Mapping of the *c-myc*, *pvt-1* and immunoglobulin kappa genes in relation to the mouse plasmacytoma-associated variant (6;15) translocation breakpoint

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A variant mouse plasmacytoma (MPC)-associated translocation chromosome has arisen by pericentric inversion and exchange of the distal segments of a Robertsonian 6;15 fusion chromosome in the CAK TEPC 1198 mouse plasmacytoma, as described earlier. *In situ* hybridization was performed on the normal and the inverted Rb chromosomes, using *myc* and kappa probes. On the normal Rb chromosome, *myc* was in the 15 D2/3 region, whereas kappa hybridized in the 6 C2 area, as expected. On the inverted Rb chromosome, *myc* remains on the centromeric side of the translocation breakpoint on the chromosome 15-derived portion, whereas kappa has moved to the chromosome 6-derived segment that joined the same breakpoint on the telomeric side. Taken together with our recent demonstration that the murine *c-myc* locus is oriented 'head up' on chromosome 15, and with the results of Cory and co-workers concerning the relationship between the kappa gene and the associated *pvt-1* region in the CAK TEPC 1198 tumor, the following conclusions can be drawn: (i) in the variant translocation of the CAK TEPC 1198 MPC, the breakage occurs 3' of the *c-myc* gene, as in the human Burkitt lymphoma-associated variant translocations; (ii) the *pvt-1* gene on chromosome 15 is distal to the *myc* gene; (iii) the kappa light chain locus is oriented 'head up' on mouse chromosome 6 and faces *pvt-1* and, beyond it, *c-myc*, in a head-to-tail configuration.

Key words: mouse plasmacytomas (MPC)/*c-myc*/*pvt-1*/Ig kappa mapping/*in situ* hybridization

Introduction

Murine plasmacytomas (MPC) and human Burkitt lymphomas (BL) are characterized by regularly occurring chromosome translocations that lead to the juxtaposition of the *c-myc* locus sequences and immunoglobulin (Ig) genes. It is believed that the highly active Ig-loci activate the *c-myc* gene constitutively (for review, see Klein, 1985; Cory *et al.*, 1985b). This leads to dysregulation, as reflected by the fact that, as a rule, translocated but not the normal *myc* is transcribed in the tumor (Nishikura *et al.*, 1983; Adams *et al.*, 1983). The regularity of the translocation event suggests that it plays an essential role in the development of the tumorigenic phenotype (Klein and Klein, 1984). The typical, i.e., the most frequent translocations involve the immunoglobulin heavy chain (IgH) locus. In both the MPC and BL systems, IgH sequences face the *myc* gene head-to-head (Shen-Ong *et al.*, 1982; Erikson *et al.*, 1985; Wirschubsky *et al.*, 1985). So far, the re-

lationship of *myc* to the Ig light-chain sequences involved in the variant translocations has only been studied in the BL system. In both the *myc*/kappa and the *myc*/lambda translocations, the human *c-myc* remains on chromosome (chr.) 8 and is joined at its 3' end by the Ig light-chain sequences derived from chr. 2 (kappa) or chr. 22 (lambda), facing the oncogene in a head-to-tail orientation (Erikson *et al.*, 1983; Croce *et al.*, 1983; Gudrun *et al.*, 1984).

Our earlier work (Ohno *et al.*, 1979, 1984) detected only one type of variant translocation in the MPC system, involving a break of chr. 15 in the *c-myc* containing D 2/3 band region and another break on chr. 6 (band C2), known to carry the kappa locus. Neither the relationship between *myc* and kappa, nor the position and orientation of kappa in relation to the breakpoint on chr. 6 are known. It is not clear whether *myc* stays on chr. 15, in analogy with the human variant BL translocations, or moves to chr. 6 that carries the kappa locus, as in the typical human and murine translocations.

Extensive molecular studies have recently been carried out on the murine 6;15 translocation (Webb *et al.*, 1984; Cory *et al.*, 1985b). At the molecular level, a reciprocal recombination was found between the C kappa gene on chr. 6 and a chr. 15-derived 4–5 kb region, designated as the *pvt-1* locus. This previously unknown locus is at a distance of at least 72 kb from the promoters of the *c-myc* gene. It was suggested that *c-myc* may be activated over a considerable distance by an as yet unknown mechanism.

The purpose of our study was to establish the relationship between *c-myc*, the kappa gene, and the *pvt-1* locus. We wished to determine the position and orientation of the three genes in relation to the chromosomal breakpoints (BP) involved in the translocation. This could be achieved by performing *in situ* hybridization with *c-myc* and kappa probes, respectively, on a MPC that carries a special form of the variant 6;15 translocation.

We previously found that plasmacytomas, induced in BALB/c mice with a Robertsonian 6;15 fusion chromosome (Rb 6;15) may either contain the typical 12;15 translocation or an equivalent of the usual 6;15 variant. Since chrs. 6 and 15 are fused at the centromere, the variant is generated by pericentric inversion within the single Robertsonian chromosome (Rb chr.) (Wiener *et al.*, 1984a). Here we have selected one of three tumors with this pericentric inversion [invRb(6;15)] designated as CAK TEPC 1198. Our newly developed technique for *in situ* hybridization on isolated chromosomes (Wiener *et al.*, 1985a) could readily be applied to the large Robertsonian marker. A further advantage was the fact that the same tumor was included in the molecular study of Cory *et al.* (1985b). It had the same C kappa-*pvt-1* recombination as other MPCs with the variant translocation. We report that the BP on chr. 15 is distal to the *c-myc* locus and the oncogene remains on this chromosome. IgK sequences attach to the break on chr. 15 telomerically. Integration of these findings with our recent evidence showing that *c-myc* is oriented 'head up' on murine chr. 15 (Wirschubsky *et al.*, 1985) and with the work of Cory *et al.* (1985b) leads to the conclusion that *pvt-1*

Fig. 1. Distribution of autoradiographic grains on the Rb(6;15) (A) and invRb(6;15) chromosome (B).

Table I. Grain counts on the Rb(6;15) and inv Rb(6;15) chromosomes after *in situ* hybridization with myc and c kappa probes, respectively

Robertsonian chromosome	DNA probe	Total number of Rb chrs. scored	Without grains	With grains	Distribution of the grains on the			
					Rb(6;15) chr	chr 6	inv Rb(6;15) chr long arm	short arm
Rb(6;15)	c-myc	253	155	60	59	18 ^a		
Rb(6;15)	C _k	256	191	52	6	46		
inv Rb(6;15)	c-myc	253	158	95			75	20
inv Rb(6;15)	C _k	256	137	89			67	22

^aMore than one grain was scored on some chrs 6

is distal to the *myc* gene on chr. 15 and proximal to the translocation BP as well. It also follows that the kappa gene is oriented 'head up' on chr. 6 and faces the *myc* gene, with the intervening *pvt-1* gene, in a head-to-tail fashion. In principle, the head-to-tail orientation in the murine variant translocations resembles the human BL variants, although it is not known whether a *pvt-1* type sequence is also interposed between the two genes in the human system.

Results

Grain counting was restricted to the isolated Rb(6;15) and invRb(6;15) chr. which are easily identified. Due to the known localization of *c-myc* to chr. 15 and of IgK to chr. 6, the short and the long arms of the Rb chr. serve as convenient reciprocal controls. The number of grains on the negative arm could be used to estimate the background level. The ease of scoring of the relevant bi-armed chromosomes permitted a rapid survey of a relatively large number and thereby increased the signal/noise ratio. We found that ~200–250 bi-armed chr. had to be examined to establish the specificity of the signals.

The *c-myc* and Ig-kappa genes were mapped by *in situ* hybridization on the MCF-B derived Rb(6;15) chr. The grain distribution on the short arm (chr. 15) and the long arm (chr. 6) of the Rb fusion chromosome is shown in Table I and Figure 1A. The *c-myc* gene mapped to chr. 15 band D, as in our previous study (Wiener *et al.*, 1985a) (Figure 1A). The C kappa probe gave a total of 52 grains on the Rb(6;15) chr. out of which 46 (88%) were on chr. 6. 191 of 242 bi-armed were unlabeled. Sixteen grains (34%) were associated with the chromosomal region corresponding to band 6C (Figure 1A). The remaining 36 grains were localized to the other major bands of chr. 6. Only six randomly distributed grains were found on chr. 15, indicating a low BP level in this hybridization experiment.

Figure 2 shows the two alternative gene localizations on the invRb(6;15) chromosome, depending on whether the *c-myc* gene stays on chr. 15 and the kappa sequences are transposed to its telomeric end (alternative A) or the opposite, the transposition of *c-myc* to the telomeric end of the kappa carrying chr. 6 (alternative B).

If alternative A prevails, both the *myc* and kappa grains will

Fig. 2. Alternative possibilities for the localization of the autoradiographic grains on the *invRb(6;15)* chromosome. **A.** Expected localization if *c-myc* stays on chromosome 15 and *C kappa* is transposed to its telomeric end. **B.** Expected localization if *C kappa* stays on chromosome 6 and *c-myc* is transposed to its telomeric end.

Fig. 3. Autoradiography of isolated *invRb(6;15)* chromosomes. **A.** *In situ* hybridization of the *c-myc* probe maps the oncogene to the breakpoint region of the long arm of the *invRb(6;15)* chromosome (alternative A in Figure 2). **B.** *In situ* hybridization with the *C kappa* probe maps the gene to the breakpoint region of the long arm of the *invRb(6;15)* chromosome (alternative A in Figure 2).

be found in the BP region of the long arm of the Rb chr., consisting of a centromeric part derived from chr. 15 and a telomeric part from chr. 6. According to alternative B, the opposite would be true and both probes would hybridize to the BP region of the short arm, consisting of the centromeric region of chr. 6 and the telomeric region of chr. 15.

Table I and Figure 1B show the distribution of the grains on the *invRb(6;15)* bi-armed chr. when hybridized with the *c-myc* and kappa probes. The former gave 95 grains on 95 *invRb(6;15)* chrs. 158 chromosomes had no grains (62%). Twenty grains (26%) were on the short arm of the *invRb* chr. and 75 grains

(74%) were on the long arm. 51/75 grains were clustered in the BP region corresponding to bands D1 and D2 on chr. 15 and C2 and C3 on chr. 6c (Figures 1B and 3A).

In situ hybridization with the *C kappa* probe gave 898 grains associated with 89 of 256 scored in *invRb* chrs. (34%), 137 chrs. were without grains (53%). The distribution of the 89 grains on both arms of the *invRb(6;15)* chr. showed a similar pattern as for *c-myc*. 67 grains were on the long arm and 22 on the short arm. 37/67 grains (55%) were clustered in the BP region similarly to grains obtained with the *myc* DNA (Figures 1B and 3B). These findings support alternative A (Figure 2).

Fig. 4. Schematic illustration of the order of *c-myc*, *pvt-1* and C kappa genes and their orientation on the variant rept (6;15) chromosome.

Fig. 5. Schematic illustration of the Rb(6;15) and the invRb(6;15) chromosomes generated by pericentric inversion. The breakpoint on the long arm of the bi-armed chromosome is flanked by the 15 D2/3 and 6C 2/3 sub-bands. On the short arm the breakpoint is flanked by sub-bands 6C1 and 15D3.

Discussion

The *in situ* hybridization results with the *myc* and kappa probes on the invRb(6;15) translocation chr. can be discussed in relation to our earlier studies on the orientation of *c-myc* on chr. 15, and to the work of Cory *et al.* (1985b) on the relationship of the kappa and the *pvt-1* locus in the translocation associated with the same CAK TEPC 1198 tumor. Concerning the orientation of *c-myc*, our cytogenetic studies on 'deletion plasmacytomas' (Wiener *et al.*, 1984b, 1985b) have suggested that the 5' end of the gene may be facing the centromere. This was directly confirmed by our recent molecular hybridization studies on separated MPC-derived 12;15 translocation chrs. (Wirschubsky *et al.*, 1985). The same conclusion was reached independently by Erikson *et al.* (1985) using a somatic hybrid approach. Cory *et al.* (1985b) have studied nine MPCs with the variant (6;15) translocation (including CAK TEPC 1198, the

tumor used in the present study) at the molecular level. They have shown that the C kappa gene has recombined with a chr. 15-derived segment, designated as *pvt-1* in 5/9 tumors. *Pvt-1* is a new, previously unknown locus that is located at least 72 kb from *c-myc*. It is not known whether *pvt-1* faces the 5' or the 3' end of the *myc* genes.

Our study shows that *c-myc* stays on chr. 15 in the variant translocation. The segment transposed from chr. 6, known to carry the Ig kappa gene, joins the telomeric end of the break. It must be concluded that the *pvt-1* gene, also on chr. 15, is distal to the *myc* gene because it joins the transposed C kappa directly. Since *c-myc* is oriented 'head up' on chr. 15, it must face the 3' end of *myc*. Our study also permits the indirect mapping of the IgK locus on chr. 6. Since the C2 and C3 sub-bands of chr. 6 are transposed onto chr. 15, the kappa gene must be within this chromosome region. The clustering of the kappa grains in the BP region of the long arm of the invRb(6;15) chr. further

Fig. 6. Metaphase plate of the CAK TEPC 1198 plasmacytoma. The *invRb(6;15)* is the sole bi-armed chromosome present in the line (arrow).

confirms that the kappa gene must be localized in the C-band region of chr. 6. Combining the cytogenetic evidence with the earlier molecular hybridization results of Cory *et al.* (1985b) concerning the relationship between *pvt-1* and the kappa gene, it also follows the orientation of the IgK on chr. 6 is centromere-5'-V kappa-C kappa-3'-telomere. Figure 4 summarizes the evidence concerning the localization and orientation of the three genes in the normal chrs. 6 and 15 and on the variant rcpt(6;15) translocation chr.

Materials and methods

Cell lines

The MCF-B line was derived from a thymic lymphoma induced in an AKR 6;15 mouse by MCF-virus (Wirschubsky *et al.*, 1984). This strain is a constitutional carrier of the Rb(6;15) chr. After adaptation to *in vitro* growth the line became hypotetraploid, with four Rb(6;15) chrs. It was propagated as a suspension culture on RPMI with 10% fetal calf serum.

The CAK TEPC 1198 line originated from a PC induced in (BALB/c × AKR 6;15) × BALB/c back-cross mouse (Wiener *et al.*, 1984a). The variant translocation associated with this tumor arose by a pericentric inversion of the AKR-derived Rb(6;15). In contrast to the usual MPC-associated variant rcpt(6;15) generated by the reciprocal exchange between the separate 6 and 15 chrs., the exchange occurred within a single Rb chr., in the CAK TEPC 1198 tumor, resulting in an invRb(6;15) chr. (Figure 5).

High resolution banding of the invRb(6;15) showed that the BP on chrs. 15 and 6 were the same as in the usual rcpt(6;15), namely D 2/3 on chr. 15 and C2 on chr. 6 (Wiener *et al.*, 1984a). As a result, the long arm of the Rb chr. is composed of the centromeric part of chr. 15 (bands A, B, C, D1 and D2) and the telomeric segment of chr. 6 (bands C3, D, E F and G). The short arm contains the centromeric part of chr. 6 (bands A, B, C1 and C2) and the telomeric end of chr. 15 (bands D3, E and F) (Figure 5).

Chromosome isolation

The normal Rb(6;15) and invRb(6;15) were isolated as described (Wiener *et al.*, 1985a). Since the Rb chr. is the only bi-armed element in the karyotype (Figure 6), it can be identified without G-banding. Conventional Giemsa staining after hybridization and autoradiography was sufficient for accurate identification.

In situ hybridization

The mouse pM-c-myc 54 probe was kindly supplied by Dr K. Marcu. It spans 2.2 kb of the *myc* locus corresponding to all the three exons of the gene. A full restriction map of the probe was described by Staton *et al.* (1983). The Ig-kappa pKB/94 probe was obtained from Dr I. Schechter. It was derived from a rat library and covers ~200 nucleotides of the 3' untranslated region of the kappa constant region. The restriction map of the probe was published by Breiner *et al.* (1982). Hybridization and autoradiography were performed according to the technique of Neel *et al.* (1979).

Staining, photography and positioning of the autoradiographic grains

Dried slides were stained with 0.25% Giemsa, diluted in phosphate buffer 0.15 M at pH 8. Each individual Rb(6;15) and invRb(6;15) chromosome that had a conspicuous silver grain on one or both of its arms was photographed with a Leitz Orthoplan photomicroscope with a green emission filter of 546 nm.

The location of the grains on the chromosome arms was determined on highly magnified prints. The relative position of the grains was recorded in relation to the centromere and telomere. The assignment of the grains to the major chromosomal bands was realized by transferring the data recorded on the prints to G-band Rb(6;15) and invRb(6;15) chromosomes as illustrated in Figure 1. Although the assignment is tentative in the absence of chromosome banding it reflects, within the limits of this approach, the approximate localization of the autoradiographic signals on the relevant chromosome bands (breakpoint regions).

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