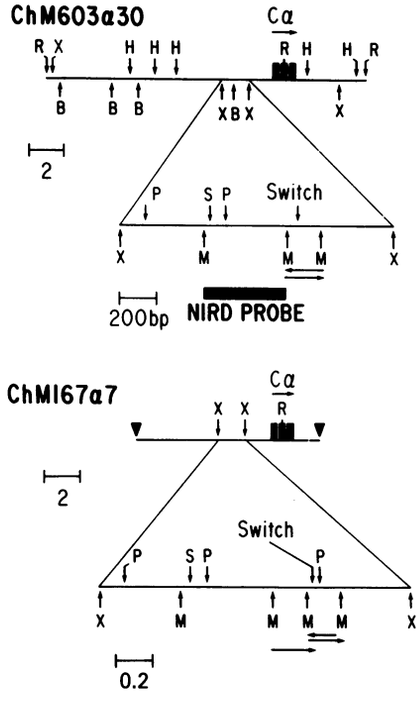

FIG. 2. Restriction map of α30 and α7 in the vicinity of the S region. The regions subjected to sequence analyses are indicated by arrows. Both clones contain one additional *Msp* I site that is not mapped. The solid bar indicates the 440-bp *Msp* I fragment used as a NIRD probe. The portion outside of the 1.7-kb *Xba* fragment in α7 was not mapped. R, *Eco*RI; X, *Xba* I; P, *Pst* I; M, *Msp* I; S, *Sac* I; B, *Bam*HI; H, *Hin*dIII. Scale markers are in kb.

genes led us to subclone a DNA fragment from this region to use as a probe in the analysis of other IgA-producing plasmacytomas to determine whether their nonproductive C_α rearrangements use NIRD sequences. A 440-bp *Msp* I restriction fragment was subcloned to use as a hybridization probe (Fig. 2). This subclone is referred to as the NIRD probe.

Fig. 4 shows the results of Southern blot analyses of genomic DNAs from five IgA-producing plasmacytomas. Embryo and liver DNAs contained NIRD on a 16-kb *Eco*RI fragment whereas the five IgA-producing plasmacytomas (M167, H8, T15, W3082, and M511) contained NIRD on the 16-kb fragment and on an additional 13-kb *Eco*RI fragment. Because the DNA from the H8, T15, and M511 tumors revealed a 13-kb *Eco*RI band when hybridized with either a NIRD or a C_α probe, we

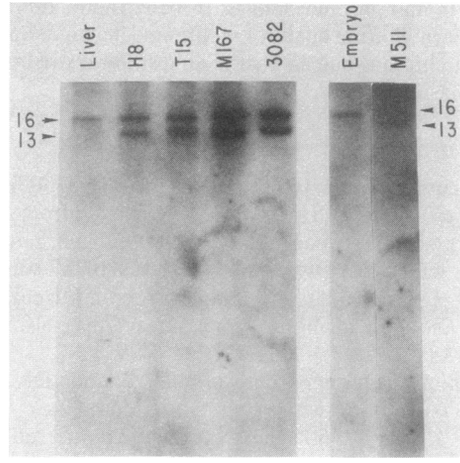

FIG. 4. Genomic blots of germ-line and plasmacytoma DNAs with the NIRD probe. DNAs were digested with *Eco*RI.

conclude that, like M603 and M167, they have NIRD sequences 5′ to the nonproductively rearranged C_α gene. In the M511 case, the juxtaposition of NIRD with C_α was further shown by using Southern blotting analysis of *Hin*dIII- and *Xba* I-digested DNAs (data not shown). By analogy, the above blotting results suggest that S63 and Y5236, which have a rearranged 13-kb *Eco*RI C_α band, and W3082, which has a rearranged 13-kb *Eco*RI NIRD band, have the NIRD/C_α rearrangement. Finally, comparison of the NIRD restriction sites from α7 or α30 to the restriction sites from nonproductive C_α gene clones from the IgA-producing plasmacytomas S107 (13) or J558 (14) showed that these clones also have a NIRD/S_α rearrangement. Thus, it appears that as many as 10 of 11 IgA-producing plasmacytomas may have nonproductively rearranged S_α regions joined to NIRD. In other words, rearrangement of NIRD to the S_α region is a frequent event in nonproductive C_H switching in IgA-producing tumors. Moreover, the rearrangements in each IgA-producing plasmacytoma appear to have occurred to nearly the same area within S_α and NIRD because the size of the restricted site fragments spanning the recombination breakpoint varied only slightly.

NIRD Rearrangements Occur in Non-IgA-Producing Cell Lines but Are Not Closely Associated with the Allelic Counterpart of the Expressed C_H Gene. The status of NIRD sequences in cells expressing isotypes other than IgA was deter-

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                                                                    Sα
                                                                    |
M603      GCGGGATTGGCGGGCGGCGACCTCCGTTGTCGGTCCCAGGCCTCCAGAAATGTACCAAGCTAAATTTAAATGCCTCTCAGAGACTGGTTGAGCTGAACTA   100
SP Sα     CTGGGATGAGACAGGCTGACTGCAGGAGGAAGACTGGAAGGGCTGGCTGAGCTAGACTAGGCTGGGCTGAGCTGGAATGAGCTG ———————————   1213
          GTATAAACTTGGCTAGGCTACAATGGATTGAGCTGAGCTAGACTTAGGGTGGAATGGGCTGAACAAGGCTGAGCTTACCTAGACGC                186
          ————————————————————————————————————————————————————————————————————————————————                  1299

                                                Sα
                                                |
M167      TTTCTTTGGCCACCACCGTAAGCGACCTCCCGGTTTGACCCAAACTAAGCTGGGATGAGACAAGCTGGACTGCAGGAGGAAGACTGGAAGGGCGTGACTG   100
SP Sα     TGAGCTGAGCTGAGCTGGGCTAAGCTGGGATGGACTAGGAT ———————————————————————————————————————————————————   1164
          AGCTAGACTAGGCTGGGCTGAGCTGGAATGAGCTGGGTTGAGCTGAACTAGTATAAACTTGGCTAGGCTACAATGGATTGAGCTGAGCTAGACTTAGGGT   200
          ————————————————————————————————————————————————————————————————————————————————————————————  1264

          GGAATGGGCTGAACAAGGCTGAGCTTACCTAGACC                                                                 235
          ———————————————————————————                                                                       1299
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FIG. 3. DNA sequence of germ-line S_α (α29), M603 rearranged S_α (α30), and M167 rearranged S_α (α7) DNAs at the S sites. Nucleotide identities are indicated by a line. Numbering of germ-line S_α sequences is from ref. 12.

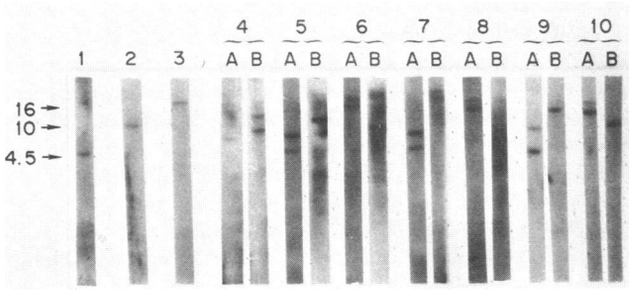

FIG. 5. Genomic blots of non-IgA-producing plasmacytomas. Lanes: 1–3, liver DNA; 4–10, plasmacytoma DNAs; A, probed with the NIRD probe; B, same filter probed with C_H cDNA probes after washing for 3 hr in 5 mM Tris, pH 8.0/0.2 mM EDTA/0.05% sodium pyrophosphate at 68°C. Details: lanes 1–3, liver DNA digested with *Hin*dIII, *Kpn* I, and *Eco*RI, respectively, and probed with the NIRD probe; 4B, E1 (IgG2b) DNA digested with *Hin*dIII, probed with a $C_{\gamma 2b}$ probe; 5B and 6B, IB7 (IgG3) DNA digested with *Hin*dIII and *Eco*RI, respectively, probed with a $C_{\gamma 3}$ probe; 7B and 8B, P3K (IgG1) DNA digested with *Hin*dIII and *Eco*RI, respectively, probed with a $C_{\gamma 1}$ probe; 9B and 10B, HPCM2 (IgM) DNA digested with *Eco*RI and *Kpn* I, respectively, and probed with a C_μ probe.

mined. Genomic DNA from an IgM-producing hybridoma (M2), an IgG3-producing hybridoma (IB7), an IgG1-producing plasmacytoma (P3K), and an IgG2b-producing plasmacytoma (E1) was analyzed by Southern blotting. Fig. 5 shows the results of several enzyme digestions in which the same filter was hybridized first with the NIRD probe, washed, and then hybridized with a cDNA probe corresponding to the isotype expressed by the cells from which the DNA was prepared. In all cases, a rearranged NIRD band was observed in addition to the germline NIRD band seen in liver DNA.

The parental cell line used in hybridoma fusions, SP2, exhibits a 13.0-kb *Eco*RI band and a 6.6-kb *Hin*dIII band when hybridized to a NIRD probe (14). Both M2 and IB7 exhibited the same size *Eco*RI and *Hin*dIII bands as SP2 when hybridized to a NIRD probe (Fig. 5; unpublished data). This similarity suggests that the rearranged NIRD bands in hybridomas M2 and IB7 were donated by the parental cell line, SP2.

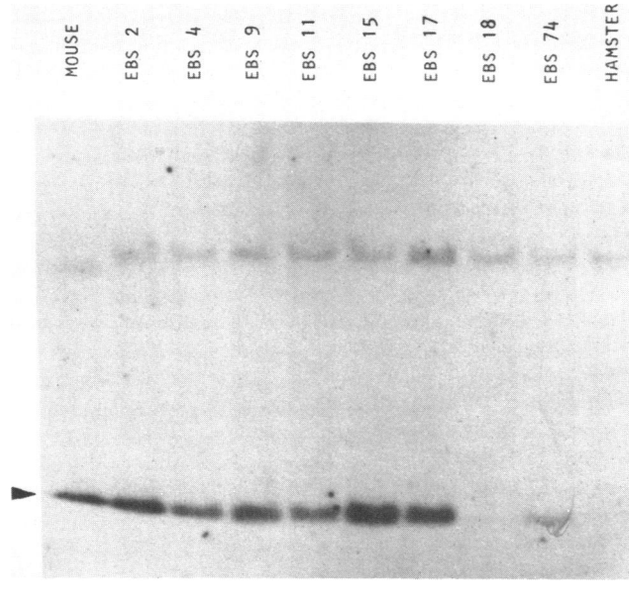

FIG. 6. Genomic blot of somatic hamster–mouse cell hybrid DNAs. Genomic DNA from mouse liver, hamster, and hamster–mouse somatic cell hybrids was digested with *Hin*dIII and hybridized to a 1.2-kb *Hin*dIII NIRD probe subcloned into pBR322 from $\alpha 30$. Arrowhead, 1.2-kb *Hin*dIII band in mouse liver DNA.

Table 1. Segregation of NIRD in mouse–hamster hybrids

Chromosome	Marker enzyme	Concordant	Discordant
1	PEP-3	3	5
2	AK-1/SODH/ACP-2	5	3
3	*	5	3
4	PGD/PGM-2	3	5
5	PEP-7/PGM-1	2	6
6	TPI	3	5
7	LDH-1/GPI/PEP-4	6	2
8	GR/APRT	4	4
9	ME/MPI	4	4
10	PEP-2/HK-1	4	4
11	GLK	1	7
12	ACP-1	6	2
13	*	6	2
14	ES-10	1	7
15	*	8	0
16	SOD-1	4	4
17	GLO	6	2
18	PEP-1	2	6
19	GOT	5	3
X	HPRT	7	1

The marker enzymes, their chromosomal assignments, and the procedures used to separate the Chinese hamster and mouse enzymes have been described (8, 9). Marker enzyme analysis and karyotype analysis agreed for each chromosome. Enzyme, karyotype, and Southern blotting analyses were performed on the same passage for each hybrid cell line.

* Chromosomes 3, 13, and 15 have no enzyme markers; their concordance/discordance was based on karyotype analysis alone.

In no case, however, was the rearranged NIRD band found on the same size restriction fragment as the relevant C_H gene. We also have found that NIRD is not rearranged to the C_α gene in these cells because the C_α gene and S_α region remain in germline configuration (data not shown). These results show that NIRD is rearranged in almost all plasmacytomas and hybridomas analyzed in our study regardless of which isotype is expressed (IgM, IgG, or IgA). Although in IgA-producing cells NIRD rearranges to the S_α region, in IgG- or IgM-producing cells NIRD rearranges neither to S_α nor to the S region used for rearrangement on the expressing chromosome.

NIRD Originates from Chromosome 15 as Shown by Somatic Cell Hybrid Mapping. Chinese hamster–mouse somatic cell hybrids that selectively lose mouse chromosomes can be used to map mouse sequences to individual chromosomes. Different hamster–mouse hybrid cell lines contain different arrays of mouse chromosomes as determined by enzyme and karyotype analysis. DNA was prepared from these hybrid cells and analyzed by Southern blots for the presence of NIRD sequences. NIRD sequences mapped concordantly with mouse chromosome 15 but discordantly with all other mouse chromosomes (Fig. 6; Table 1). Since the C_α genes originate from chromosome 12 and the NIRD sequences originate from chromosome 15, the $\alpha 30$ and $\alpha 7$ clones, from the plasmacytomas M603 and M167, span a translocation involving chromosome 15 and chromosome 12.

DISCUSSION

This paper describes clones isolated from two IgA-producing murine plasmacytomas in which the same DNA sequence is joined to the 5′ side of the C_α gene by a nonproductive C_α switch rearrangement. This sequence, termed NIRD, has been

mapped to chromosome 15, thus showing that these two clones represent the junction of a translocation involving chromosomes 12 and 15. Nonproductively rearranged C_α genes from the plasmacytoma tumors S107 (13) and J558 (14) have been cloned and, by restriction site similarity, these clones also contain a NIRD/S_α rearrangement. Furthermore, by Southern blot analysis presented here and elsewhere (13, 14), NIRD sequences are rearranged in 40 of 48 plasmacytomas. Previous cytogenetic evidence has shown that the distal portion of chromosome 15 is frequently translocated to either chromosome 12 or chromosome 6 in murine plasmacytomas (15, 16). The simplest explanation of these observations is that the α30 and α7 clones represent the T(12;15) translocations and that, in murine plasmacytomas, the NIRD sequence on chromosome 15 is frequently translocated, along with the distal portion of the chromosome, to the heavy chain immunoglobulin locus on chromosome 12 or to the κ chain immunoglobulin locus on chromosome 6.

Several lines of evidence suggest that NIRD rearrangement is limited to plasmacytomas. First, thymus, liver, and embryo DNAs show only the germ-line NIRD band (Fig. 4; unpublished data). Second, NIRD rearrangement is not observed in *Eco*RI-digested DNAs from a helper T-cell line (HT-1), a cytotoxic T-cell line (CTLL16), or two suppressor T-cell hybridomas (B3B5 and C4#4) (unpublished data). Third, except for the NIRD rearrangement donated by the parental cell line, SP2, NIRD rearrangement was not observed in 10 hybridomas, suggesting that NIRD is not rearranged in normal B lymphocytes (Fig. 5; ref. 14). Thus, these results suggest that NIRD rearrangement is specific for plasmacytomas.

One plausible explanation for NIRD rearrangement in plasmacytomas is that NIRD may contain an oncogene residing on chromosome 15 which can be activated by translocation to chromosome 12. For example, when B cells undergo C_H switching, DNA rearrangement of S regions may predispose the immunoglobulin heavy chain genes to translocation. In those cases in which an S region joins to NIRD, the NIRD oncogene may become activated, contributing to neoplastic transformation. A precedent for this hypothesis can be found in avian leukosis virus-transformed cells (17). In this case, a cellular oncogene, c-*myc*, is expressed at a higher level when it is activated by the insertion of a retrovirus, avian leukosis virus. Increased expression of c-*myc* by retrovirus-mediated promotor insertion is believed to cause neoplastic transformation in these cells.

Several points are worth noting regarding NIRD rearrangements. First, although promotor insertion seems to be the simplest explanation, it is not known how T(12;15) translocations can lead to neoplastic transformation. For example, translocations could derepress the NIRD gene. Alternatively, the NIRD gene could be a regulatory gene whose activation or repression could control oncogenes residing elsewhere. Furthermore, the translocated immunoglobulin gene is missing its normal promotor that resides with the V_H gene. Thus, even if T(12;15) translocations activated an oncogene residing at NIRD, the mechanism of activation is not clear. Perhaps the translocation is actually reciprocal so that a 5′ V_H-NIRD 3′ sequence resides in the plasmacytomas. Alternatively, the translocated C_α gene may activate nearby sequences via enhancer or chromatin effects (18).

Second, because some plasmacytomas show an unrearranged NIRD band on Southern blots, there may be a different mechanism for transformation in these cells. Alternatively, rearrangements may occur on chromosome 15 that are undetected by the NIRD probe. For example, by Southern blotting analysis, MOPC315 is reported to have no NIRD rearrangement (14) whereas a translocation involving chromosome 15 (D3/E) is detected cytogenetically (15).

Third, because chromosome 15 is observed to translocate to chromosome 6 (16) (which contains the immunoglobulin κ chains) and because light chains do not undergo C_H switching, it is possible that translocations may be induced by *V-J* or *V-D-J* joining as well as by C_H switching.

Fourth, the type of NIRD rearrangement found in plasmacytomas seems to correlate with the expressed isotype. For example, cloning and Southern blotting analysis suggest that NIRD is joined to S_α in 10 of 11 IgA-producing plasmacytomas. However, NIRD is not joined to S_α in four IgM- and IgG-producing lines.

Fifth, because trisomy of chromosome 15 is observed in murine T- and B-cell leukemias (3), it is possible that NIRD is involved in leukemogenesis. In addition, in human Burkitt lymphoma, translocations involving chromosome 8 and chromosome 2, 14, or 22 are known to occur (3). Because chromosome 2 contains the κ immunoglobulin genes, chromosome 14 contains the heavy chain genes, and chromosome 22 contains the λ light chain genes, it is possible that an analogous situation exists in these tumors in which a sequence similar to NIRD resides on human chromosome 8.

Finally, NIRD rearrangement in murine plasmacytomas may not be involved in oncogenesis at all. These rearrangements may have significance in a way not yet identified.

While this manuscript was in preparation, Marcu and coworkers (19) reported that a similar T(12;15) chromosomal translocation event occurs in the murine plasmacytoma J558.

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