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# The c-*myc* oncogene is translocated to the involved chromosome 12 in mouse plasmacytoma

(genetics of B-cell neoplasia/chromosomal translocations/gene orientation/immunoglobulin heavy chain locus)

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ABSTRACT **Although it is known that the c-*myc* oncogene is rearranged in a head-to-head fashion with the immunoglobulin heavy chain locus in mouse plasmacytomas, it has not been clear whether the c-*myc* oncogene is translocated to the heavy chain locus on mouse chromosome 12 or whether the heavy chain locus is translocated to the c-*myc* locus on mouse chromosome 15. To determine which of these two possibilities is correct, we hybridized Chinese hamster fibroblasts with J558 mouse plasmacytoma cells that carry a reciprocal chromosome translocation between chromosomes 12 and 15, and we examined the segregating hybrids for the presence of the normal and rearranged mouse c-*myc* genes, for the presence of different regions of the mouse heavy chain locus, and for the presence of genes located on mouse chromosomes 12 and 15. The results of this analysis indicate that, as in human Burkitt lymphomas with the 8;14 chromosome translocation, the c-*myc* gene is translocated to the heavy chain locus in mouse plasmacytomas. Thus the orientation of the heavy chain locus on mouse chromosome 12 and of the c-*myc* gene on mouse chromosome 15 is the same as the orientation of the homologous loci in man.**

Molecular studies have shown that in mouse plasmacytomas, the c-*myc* oncogene is rearranged in a head-to-head fashion with the immunoglobulin heavy chain locus (1–5). A similar situation is observed in Burkitt lymphomas with the 8;14 chromosome translocation (6, 7). By use of somatic cell hybrids between mouse cells and Burkitt lymphoma cells, it was shown that the c-*myc* oncogene moves from its normal position on human chromosome 8 to the heavy chain locus on human chromosome 14 (8), that the breakpoint directly involves the heavy chain locus on chromosome 14, and that the variable region genes are distal to the constant region genes at band q32 of human chromosome 14 (9). Thus the 5′ end of the c-*myc* gene is more proximal than the 3′ end of the gene at band q24 of chromosome 8 (6, 8, 10). These findings were confirmed by examining Burkitt lymphomas with the variant chromosome translocations (11, 12). In these cases the c-*myc* oncogene remains in its germ-line configuration at band q24 of human chromosome 8, while either the λ or the κ immunoglobulin light chain locus translocates to a region that is distal (3′) to the involved c-*myc* oncogene (11, 12). It is not known whether in mouse plasmacytomas, the c-*myc* oncogene, which resides on mouse chromosome 15, is translocated to the heavy chain locus on chromosome 12 (13) or whether the heavy chain locus is translocated to the c-*myc* locus on chromosome 15. To determine which one of these two possibilities is correct and, therefore, the orientation of the c-*myc* oncogene and of the heavy chain locus on mouse chromosomes 15 and 12, respectively, we have hybridized J558 mouse plasmacytoma cells carrying a translocation between chromosome 12 and 15 (3) with Chinese hamster cells. Since Chinese hamster fibroblast–mouse cell hybrids segregate mouse chromosomes, analysis of the segregating hybrids for the presence of the rearranged c-*myc* and heavy chain genes and for the presence of mouse chromosomes should result in the characterization of the 12;15 chromosome translocation in mouse plasmacytomas.

## MATERIALS AND METHODS

**Cells.** J558 mouse myeloma cells were obtained from Litton Bionetics and were fused with either A3 (14) or Wg3 (15) Chinese hamster cells in the presence of polyethelene glycol 1000 according to established procedures (16). A3 cells are deficient in thymidine kinase and Wg3 cells are hypoxanthine phosphoribosyl transferase-deficient. The hybrids were selected in the presence of hypoxanthine/aminopterin/thymidine medium (16). Independent hybrid clones were picked and expanded for chromosomal and DNA analysis. Two of the clones, J558–Wg3 13 and J558–Wg3 8, were subcloned by limiting dilution. Subclones were picked and expanded for analysis.

**Chromosome Preparation.** Fresh medium was added to the cells about 18 hr before harvesting. Metaphase cells were detached by vigorous shaking, collected by centrifugation, treated with 0.5% KCl for 15 min at 37°C, and fixed in repeated changes of methanol/glacial acetic acid (3:1, vol/vol). The cells were dropped onto cold, wet slides and stored at 4°C. Slides were stained with quinacrine mustard to produce Q-banding (17) and photographed. The slides then were passed through fixative, treated with saturated $Ba(OH)_2$ solution for 6 min, incubated in 0.3 M sodium chloride/0.03 M sodium citrate, pH 7, for 4 hr at 60°C, and stained with Giemsa to produce C-banding (17). Previously photographed cells were relocated and photographed.

**Gel Electrophoresis of DNA and Southern Transfer.** Agarose gel (0.7% or 1%) electrophoresis was carried out in 40 mM Tris·HCl/5 mM NaOAc/2.0 mM EDTA, pH 8.0. *Hin*dIII-digested λ phage DNA molecular weight markers (0.75 μg per lane) (Bethesda Research Laboratories) were included on every gel. Cellular DNA samples were digested with *Eco*RI and then subjected to electrophoresis in a horizontal agarose (Sigma) slab gel (10 μg of DNA per lane). Gels were stained for 10 min with ethidium bromide (1 μg/ml) and photographed under UV light. Transfer of DNA from gel to nitrocellulose sheet (Millipore) was performed essentially as described by Southern (18).

**Preparation of Labeled Probe DNAs.** The following oncogene probes were used: a c-*myc* cDNA clone, Ryc 7.4,

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Abbreviations: *C*, constant region gene; *V*, variable region gene; *J*, joining segment gene; *S*, switch region; H, immunoglobulin heavy chain; kb, kilobase(s).

Table 1. Mouse oncogenes and immunoglobulin heavy chain (*Igh*) genes in J558 × Chinese hamster cell hybrids

| Cells | Oncogenes | | | | *Igh* Genes | | | | | Chromosomes | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | c-*myc** | | $C_\alpha$† | | $J_H$ | | | No. of cells analyzed | No. of cells with | | | |
| | c-*fos* | c-*sis* | gl | R | R1 | R2 | $V_H$ | 6-kb | 7-kb | | 12 | 12q+ | 15 | 15q− |
| J558 | + | + | + | + | + | + | + | + | + | | | | | |
| Wg3 | − | − | − | − | − | − | − | − | − | | | | | |
| A3 | − | − | − | − | − | − | − | − | − | | | | | |
| J558–Wg3 MC | + | + | − | + | + | + | + | + | + | | | | | |
| J558–Wg3 13 | + | + | + | + | − | + | − | − | − | | | | | |
| J558–Wg3 9 | + | + | − | + | − | + | + | − | + | 17 | − | 10 (3)‡ | − | 11 |
| J558–Wg3 8-3 | + | + | − | + | − | + | + | − | + | 26 | − | 11 | − | 25 (10) |
| J558–Wg3 8-6 | + | + | − | + | − | + | + | − | + | 40 | − | 16 | 2 | 33 (11) |
| J558–Wg3 8-7 | + | + | − | + | − | + | + | − | + | 14 | − | 14 | − | 11 (6) |
| J558–Wg3 13-13 | + | + | − | + | − | + | − | − | − | 21 | − | 14 (7)‡§ | − | − |
| J558–A3 2 | − | − | − | − | − | − | + | − | + | | | | | |
| J558–A3 3 | + | + | − | + | − | + | − | − | − | | | | | |

*gl, 21.0 kb germ-line c-*myc* band; R, 17.0-kb rearranged c-*myc* band.
†R1, productively rearranged; R2, unproductively rearranged.
‡Number of cells with two copies of this chromosome.
§An isochromosome of 12q+ was present in 7 cells.

derived from a human K562 cDNA library (19); a 3.1-kilobase (kb) *Nco* I–*Xho* I human c-*fos* genomic fragment (20); and a 1.7-kb *Bam*HI fragment representing the 3′ exon of the human c-*sis* gene (21). The mouse immunoglobulin probes used in this study were: pαJ558, a cDNA probe that contains the entire IgA α heavy chain constant region gene ($C_\alpha$) (22); pVhS107, a *Hin*fI–*Bst*EII genomic fragment specific for the immunoglobulin heavy chain variable region genes ($V_H$) of the S107 family ($V_H$S107) (23, 24); p107αR5, a 960-base-pair *Eco*RI cDNA fragment that contains both $V_H$S107 and $C_\alpha$ sequences (24); and p$J_H$11, a genomic fragment containing the heavy chain joining segment genes $J_{H3}$ and $J_{H4}$ (25).

Hybridization probes were prepared with calf thymus primers (26). Specific activities were routinely $10^9$ cpm/μg. Unincorporated [α-$^{32}$P]dNTPs were removed by centrifugation through a Sephadex G-100 minicolumn.

**Hybridization.** Southern blot hybridizations were carried out in 4× standard saline citrate/50% formamide at 37°C for 36 hr. Final washes were in 0.1× standard saline citrate/0.1% $NaDodSO_4$ at 65°C. (Standard saline citrate is 0.15 M NaCl/15 mM sodium citrate, pH 7.) After air-drying, the filters were exposed to Kodak XAR film for various periods.

## RESULTS

**Presence of Immunoglobulin Gene Sequences and of the c-*myc* Oncogene in Hybrid Clones.** In mouse plasmacytomas, the 12;15 translocation represents a reciprocal exchange involving the c-*myc* locus and the $C_H$ locus. Molecular cloning of both exchange products from J558 plasmacytoma cells has shown that a portion of exon 1 and exons 2 and 3 of the c-*myc* oncogene are associated with the α heavy chain switch region ($S_\alpha$) and that the reciprocal clone contains c-*myc* 5′ flanking sequences adjacent to $S_\mu$–$J_{H3}$ sequences (27).

As shown in Table 1, most of the hybrids we obtained have lost one of the α heavy chain genes and one of the $J_H$ bands observed in the parental J558 mouse plasmacytoma cells. Using the $C_\alpha$-specific probe pαJ558 and the $V_H$-specific probe p$V_H$S107, we observed that hybrid 13-13 contained $C_\alpha$ sequences but neither $V_H$ (Table 1) nor $J_H$ (Fig. 1, Table 1) sequences. This was confirmed by using the $V_H/C_\alpha$ probe p107αR5, as shown in Fig. 2. We conclude, therefore, that this hybrid has retained only the unproductively rearranged $C_\alpha$ gene and has lost the reciprocal chromosome harboring the $V_H$ and $J_H$ sequences. In addition, as shown in Fig. 3, hybrid 13-13 contains the rearranged c-*myc* oncogene. Therefore, chromosome analysis of the clone should indicate whether the c-*myc* gene has been translocated to chromosome 12q+ or has remained on chromosome 15q−.

**Chromosome Analysis of Hybrid Cells.** As shown in Fig. 4 and Table 1, hybrid 13-13 has retained the 12q+ chromosome but has lost the normal chromosome 15, the 15q− chromosome, and the normal chromosome 12. Since this hybrid has retained the rearranged c-*myc* oncogene and the $C_\alpha$ gene that has rearranged with it, we conclude that the c-*myc* oncogene has moved from its normal position on chromosome 15 to the immunoglobulin heavy chain locus on chromosome 12 in J558. Hybrids, such as 8-6 and 8-3, that retained $V_H$ and the 7.0-kb $J_H$-containing fragment also retained the 15q− chromosome, confirming that the chromosome break point occurred between $J_H$ and $C_\alpha$ [in fact, the break point in J558 has been mapped to the $S_\alpha$ region (3)] and shows that the $V_H$ and $J_H$ regions translocated to the 15q− chromosome (Table 1). These results indicate that in mouse plasmacytomas with the 12;15 chromosome translocation, as in Burkitt lymphomas with the 8;14 translocation, the involved c-*myc* oncogene is translocated to the heavy chain locus (Fig. 5).

**Presence of Mouse c-*fos* and c-*sis* Oncogenes in Hybrids.** The c-*fos* oncogene has been assigned to a region of mouse chromosome 12 immediately proximal to the heavy chain locus (28). In addition, the mouse cellular homologue (c-*sis*) of the viral *sis* oncogene (v-*sis*) has been mapped to mouse

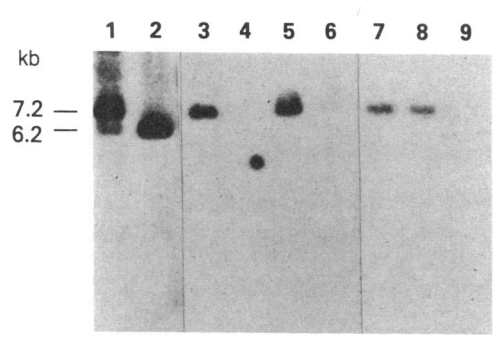


FIG. 1. Southern blot hybridization of *Eco*RI-digested cellular DNA with the mouse p$J_H$11 probe. DNAs analyzed were from the parental J558 and Wg3 lines (lanes 1 and 9, respectively); mouse fibroblasts, showing the unrearranged $J_H$ pattern (lane 2); J558–A3 hybrid 2 (lane 3); and J558–Wg3 hybrids 13-13 (lane 4), 8-3 (lane 5), 8-6 (lane 7), and 9 (lane 8).

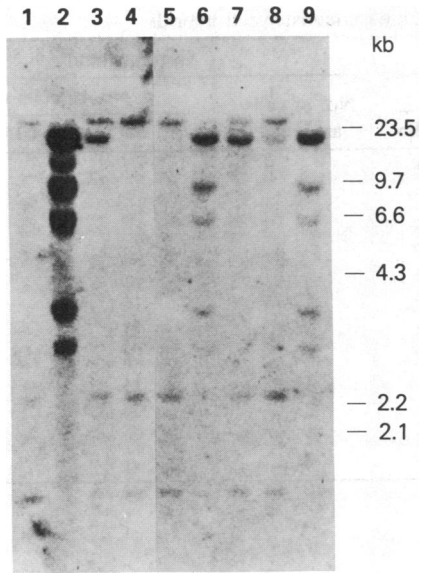


FIG. 2. Southern blot hybridization of *Eco*RI-digested cellular DNA with the p107$\alpha$R5 probe, which detects both $C_\alpha$ and $V_H$S107 sequences. DNAs were from the Chinese hamster line A3 and the mouse plasmacytoma line J558 (lanes 1 and 2, respectively) and J558–Wg3 hybrids (lanes 3–9). Hybrid 13-13 (lane 7) shows only the $C_\alpha$ hybridization pattern, whereas hybrids 8-3 (lane 6) and 8-6 (lane 9) show both $C_\alpha$ and $V_H$S107 bands.

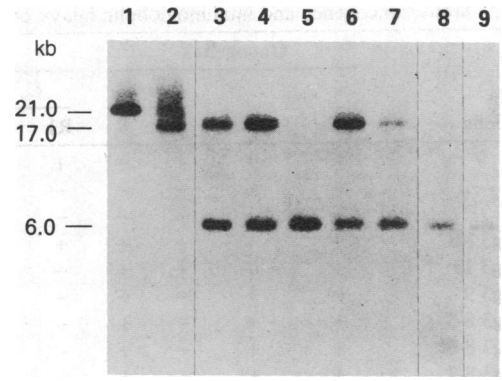


FIG. 3. Southern blot hybridization of *Eco*RI-digested cellular DNA with the c-*myc* (Ryc 7.4) probe. Lane 1: mouse fibroblast DNA, showing the germ-line c-*myc* band. Lane 2: J558 cell DNA, showing a rearranged 17.0-kb band in addition to the 21.0-kb germ-line band. Lane 9: Chinese hamster cell DNA. Lanes 3–8: DNAs from J558–Wg3 hybrids MC (lane 4), 13-13 (lane 3), and 8-6 (lane 6) and from J558–A3 hybrids 2 (lane 5) and 3 (lane 7). These hybrids have all segregated the germ-line c-*myc* gene on chromosome 15 while retaining the rearranged c-*myc* gene. Hybrid A3-2 has segregated both c-*myc* genes.

chromosome 15 (29), but its regional position on this chromosome is not known.

We have examined the Chinese hamster fibroblast–J558 hybrid cells for the presence of these two genes and found that the hybrid cells carrying the 12q+ chromosome contained the mouse c-*fos* oncogene, whereas hybrids that have retained only the 15q− chromosome did not (Fig. 6, Table 1). In addition, since we found that the hybrids containing the 12q+ chromosome (Table 1) retain the mouse c-*sis* oncogene (Fig. 7), we also conclude that this gene is located in that segment of chromosome 15 that is translocated to the involved chromosome 12.

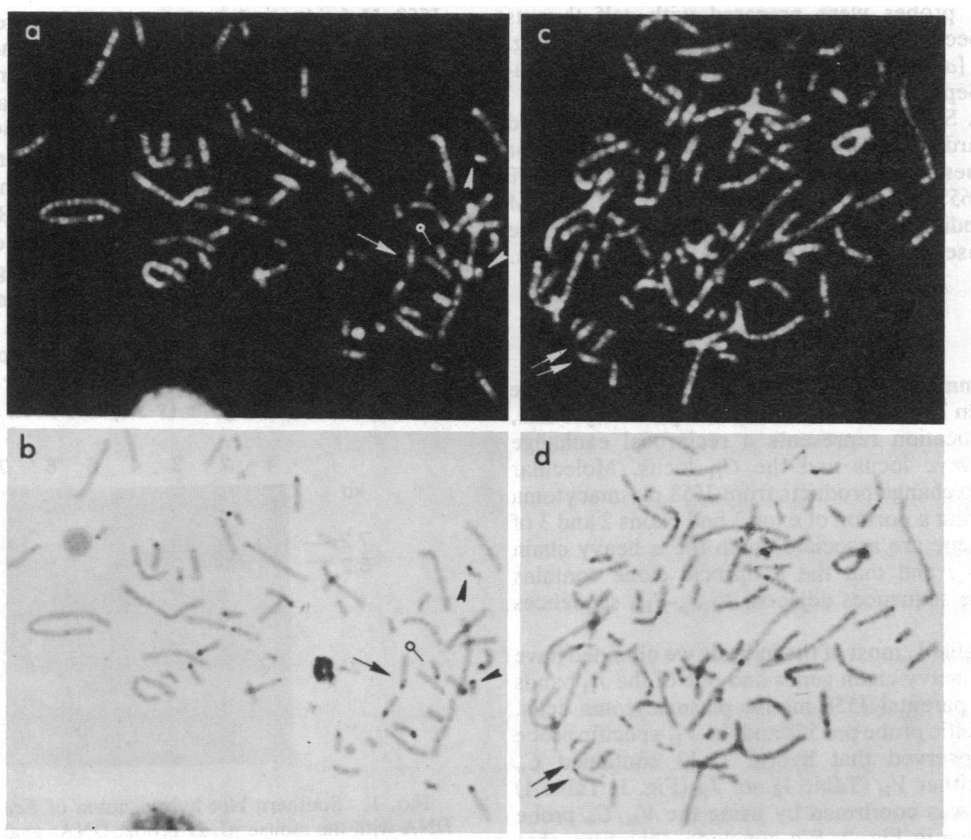


FIG. 4. Metaphase chromosome spreads from J558–Wg3 hybrid lines 8-6 (*a*) and 13-13 (*c*) were stained with quinacrine mustard to produce Q-banding (17). The same cells were treated to show C-bands (17) (*b* and *d*), which are present on mouse but not hamster chromosomes. Symbols: arrow, 12q+; ○→, 15; arrowhead, 15q−.

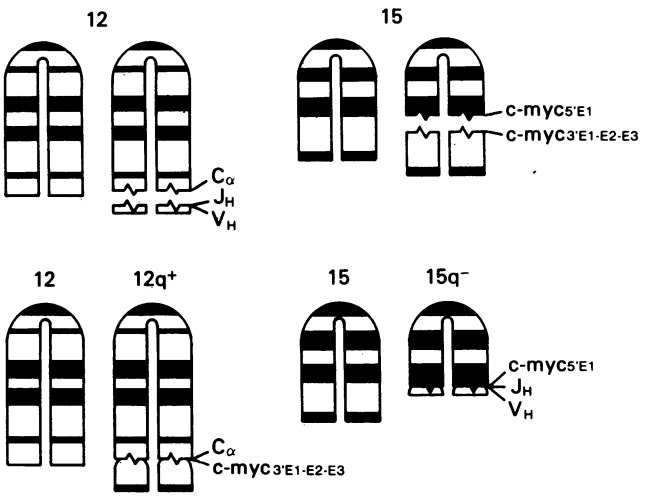


FIG. 5. Diagram of the 12;15 translocation occurring in mouse plasmacytomas. As shown, $V_H$–$J_H$ genes are distal to $C_\alpha$ on chromosome 12 and translocate to the 15q− chromosome. The c-*myc* gene on chromosome 15 is interrupted by the translocation with some of exon 1 (E1) remaining on 15q− while the rest of the gene translocates to 12q+.

## DISCUSSION

Although there is considerable molecular biological experimental evidence establishing the order, linkage, and distances between $C_H$ and $J_H$, physical linkage between the many $V_H$ genes and the $J_H$–$C_H$ regions has not yet been determined. Genetic and molecular experiments have positioned $V_H$ proximal to $J_H$–$C_H$ on chromosome 12, but the proposed orientation relative to the centromere (cen–$V_H$–$C_H$) (30) has never been confirmed. If this orientation were correct, and since the heavy chain locus and the c-*myc* gene rearrange in a head-to-head fashion, then the c-*myc* oncogene should have remained on chromosome 15 and the $C_\alpha$ gene should be translocated to the involved chromosome 15 (15q−) in mouse plasmacytomas such as J558, which carry the 12;15 chromosome translocation. We find that the rearranged c-*myc* gene and the involved $C_\alpha$ gene are on the 12q+ chromosome and that $V_H$ and $J_H$ sequences are translocated to the involved chromosome 15 (15q−). We conclude, therefore, that the orientation of the heavy chain locus is opposite to that proposed by Owen *et al.* (30) and that the rearranged and activated c-*myc* gene is on the 12q+ chromosome and not on the 15q− chromosome. A corollary to these findings is that the orientation of the c-*myc* oncogene on mouse chromosome 15 is the same as the orientation of the human c-*myc* oncogene on band q24 of human chromosome 8 (8). The widespread assumption that the mouse c-*myc* gene is translocated to the heavy chain locus on mouse chromosome 12 was based on somatic cell genetic analysis of Burkitt lymphomas and not on experimental evidence concerning mouse plasmacytomas (8, 9). The results presented in this paper address this issue and conclusively show that the involved c-*myc* oncogene is translocated and resides on the 12q+ chromosome in mouse plasmacytoma.

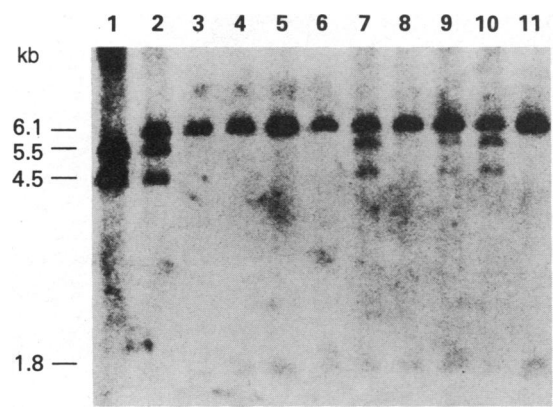


FIG. 6. Southern blot hybridization of *Eco*RI-digested cellular DNA with the c-*fos* probe. DNAs were from J558 (lane 1), hybrid 13-13, (lane 2), the Chinese hamster fusion partners Wg3 and A3 (lanes 3 and 4, respectively), and various hybrids (lanes 5–11), including A3 2 (lane 5), 8-3 (lane 7), and 8-6 (lane 10).

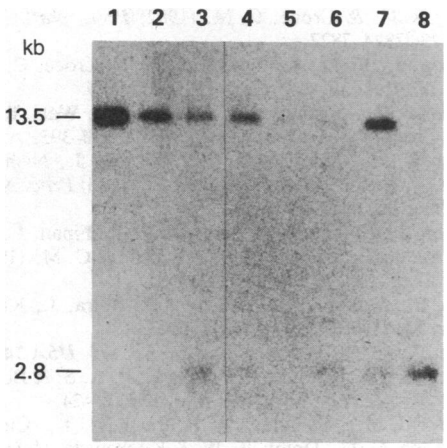


FIG. 7. Southern blot hybridization of *Eco*RI-digested cellular DNA with the c-*sis* probe. DNAs were from the parental lines J558 and Wg3 (lanes 1 and 8, respectively) and from hybrids (lanes 2–7): MC (lane 2), 8-3 (lane 3), A3 3 (lane 4), A3 2 (lane 5), and 13-13 (lane 7).

Our findings concerning the chromosomal localization of the c-*fos* oncogene are consistent with previous reports concerning the mapping of this gene (28). In addition, we also find that the c-*sis* oncogene is located on the distal segment of mouse chromosome 15. Since, as a result of this study, the orientation of the c-*myc* oncogene in the mouse is known, it should also be possible to determine the orientation of the κ light chain locus on mouse chromosome 6 by taking advantage of mouse plasmacytoma cells carrying a translocation between chromosomes 6 and 15.

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