Multiple chromosomal rearrangements in a spontaneously arising t(6;7) rat immunocytoma juxtapose c-myc and immunoglobulin heavy chain sequences

(chrromosome translocation/immunoglobulin switch region)

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ABSTRACT Spontaneously arising immunocytomas in Lou/Wsl rats contain a consistent translocation between chromosomes 6 and 7. The c-myc gene has been localized to chromosome 7 and has been shown to be rearranged in the majority of the rat immunocytomas. We now report the cloning of the rearranged 11-kilobase EcoRI c-myc fragment from the IgE-secreting IR75 tumor. Sequence analysis revealed that the cytogenetically visible t(6;7) translocation must have involved several events in this tumor. One event has led to the juxtaposition of c-myc and the switch μ region, in a head-to-head orientation. The breakpoint is approximately 850 base pairs upstream from the proximal c-myc promoter on chromosome 7. This area is distinct from the more common mouse plasmacytoma- and Burkitt lymphoma-associated translocation breakpoints and also differs from the known murine retroviral insertion sites. A second rearrangement has led to the transposition of sequences upstream from the switch γ1 region to the c-myc-distant end of the switch μ region, tail-to-tail. This requires at least two events, including one inversion. In addition to showing that identical loci (c-myc, immunoglobulin) are juxtaposed via chromosomal translocations in three different tumors (Burkitt lymphoma, mouse plasmacytoma, and rat immunocytoma) in different species (human, mouse, and rat), the multiple rearrangements in IR75 and some other tumors emphasize the selective value of c-myc activation by an immunoglobulin locus in the tumorigenic process.

Activation of a cellular oncogene by chromosomal translocation appears to play a total role in the genesis of certain hematopoietic tumors (1, 2). This is most consistently seen in two B-cell tumors, Burkitt lymphoma (BL) and mouse plasmacytoma (MPC), where chromosomal translocation juxtaposes the c-myc oncogene and one of the three immunoglobulin loci in 100% of typical BLs and in 95% of MPCs studied (3). Eighty-five percent of the BL- and 90% of the MPC-associated translocations juxtapose c-myc and the immunoglobulin heavy chain locus. In the rest of the BLS, c-myc is juxtaposed to either the κ or λ locus, while only κ appears to be involved in MPC.

In a review, Cory (2) has classified the c-myc/heavy chain translocation breakpoints. They can occur in the following three regions of the c-myc-carrying chromosome: (i) within the myc gene, (ii) immediately to the 5' side of the gene, and (iii) distant from the gene. The breakpoint is located within the first c-myc exon or intron in approximately 70% of the BALB/c MPCs and 50% of the BLs. In the remaining 30% of BALB/c MPCs, it is distributed almost equally between regions II and III. Region III predominates in BL. No tumors have been found where the translocations would interrupt the coding exons 2 and 3.

The switch (S) regions were the recombination targets inside the heavy chain gene cluster in all BALB/c MPCs studied, with Sα as the preferred site in more than 80% (2). Sα is the favored BL heavy chain target, but it is only associated with the third of the typical BL breakpoints. Recombination sites were also found in or upstream from the heavy chain joining region. The target in the heavy chain locus is unknown in approximately 25% of the BLS.

Our group has previously shown that spontaneous immunocytomas in Lou/Wsl rats (RIC) contain a reciprocal translocation between chromosomes 6 and 7 that resembles the MPC-associated typical translocation between chromosomes 12 and 15 (5). We have also shown that the c-myc gene of the rat is located on chromosome 7 and is rearranged in the majority of these I(6;7) tumors (6). The purpose of the present study was to explore whether the rat immunocytoma-associated translocations also act by juxtaposing c-myc to an immunoglobulin locus. Our detailed analysis of a rearranged c-myc DNA fragment isolated from the IR75 RIC gives an affirmative answer, but it also shows that the translocation involved multiple rearrangements in this particular tumor. One event has juxtaposed the c-myc gene and the Sα region in opposite transcriptional orientations. A second event has placed sequences upstream of the Sα region (5' SWIG 1) next to the c-myc-distant side of the Sα region, tail-to-tail in opposite transcriptional orientation. Consequently, the 5' SWIG 1 sequences are in the same transcriptional orientation as the c-myc gene. Neither heavy chain constant region coding sequences nor the heavy chain enhancer could be detected within at least 2.3 kilobases (kb) of the c-myc gene proximal promoter.

MATERIALS AND METHODS

Hybridization Probes. c-myc probes were as follows: (i) pMC41-3RC (7), 1.5-kb Cla I-EcoRI fragment containing the third exon of the human MYC gene; and (ii) RB-2 (8), 1.1-kb Sac I-Sma I fragment containing the 5' portion of the first c-myc exon and approximately 580 base pairs (bp) of 5'-flanking sequences cloned from the mouse c-myc gene. Immunoglobulin probes were as follows: (i) L3B (9), 10.5-kb Sac I fragment containing the entire rat joining region; (ii) pR14 (9), 3.5-kb Sac I-HindIII fragment containing the rat Sα, and (iii) yl probes were as follows [all probes were

Abbreviations: RIC, rat immunocytoma; BL, Burkitt lymphoma; MPC, mouse plasmacytoma; S, switch; kb, kilobase(s); bp, base pairs; 5' SWIG 1, DNA sequences 5' proximal to Sα.

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derived from the mouse γ1 clone, IgH2 (10): (i) Sγ1 probe, 2.2-kb EcoRI–Sac I fragment containing the Sγ1 region and Cγ1 probe, 2.4-kb Sac I fragment containing coding domains for Cγ1 and 3′- and 5′-flanking sequences. Cloning. The IgE-secreting IR75 rat immunocytoma has arisen spontaneously in the Lou/Wsl strain, and contains a reciprocal t(6;7) translocation (5). An 11-kb EcoRI fragment containing the rearranged c-myc gene of IR75 was cloned by standard techniques (11). The Rgam9.8 and Rgam9.6 clones were obtained by similar procedures except that the EcoRI-digested fragments were obtained from Lou/Wsl kidney DNA. The cloning of the 17-kb EcoRI c-myc clone Rmycl will be described elsewhere (D.S., unpublished results).

DNA Analysis. DNA was prepared and blotted onto nitrocellulose as described (6). Hybridizations, washings, and autoradiography were carried out as described (6). DNA sequences were determined using the method of Sanger et al. (12). Sequences were analyzed using the programs developed by Staden (13) and the University of Wisconsin Genetics Computer Group (14).

RESULTS

Cloning and Structure of the Rearranged c-myc Gene in the IR75 RIC. When cleaved with EcoRI, 15 of 16 t(6;7) RICs (5) contained the 17-kb germ-line myc band (Fig. 1A) plus an additional c-myc-specific fragment (ref. 6 and W.S.P., unpublished data). In two tumors, IR75 and IR209, the rearranged c-myc EcoRI fragments were 11 and 14 kb in size, respectively. Both of these tumors had high c-myc mRNA levels, comparable to the X24C and ABPC45 MPCS (data not shown). The 11-kb fragment from the IR75 was inserted into the EMBL4 vector and mapped with restriction enzymes (Fig. 1B).

Comparison of the maps of the rearranged and nonrearranged c-myc fragments showed that the Xba I site, located 600 bp upstream from the BamHI site flanking the first exon, was missing from the IR75 rearranged c-myc (Fig. 1A and B). The likelihood that the translocation break occurred in this region was confirmed by the finding that the 300-bp BamHI fragment from IR75RMyc (IR75.300) hybridized to the 600-bp Xba I–BamHI fragment (Rync.6XB) from the full-length rat c-myc clone Rmycl; whereas, the adjacent BamHI fragments in IR75RMyc (200 bp, 120 bp, and 700 bp) showed no homology to the rat c-myc clone. Both IR75.300 and Rmyc.6XB were subcloned into M13mp18 and M13mp19 vectors and sequenced. There was perfect homology between IR75.300 and Rmyc.6XB for the first 95 bp from the BamHI site (Fig. 2). Beyond this point, there was virtually no homology. Thus, the break has occurred approximately 850 bp upstream from the most proximal c-myc promoter, which is located 143 bp upstream from the BamHI site that divides the 0.9- and 1.1-kb BamHI fragments (L. Nacar and D.S., unpublished results).

The DNA Fragments Juxtaposed to c-myc Origin from Chromosome 6. To determine the identity of the ≈1.5-kb c-myc juxtaposed region in IR75, the 700-bp EcoRI-BamHI terminal fragment (IR75.700) was hybridized to a rat–mouse somatic cell hybrid panel (16). The pattern shown in Table 1 was consistent with the localization of IR75.700 to rat chromosome 6, known to contain the heavy chain locus (17). Hybridization of IR75.700 to a mouse–Chinese hamster hybrid panel (18, 19) showed that the murine homologue of this DNA fragment lies on chromosome 12, known to carry the heavy chain locus (data not shown).

To explore the likely possibility that the 1.5-kb c-myc juxtaposed fragment originated from the heavy chain gene cluster, we attempted to locate IR75.700 homologous sequences within the well-characterized mouse heavy chain constant region. A complete map of the region has been published, including the restriction fragment length polymorphisms between BALB/c and C57/BL (20). IR75.700 was found to be polymorphic between these mouse

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**Fig. 1.** Restriction map of the rearranged IR75 c-myc-carrying fragment and its germ-line components. (A) Germ-line c-myc. The structure of the rat c-myc gene was deduced by hybridization to genomic DNA of Lou/Wsl and Fisher rats (refs. 6 and 15, and W.S.P., unpublished observations) and cloning of the 17-kb unarranged EcoRI fragment from the Fisher strain (D.S., unpublished results). The c-myc restriction maps of Lou/Wsl and Fisher rats are identical (W.S.P. and D.S., unpublished observations). No differences have been found in overlapping regions that have been sequenced. The approximate location of the three c-myc exons is indicated by the bar above the map. (B) IR75RMyc, IR75 c-myc rearrangement. Homology to germ-line fragments is shown by connecting lines. The c-myc homologous region of IR75RMyc extends at least to the 3′-EcoRI site. Only the 900-bp fragment that contains the 5′ part of exon 1 is shown. The transcriptional orientations of the transposed fragments are shown by arrows. (C) RGAM9.8 and RGAM9.6, restriction fragments carrying homologous sequences to Sγ1 and Cγ1 probes are indicated. Only one Bgl II site in RGAM9.6 is indicated. (D) Germ-line rat Sγ1 and Cγ1 regions (9).
strains. Comparison of the mouse heavy chain gene map with the pattern obtained after hybridization of IR75.700 with EcoRI, XbaI, SacI, KpnI, or HindIII-digested BALB/c and C57BL/6J DNA showed that the hybridizing sequences were within the 6.2-kb Sac I-KpnI fragment, located in the intervening region between the y3 and y1 loci (Fig. 3A). This was confirmed by comigration of IR75.700 and C3H probe hybridizing sequences within HindIII-digests of BALB/c and C57BL/6J DNA (Fig. 3B).

Cloning of the Germ-Line Fragment of IR75.700. IR75.700 identified two λ phage clones in an EcoRI-cleaved Lou/Wsl kidney library. The two positive clones, 9.8 kb (Rgam9.6) and 9.6 kb (Rgam9.6) (see Fig. 1C for restriction maps), correspond to the IR75.700 germ-line fragments (W.S.P., unpublished observations). The 4.6-kb BamHI fragment of both clones hybridized to a murine C4 probe and a murine S4 probe. IR75.700 hybridized to the terminal 800-bp EcoRI-BamHI fragments of Rgam9.6 and Rgam9.6, and also to the 600- and 1300-bp BamHI fragments of Rgam9.8. Similarly to the location of the corresponding sequences in the mouse, the origin of IR75.700 can thus be traced to a region in the rat heavy chain gene cluster approximately 2 kb upstream from the S4 region.

To localize the breakpoint in Rgam9.8, the c-myc breakpoint-containing fragment, IR75.300, was hybridized to the rat y1 clones. IR75.300 hybridized to the 600-bp and 1300-bp BamHI fragments of Rgam9.8, but not to Rgam9.6. The 120-bp and 200-bp BamHI fragments of the IR75 clone were also present in Rgam9.8, located between the 800-bp and 600-bp BamHI fragments. Since the 600-bp BamHI fragment was closest to the 120- and 200-bp BamHI fragments conserved in the translocation, the breakpoint site in Rgam9.8 was most probably located in this fragment.

DNA sequencing showed that there was perfect homology between Rgam98.600 and IR75.300 for the first 40 bp, beginning at the BamHI site abutting the 200-bp BamHI fragment (Fig. 2). After this point, the homology was very low. In addition, the sequenced regions of the 200-bp BamHI fragments of Rgam9.8 and IR75RMyc showed perfect homology (Fig. 2). These results also showed that the translocation has placed the S4-WIG 1 sequences and the c-myc gene in the same transcriptional orientation (Fig. 1B).

Table 1. Correlation of IR75.700 with rat chromosomes in rat-mouse somatic cell hybrids

| Hybrid | IR75.700 | X | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| LB20   | +       | + | + | + | (−) | + | − | + | − | + | − | + | − | + | + | − | + | + | + | + | + |
| LB251  | +       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB161  | +       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB510  | +       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB571  | +       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB780  | +       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB860  | +       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB1040 | +       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

Discordant clones: 6 4 7 4 5 2 0 5 2 2 2 4 5 6 3 2 4 7 5 3 4.

+; Chromosome dosage factor, 0.6; (+); Chromosome dosage factor, 0.3-0.5; (−); Chromosome dosage factor, 0.1-0.2; −; Chromosome dosage factor, 0.
Because Fok I recognizes the sequence

\[ 5' \text{GGATG(N)}_9 3' \]
\[ 3' \text{CCTAC(N)}_{13} 5' \]

and cleaves either 9 or 13 nucleotides away from the recognition site (22), one Fok I recognition site should be found within any Fok I-digested fragment. The sequencing results showed that the Fok I recognition sequence GTAGG is located exactly 13 bp away from the Sma I site in the M13mp18 vector, showing that this is the 3' end of this fragment (Fig. 2). The 3' end of the Sµ subclone, pR14 (denoted by the HindIII site in Fig. 1D), points towards the heavy chain constant region. Since the conserved Fok I site in IR75.300 is located at the 5'-SWIG 1 junction, rather than the c-myc junction, the Sµ and c-myc regions must be facing each other head-to-head (Fig. 1B). Conversely, the Sµ and 5'-SWIG 1 regions are located tail-to-tail (Fig. 1B).

**DISCUSSION**

Chromosomal Translocations Juxtapose c-myc and Heavy Chain Sequences in Three B-Cell Tumors Occurring in Three Different Species. The documented juxtaposition of c-myc and heavy chain sequences in IR75 adds a third tumor and a third species to the already impressive evidence of closely homologous translocations in BL and MPC. These three tumors represent at least two different stages of B-cell maturation, and their natural histories and modes of induction are very different. Unlike BLs and MPCs, most RICs, including IR75, produce IgE (4).

Cytogenetic analysis of IR75 and 15 other RICs revealed the presence of an identical (t6;7) translocation in all 16 tumors (5). Southern blot hybridization showed that c-myc, known to be located on chromosome 7 (6), was rearranged in 15 of 16 tumors (ref. 6 and W.S.P., unpublished results). With the localization of the rat heavy chain locus to chromosome 6 (17) and with the analysis of the IR75 translocation reported in this paper, it is likely that the RIC-associated (t6;7) translocation contributes to the tumorigenic process by a similar c-myc/heavy chain juxtaposition as in MPC and BL. This is further supported by our findings that the rearranged c-myc fragments comigrate with heavy chain-specific sequences in several other RICs (W.S.P. and G. Wahlström, unpublished results).

The Chromosome 7 Breakpoint Is Outside the c-myc Gene in IR75. The chromosome 7 breakpoint in the IR75 is approximately 850 bp upstream from the c-myc proximal promoter. This is further upstream than the breakpoints in all analyzed MPCs with a typical (heavy chain/c-myc) translocation that have a rearranged c-myc-containing EcoRI fragment and the majority of BLs in the corresponding category. It is also upstream from the c-myc-associated viral integration sites in AKR-derived T-cell lymphomas (2) and Moloney virus-induced rat thymomas (ref. 15 and D.S., unpublished data).

As in the majority of BLs, the RIC-associated translocations do not appear to depend on the removal of postulated exon 1-associated regulatory sequences (23-25).

A Heavy Chain Switch Region Is Juxtaposed to the c-myc Gene in a Head-to-Head Orientation in All Three Typical Translocation B-Cell Tumors. Similarly to all molecularly analyzed MPCs and BLs with typical (c-myc/heavy chain) translocations associated with rearranged c-myc EcoRI fragments, the IR75 translocation has led to the juxtaposition of c-myc and a heavy chain switch region in a head-to-head orientation. This consistent topology may merely reflect the chromosomal orientation of the participating genes (26, 27). Alternatively, it is possible that the predominance of the head-to-head juxtaposition has functional reasons. As with the analyzed, exceptional MPC ABPC45 (28), our present
findings on IR75 show that complex rearrangements may occur and can include inversions. It is, therefore, unlikely that chromosomal geometry is the limiting factor as such, although it may serve as the basis for the most frequent configurations in the uncompleted reciprocal translocations.

The IR75 (6;7) Translocation Involves Multiple Rearrangements. Unlike the majority of BL and MPC translocations, the IR75 must have arisen by several cytogenetic events. Only one of them, the reciprocal t(6;7) translocation, is visible at the chromosomal level. Conceivably, and in analogy with the majority of the MPC- and BL-associated translocations, this event may have been responsible for the head-to-head juxtaposition of c-myc and an Sγ region. At least one additional event is necessary to explain the juxtaposition of the Sγ and S′-SWIG 1 region in opposite orientations. One possibility is that inversion in the heavy chain gene cluster may have occurred as an aberrant event in the looping-out model of switch recombination proposed by Honjo and Kataoka (29). It is also possible, however, that an inversion occurred independently of switch recombination.

Unlike recombination in the T-cell receptor (30), inversion does not appear to be a normal mechanism of heavy chain switch recombination. Greenberg et al. (31) have described an inversion within the Sγ region of MPC11, known to contain a translocated c-myc gene. We have analyzed an exceptional mouse plasmacytoma, ABPC45, where an inversion has occurred in the region juxtaposed to the rearranged c-myc fragment (28). This case is very similar to IR75, but with some important differences. ABPC45 carries no cytogenetically detectable translocation, but has an interstitial deletion in the c-myc region of chromosome 15, and carries a rearranged EcoRI c-myc fragment (32). Molecular analysis has revealed a complex rearrangement. The intact c-myc gene was juxtaposed head-to-head to the Sγ region. This was not followed by a Cγ region, as expected, but by another heavy chain derived, e.g. containing Sγ sequences and the heavy chain enhancer, in that order, joined to Sγ tail-to-tail (28). This requires at least two translocations and one inversion. An important difference between ABPC45 and IR75 is the presence of the heavy chain enhancer in the rearranged c-myc structure in the former. Although the heavy chain enhancer is not present within 2.3 kb of the c-myc promoters in the IR75, probing further upstream from the transposed S′-SWIG 1 sequences would be required to rule its presence out.

It has been demonstrated that a variety of regions in the immunoglobulin loci can exert B-cell-specific enhancer or promoter activity (33–35). It is conceivable that the S′-SWIG 1, or sequences further upstream from it, may play a similar activating role. Alternatively, the active state of the chromatin, rather than specific enhancer sequences, may bear the responsibility for the constitutive activation of the transposed c-myc sequences. If so, it is conceivable that activation requires a certain "minimum length" of immunoglobulin locus-associated chromatin in the neighborhood of c-myc. The most frequent representations of certain regions may be due to topographical and/or enzymatic limitations, rather than to the requirement for a limited number of specific sequences.

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