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Linkage analyses of murine immunoglobulin heavy chain and serum prealbumin genes establish their location on chromosome 12 proximal to the *T*(5;12)*31H* breakpoint in band 12F1

(immunoglobulin allotypes/mouse chromosomal translocations/duplication–deficiency mapping)

T. MEO*, J. JOHNSON*, C. V. BEECHEY†, S. J. ANDREWS†, J. PETERS†, AND A. G. SEARLE†

*Institute for Immunology of the University of Munich, D-8000 Munich 2, West Germany; and †M.R.C. Radiobiology Unit, Harwell, Oxon OX11 ORD., Great Britain

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ABSTRACT **Analysis of backcross mice carrying the Harwell translocation *T*(5;12)*31H* has led to the definitive localization of the immunoglobulin heavy chain gene cluster. Both *Igh-1* and *Pre-1* loci were found to segregate in tight linkage with the chromosomal markers 5^{12} and 12^{5}, which define the balanced translocation *T*(5;12)*31H*. Additional data establish the location of these genes at the telomeric end of chromosome 12. That both loci are proximal to the chromosomal breakpoint in band 12F1 is shown by the phenotypes of segregants aneuploid for the presence or absence of the small marker 5^{12}. The order of loci inferred from a single recovered recombinant is: centromere–*Igh-1*–*Pre-1*–*T*(5;12)*31H*.**

The genes coding for the immunoglobulin polypeptides present unique features with respect to their organization, structure, and expression (1). Interest in their chromosomal location has been rekindled by the evidence that the codon sequences of each light chain, κ and λ, are constructed during differentiation of plasma cell precursors by the joining of DNA segments that were previously far apart (2–4). Aside from other general implications, information on the map position of the immunoglobulin genes may allow direct investigation on the nature of the relationship between somatic gene rearrangement and allelic exclusion (3). Particularly with regard to the heavy chain genes, knowledge of their location can be used to explore other phenomena, such as switching of heavy chain gene expression (5) and somatic crossovers between variable and constant region determinants (6). Nevertheless, none of the three unlinked clusters of the κ, λ, and heavy chain immunoglobulin genes has been unequivocally mapped in any species (7, 8).

In the mouse, the chromosomal segment comprising the heavy chain variable and constant regions, *Igh-V* and *Igh-C*, respectively, (9), extends for at least 7–11 units (10) and is linked, probably at its *Igh-C* end, with the serum prealbumin locus *Pre-1* at a distance of about 11 units (11). Although more than 80% of the mouse genetic map has been examined, linkage analyses have failed to position either locus (12). Recently, conflicting reports have been published on the assignment of the *Igh* genes. The results obtained by renaturing specific cDNA transcripts with chromosomal DNA preparations from human fibrosarcoma–mouse macrophage cell hybrids led Valbuena *et al.* (13) to conclude that the most probable location of the *Igh-C* genes was on mouse chromosome 15. In contrast, Hengartner *et al.* (14), analyzing the immunoglobulin production of intraspecific cell hybrids, have tentatively assigned the *Igh-V* and *Igh-C* group to mouse chromosome 12, which was clearly excluded in the former study. Interestingly, both assignments failed to match with the probable locations inferred from negative linkage data.

The resolving power of somatic cell genetics has some inherent limitations associated with the analysis of phenotypes at a clonal level and with the nonrandom or limited loss of chromosomes. For instance, in the hybrids studied by Hengartner *et al.* (14), the possibility of allelic exclusion and the infrequent loss of metacentrics hinder a direct correlation between immunoglobulin expression and karyotype composition. Consequently, those data allow gene mapping by exclusion rather than a direct chromosomal assignment. In order to clarify the discrepant observations made with cell hybrids, we sought to extend the linkage analyses in the mouse by availing ourselves of previously unexploited markers. We now report the conclusive mapping of *Igh-1* and *Pre-1* loci on mouse chromosome 12 in close proximity to band 12F1.

EXPERIMENTAL

Animals. The wild-derived mice, CD (15), were obtained from E. Capanna, Istituto di Anatomia Comparata, University of Rome, Rome, Italy. These mice are homozygous for nine metacentric marker chromosomes derived by Robertsonian fusions among autosomes 1–18, whereas the sex chromosome and chromosome 19 remain structurally unmarked. The inbred strains C57BL/6J, LP/J, and BALB/cJ were purchased from the Jackson Laboratory, whereas all other mice were from the colonies maintained at the Medical Research Council Radiobiology Unit, Harwell. The translocation stock *T*(5;12)*31H* (*T31H*) was maintained by mating translocation heterozygotes to F1 (C3H/HeH ♀ × 101/H ♂) males. Chromosomal breakpoints of *T31H* are at 5B and 12F1 (16, 17), giving rise to long (12^{5}) and short (5^{12}) marker chromosomes (Fig. 1). Male heterozygotes are usually sterile but female heterozygotes breed freely, though with the expected decrease in litter size. Because of the small size of one translocation product, numerical nondisjunction is frequent, with production (Fig. 2) of viable tertiary trisomics, *Ts*(5^{12})*31H*, and monosomics, *Ms*(5^{12})*31H*, which cause sterility only in the male. For linkage studies, female *T31H*/+ mice were crossed with strain BALB/c males. F1 females, shown cytologically to carry *T31H*, were found by progeny testing to be *Igh-1*^{a} *Pre-1*^{a}/*Igh-1*^{b} *Pre-1*^{b}. Because the BALB/c strain was typed as homozygous for *Igh-1*^{a} and *Pre-1*^{a}, the *T31H* genome must have carried *Igh-1*^{b} and *Pre-1*^{b}.

Karyotype Identifications. The chromosomal markers segregating in the CD backcross progeny were individually identified in banded karyotypes. Animals were splenectomized and lymphocyte suspensions were prepared (18) and distributed at 2–3 × 10^{6} viable cells per ml (per well) in tissue culture plates

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Abbreviations: *Igh*, immunoglobulin heavy chain gene cluster; *Igh-1*, IgG2a isotype locus; *Pre-1*, prealbumin locus; *T31H*, *T*(5;12)*31H* translocation; *de*^{H}, droopy ear mutant; +, wild-type allele.

(Costar). The medium (18) contained pokeweed mitogen at a 1:100 dilution of a reconstituted commercial preparation (GIBCO). Colcemid (GIBCO) was added to a final concentration of 0.1 μg/ml after 66–68 hr of culture. Chromosome preparation and Giemsa banding methods were as reported (14).

Karyotyping of progeny from crosses involving the translocation *T31H* did not require chromosome banding. Accordingly, the 5^{12} and 12^{5} markers were identified in metaphases prepared from corneal tissues (19) or from whole blood cultures (20).

Genotype Determinations. Allotypes of the *Igh-1* locus were determined by immunodiffusion or by inhibition of passive hemagglutination (21). Antisera were prepared in LP/J and BALB/cJ mice and tested on *Igh* congeneic pairs. Indicator cells were conjugated with the protein A binding fraction (22) or the 7S fraction (prepared by gel filtration) of sera from *Igh-1*^{a} and *Igh-1*^{b} inbred strains.

Prealbumin typing was according to the method of Wilcox (23), except that serum instead of plasma was used and electrophoresis was over 4.5 hr. Strain 101/H mice appeared to carry the *Pre-1*^{b} allele (like SWR/J) (23), but they have not been tested against the latter strain. Note that, as recommended, *Pre-1*^{a}, *Pre-1*^{b}, and *Pre-1*^{c} are used in place of Wilcox's original symbols *Pre-2*^{c}, *Pre-2*^{b}, and *Pre-2*^{a} (24).

Alleles at both *Igh-1* and *Pre-1* loci are codominant.

RESULTS AND DISCUSSION

Simultaneously with the production of CD and CB somatic hybrids (14), we used the same nine Robertsonian markers in a formal linkage analysis with *Igh-1* and *Pre-1*. CD mice, shown to carry *Igh-1*^{a} and *Pre-1*^{a}, were mated to C57BL/6J (*Igh-1*^{b} *Pre-1*^{c}) females. Genotyped F1 males were then backcrossed to C57BL/6J to examine the centromeric ends of autosomes 1–18 for linkage with either *Igh-1* or *Pre-1*. Thirty male and 29 female offspring were typed for the 11 markers. Although *Igh-1* and *Pre-1* were found to segregate together [recombination frequency = 0.136 ± (SEM) 0.045], no evidence of linkage between either locus and any of the nine marker chromosomes was observed (Table 1). These results extend previous studies, including an analogous one made by Klein *et al.* using the Robertsonian markers of *Mus poschiavinus* (reported in ref. 25). On the basis of the estimated "swept radius" of a marker gene (26) and with the limitations implied in this concept, the data obtained with CD mice can be used to exclude, with a 95% probability, location of the *Igh-1* and *Pre-1* segment within 47 centimorgans from the centromere of all autosomes except chromosome 19. Thus, our attention was focused on the telomeric ends of the chromosomes. On the basis of the findings with the cell hybrids, we sought to extend the analysis of chromosome 12 by using the marker *de*^{H} (droopy ear) on linkage group XVI, previously assigned to this chromosome. Forty-nine backcross offspring were typed (data not shown) and no evidence of linkage was found between *de*^{H} and *Igh-1*. However, the meaning of the independent assortment of *Igh-1* with linkage group XVI markers (12, 27), which were thought to cover the estimated genetic length of chromosome 12 (about 75 centimorgans), is altered by the discovery that linkage group XVI belongs elsewhere (28, 29). This has left chromosome 12 devoid of mapped genes.

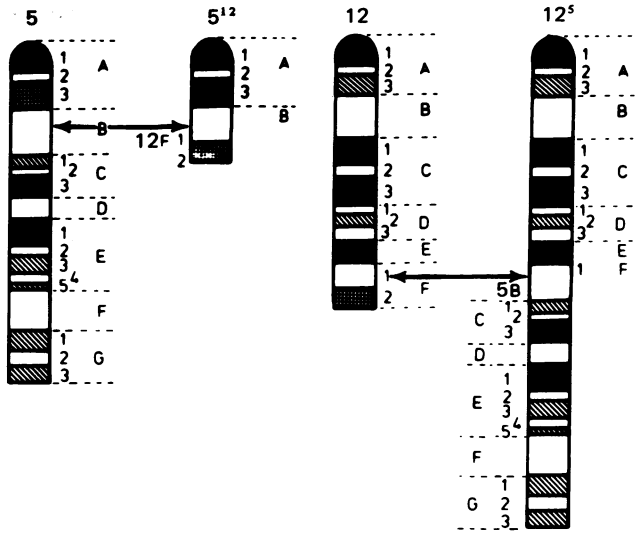

FIG. 1. Idiogram of a heterozygote for the *T31H* translocation. Arrows designate breakpoints in bands 12F1 and 5B. Banding nomenclature is after Nesbitt and Francke (17).

The Harwell translocation *T31H* (ref. 16; Fig. 1) provides an unquestionable structural marker for the telomeric end of chromosome 12. Use of this balanced translocation proved decisive in the mapping of *Igh-1* and *Pre-1*. Heterozygous females with the genotype *Igh-1*^{b} *Pre-1*^{b} *T31H*/*Igh-1*^{a} *Pre-1*^{a} + were crossed to strain C3H/HeH males homozygous for *Igh-1*^{a} and *Pre-1*^{c}. Only one recombinant between *Igh-1* and the translocation breakpoint was observed on 89 typed progeny, whereas no recombinants were found between *T31H* and the *Pre-1* locus in 59 classified males (Table 2). Because translocation *T31H* involves chromosomes 5 and 12, close linkage of

Table 1. Number of recombinants in 59 tested progeny from the cross (CD × C57BL/6J)F_1 ♂ × C57BL/6J ♀ segregating for nine Robertsonian marker chromosomes and alleles at *Igh-1* and *Pre-1* loci

	Robertsonian marker chromosome									Gene markers	
	1.7	3.8	6.13	4.15	10.11	2.18	5.17	12.14	9.16	*Igh-1*^{a}	*Pre-1*^{a}
1.7	—	29	25	26	30	30	27	26	29	26	30
3.8	—	—	26	25	25	27	23	43*	21*	27	32
6.13	—	—	—	23	23	32	31	27	20*	30	28
4.15	—	—	—	—	31	27	34	26	26	27	25
10.11	—	—	—	—	—	27	19*	32	21*	29	30
2.18	—	—	—	—	—	—	28	28	34	31	32
5.17	—	—	—	—	—	—	—	29	27	30	25
12.14	—	—	—	—	—	—	—	—	27	32	23
9.16	—	—	—	—	—	—	—	—	—	30	27
Igh-1^a	—	—	—	—	—	—	—	—	—	—	8*

* Combinations for which χ^2 values testing deviation from random assortment are significant at the 0.05 probability level. However, the significant values for the Robertsonian markers 3.8 vs. 12.14 ($\chi^2 = 11.5$), 3.8 vs. 9.16 ($\chi^2 = 4.3$), 6.13 vs. 9.16 ($\chi^2 = 5.5$), 10.11 vs. 5.17 ($\chi^2 = 6.8$), and 10.11 vs. 9.16 ($\chi^2 = 4.3$) cannot be regarded as more than suggestive in view of the large number of comparisons. Segregation of each marker (not reported) did not show significant deviation from expected. All χ^2 values were calculated with a correction for continuity.

Table 2. Progeny from crosses of *Igh-1^b Pre-1^b T31H/Igh-1^a Pre-1^a* + females to males homozygous for *Igh-1^a Pre-1^c*

Karyotype	Loci *Igh-1* *Pre-1**	Number of offspring with different allelic combinations: Females *aa* ND	Females *ba* ND	Males *aa* *ac*	Males *aa* *bc*	Males *ba* *ac*	Males *ba* *bc*	Males *ND* *ac*	Males *ND* *bc*	Males *ba* ND	Total
+/+		11	0	22	0	1†	0	3	0	0	37
T31H/+		0	12	0	0	0	20	0	1	1	34
Ts(5^{12})31H		9	0	7‡	0	0	0	1	0	0	17
Ms(5^{12})31H		0	2	0	0	0	4	0	0	0	6

ND, not determined.

* PRE-1 phenotypes were scored only in males because of difficulties of detection in females.

† Recombinant.

‡ Because two distinct PRE-1 bands were seen only infrequently in *Pre-1^a Pre-1^b* males, it seems doubtful whether we could have distinguished the PRE-1AC phenotype from PRE-1ABC had the latter occurred in these trisomics.

Igh-1:Pre-1 with the breakpoints means that the loci are on one of these chromosomes. Our failure to find linkage between *Igh-1:Pre-1* and the centromere of chromosome 5 (Table 1) excludes a proximal location on this chromosome, and a distal one is ruled out by previous negative linkage data (11, 12, 30). Thus, the loci must be on chromosome 12, confirming the assignment made by Hengartner *et al.* (14). *T31H, Pre-1,* and *Igh-1* therefore define the linkage group of chromosome 12.

The position of *Igh-1* and *Pre-1* relative to the *T31H* breakpoint in band 12F1 can be determined by considering the tertiary monosomics and trisomics—i.e., by duplication–deficiency mapping (31). Due to the nature of the cross, duplicated and deficient phenotypes can be qualitatively distinguished. Aside from the single recombinant, all the euploid offspring that inherited the translocation were shown to carry the *Igh-1^b Pre-1^b* alleles, whereas those with the normal chromosome 12 carried *Igh-1^a Pre-1^a*. If the loci were centromeric to the chromosome 12 breakpoint, they would be carried by the long 12^5 marker but not by the short 5^{12} one (Fig. 2). The tertiary monosomic has this long marker and therefore would inherit *Igh-1^b* and *Pre-1^b* from the maternal translocation and, in the absence of recombination, would show the *Igh-1^a Pre-1^c/Igh-1^b Pre-1^b* genotype. The tertiary trisomic, however, has only the short marker and would be *Igh-1^a Pre-1^c/Igh-1^a Pre-1^a*. Similarly, if the loci were distal to the breakpoint, the monosomics would be *Igh-1^a Pre-1^c/− −* (double hemizygous), whereas the trisomics would be *Igh-1^a Pre-1^c/Igh-1^a Pre-1^a/Igh-1^b Pre-1^b*. Location of *Igh-1* between the centromere and the *T31H* site is established by evidence that the trisomic animals are homozygous and the monosomics are heterozygous for this locus. Due to the difficulty of clearly distinguishing between the alternative *Pre-1* genotypes expected for the trisomics, positioning of this locus proximally to *T31H* relies solely on the demonstration that the monosomics are heterozygous.

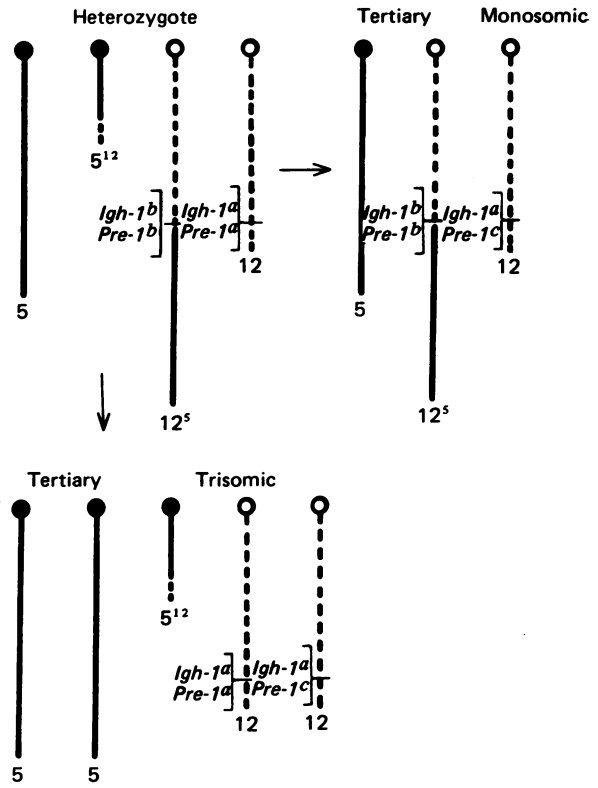

FIG. 2. Genotypes and line drawn idiograms of an *Igh-1 Pre-1 T31H* heterozygous female and her tertiary monosomic and trisomic progeny from a cross to strain C3H/HeH.

The most probable order of the loci is centromere–*Igh-1–Pre-1–T31H,* because the one recombinant found (Table 2) would then be the result of a single crossover event. The estimated recombination frequency between *Pre-1* and *Igh-1* is 0.019 ± (SEM) 0.018, significantly lower than that observed in the CD cross (Table 1) and that of Taylor *et al.* (11). This suggests that *T31H* inhibits crossing over around its 12F1 breakpoint. Thus, the actual genetic distance between *Pre-1* and the *T31H* breakpoint may be greater than the zero recombination frequency (upper 95% fiducial limit = 6.7%) would imply.

The map position of *Igh-1* established by these data conflicts with the results obtained by using specific cDNA probes (13) which unequivocally identify structural genes. A possible explanation is that the observations of Valbuena *et al.* are contingent on the particular cell hybrids examined and therefore lack general validity. This could also explain the surprising observations on the position of constant region κ genes which were shown in the same hybrids to be uncorrelated with the chromosomal location of variable region κ markers (32). On the other hand, the data pertaining to the heavy chains would be consistent if chromosome 12 does not carry structural genes but carries regulatory ones, which must also be allele specific and allelically excluded. The hypothesis of polymorphic genes regulating immunoglobulin heavy chain expression was previously invoked (33, 34) but has not been formulated into a simple model that accounts for all available data.

The positioning of *Igh* genes on chromosome 12 in germ line and somatic cells may now lead to a clearer understanding of the regulation of immunoglobulin expression in normal differentiated lymphocytes, by combining the tools of cell hybridization and molecular genetics. This investigation has also demonstrated the value of male-sterile translocations for mapping studies because of two special properties: their association with easily identifiable long and short marker chro-

mosomes and their tendency to generate viable aneuploid progeny (35).

Note Added in Proof. E. M. Eicher *et al.* (36) also report that *Igh-1* and *Pre-1* are linked to *T31H*.

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