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Interchromosomal recombination of the cellular oncogene c-*myc* with the immunoglobulin heavy chain locus in murine plasmacytomas is a reciprocal exchange

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The 15;12 chromosome translocations found in most murine plasmacytomas involve the cellular gene (c-*myc*) homologous to the oncogene (v-*myc*) of avian retrovirus MC29. Translocation links the c-*myc* gene of chromosome 15 to the immunoglobulin heavy (H) chain locus of chromosome 12, often within the switch recombination (S) region 5′ to the α constant region (C_α) gene. We have investigated c-*myc* rearrangements in 21 BALB/c plasmacytomas and three B lymphomas by Southern blot analysis. We show that the t(15;12) is a reciprocal chromosome exchange since most tumours contain not only a c-*myc* gene linked to the $S_\alpha C_\alpha$ region but also a separate structure with S_μ or S_α linked to the c-*myc* 5′-flanking region. Analysis of the two rearrangement products cloned from plasmacytoma J558 suggests that one type of H locus target for translocation is an S_α region recombined with S_μ; two other targets appear to be other switched heavy chain genes and an unrearranged C_α gene. Nearly all the chromosome 15 breakpoints fall within a 1.1-kb region spanning a 5′ c-*myc* exon; hence scission of the transcriptional unit by translocation can account for the altered c-*myc* transcription in plasmacytomas. The c-*myc* breakpoint region lacks substantial homology with S_μ or S_α, arguing against homologous recombination as the translocation mechanism.

Key words: B lymphocyte oncogenesis/chromosome breakpoints/15;12 chromosome translocations/immunoglobulin switch recombination regions

Introduction

Specific chromosome translocations probably play a key role in certain neoplasms. Those in two malignancies of B lymphocyte lineage, murine plasmacytomas and human Burkitt lymphomas (reviewed by Klein, 1981; Rowley, 1982), involve chromosomes bearing immunoglobulin loci. In murine plasmacytomas, the distal region of chromosome 15 is translocated to the end of chromosome 12, where the immunoglobulin heavy (H) chain locus resides or, less frequently, is involved in a reciprocal exchange with chromosome 6, which bears the κ locus (Ohno *et al.*, 1979). Burkitt lymphomas display translocations between human chromosome 8 (band q24) and the bands on chromosome 14, 2 and 22 which bear, respectively, the H chain, κ and λ loci (Malcolm *et al.*, 1982; Kirsch *et al.*, 1982; Taub *et al.*, 1982). Klein (1981) proposed that a potentially lymphomagenic region on murine chromosome 15, and human chromosome 8, was activated when translocated near an immunoglobulin locus.

Converging streams of work (summarised by Klein, 1983) have recently revealed that breakpoints on mouse chromosome 15 and human chromosome 8 are near the cellular homologue (c-*myc*) of the oncogene (v-*myc*) in avian retrovirus MC29. Firstly, non-immunoglobulin DNA which has recombined near the α constant region (C_α) gene in many plasmacytomas (Kirsch *et al.*, 1981; Harris *et al.*, 1982b; Adams *et al.*, 1982; Calame *et al.*, 1982) derives from chromosome 15 (Harris *et al.*, 1982a; Calame *et al.*, 1982; Cory *et al.*, 1983) and bears the c-*myc* gene (Crews *et al.*, 1982; Taub *et al.*, 1982; Adams *et al.*, 1983; Marcu *et al.*, 1983). Rearrangements of c-*myc* in plasmacytomas were also detected by Shen-Ong *et al.* (1982) and Mushinski *et al.* (1983). Moreover, the human c-*myc* gene resides at the relevant region of chromosome 8 (Dalla Favera *et al.*, 1982; Neel *et al.*, 1982b; Taub *et al.*, 1982) and is rearranged in many Burkitt lymphomas with the 8;14 translocation (Taub *et al.*, 1982; Adams *et al.*, 1983; Marcu *et al.*, 1983). Plasmacytomas contain new species of c-*myc* mRNA (Adams *et al.*, 1982, 1983; Shen-Ong *et al.*, 1982; Mushinski *et al.*, 1983; Marcu *et al.*, 1983), apparently because translocation alters the c-*myc* transcriptional unit (Adams *et al.*, 1983), and these altered transcripts may feature in plasmacytoma oncogenesis (Adams *et al.*, 1982, 1983; Shen-Ong *et al.*, 1982).

The 15;12 chromosome translocation may be related mechanistically to the 'switch recombination' by which a lymphocyte switches H chain class expression: Figure 1A

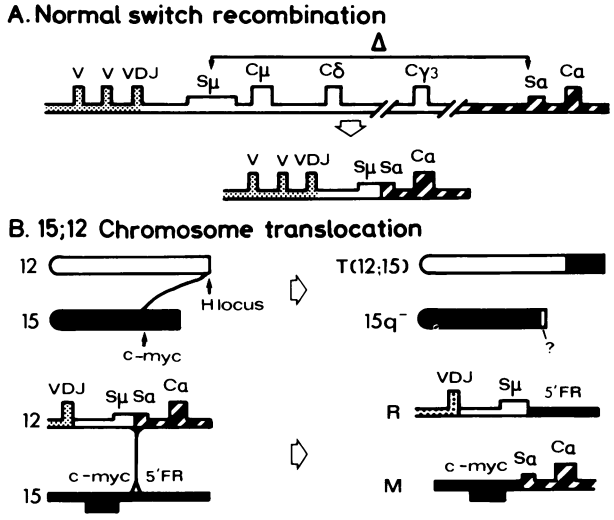

Fig. 1. Relation of switch recombination in the H chain locus to plasmacytoma-associated 15;12 chromosome translocation. **A** depicts the switch from μ to α expression *via* recombination between S_μ and S_α regions, with deletion (Δ) of intervening C_H genes. **B** illustrates the 15;12 translocation at the cytogenetic and DNA level. The question mark at the end of $15q^-$ indicates that no chromosome 12 region could be detected cytogenetically (Ohno *et al.*, 1979). To aid comparison of **A** and **B**, orientation here is such that immunoglobulin gene sequences are transcribed from left to right; hence the 5′-flanking region (5′FR) of the c-*myc* gene is on its right. Recombination is depicted for c-*myc* and a 'switched' C_α gene (see text and Figure 4). The two products expected from a reciprocal exchange are denoted M, for c-*myc*-bearing, and R, for the 'reciprocal product', shown here to bear the c-*myc* 5′FR. If V_H is proximal to the centromere of chromosome 12 (Owen *et al.*, 1981), c-*myc*-C_α structures (M) must derive from $15q^-$ and R structures from t(12;15).

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shows that a variable region gene assembled near the C_μ gene from V (variable), D (diversity) and J (joining) elements can be switched to a downstream C_H gene (C_α in Figure 1A) *via* recombination between switch (S) regions 5′ to C_μ and the other C_H gene (for review, see Davis *et al.*, 1980). Since the most common c-*myc* translocation in BALB/c plasmacytomas is to S_α (Adams *et al.*, 1982; Calame *et al.*, 1982; Shen-Ong *et al.*, 1982) and c-*myc* rearrangements in some Burkitt lymphomas apparently also involve switch regions (Taub *et al.*, 1982; Adams *et al.*, 1983; Marcu *et al.*, 1983), the switch recombination machinery may be implicated.

We address here several basic questions about the plasmacytoma translocations. The first is whether the t(15;12) is a reciprocal exchange, as depicted in Figure 1B. This could not be determined cytogenetically (Ohno *et al.*, 1979), although the analogous human t(8;14) is reciprocal (Manolova *et al.*, 1979; Adams *et al.*, 1983). We have also investigated where chromosome 15 breakpoints lie with respect to the c-*myc* gene, the nature of H locus targets, and whether the translocation represents homologous recombination. To approach these questions, we have analysed breakpoint regions in 21 plasmacytomas and three B lymphomas by Southern blot analysis with cloned c-*myc* and H locus probes. We show that breakpoints occur within a region spanning a 5′ exon of the c-*myc* gene, a finding which can account for the altered c-*myc* transcripts detected in plasmacytomas. We describe clones from plasmacytoma J558 representing the structures expected from a reciprocal chromosome exchange and show that most plasmacytomas contain such products. We distinguish three types of targets for translocation in the H locus and show that some chromosome exchanges may not be precise cross-overs.

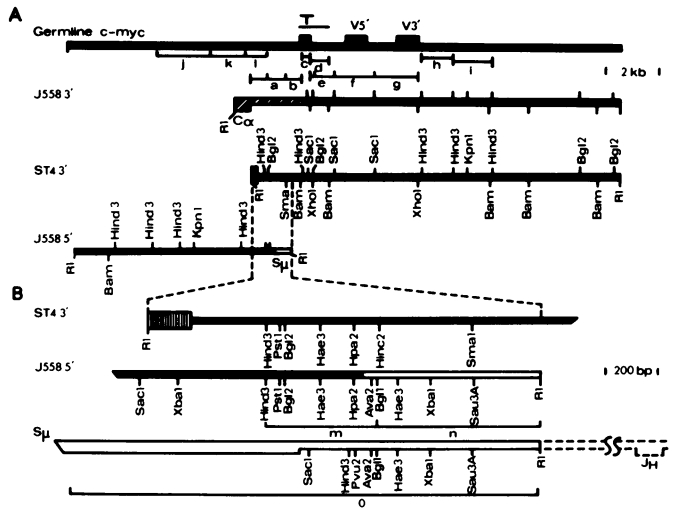

Fig. 2. Relation of three rearranged c-*myc* clones to the germline c-*myc* locus (top). Filled bars denote sequences from the c-*myc* locus and filled boxes, apparent c-*myc* exons, v5′ and v3′ being those homologous to 5′ and 3′ regions of v-*myc* (Adams *et al.*, 1983). In this and subsequent figures, maps are oriented such that c-*myc* is transcribed from left to right (unlike Figure 1). **A** shows restriction maps of three cloned *Eco*RI fragments from rearranged c-*myc* loci: for J558 3′ (Adams *et al.*, 1982; 1983) linkage is with the C_α locus (hatched); in ST4 3′, linkage is with an unknown region (striped box); and J558 5′ bears c-*myc* 5′-flanking sequences linked to the S_μ region (open bar). Lines denote subclones and letters indicate fragments used as probes. T denotes the region in which most chromosome 15 breakpoints occur (see text and Figure 6). Not all *Bgl*II sites have been mapped on J558 5′. **B** compares expanded maps of part of the ST4 3′ and J558 5′ clones with the published map of the sequenced S_μ region (Takahashi *et al.*, 1980).

Results

The 15;12 chromosome translocation is reciprocal

Figure 2A (top) depicts the murine c-*myc* locus on chromosome 15, orientated such that c-*myc* is transcribed from left to right (i.e., opposite to that in Figure 1B); filled boxes denote exons homologous to 5′ and 3′ regions of v-*myc* (v5′ and v3′) and an upstream exon (Adams *et al.*, 1983). The 20.5-kb region shown corresponds to the germline c-*myc* *Eco*RI fragment and was characterised by mapping the three overlapping clones of rearranged c-*myc* DNA indicated in Figure 2A, and by using various fragments from them as probes in hybridization experiments with embryo DNA (not shown). In the J558 3′ clone, originally isolated as a rearranged C_α gene from plasmacytoma J558, the c-*myc* region (filled bar) is joined to S_α (cross-hatched) *via* a site near or within the 5′ c-*myc* exon. To indicate that the linked c-*myc* and C_α genes have opposite transcriptional orientation (Adams *et al.*, 1982, 1983; Shen-Ong *et al.*, 1982; Marcu *et al.*, 1983), their respective exons are placed on opposite sides of the bar in the Figures.

To look for the predicted reciprocal exchange product (R in Figure 1B), a c-*myc* probe upstream from the J558 recombination point was required. This was provided by the ST4 3′ clone from T lymphoma ST4 in which recombination (with as yet unidentified DNA, indicated as a striped box) has occurred 1.4 kb further upstream from the c-*myc* gene than in J558. Probes from this region (a and b in Figure 2A) revealed a second rearrangment product in J558 DNA (see below) and the corresponding *Eco*RI fragment was isolated as the J558 5′ clone in Figure 2A.

The J558 5′ clone bears the c-*myc* 5′-flanking region linked to S_μ region sequences (open bar), the right hand *Eco*RI site corresponding to that between J_H and C_μ in germline DNA. Figure 2B shows that restriction sites in the J558 5′ clone match known sites in the S_μ region (Takahashi *et al.*, 1980) for a 0.6-kb region from this *Eco*RI site and thereafter match those in the germline c-*myc* region (filled bar) of the ST4 clone. Moreover, in genomic blots (not shown) all probes from the J558 5′ c-*myc* segment (j, k, and l in Figure 2A and m in 2B) hybridized to the expected ~21-kb *Eco*RI fragment of embryo DNA, while probe n (Figure 2B), from within the putative S_μ segment, hybridized solely to the 12.5-kb *Eco*RI fragment of embryo DNA detected by an authentic S_μ probe (o in Figure 2B) and to rearranged S_μ regions in various plasmacytomas. Thus, in J558, the c-*myc* locus has been disrupted, its 5′ and 3′ segments being separately linked to S region sequences, as expected for a reciprocal chromosome exchange (Figure 1B).

Translocation of c-*myc* probably always involves a reciprocal exchange, because all but one line previously shown to have a rearranged c-*myc* gene (Adams *et al.*, 1982, 1983) contain a different rearrangement product bearing c-*myc* 5′-flanking sequences. Figure 3A shows five lines in which the *Eco*RI fragment bearing the rearranged c-*myc* gene clearly is larger than that bearing its 5′-flanking region (Figure 3B). Table I (second and third columns) summarizes data for five B lymphomas and 23 plasmacytomas. Of the 24 lines with a rearranged c-*myc* product (denoted M), 20 had an *Eco*RI fragment of a different size detected by c-*myc* 5′-flanking sequences (denoted R). In three others (HOPC 1, MOPC 104E and MOPC 41A) we surmise that the M and R fragments are the same size, as confirmed by cloning from

Table I. Analysis of products of reciprocal chromosome translocation

Cell line[a]	Rearranged product[b]		Product detected by[e]					Target[f]
	c-*myc* (M)[c]	5′FR (R)[d]	a	b	c	d,e	h	
B lymphoma								
WEHI-279 (μ, NZC)	14.3 U	10.7 S_μ	R	R	R,M	M	nd	$S_\mu S_x C_x$
WEHI-231 (μ, NZB F1)	–	–	nd	–	nd	nd	–	–
*BALTNLM 17 (μ)	12.6 C_α	14.1 5′S_α	R	R	R,M	M	M	$S_\alpha C_\alpha$
BALTELM 1131	13.1 C_α	10.3 (S_μ)	R	R	R	R,M	M	($S_\mu S_\alpha C_\alpha$)
2PK3 (γ2a)	–	–	nd	–	nd	nd	–	–
Plasmacytoma								
HPC 76 (μ)	–	–	–	nd	nd	nd	–	–
*MOPC 104E (μ)	14.5 U[g]	14.5 5′S_α[g,h]			[i]			($S_\alpha C_\alpha$)[f]
TEPC 1033 (δ)	~34 U	9.5 S_μ	R	R,M	M	M	M	$S_\mu S_x C_x$
*TEPC 1017 (δ)	12.6 C_α	–[j]	–	–	M	M	M	C_α
Y5606 (γ3)	14.0 C_α	12.0 (S_μ)	R	–	nd	M	M	($S_\mu S_\alpha C_\alpha$)
*P3 (γ1)	14.0 U	10.9 S_μ	R	R	R,M	M	M	$S_\mu S_x C_x$
*MPC 11 (γ2b)	16.5 U	9.2 U	R	R	R,M	M	M	?
*MOPC 173 (γ2a)	16.1 C_α	14.9 5′S_α	R	R	R	M	M	$S_\alpha C_\alpha$
*HOPC 1 (γ2a)	14.7 C_α[g]	14.7 5′S_α[g]			[i]			$S_\alpha C_\alpha$
*SAMM 368 (γ2b,α)	13.0 C_α	14.2 5′S_α	R	R	R,M	M	M	$S_\alpha C_\alpha$
*TEPC 609 (γ2b,α)	16.8 C_α	13.6 5′S_α	R	R	R,M	M	M	$S_\alpha C_\alpha$
ABPC 4 (α)	–	–	–	–	nd	–	nd	–
J558.2BU.1 (α)	14.9 C_α	8.3 S_μ	R	–	M	M	M	$S_\mu S_\alpha C_\alpha$
*S107 (α)	16.2 C_α	10.8 S_μ	R	R	R	M	M	$S_\mu S_\alpha C_\alpha$
MOPC 315 (α)	14.9 C_α	11.3 S_μ	R	R	R	M	M	$S_\mu S_\alpha C_\alpha$
McPC 603 (α)	13.6 C_α	11.8 S_μ	R	R	R	R,M	M	$S_\mu S_\alpha C_\alpha$
S117 (α)	15.5 C_α	12.5 S_μ	R	R	R	R,M	M	$S_\mu S_\alpha C_\alpha$
*S194 (α)	14.0 C_α	10.0 S_μ	R	R	R	M	M	$S_\mu S_\alpha C_\alpha$
*EPC 109 (α)	14.5 C_α	12.8 S_μ	R	R	R	R,M	M	$S_\mu S_\alpha C_\alpha$
*BFPC 61 (α)	14.3 C_α	11.8 S_μ	R	R	R	M	M	$S_\mu S_\alpha C_\alpha$
WEHI-267 (α)	15.6 C_α	12.3 S_μ	R	R	R	R,M	M	$S_\mu S_\alpha C_\alpha$
A (α)	~15 C_α	11.0 S_μ	R	R	nd	M	M	$S_\mu S_\alpha C_\alpha$
*MOPC 41A (K)	18.5 C_α[g]	18.5[g] nd			[i]			$S_\mu S_\alpha C_\alpha$[k]

nd, not determined.

[a]Strain of origin is BALB/c unless indicated otherwise. Where known, the heavy chain synthesized is indicated in brackets. An asterisk indicates that the breakpoint was also analyzed in *Bam*HI digests (see text).

[b]Size of the rearranged *Eco*RI fragment bearing the c-*myc* gene (M) (Adams *et al.*, 1982) and that bearing its 5′-flanking region (R) is given in kb. A dash indicates that only the 21-kb germline fragment, found with each probe, was detected.

[c]C_α indicates that translocation has apparently linked the c-*myc* gene to C_α, because the same size *Eco*RI fragment hybridized to probe h (Figure 2A) and to the 5′ C_α gene probe (Adams *et al.*, 1982). Additional evidence for linkage is that probe e and the 5′ C_α probe both hybridize to the same sized rearranged *Bam*HI fragment in BALTNLM 17, MOPC 173, HOPC 1, SAMM 368, TEPC 609, S194, BFPC 61 and MOPC 41A. U indicates that no C_α sequences were present in M and the recombining DNA region is unknown.

[d]Linkage of the c-*myc* 5′-flanking region to either S_μ or sequences 5′ to S_α is inferred because the same size *Eco*RI fragment hybridized to flanking region probe a (Figure 2A) and to either S_μ probe o (Figure 2B) or region p 5′ to S_α (Figure 4B), respectively, both of which hybridize solely to their respective switch regions and not to other areas of the genome. Brackets indicate uncertainty about linkage, either because the S_μ probe hybridized only very weakly (BALTELM 1131), or because R did not clearly resolve from the germline S_μ fragment (Y5606).

[e]Rearranged fragment (M or R) detected by the indicated c-*myc* probes (see Figure 6). A dash indicates that no rearranged fragment was detected.

[f]Proposed target within the H chain locus for c-*myc* translocation (see Figure 4 and text). X is unknown, but presumably γ or ϵ.

[g]Probes a–i all hybridized to the same size *Eco*RI fragment, but the existence of separate M and R fragments is inferred (see text).

[h]It is assumed that R rather than M is linked to 5′ Cα sequences, by analogy with, for example, MOPC 173.

[i]For MOPC 104E, HOPC 1 and MOPC 41A, recombination occurred within region c or d because a rearranged *Bam*HI fragment was detected by probe e as well as the expected 1.1-kb fragment from the germline allele.

[j]No R fragment was detected, perhaps due to chromosome loss (see text).

[k]No sequences 5′ to S_α (region p) are retained in MOPC 41A, so presumably the translocation target was a switched α gene.

HOPC 1 (unpublished results). Only TEPC 1017 gave no evidence of a reciprocal event (see below).

The rearranged c-*myc* 5′-flanking region in many lines appears to be linked to S_μ, as in J558. Figure 3C indicates that an S_μ probe hybridized to a fragment of the same size (arrowed) as that bearing c-*myc* 5′-flanking sequences (Figure 3B). (Other rearranged fragments detected by S_μ reflect rearrangement on the other allele; Cory *et al.*, 1980). The arrowed fragments in Figure 3C which hybridized more strongly than that in J558 probably contain a larger portion of the S_μ region. Three lines were analyzed further to confirm that the S_μ sequences remain linked to a J_H region, as postulated in Figure 1B. Since the J_H-S_μ region has no *Kpn*I site and that in the c-*myc* 5′-flanking region lies well upstream from the J558 recombination point (Figure 2A), a single *Kpn*I fragment should bear c-*myc* 5′-flanking sequences, J_H and S_μ sequences, as diagrammed for J558 in Figure 3D. Figure 3E shows that all three probes labelled an 8.3-kb fragment in J558 and an 18.7-kb fragment in plasmacytoma S107, as they did a 17-kb fragment in WEHI 267 (not shown). These different sizes, which cannot be accounted for by different c-*myc* breakpoints (see below), presumably reflect different V and/or D regions recombined at the J_H locus. Thus, one J_H allele in these lines is linked *via* S_μ to c-*myc* 5′-flanking se-

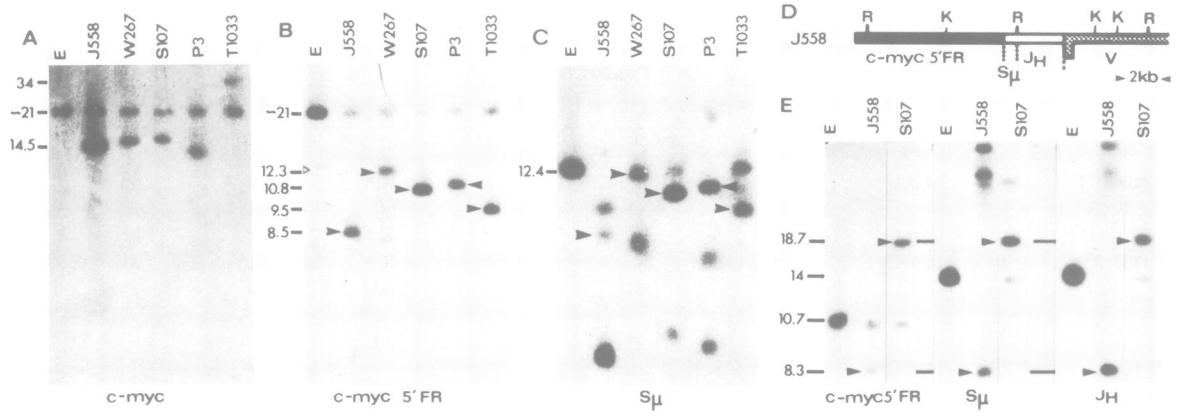

Fig. 3. Reciprocal products of c-*myc* translocation. Southern blots **A** and **B** demonstrate that fragments bearing the c-*myc* gene differ from those bearing its 5′-flanking region (FR), while **C** shows that the latter bear S_μ sequences. **D** depicts the rearranged c-*myc* 5′FR in J558; linkage of the indicated *Eco*RI fragments is supported by the *Kpn*I analysis of genomic DNA shown in **E**. The rearrangement at J_H is assumed to be a VDJ fusion. For **A**–**C**, *Eco*RI digests of DNA (15 μg) from BALB/c embryos **(E)** and the indicated plasmacytomas were fractionated on a 0.7% agarose gel and gel blots hybridized with c-*myc* probe h **(A)**, c-*myc* 5′FR probe m **(B)** and S_μ probe o **(C)**. For **E**, *Kpn* digests were hybridized to c-*myc* 5′FR probe b, S_μ probe o and a J_H probe bearing sequences extending 1.7 kb to the right of the *Eco*RI site between S_μ and J_{zh} in **D**. Fragment sizes are in kb. R and K in **D** indicate *Eco*RI and *Kpn*I sites.

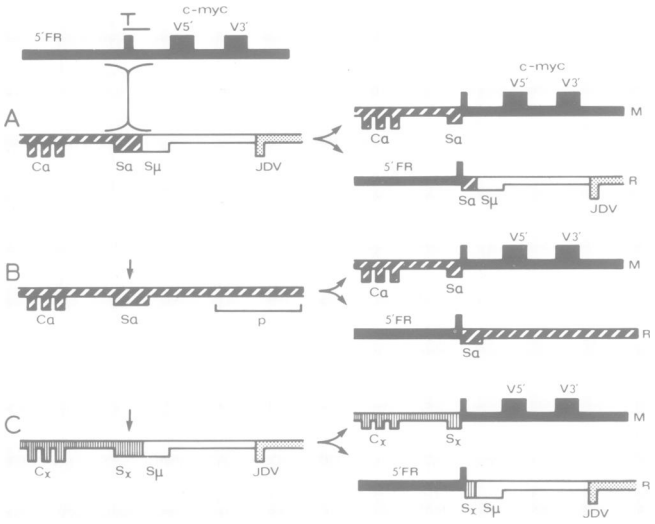

Fig. 4. Three types of c-*myc* translocation to the H chain locus. Cross-over between the c-*myc* locus (filled bar at top) and H locus structure **A**, **B** or **C** will give the two products shown on the right (see text). A single recombination point within translocation region T, which spans a 5′ c-*myc* exon, is illustrated. In **B**, p denotes a probe 5′ to the S_α region (a 3.1-kb *Hind*III-*Hha*I fragment lying 6.2 kb 5′ to the *Eco*RI site within the germline C_α gene; see Figure 1 of Adams *et al.*, 1982). In **C**, the putative S_x and C_x denote any of the six S_H and C_H regions between C_μ and C_α (C_δ, $C_{\gamma 3}$, $C_{\gamma 1}$, $C_{\gamma 2b}$, $C_{\gamma 2a}$, C_ϵ).

quences, while the other is part of the functional immunoglobulin gene.

Three types of c-myc translocation

As in J558, most lines having S_μ linked to the c-*myc* 5′-flanking region have c-*myc* linked to $S_\alpha C_\alpha$ (Table I). The simplest interpretation of these results is that the target for c-*myc* translocation was an assembled α gene, as depicted in Figure 4A. Ten α-expressing plasmacytomas, and possibly a γ-expressing plasmacytoma (Y5606) and a B lymphoma (BALTELM 1131), had this apparent $S_\mu S_\alpha$-C_α target (Table I).

A second type of translocation appears to have a germline S_α target rather than a switched S_α (Figure 4B). This was inferred from the observation that sequences 5′ to germline S_α (probe p in Figure 4B) hybridized to the fragment bearing c-*myc* 5′-flanking sequences, as shown for four lines in Figure 5, whereas S_μ sequences did not (not shown). This category with an apparent $S_\alpha C_\alpha$ target includes five lines (Table I): B-lymphoma BALTNLM 17, which expresses μ (Kim *et al.*, 1979), two plasmacytomas that express γ2a (MOPC 173 and HOPC 1), and two (SAMM 368 and TEPC 609) that express both γ2b and α (Morse *et al.*, 1976).

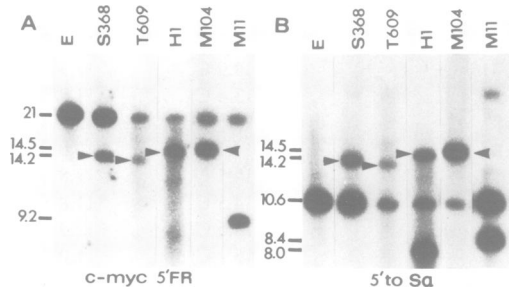

Fig. 5. Southern blots showing apparent linkage in some plasmacytomas of the c-*myc* 5′-flanking region and sequences 5′ to the S_α region (see Figure 4B). Blots of fractionated *Eco*RI digests of DNA from embryos and plasmacytomas were hybridized in **A** with probe a of Figure 2 and in **B** with p of Figure 4.

The third type of translocation probably involves an S_H target other than S_α (Figure 4C). In B lymphoma WEHI 279 and plasmacytomas TEPC 1033 and P3, the c-*myc* 5′-flanking sequence appears to be linked to S_μ, establishing that recombination has occurred within the C_H locus, but the c-*myc* gene is linked to unknown region(s) (U) rather than to C_α (Table I). Involvement of S_μ suggests that the target probably was another switched heavy chain gene, as depicted in Figure 4C, where C_x denotes any of the six C_H genes between C_μ and C_α.

Plasmacytomas MOPC 104E (μ expressor) and MPC 11 (γ2b) do not fit into the three categories in Figure 4. In MOPC 104E, sequences 5′ to S_α hybridized to the fragment bearing the rearranged c-*myc* 5′-flanking region in both *Eco*RI (Figure 5) and *Bam*HI digests (not shown), suggesting that translocation originally occurred to the germline S_α

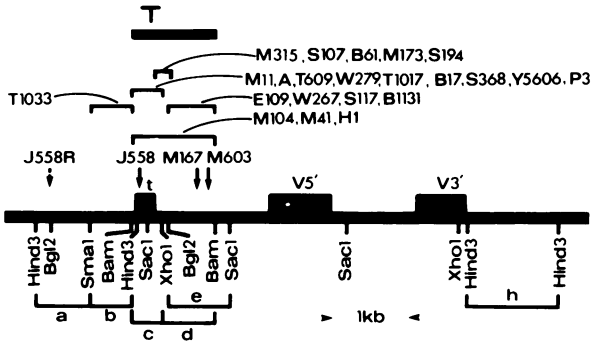

Fig. 6. Localization of the chromosome 15 breakpoints in 22 plasmacytomas and three B lymphomas. Recombination points in J558 (Adams *et al.*, 1982) and in McPC 603 and MOPC 167 (Calame *et al.*, 1982) are fixed by published sequences, and the others are localized by hybridization with the lettered probes (see text and Table I). Restriction data on an S107 clone (Kirsch *et al.*, 1981) places its recombination point within a few hundred bp of that in J558. For J558, which like Y5606, has suffered a deletion encompassing region b (see text), broken arrow J558R indicates the apparent recombination point between c-*myc* 5'-flanking sequences and the S_μ region as determined in clone J558 5' (Figure 2).

region (as in Figure 4B), but the only M104E C_α gene, which is rearranged, lies on a different *Eco*RI fragment than the c-*myc* gene (4 kb *versus* 14.5 kb), perhaps as a result of a secondary recombination event. In MPC 11, the 5' S_α probe did not detect the rearranged c-*myc* 5'-flanking sequence (Figure 5), nor did S_μ (not shown), and the sequence linked to the c-*myc* gene is unknown (Table I). Thus, the target in this line remains undetermined and might not involve the H chain locus (see also Harris *et al.*, 1982b).

Chromosome 15 breakpoints within the c-myc locus

To map c-*myc* breakpoints, we analyzed DNA from each line with a set of c-*myc* region probes near the J558 breakpoint (a – e in Figure 6). By determining which regions lie on the fragment bearing the c-*myc* gene (M) and which on that bearing its 5'-flanking sequences (R) (Table I), we localize the cross-over points to the bracketed regions in Figure 6. In 23 of 24 lines, the breakpoint fell within a region designated T in Figure 6, which corresponds to a 1.1-kb *Bam*HI fragment located 0.7 – 1.8 kb upstream from v5'. This conclusion was confirmed for 13 of the lines (indicated by an asterisk in Table I) by detection of a rearranged *Bam*HI fragment hybridizing to probe e. In TEPC 1033, recombination occurred up to 0.4 kb further 5', within region b. A 'hot-spot' within region T is the 408-bp region c, within which breakpoints for J558 and at least 14 of the other lines mapped. In most of the remaining lines, the breakpoint was closer to v5', within the 650-bp region d, and arrows mark those fixed by nucleotide sequences for McPC 603 and MOPC 167 (Calame *et al.*, 1982). Moreover, five of six breakpoints analysed by Shen-Ong *et al.* (1982), including MOPC 104E and MOPC 315, fell within c or d, and that of TEPC 15 just to the right of d, within e. Thus, all the breakpoints fell near or within the 5' exon designated t in Figure 6, a finding with important implications for c-*myc* transcription (see Discussion).

Deletions may be associated with some translocations

Recombination may result in loss of sequences. About 1 kb of germline c-*myc* sequences near the J558 breakpoint, including all of region b, is not present in either J558 clone (Figure 2A). This deletion is not a cloning artefact, because probe b did not hybridize to either rearrangement product in J558 tumour DNA, and region b is also deleted in Y5606 (Table I). Moreover, the reciprocal product R would be expected to contain some S_α as well as S_μ sequences, as in Figure 4A, but the J558 5' clone has no substantial S_α region. While sequence analysis will be required to exclude the presence of less than ~60 bp of the S_α region, a fragment extending from the c-*myc* portion of J558 5' into its S_μ region (m in Figure 2B) did not hybridize to the 10.4-kb S_α-bearing fragment in embryo DNA, even though it hybridized (weakly) to the 12.5-kb S_μ-bearing fragment (indicated by an open arrowhead in Figure 3B), as well as strongly to the ~21 kb c-*myc*-bearing fragment. In TEPC 1017, no rearranged fragment was detected by regions a, b, j, k or l (not shown). We conclude that TEPC 1017 has either suffered a larger deletion than J558 or Y5606 (>5 kb), or has lost the chromosome bearing the R product.

The c-myc translocation region is not homologous with switch regions

The apparent analogy with switch recombination (Figure 1) raises the question of whether 15;12 translocations involve sequences near c-*myc* that are homologous to C_H switch regions. We previously reported that fragments of the c-*myc* locus extending 3.7 kb downstream from the J558 recombination point did not hybridize to cloned S_μ, S_α, $S_{\gamma 1}$ and $S_{\gamma 3}$ regions (Adams *et al.*, 1982). We have now tested probes upstream from the J558 3' recombination point (a and b in Figure 1) against these switch regions, and have retested probes c and d from region T at very low stringency, under conditions where even 50 bp of closely related sequences would have been detected. No hybridization was observed. These results exclude any translocation mechanism requiring substantial homology between the recombining regions.

Discussion

We have shown that the recombination between c-*myc* on chromosome 15 and the immunoglobulin H locus on chromosome 12 involves a reciprocal exchange. Three B lymphomas and 20 of 21 BALB/c plasmacytomas with a c-*myc* rearrangement had the structures expected for a reciprocal rearrangement and, in 23 of these lines, one or both products appears to contain either S_μ or S_α sequences. Thus, nearly all c-*myc* translocations in BALB/c plasmacytomas represent recombination within a C_H switch region.

The three types of reciprocal rearrangement products distinguished here presumably reflect different 'targets' for c-*myc* translocation within the H locus. Structures like those cloned from J558 were detected in at least 10 other lines and, as indicated in Figure 4A, could result from recombination of the c-*myc* gene with a S_α region already fused to S_μ. Five lines appear instead to have had a germline S_α target (Figure 4B), and three to involve other (unidentified) S_H region(s) fused to S_μ (Figure 4C). Figures 4A and 4C assume that S_μ-S_H recombination occurred prior to translocation, but it may have occurred afterwards. Alternatively, no $S_\mu S_H$ fusion may have occurred: breaks created within S_μ and the other S_H region by the switch machinery may have recombined directly with the two segments of the c-*myc* locus. On this model the R product in Figure 4A (and 4C) would bear no S_α (S_X) sequences.

The predominance of translocations to S_α in these BALB/c tumours may relate to the prevalence of α-expressing tumours in this strain (Potter, 1972). For instance, deletion of all S regions between S_μ and S_α on both alleles in some α-

expressing lines (Cory *et al.*, 1980) might restrict c-*myc* translocation to S_α, assuming that the deletions occurred prior to translocation. Nevertheless, at least five non-α-expressing lines exhibited c-*myc*-S_α recombination (Table I), so S_α is clearly favored as a target. Since rearrangements of c-*myc* in NZB tumours are not to S_α or seemingly to any other S_H region (Harris *et al.*, 1982b), the preferred target apparently differs for the two strains in which plasmacytomas are readily induced.

Since nearly all chromosome 15 breakpoints (Figure 6) fall either within the 408-bp region c, which contains a 5′ c-*myc* exon, or downstream from it, translocation disrupts the normal c-*myc* transcription unit (Adams *et al.*, 1983), and thus must separate the bulk of the gene from its normal 5′ regulatory signals. Hence the altered c-*myc* mRNAs detected in plasmacytomas (Adams *et al.*, 1982, 1983; Shen-Ong *et al.*, 1982; Mushinski *et al.*, 1983) must result from activation of a cryptic promoter, as discussed previously (Adams *et al.*, 1983). The proposal that these mRNAs encode altered *myc* polypeptides (Shen-Ong *et al.*, 1982; Adams *et al.*, 1983) contrasts with models for avian leukosis virus-induced chicken B lymphomas (Haywood *et al.*, 1981; Payne *et al.*, 1982), which emphasize the elevated levels of the presumably normal c-*myc* gene product. However, like the mouse translocations, the great majority of avian retroviral insertions occur within a small upstream region proposed to contain 5′ c-*myc* exon(s) (Neel *et al.*, 1982a), so the avian c-*myc* protein might also be altered.

Some constraints can be set on the c-*myc* translocation mechanism. Sequences for several kilobases around the translocation region T hybridized to genomic DNA like unique sequences, ruling out involvement of repeated sequences such as insertion elements (Rechavi *et al.*, 1982), or endogenous proviruses. No homology with S_μ, S_α or S_γ regions was found by hybridization, and the sequence of region c (Adams *et al.*, 1982), within which at least 15 breakpoints lie (Figure 6), lacks homology with published sequences of S_μ (Takahashi *et al.*, 1980) and S_α (Davis *et al.*, 1980). Thus, recombination based upon homologous regions around the breakpoints appears to be excluded. The preference for switch region targets, and the apparent restriction of the t(15;12) to tumours of relatively mature B lymphocytes (Klein *et al.*, 1980; Adams *et al.*, 1982), implicate switch recombination enzymes, but the known portion of the T region sequence lacks the features so far associated with switch regions (Adams *et al.*, 1982). Thus, the switch machinery may well make the scission(s) on chromosome 12 while some other mechanism breaks chromosome 15. Apparent deletions near the J558 and Y5606 breakpoints may reflect either the recombination mechanism or subsequent selection due to some effect on c-*myc* transcription.

To date, t(15;12) has not been detected cytogenetically in B lymphomas, although trisomy of chromosome 15 is frequent (Wiener *et al.*, 1981). However, three of five B lymphomas analyzed here had reciprocal c-*myc* rearrangements (Table I), including one, BALTNLM 17, analyzed by Wiener *et al.* (1981). Presumably, translocation in this line occurred subsequent to karyotyping. Although translocations appear to be confined to the B lymphocyte lineage, 3 of 18 T lymphomas had a c-*myc* rearrangement (Adams *et al.*, 1982), which may have had an oncogenic role (Cory *et al.*, 1983).

Alteration of c-*myc* is probably not sufficient for plasma cell oncogenesis, because plasmacytomas also contain a different oncogene which is capable of transforming fibroblasts (Lane *et al.*, 1982; Crews *et al.*, 1982; Adams *et al.*, 1983). Nor can c-*myc* rearrangement be essential, because we observed neither of the expected reciprocal products in plasmacytomas HPC 76 (a μ expressor) or ABPC 4 (an α expressor), or two B lymphomas (Adams *et al.*, 1982 and Table I), and a number of others are negative (e.g., Mushinski *et al.*, 1983). Hence another oncogene, such as c-*mos*, may function in some plasmacytomas (Rechavi *et al.*, 1982). Since ABPC 4 has a 15;6 translocation involving the band of chromosome 15 involved in 15;12 translocations (S.Ohno *et al.*, personal communication), another gene within that band may be implicated, as may also be the case for those Burkitt lines displaying translocations but lacking c-*myc* rearrangement (Taub *et al.*, 1982; Adams *et al.*, 1983).

Materials and methods

Tumour cell lines

Many of the lines, kindly provided by M.Potter, are described in the catalogue of Litton Bionetics (Kensington, MD). WEHI-279 and WEHI-231 are described by Gutman *et al.* (1981); 2PK-3 by Warner *et al.* (1975); BALTNLM 17 by Mathieson *et al.* (1978); EPC 109 and ABPC 4 by Lieberman *et al.* (1975); S194 and P3 by Horibata and Harris (1970); S117 and J558 by Ralph (1973); Samm 368 and TEPC 609 by Morse *et al.* (1976); MPC 11 by Laskov and Scharff (1970); and HPC 76 by MacKenzie *et al.* (1978). WEHI-267 is an IgA-producing plasmacytoma induced in a BALB/c mouse by injection of Abelson murine leukaemia virus and oil (Warner and Harris, unpublished data), as was ABPC 4. BALTELM 1131 is a B cell tumour from M.Potter. A is a BALB/c plasmacytoma (of unknown origin) in our WEHI collection. Nearly all the tumours were originally induced with mineral oil (or pristane), except MPC 11 which arose in a mouse implanted with a Millipore diffusion chamber; WEHI-279, which was X-ray induced; and BALTNLM 17, which arose in a mouse injected with pristane and ethyl nitrosourea.

DNA extraction and Southern blotting

DNA was extracted by a modification of Gross-Bellard *et al.* (1973). A washed suspension of ~10^9 cells, usually from tumour tissue, was resuspended in 20 ml of 10 mM NaCl, 10 mM Tris-Cl, 10 mM EDTA, pH 7.5. After the addition of an equal volume of 0.5 M EDTA (pH 7.5), the solution was made 0.5% in sarkosyl and 200 μg/ml in proteinase K (Merck), and incubated at 50°C for 3 h and then at 37°C overnight. The highly viscous solution was extracted three times with phenol saturated with buffer A (0.5 M Tris-Cl pH 8.0, 10 mM EDTA, 10 mM NaCl, 0.5% SDS) by mixing with a stirring bar for 15 min. The aqueous phase was extracted twice with $CHCl_3$-isoamyl-alcohol (24:1) saturated with buffer A and then dialysed at room temperature for 24 h against 0.2 x SSC (SSC = 0.15 M NaCl, 0.15 M trisodium citrate). RNA was removed by isopropanol precipitation (Marmur, 1961): 0.1 vol 3 M sodium acetate, 10 mM EDTA pH 7 was added and then 0.54 vol of isopropanol added dropwise while stirring vigorously; DNA was wound out on a rod and dissolved in 10–20 ml of 0.2 x SSC at 37°C overnight. Isopropanol precipitation was repeated, the DNA dissolved overnight at 37°C in 10 mM Tris-Cl, 1 mM EDTA (pH 7.5) and stored at 4°C.

The Southern (1975) blotting procedure was carried out as detailed by Cory and Adams (1980). Fragments used as probes were nick-translated (Rigby *et al.*, 1977) except for M13 probes c and d which were labelled by primer extension (Messing, 1981).

Clones and probe fragments

The ST4 3′ clone and J558 5′ clone (Figure 2) were isolated from T lymphoma ST4 and plasmacytoma J558 by the procedure used for the J558 3′ clone (Adams *et al.*, 1982): *Eco*RI digests were sedimented on a glycerol gradient and fragments of selected sizes packaged (Hohn, 1979) into phage Charon 4A. Recombinant phage were screened (Benton and Davis, 1977) with the probes indicated in Results. Fragments used as probes (lettered in Figure 2) were derived from subclones in pBR322, except for probes c and d, which were cloned into M13mp8 and M13mp9 (Messing, 1981).

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