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H-2 hemizygous mutants from a heterozygous cell line: role of mitotic recombination

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Variants that no longer express an entire *H-2* haplotype were readily isolated, by immunoselection with antisera directed against the haplotype, from an *H-2^b/H-2^d* heterozygous Friend leukemia cell line carrying a Robertsonian translocation of the chromosomes bearing the *H-2* genetic region. These variants can be denoted as being of the phenotype H-2^b⁻H-2^d⁺ or H-2^b⁺H-2^d⁻. Some of the H-2^b⁻H-2^d⁺ variants: (1) lack the restriction enzyme fragments characteristic of the missing *H-2^b* haplotype, as assessed by Southern blot analysis; (2) express more cell surface *H-2^d* antigens than wild-type cells, as assessed by flow microfluorimetry; and (3) appear to have become homozygous for the more active *H-2^d*-linked allele at the *Glyoxalase I* locus. These variants thus seem to have lost genetic material corresponding to the *H-2^b* haplotype and may have gained genetic material corresponding to the *H-2^d* haplotype. These results are consistent with the possibility that these variants were generated by mitotic recombination.

Key words: H-2/mitotic recombination/Robertsonian translocation/somatic cell genetics

Introduction

We have been investigating the emergence of variants from a mouse cell line that is heterozygous for the murine Major Histocompatibility Complex (MHC), *H-2*. Antigens coded for by genes in the MHC are expressed stably by cultured cell lines even after decades of *in vitro* maintenance. In heterozygous cells, these antigens are expressed co-dominantly, i.e., antigens coded for by both the parental chromosomes can be detected on the surface of all cells of the heterozygous population. With appropriate immunoselection, mutants exhibiting various combinations of MHC-coded antigen loss can be isolated (Rajan, 1977; Pious *et al.*, 1982; Orr *et al.*, 1982). We have shown, for instance, that variants which do not express one of the two parental sets of *H-2* haplotypes can be readily obtained from an F_1 cell line (Rajan, 1977). Some of the earliest mutants that we have isolated were obtained by subjecting the culture to a single step selection with alloantisera directed against the gene products of an entire haplotype (Rajan, 1977); following such immunoselection, the survivors were cloned and random clones retained for analyses. More recently, mutants have been isolated using mixtures of monoclonal antibodies, some of which are directed against the gene product of the *H-2K* locus and others against the product of the linked *H-2D* gene. For further reference, the mutants that we will be discussing and their method of derivation are listed in Table I. It is worthwhile pointing out that though the mutants described in this communication are those derived by antibodies directed against an entire haplotype (be they allosera or mixtures of monoclonal antibodies), mutants with essentially the same phenotype can be obtained using antibodies against only the *H-2K* locus gene product or only the *H-2D* gene product (Flores and Rajan, 1977; Rajan and Flores, 1977). The general properties of the mutants have been described in detail in earlier publications and need only be summarized here. (1) The hemizygous mutants retain both the copies of chromosome 17 present in the wild-type cells. In the wild-type cells and in the mutants, these two copies are present as a small metacentric marker chromosome, formed by Robertsonian fusion. Thus, these 'hemizygous' mutants seem not to be due to simple chromosome loss. (2) The mutants preexist the selection and arise at 10^{-5}–10^{-6} per cell per generation (Rajan and Halay, 1980). (3) They are stable. We have been unable to obtain revertants that reexpress the suppressed haplotype. (4) The products of the suppressed haplotype cannot be detected to the limits of detection of serological or cell mediated methods.

Here we present data that bear on the mechanism by which these mutants arise.

Results and Discussion

Quantitation of H-2 antigen expression on wild-type and variant cells

Two general hypotheses can be invoked to explain how cells that appear diploid by karyotype fail to express one of the two parental sets of genes at any locus or set of loci. One group of mechanisms includes inactivation of the gene(s) or interstitial loss of part of the chromosome bearing the marker. Simplistically, one might expect that the level of expression of the other, expressed parental allele(s) might be the same as in the wild-type, since there is only one copy of that allele in the variant cell. Another group includes chromosome loss, followed by reduplication of the remaining chromosome or mitotic recombination resulting in homozygosity. In this latter group of mechanisms, the variant cell has in fact become homozygous for the other, expressed parental allele and one might expect the level of expression of this allele to

Table I

Mutant	H-2 phenotype	Selected with	Specificity
H-2^b⁻ clone 1	H-2^b⁻H-2^d⁺	Alloantiserum	H-2K^b and H-2D^b
H-2^b⁻ clone 5	H-2^b⁻H-2^d⁺	Monoclonals	H-2D^b* and H-2K^b§
H-2^b⁻ clone 8A	H-2^b⁻H-2^d⁺	Monoclonals	H-2D^b* and H-2K^b§
H-2^d⁻ clone 11#	H-2^d⁻H-2^b⁺	Alloantiserum	H-2K^d and H-2D^d

*The monoclonal used was H141-51, a kind gift from Dr. G.J. Hammerling, DFKZ, Heidelberg.
§The monoclonal used for this specificity was Y3, a generous gift from Dr. S.G. Nathenson, AECOM,NY.
#This and all other H-2^d⁻ mutants were selected with an alloantiserum.

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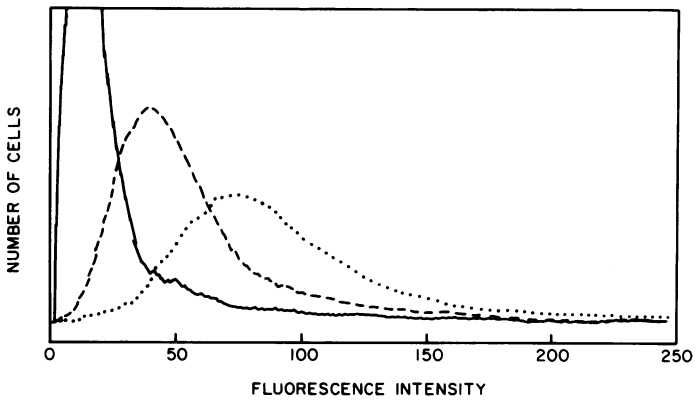

Fig. 1. FACS profiles on normal diploid nucleated spleen cells from BABL/c (*H-2*^{d} homozygous, ...), C57Bl/6 (*H-2*^{b} homozygous, ——) and F_1 mice (*H-2*$^{b/d}$, - - - -) by direct immunofluorescence using fluoresceinated monoclonal antibody 34-2-12 (anti-H-2^d). The x-axis is fluorescence in arbitrary units, y-axis the number of cells per channel.

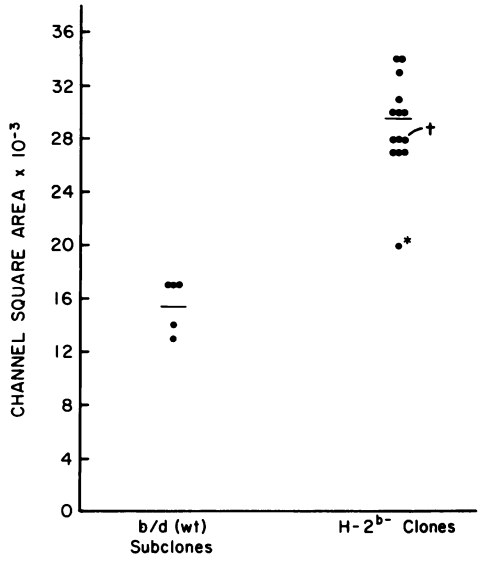

Fig. 2. Dot plot of level of expression of *H-2D*^{d} encoded antigens by b/d 4.4.1 and four subclones and a number of independently derived H-2^{b-} mutants. The H-2^{b-} mutant indicated by (*) is H-2^{b-} clone 5. In two out of five experiments, its position was similar to that shown in this figure, expressing more H-2D^d than wild-type cells and less than the other H-2^{b-} mutants; in the other three experiments it was indistinguishable from wild-type cells. The mutant indicated by (+) is H-2^{b-} clone 8A. It is highlighted to show that it resembles other H-2^b loss mutants in expressing more *H-2*^{d}-coded antigens than wild-type cells.

be higher than in the wild-type cell. To investigate which of these two general groups of mechanisms is involved in the generation of the 'hemizygous' mutants, we quantitated the level of expression of antigens coded for by the expressed haplotype by both direct and indirect immunofluorescence using a FACS II.

Although absolute amounts of cell surface antigen are difficult to determine, quantitative immunofluorescence using flow microfluorimetry allows a reasonable estimate of relative quantities of expressed molecules. Figure 1 shows this conclusion. The reactivity of fluorescein-labelled 34-2-12 antibody (anti-H-2D^d) on (BALB/c x C57Bl/6)F_1 (*H-2*^{b}/*H-2*^{d} heterozygous) spleen cells was intermediate between that in the homozygous positive BALB/c parental and the homozygous negative C57Bl/6 parental spleen cells. This result

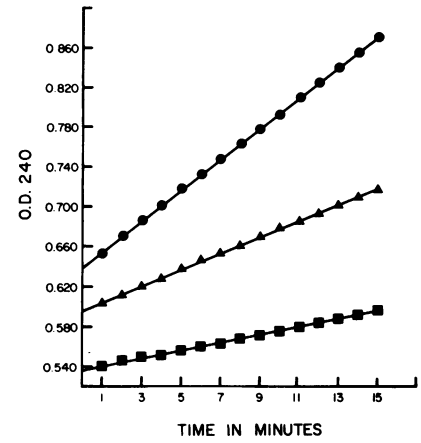

Fig. 3. Kinetics of formation of OD_{240} absorbing intermediate from methylglyoxal and reduced glutathione by cell lysates containing 100 mg/ml of protein. The lines were determined by linear regression analyses on OD readings recorded for 15 min. The cells shown are: b/d 4.4.1 (▲——▲); H-2^{b-} clone 1 (●——●); H-2^{d-} clone 11 (■——■).

confirms the published observations of Potter *et al.* (1981). We then used 34-2-12 to quantitate the expression of H-2D^d on the wild-type cells (b/d 4.4.1), a number of subclones of the wild-type cells and a number of H-2^{b-} variants. All subclones of the wild-type cell exhibited the same fluorescence intensity as each other and the uncloned wild-type cells; in contrast, in a total of 37 analyses on 17 H-2^{b-} variants, all but one (H-2^{b-} clone 5) showed a consistent increase of ~2-fold compared with the wild-type cells (Figure 2). The variant H-2^{b-} clone 5 could not be consistently distinguished from the wild-type cells. Using a similar procedure, we quantitated the expression of H-2K^d on wild-type cells, subclones of wild-type cells and the 17 H-2^{b-} mutants, using a directly fluoresceinated anti-H-2K^d monoclonal antibody (15-5-5). Once again, all mutants except H-2^{b-} clone 5 showed an increased expression of this antigen compared with wild-type cells or its subclones (data not shown).

Expression of glyoxalase in the wild-type and variant cells

The enzyme glyoxalase I is encoded by a locus *GLO-1* that has been mapped 3 cM proximal to the *H-2* complex on chromosome 17 (Meo *et al.*, 1977). In the course of studying *H-2* variants from b/d 4.4.1 we observed that amongst the *H-2* congenic mice of the BALB background, the *H-2*^{d} haplotype is linked to an allele of *GLO-1* that is associated with about twice the specific activity of the enzyme as that linked to *H-2*^{b}. Thus, we found that BALB/c (*H-2*^{d} homozygous) erythrocytes expressed 1.6–2.6 times the level of activity as did the BALB.B (*H-2*^{b} homozygous) erythrocytes and the F_1 erythrocytes expressed an intermediate level of activity (Rajan, 1979). Based on this finding, we felt that the examination of the level of glyoxalase activity in the H-2^{b-} variant from b/d 4.4.1 might be particularly useful. Thus, if these mutants had inactivated the *H-2*^{b} haplotype (including the closely linked, unselected marker *GLO-1*), then the level of the enzyme in these cells would be lower than that in the wild-type cells. If, however, they had become homozygous for the *H-2*^{d} haplotype (including the closely linked *GLO-1* locus), then the level of the enzyme would be higher than that in the wild-type cells. It is important to point out that the other mechanism that could give rise to the latter result would be if the *H-2*^{b} haplotype were to be associated with an in-

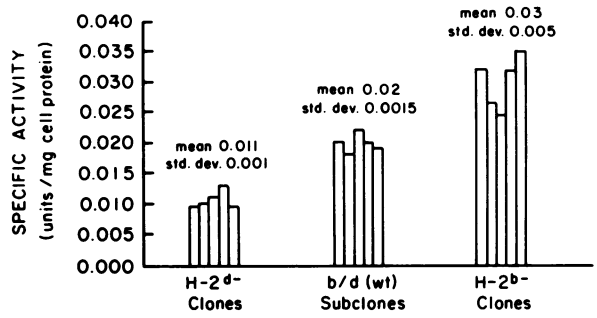

Fig. 4. Histograms of specific activities of b/d 4.4.1 and four subclones and five independently derived H-2^{b-} and H-2^{d-} mutants. In each case, the specific activity was determined by linear regression analyses on data similar to those shown in Figure 1.

hibitor of glyoxalase. We have performed mixing experiments which suggest that the lower level of activity in the *H-2*^{b} haplotype is not due to such a mechanism (Rajan, 1979). The reason for choosing the *H-2*^{b} loss mutants rather than the *H-2*^{d} loss mutants is that irrespective of the mechanism involved, the latter mutants should have lower levels of activity than the wild-type cells.

Figure 3 shows the kinetics of formation of the intermediate OD_{240} absorbing material formed from methylglyoxal and glutathione by the wild-type cell line and the two reciprocal variants H-2^{b-} clone 1 and H-2^{d-} clone 11. The H-2^{b-} clone expresses more activity than the wild-type cell; the H-2^{d-} clone expresses considerably less. Thus, in the particular experiment shown, the specific activity of the enzyme in the wild-type cell is 0.024 units/mg cell protein; that in the H-2^{b-} variant is 0.046 (the ratio of specific activity of mutant to wild-type is 1.9; in four separate experiments, the ratio was 1.48 ± 0.2); that in the H-2^{d-} mutant is 0.012 (the ratio of specific activities of wild-type to mutant is 2; in four separate experiments, the ratio was 2.02 ± 0.35). It is interesting to note that the level of activity of the wild-type heterozygous cell approximates the mean of the level of activities of the two reciprocal variants.

To determine whether this finding was merely a reflection of clonal variation in the expression of this enzyme, we have compared the activity of several subclones of b/d 4.4.1 as well as b/d 4.4.1 cells that were frozen several years ago. Data from one such experiment are shown in Figure 4. It can be seen that all the subclones of b/d 4.4.1 have essentially the same level of activity. We have tested a total of 10 subclones of b/d 4.4.1 and cells frozen 4 years ago and the data are similar to those shown in Figure 4. Thus, the specific activity of glyoxylase I is a stable phenotype for this cell line and the observation of increased activity in the H-2^{b-} mutant is not due to random clonal variation. On the other hand, the increase may not be related to the alteration in the H-2 phenotype. To determine this, we picked independent mutants of the same phenotype and quantitated glyoxalase in these mutants. As was the case with the first H-2^{b-} and H-2^{d-} mutants discussed earlier, the mutants shown here also demonstrate the same phenomenon. The mean specific activity of the five H-2^{b-} mutants shown in Figure 4 is 0.0305 units/mg cell protein (±0.0053 units) in comparison with that of the wild-type cells (0.02 ± 0.0015 units/mg cell protein; $t = -4.23$; $p < 0.005$); the mean specific activity of the five H-2^{d-} mutants is 0.011 ± 0.0012 units/mg cell protein ($t = -10.02$; $p < 0.001$). Once again it is interesting to note that the mean specific activity of the clones of the wild-type (heterozygous) clones (0.02) is the average of the means of the specific activities of the two groups of mutants of reciprocal phenotypes (0.03 and 0.011: the average is 0.02). We have analysed a total of 14 independent H-2^{b-} mutants and 13 of these express higher levels of activity than the wild-type cells. The enzyme activity of one mutant, H-2^{b-} clone 8A, could not be distinguished from wild-type cells. Since the phenotype of this mutant is of some importance in attempting to understand the possible mechanism of emergence of these mutants, we have analysed it repeatedly, and in multiple experiments the glyoxalase activity of this mutant was similar to that of wild-type cells.

Southern blot analyses of variants

To determine the genotype of the mutants that are phenotypically hemizygous, we performed Southern blot analyses of genomic DNA using a 3′ probe derived from an *H-2L*^{d} genomic clone (Margulies *et al.*, 1982). This probe contains part of the long intervening sequence and 30 bp of the coding sequence from the third extracellular domain of the *H-2* gene (Evans *et al.*, 1982). Previous work (Steinmetz *et al.*, 1981; Pease *et al.*, 1982; Margulies *et al.*, 1982) has shown that Southern blot analysis of murine genomic DNA generates a rather complex picture with multiple fragments of varying molecular size because of the large number of genes that constitute the MHC. To determine the significance of any changes we might observe on the Southern blot analyses of the hemizygous mutants, we first attempted to map some of the fragments that are visualized on a Southern blot analysis with the 3′ probe, using genomic DNA from several mouse strains that have undergone recombinations within the MHC. Such an approach has been shown to be useful in mapping polymorphic fragments (Steinmetz *et al.*, 1981; Pease *et al.*, 1982; Margulies *et al.*, 1982). Figure 5 is a Southern blot analysis of *Bam*HI-digested DNA hybridized to the probe. Lane 2 contains DNA from the wild-type cell line b/d 4.4.1 flanked by DNA from BALB/c (lane 3) and C57Bl/6 (lane 1) spleen cells. It should be noted that the restriction pattern of b/d 4.4.1 DNA is essentially the sum of the patterns of C57Bl/6 or BALB/c genomic DNA. This result was anticipated since b/d 4.4.1 represents the F_1 hybrid between the C57Bl/6 and BALB/c parental strains. Of the bands that are visualized in the C57Bl/6 (*H-2*^{b}) genomic DNA, several are not seen in the BALB/c (*H-2*^{d}) DNA. Two of these fragments, of 8 kb and 2.2 kb, are also present in B10.A(5R) (lane 5), but not seen in B10.GD. B10.A(5R) contains the *K* region from *H-2*^{b} while B10.GD contains the *K* region from *H-2*^{d}. Thus, since those strains that have in common the *K* end of *H-2*^{b} contain these fragments and those that do not have the *K* end of *H-2*^{b} do not, these two fragment can be mapped to the *K* end of *H-2*^{b} haplotype. Reciprocally, the faster migrating of the doublet at 3.8 kb is not seen in the B10.A(5R) strain but is present in B10.GD. Since the C57Bl/6 and B10.GD strains share the *D* end of the *H-2* complex and BALB/c and B10.A(5R) differ from them at this region, this fragment can be mapped to the *D* end of the *H-2*^{b} haplotype. With the particular strains that we have used in this analysis, we cannot rule out the possiblity that this fragment is from the *Tla* region.

None of the three fragments noted above, the 8 kb fragment, the faster migrating of the doublet at 3.8 kb or the 2.3 kb fragment is seen in the genomic restriction pattern of

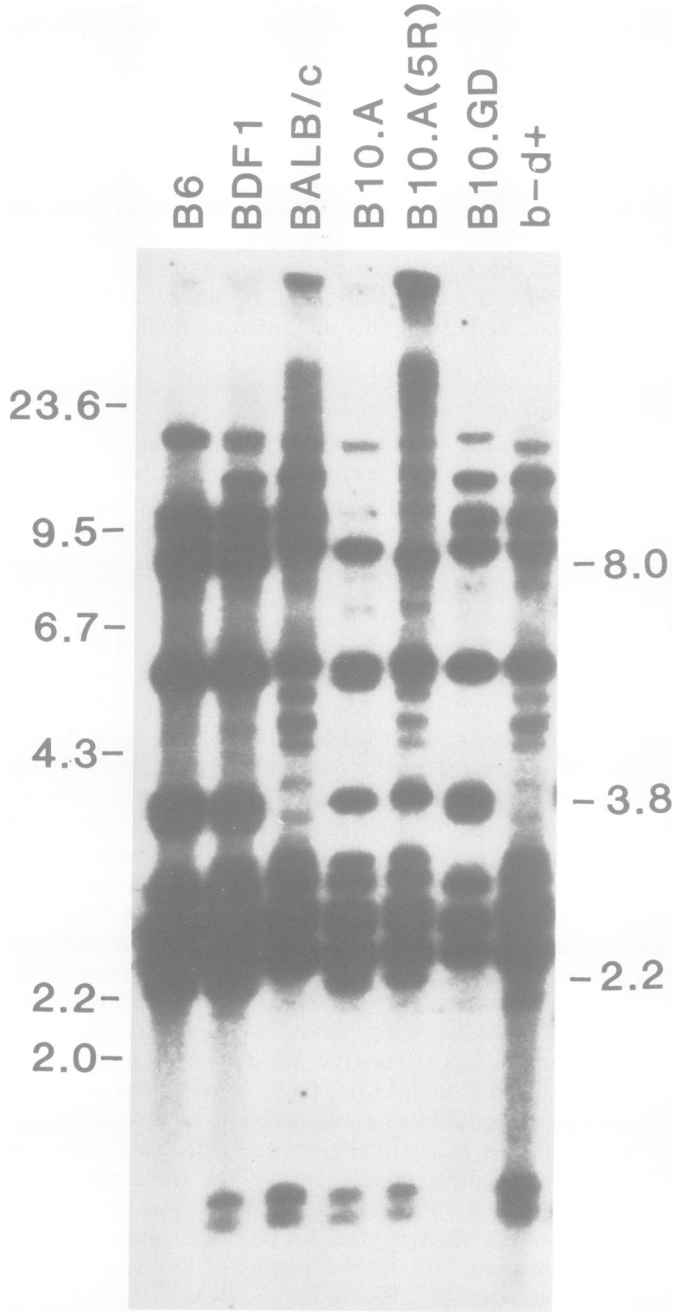

Fig. 5. Southern blot of *Bam*HI-restricted genomic DNA probed with 92S15. Lanes are, from left to right, DNA from C57Bl/6, b/d 4.4.1, BALB/c, B10.A, B10.A(5R), B10.GD and H-2^{b-} clone 1. The fragments that are polymorphic between C57Bl/6 and BALB/c are indicated on the right margin, the mol. wt. markers on the left.

the hemizygous mutant H-2^{b-} clone 1 (lane 7). Thus, the H-2^{b-} clone 1 appears to have lost a rather extensive region of the MHC gene complex, including at least some fragments from the *H-2K* region and some from the *H-2D* or *Tla* region. To determine whether this genotype is unique to H-2^{b-} clone 1, or is a general phenomenon in the H-2^{b-} hemizygous mutants we have performed Southern blot analyses on a total of 13 independent mutants. With one exception, all the mutants are similar to H-2^{b-} clone 1 in exhibiting a loss of all those fragments that are specific for the *H-2*^{b} haplotype and missing from the *H-2*^{d} haplotype, while

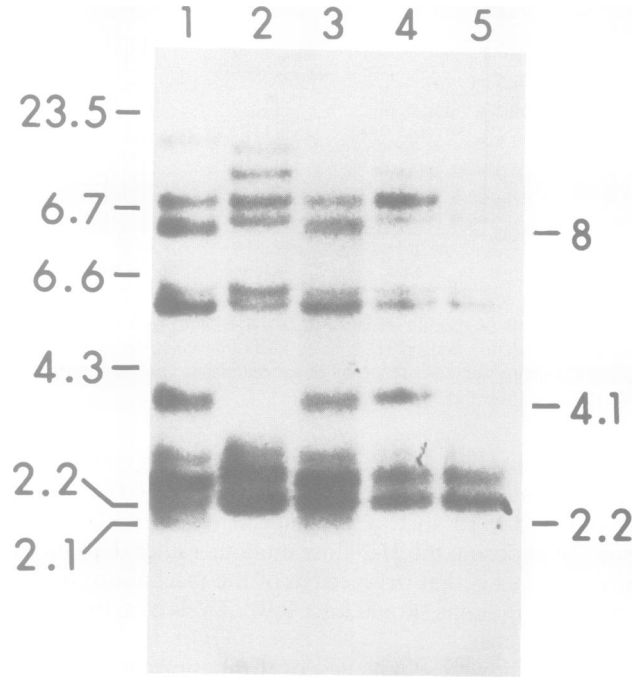

Fig. 6. Southern blot analyses of a C57Bl/6 **(lane 1)** , BALB/c **(lane 2)**, b/d 4.4.1 **(lane 3)**, H-2^{b-} clone 5 **(lane 4)** and H-2^{b-} clone 8A **(lane 6)**; all genomic DNAs were restricted with *Bam*HI and probed with 92S15. The size markers are indicated on the left. The fragments that are polymorphic between *H-2*^{b} and *H-2*^{d} and are present in the former and missing in the latter are indicated on the left.

retaining all the fragments that are specific for the *H-2*^{d} haplotype and those fragments that are shared between the *H-2*^{b} haplotype and the *H-2*^{d} haplotype. The one exception to this is a mutant, H-2^{b-} clone 5, shown in Figure 6. In this figure, C57Bl/6 (*H-2*^{b}), BALB/c (*H-2*^{d}) spleen cell DNAs, and DNA from b/d 4.4.1 and two hemizygous mutants H-2^{b-} clone 5 and H-2^{b-} clone 8A were subjected to Southern blot analyses after restriction cleavage with *Bam*HI. H-2^{b-} clone 8A (lane 5) resembles H-2^{b-} clone 1 described above. However, H-2^{b-} clone 5 (lane 4) differs in an interesting way. Thus, while it is missing the 8-, 3.8- and 2.2-kb *H-2*^{b} specific fragments, it now has a 4.1-kb fragment not present either in the C57Bl/6 or the BALB/c DNAs.

Possible mechanisms of derivation of the hemizygous mutants

Based on these data we feel that there are at least three phenotypes associated with the loss of expression of a haplotype. (1) The majority of H-2^{b} loss mutants express higher levels of the enzyme glyoxalase I and seem to express more H-2D^{d} and H-2K^{d} than the wild-type cells. We believe that these increases are not the trivial result of changes in H-2 expression, since for each of these two assayed markers there is at least one mutant of identical H-2 phenotype which does not manifest these changes. Both these phenotypes are consistent with there being two copies of the expressed haplotype in these cells. (2) At least one mutant, H-2^{b-} clone 8A, is not distinguishable from wild-type with regard to glyoxalase I but has more H-2D^{d} and H-2K^{d} than the wild type. This is suggestive of there being two copies of H-2D^{d} and H-2K^{d} in these cells; however, since the specific activity of glyoxalase I is similar in this mutant to that in the wild-type cells, this mutant must be regarded as heterozygous for the *GLO-1* locus.

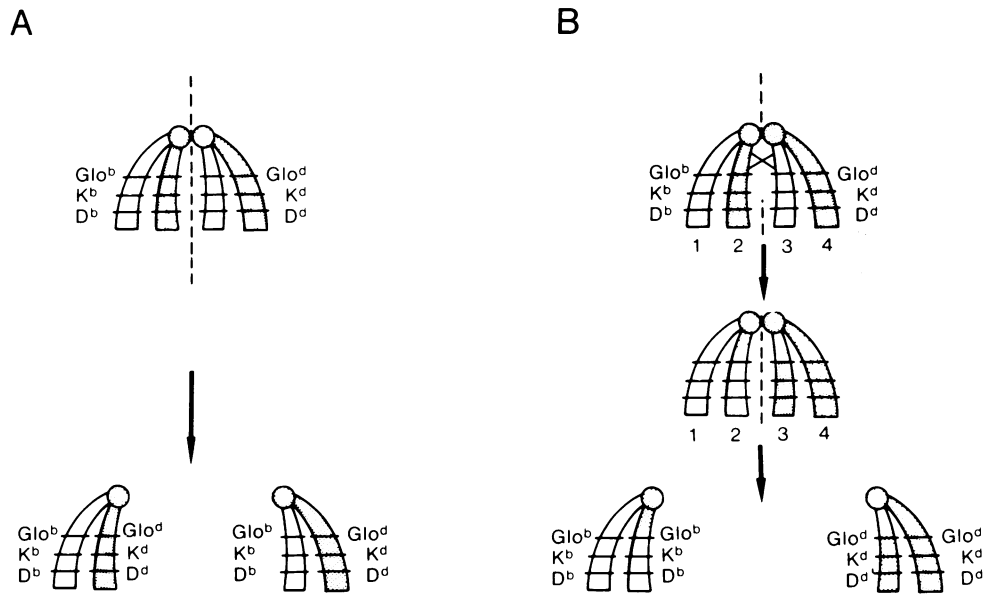

Fig. 7. Model suggesting how hemizygous mutants may be generated by somatic crossing-over in a cell line with a Robertsonian chromosome. **Panel A** shows a normal mitotic event where the two daughter cells generated would be identical to each other and the parent cell; in **panel B**, somatic crossing-over between the centromere and *GLO-1* has generated two daughter cells that have become homozygous for *GLO-1* and the *H-2* complex.

(3) The third class of mutants has at the moment one member, H-2^{b-} clone 5. This mutant is not distinguishable from wild-type cells in the expression of *H-2*^{d}-coded antigens, but expresses more glyoxalase I than the wild-type cells and has a complex change in its *H-2* gene constitution.

Previous work from our laboratory has shown that: (a) the b/d 4.4.1 has a Robertsonian translocation between the two copies of chromosome 17 and these seem to be the only copies of chromosome 17 in this cell line; and (b) all the mutants whose karyotype have been analysed retain this Robertsonian translocation (Rajan, 1977; Flores and Rajan, 1977). Because of this, we feel that simple chromosome loss is not likely to have generated these mutants, since loss of the Robertsonian chromosome would result in the loss of all the H-2 antigens. Furthermore, the fact that most mutants express more glyoxalase I than the wild-type strongly argues against loss of the chromosome. This increase in the activity of an unselected, outside marker is in favour of reduplication of the chromosome bearing the allele associated with higher specific activity. Loss of H-2^b alone cannot explain the higher level of activity of this enzyme, since mutant H-2^{b-} clone 8A also lacks H-2^b but does not express more glyoxalase I.

Based on these data we feel that the model shown in Figure 7 is a possible way in which the mutants in class (1) above are derived. Thus, in this particular cell line, since the homologous chromatids are in close proximity in each mitotic division, mitotic recombination may be favoured. Normal cell division is indicated in panel A, where the two daughter cells formed would be identical to each other and the parent cell. Recombinational events close to the centromere between appropriate chromatids would result, as shown in panel B, in progeny cells that differ from the parental cell and would be homozygous for both glyoxalase I and the *H-2* complex. In case of the daugher cell that has become *H-2*^{d} homozygous, the phenotype would be H-2^{b-}, and the cell would express more glyoxalase I since it is also homozygous for the allele of *GLO-1* associated with a higher level of specific activity. Further, if the cross-over in the cell line were to occur between *GLO-1* and the *H-2* complex, a phenotype such as H-2^{b-} clone 8A would result – the cell would be H-2^{b-}, express more H-2^d than wild-type cells but, being still heterozygous for *GLO-1*, would not be distinguishable from wild-type cells for this phenotype. Two predictions of the model might be that: (a) these mutants should have lost all genetic material of the *H-2*^{b} haplotype and this is borne out in part by the Southern analyses; and (b) that the mutants must not be capable of reverting to wild-type and this has been our observation (unpublished results). The model would further predict polarity of loss of the *H-2* markers – selection against the proximal marker *H-2K* should invariably result in the loss of the distal marker *H-2D*, while the reverse selection, against *H-2D* would not necessarily result in the loss of *H-2K*.

Mitotic recombination has not been demonstrated in mammalian cells in earlier studies. Rosenstauss and Chasin (1978) have conducted an exhaustive analysis of segregation of linked markers in hybrids of Chinese hamster cell lines and have concluded that mitotic recombination is not involved. The data we present here might well be unique to the cell line we have chosen to study and the Robertsonian translocation might facilitate mitotic recombination which might otherwise not be a common event in animal cells. Two corollaries may be drawn: (1) H-2 hemizygous mutants from cell lines that do not have the 17:17 Robertsonian translocation may not share the properties demonstrated by b/d 4.4.1; and (2) cell lines that have undergone Robertsonian translocations of other chromosomes to generate isochromosomes may be useful in studying the generality of the phenomenon. Finally, the model does not explain the mutant H-2^{b-} clone 5. A mutant that expresses higher level of glyoxalase I than wild-type, expresses wild-type levels of *H-2*^{d} and may contain some *H-2*^{b}-like genomic sequences cannot be explained by this model without invoking complex modifications such as a double cross-over event. However, since this mutant has a unique *H-2* sequence-containing fragment not present in either of the parental strains, it is potentially of great interest since this fragment may be cloned. Sequence analysis of this fragment

may shed considerable light on this model and the mechanism of emergence of all the variants.

Materials and methods

Cell lines

The Friend leukemia cell line b/d 4.4.1 was used in all these studies. It has been described earlier (Flores and Rajan, 1977). Various H-2 antigen loss variants derived from this cell line have been described in earlier publications (Rajan, 1977; Flores and Rajan, 1977; Rajan and Flores, 1977). Rcsb is a Friend leukemia cell line of the BALB.B ($H\text{-}2^b$) strain.

Immunoselection

Immunoselection to obtain variants that have lost either *H-2* haplotype were obtained as described earlier (Rajan, 1977). To obtain independent events of the same phenotype, small cultures of b/d 4.4.1 were initiated, each by inoculating 100 cells into 5 ml of growth medium. When the cultures grew to contain nearly 5×10^5 cells, each was separately subjected to immunoselection and cloned. One clone was picked from each plate and retained for analyses.

FACS analyses

Cells were treated with antibodies directed against the antigen whose expression was to be determined for 1 h at 4°C. They were then washed extensively to remove unbound antibody and treated with a fluorescein tagged goat anti-mouse Ig for another hour at 4°C. They were again washed extensively to remove unbound antibody. The fluorescence was then analysed on a FACS II. Quantitation of fluorescence was expressed by the function called Channel Square Area. This number is the product of the channel number and the number of cells registered in the channel.

Glyoxalase I assays

The assays for this enzyme have been described earlier (Rajan, 1979). Specific activity of the enzyme activity was calculated using the known molar extinction coefficient of the intermediate product (ϵ_{240} = 3.37/mM/cm); a unit of activity is defined as the amount catalysing the formation of one μmol of intermediate per minute in the routine enzyme assay (Kester and Norton, 1975).

Southern blot analyses

High mol. wt. DNA was obtained from cells (Margulies *et al.*, 1982) and the DNA was digested to completion with restriction endonucleases under conditions suggested by the manufacturer. The restricted DNA was subjected to electrophoresis in 0.75% agarose gels and transferred to nitrocellulose paper according to Southern (1975). The blots were probed with nick-translated (Rigby *et al.*, 1977) *H-2* genomic probe (Evans *et al.*, 1982), with minor modifications. The reaction mixture with 0.25 μg of DNA in a final volume of 30 μl contained 50 mM Tris (pH 7.8), 10 mM dithiothreitol, 500 μg/ml nuclease-free bovine serum albumin, 5 mM $MgCl_2$, 20 μM each of dTTP, dGTP and dATP, 1 unit of DNA polymerase I (Boehringer-Mannheim) and 100 μCi of dCTP (specific activity 3000 Ci/mmol). Reactions were carried for 60 min at 150°C.

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