

Hemizygous interstitial deletion of chromosome 15 (band D) in three translocation-negative murine plasmacytomas

(high-resolution banding/*c-myc* expression)

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ABSTRACT Three murine plasmacytomas that were exceptional in lacking the characteristic (12;15) or (6;15) translocations were studied by G banding and high-resolution banding. One of every two chromosomes 15 (two of four in tetraploid tumors) was shortened in all three tumors. High-resolution banding analysis revealed that this was due to an interstitial deletion in the 15D band region. The two breaks responsible for the deletion have been tentatively localized to the interface of bands D2/3 and within band D2. One of the three plasmacytomas, ABPC45, had a rearranged *c-myc* gene. All three tumors contained a greater abundance of 2.4-kilobase *myc* RNA transcripts than normal spleen or thymus. The *c-myc* gene is located in the 15 D2/3 band region. We suggest that it may have joined the centromeric portion in the deletion plasmacytomas. This transposition may have led to its constitutive activation, as in the more frequent translocation-carrying plasmacytomas.

Murine plasmacytomas induced in the BALB/c or NZB strain of mice consistently contain one of two alternative translocations, $rcpt(12;15)$ or $rcpt(6;15)$ (1, 2). $rcpt(12;15)$ is referred to as the typical translocation because it is found in the majority of the murine plasmacytomas, including both κ and λ chain producers. The variant $rcpt(6;15)$ is only seen in a minority of the κ chain producers. Chromosomes 12 and 6 are known to carry the Ig heavy chain and the κ light chain locus, respectively (3-5). Therefore, we have suggested (2) that the translocations contribute to the carcinogenic process by transposing an oncogene, located in the distal segment of chromosome 15, into the highly active neighborhood of the immunoglobulin genes (6). This hypothesis was further supported by closely similar findings on a human B-cell-derived tumor, Burkitt lymphoma (for review see ref. 6). Molecular analysis of tumors containing the typical translocations has confirmed this hypothesis and also showed that the two systems are homologous, because they involve the same oncogene, *c-myc*. In the mouse, the *c-myc* is located at the translocation breakpoint on chromosome 15. In the majority of the cases it is transposed to the $S_{\alpha}-C_{\alpha}$ region of the IgH locus on chromosome 12, where it is actively transcribed (7-16).

Pristane oil-induced plasmacytomas show a great preponderance of the $rcpt(12;15)$ translocation ($\approx 85\%$). Plasmacytomas induced by a combination of pristane oil and Abelson murine leukemia virus (A-MuLV) carry (12;15) and (6;15) in approximately equal proportions (17).

In our previous study on 14 pristane oil-induced early-passage generation plasmacytomas, we have encountered 2 tumors that did not contain any translocations (1). In another karyotypic study of 8 diffusion chamber- and 17 A-MuLV-

induced plasmacytomas, 1 and 2 tumors were translocation negative, respectively (unpublished data).

In a total of 39 murine plasmacytomas we have thus encountered 5 translocation-negative tumors (13%). Although some of them were near diploid and others near tetraploid, they appeared to lack any obvious chromosomal marker. The question arose whether they had been generated by different mechanisms.

We now report that G-banding studies on three translocation-negative tumors, combined with high-resolution banding (HRB) analysis, revealed the same type of aberrant banding pattern in three tumors affecting part of the major D band. We previously found (1, 2) that the breakpoint on chromosome 15, involved in the plasmacytoma-associated $rcpt(12;15)$ and $rcpt(6;15)$ translocations, can be localized to the same band.

MATERIALS AND METHODS

Plasmacytomas. ABPC26 and ABPC45 were induced in BALB/c mice by injecting 0.5 ml of pristane oil intraperitoneally and infecting the mice intraperitoneally with 0.1 ml of A-MuLV 23-56 days later. CBPC112 was induced by 0.5 ml of pristane oil intraperitoneally as described in ref 1.

Chromosome Preparation. Metaphase plates were prepared from the ascites of plasmacytomas without Colcemid treatment. G banding was performed by a slight modification of Wang and Federoff's method (18). Chromosomes were identified according to the standard mouse karyotype (19).

HRB. High quality prometaphase and prophase chromosome plates were prepared according to the method of Yu *et al.* (20) adapted to mouse chromosomes. Major and minor band identification followed the nomenclature of Nesbitt and Francke (21).

Isolation and Hybridization of RNA and DNA Blots. Genomic DNAs were prepared, digested with restriction endonuclease, electrophoresed in 0.7% agarose gels, transferred to nitrocellulose filters, and hybridized as described (22). Total poly(A)⁺ RNA was prepared, electrophoresed in 1% agarose gels containing 5 mM methylmercury hydroxide, transferred to 2-aminophenylthioether (APT) paper, and hybridized as described (22). In both cases, the hybridization probe was a nick-translated 5.5-kilobase-pair *Bam*HI fragment excised from a plasmid containing *c-myc* from plasmacytoma S107 (14).

RESULTS

Table 1 summarizes the main immunoglobulin and karyotype characteristics of the three translocation-negative plasmacytomas. More detailed karyotypes will be reported elsewhere (unpublished data).

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Abbreviations: A-MuLV, Abelson murine leukemia virus; HRB, high-resolution banding.

Table 1. Ig production and karyotype of three translocation-negative plasmacytomas

Plasmacytoma	Mode of induction	Ig chain type		Modal chromosome no.	Interstitial deletion at chromosome 15*	Trisomy 11 [†]	No. of diploid/ no. of tetraploid plates
		Heavy	Light				
ABPC26	Pristane + A-MuLV	α	?	41;76	Yes	4/5	8/20
ABPC45	Pristane + A-MuLV	α	κ	41;80	Yes	8/11	15/25
CBPC112	Pristane	μ	κ	79	Yes	None	0/12

*One copy in near-diploid cells; two copies in near-tetraploid cells.

[†]Identified in near-diploid cells only. Each value is the number of metaphase plates of near-diploid cells with three chromosomes 11/total metaphase plates examined.

Conventional G banding showed no detectable changes in chromosome 6, 12, or 15. However, detailed analysis of some plates with well-preserved and elongated chromosomes focused our attention on a regular size difference between the two chromosomes 15. Diploid tumor cells usually contained one chromosome 15 that was shorter than its normal homologue (Fig. 1), whereas tetraploid tumor cells contained two shortened and two normal chromosomes 15 (Fig. 2). The difference in length could be related to a size difference in the major band 15D.

To obtain more precise information, we have analyzed the pairs of chromosome 15 by HRB in two plasmacytomas, ABPC26 and ABPC45. Fig. 3 shows the findings in ABPC26. In the normal chromosome 15, the major D band is composed of two minor dark bands (D1 and D3) of approximately equal size. A minor white band (D2), thinner than D1 and D3, is intercalated between the dark bands. In contrast, the

aberrant chromosome 15 contained only one thin D band, corresponding in size to either the D1 or D3 band. There was no intercalated white band visible at all. This was more easily recognizable on the chromosome pairs subjected to HRB analysis (Fig. 3 *a-c*) than on the conventional G-banded chromosomes (Fig. 3 *d-f*). We interpret these findings to mean that an interstitial deletion has occurred in these tumors, resulting in a smaller D band and a correspondingly shorter chromosome 15.

Southern blots of *EcoRI*, *BamHI*, and *HindIII* digests of genomic DNAs were hybridized to a *c-myc* probe (Fig. 4*a*) to test whether the interstitial deletion affects the *c-myc* transcriptional unit. The *c-myc* locus was rearranged in ABPC45 but not in CBPC112 or ABPC26, according to the *EcoRI* DNA digest. *BamHI* and *HindIII* digestion did not reveal the rearrangement in ABPC45, suggesting that the breakpoint site was relatively far from the *c-myc* structural gene. This

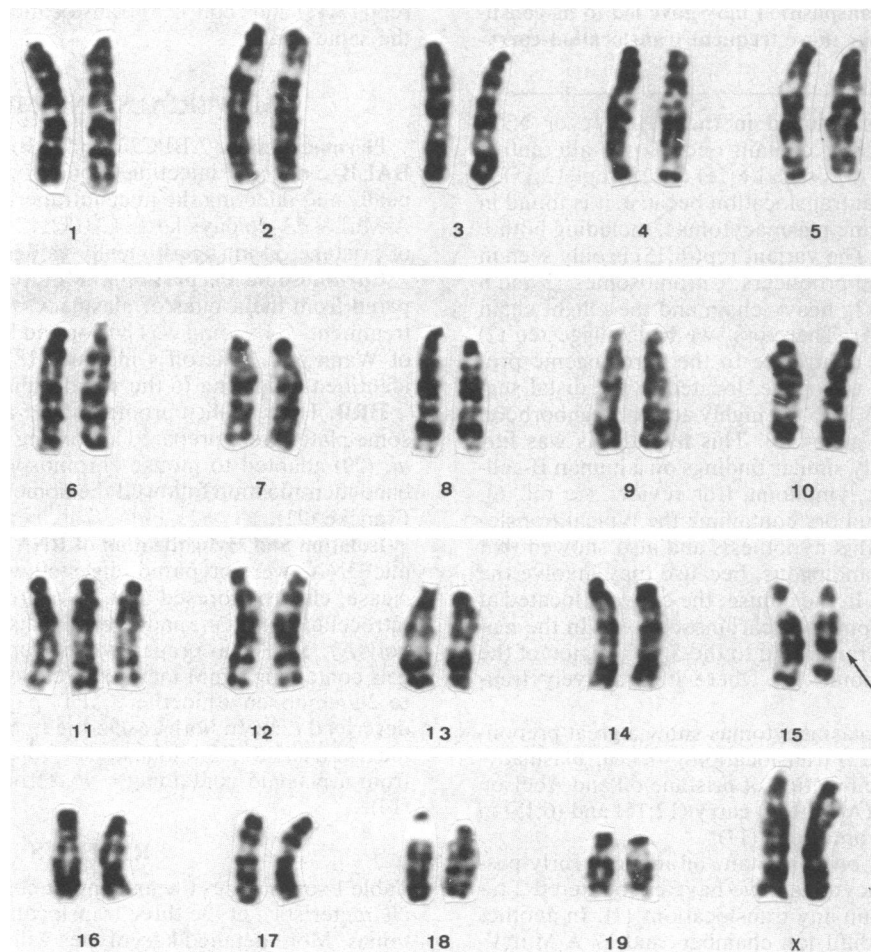


FIG. 1. G-banded metaphase plate of a near-diploid plasmacytoma (ABPC26). Note the absence of the plasmacytoma-associated translocation chromosomes. The aberrant chromosome 15 (arrow) is shorter than its corresponding homologue. Each form of chromosome 15 is present in a single copy.

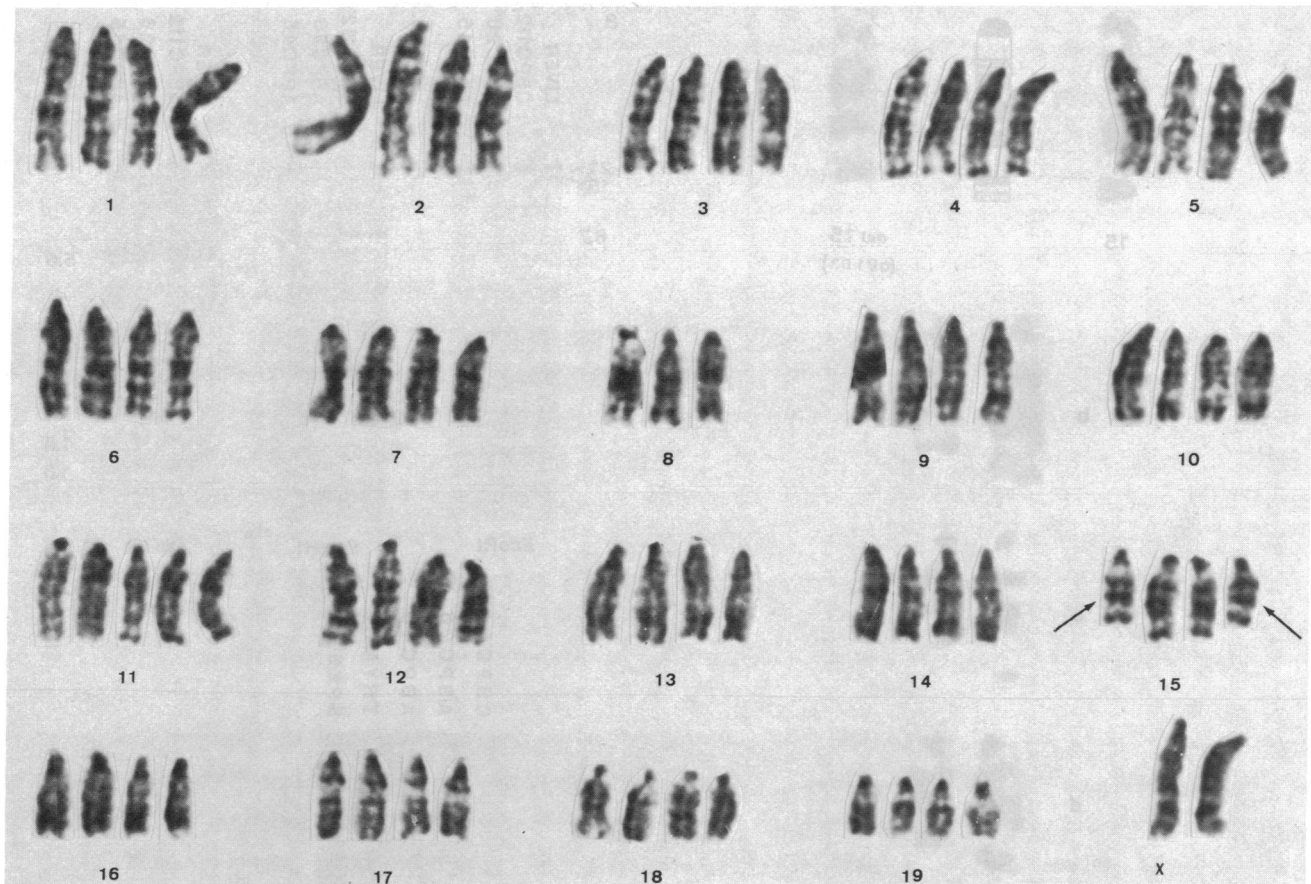


FIG. 2. G-banded metaphase plate of a near-tetraploid plasmacytoma (ABPC45). Note the absence of the plasmacytoma-associated translocation chromosomes. Two copies of the normal and two copies of the shorter aberrant chromosomes (arrows) are present.

was also indicated by the RNA transfer blots (Fig. 4*b*). Only the normal-sized 2.4-kilobase *myc* RNA was present in all three tumors, including ABPC45 (Table 2). The intensity of the RNA band suggested a 3- to 10-fold increased level of the *c-myc* transcription product, compared to normal spleen cell RNA, and a 2- to 3-fold increase over normal thymus RNA.

DISCUSSION

Using HRB, we have found (23) that the breakpoint on chromosome 15 in plasmacytomas with the typical (12;15) or the variant (6;15) translocation maps to the junction (interface) between bands D2 and D3. Molecular studies have shown (9, 11, 16) that the break usually cuts across the 5' end exon of the *c-myc* gene or its immediate neighborhood, and the severed oncogene is translocated to the immunoglobulin heavy chain locus (IgH). Frequently, it faces the α switch region immediately 5' to the IgA heavy chain constant region, although occasionally it may face other regions. In all of these cases, the *c-myc* and IgH sequences face each other head to head, 5' to 5' (9)—i.e., in the reverse transcriptional orientation.

In the present study, three translocation-negative plasmacytomas were analyzed. In each of these tumors, one of the two homologous chromosomes 15 (or two of four in the tetra-

ploid tumors) appeared shortened in G-banding studies. The HRB analysis showed that the minor white D2 band was deleted. Because the HRB technique did not reveal any discernible minor "sub-bands" in the D1 or the D3 band, it was difficult to determine which of the dark bands was deleted in addition to the white D2 band. However, a joint consideration of the cytogenetic and the molecular data permits a tentative definition of the breakpoints and the band regions removed by the deletion.

Interstitial deletions require two breaks, followed by the loss of the interstitial piece and the joining of the centromeric and telomeric chromosome segments. In the present case, the deletion may, in principle, lead to a complete elimination of the *c-myc*-containing chromosomal segment or it may affect a chromosomal region upstream or downstream of the *c-myc* gene.

The cytogenetic mapping of the breakpoints flanking the deleted segment is determined by the orientation of the *c-myc* locus in relation to the centromere. As drawn schematically in Fig. 5, the 5' end of *c-myc* gene on chromosome 15 could be centromere proximal (alternative A) or distal (alternative B).

According to alternative A, the first break would occur outside the 5' exon of the oncogene through the interface of bands D2/3. This would be consistent with the normal-sized *c-myc* transcripts and also with the previously defined breakpoint on chromosome 15 in translocation-carrying plasmacytomas. Because HRB has revealed that the minor white D2 band was deleted, the second break—proximal to centromere—has to occur either on the interface of bands D2/1 or within the D1 band (Fig. 5*a*). This would suggest that the dark band on the deleted chromosome 15 arose from the joining of the D3 band—the *c-myc*-carrying chromosomal re-

Table 2. Occurrence of *c-myc* rearrangement and level of *c-myc* transcription

Plasmacytoma	Rearranged <i>c-myc</i>	<i>c-myc</i> RNA	
		1.8 kilobases	2.4 kilobases
ABPC26	—	—	+++
ABPC45	+	—	+++
CBPC112	—	—	+++

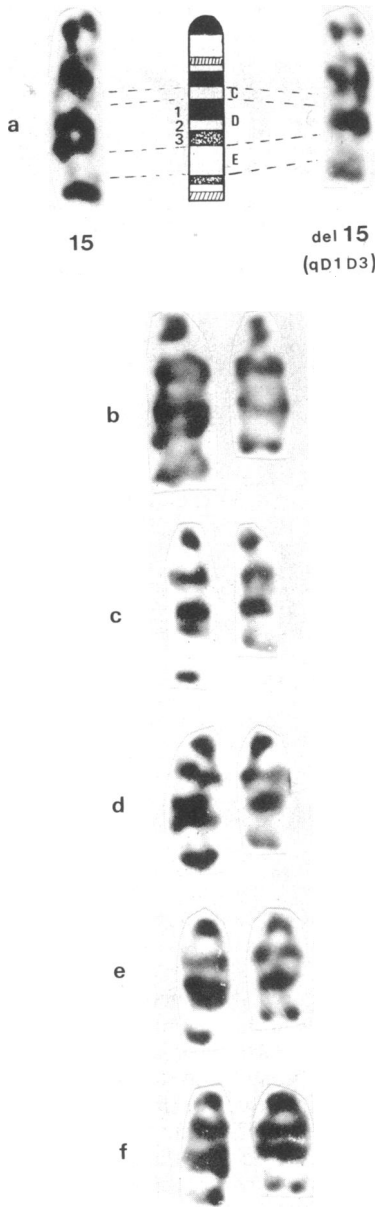


FIG. 3. Selected pairs of homologous chromosome 15 from metaphase plates of ABPC26. Pairs *a-c* are derived from plates prepared by HRB; pairs *d-f* are derived from plates prepared by conventional G banding. For details, see text.

gion—with the D1 band or part of the latter (Fig. 5*b*).

The other possibility is illustrated in alternative B. Here, the same two assumptions are made: the first break occurs

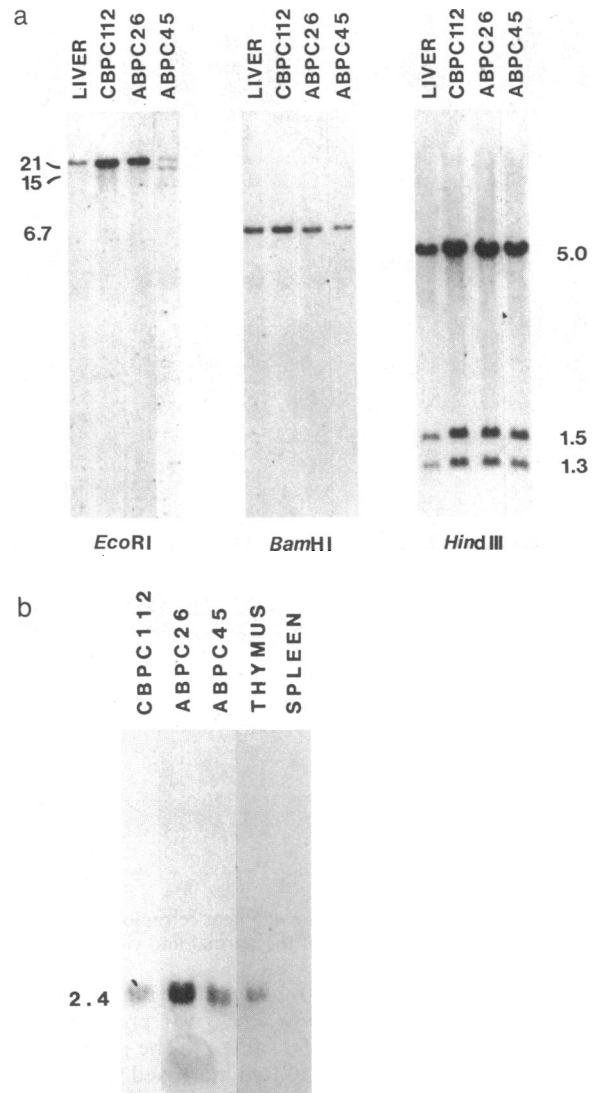


FIG. 4. DNA and RNA blots hybridized with mouse *c-myc*. (a) Twenty-five micrograms of genomic DNA was digested with the restriction endonuclease indicated, electrophoresed on a 0.7% agarose gel, blotted onto nitrocellulose, and hybridized as described (22). (b) Five micrograms of poly(A)⁺ RNA was denatured and electrophoresed on 1% agarose gels containing 5 mM methylmercury hydroxide, blotted onto diazotized paper, and hybridized as described (22). Sizes are given in kilobases.

just outside the 5' exon of *c-myc* gene and affects band D2/3. It represents the centromere-proximal break in this case. The second break would occur distally to the first, on the telomeric side (Fig. 5*c*), resulting in a narrow dark band (D1)

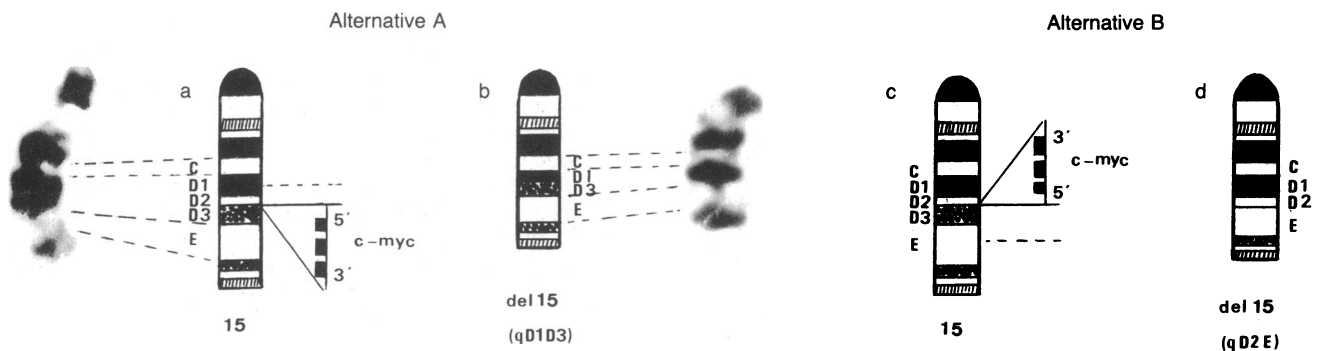


FIG. 5. Schematic illustration of two alternative possibilities that could explain the cyto-genetic mapping of the breakpoints on chromosome 15 in translocation-negative plasmacytomas. For details, see text.

and a wide band (D2 and DE), as shown in Fig. 5*d*. An opposite, centromere-proximal deletion would remove the *c-myc*-containing chromosomal segment. This would be unlikely, both in view of the HRB pattern of the deleted chromosome and the high *c-myc* transcription in the tumor.

It has been suggested that the deletion of the 5' exon of *c-myc* or the translocation of the oncogene to an active Ig region (or both) may be responsible for the constitutive switch-on of the oncogene. The existence of the "deletion" plasmacytomas shows that the gene can also be activated by rearrangement within a single chromosome 15. It is most likely that this happens by the joining of the 5' end of the *c-myc* oncogene to an actively transcribed chromatin region in the D1 band area of chromosome 15.

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