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Length of the chromosomal segment marked by galactose-1-phosphate uridyl transferase and soluble aconitase and conserved since divergence of lineages leading to mouse and man

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Summary

Standard linkage testing crosses and ovarian teratoma mapping were used to estimate the length of the chromosomal segment that is marked by galactose-1-phosphate uridyl transferase and soluble aconitase and that has been conserved since divergence of lineages leading to mouse and man. These experiments were also used to determine whether the *Rb(4·6)2Bnr* Robertsonian translocation suppresses recombination on the proximal portion of mouse Chromosome 4. The estimated length of the conserved segment marked by galactose-1-phosphate uridyl transferase and soluble aconitase in mouse and man was estimated to be 24 cM. It was also shown that *Rb(4·6)2Bnr* strongly suppressed recombination on the centromeric portion of mouse Chromosome 4.

1. Introduction

Lengths of chromosomal segments that have been conserved since divergence of lineages such as those leading to mouse and man provide valuable information concerning the organization and evolution of the mammalian genome. These lengths can be used to estimate the rate and number of linkage disruptions that have occurred since divergence of lineages and to test whether chromosomal rearrangements that disrupt linkage are randomly distributed in the genome (Nadeau & Taylor, 1984; Nadeau & Reiner, 1988). Accurate estimates of segment length are essential for these analyses. One of these putative conserved autosomal segments is the segment marked by galactose-1-phosphate uridyl transferase (*Galt*), soluble aconitase (*Aco-1*), aminolevulinate dehydratase (*Lv*), and orosomucoid-1 (*Orm-1*). These genes are located on mouse Chromosome (Chr) 4 (Nadeau & Eicher, 1982; Baumann, Held & Berger, 1984; Baumann & Berger, 1985; Nadeau *et al.* 1986). The homologues of *Galt* and *Aco-1* are located on Chromosome 9p in man, *Orm-1* is located on Chromosome 9q, and although the arm assignment for the homologue of *Lv* has not been confirmed, it is probably 9q (Westerveld *et al.* 1975; Mohandas *et al.* 1977, 1979; Bruns *et al.* 1978; Cook *et al.* 1978; Aitken & Ferguson-Smith, 1979; Mulcahy & Wilson, 1980; Eiberg, Mohr & Staub-Nielsen, 1982, 1983). There is uncertainty about the length of the segment marked by *Aco-1* and *Galt* in the mouse, however, because the cross used to measure the recombination frequency involved a Robertsonian translocation *Rb(4·6)2Bnr* (hereafter abbreviated *Rb2*) (Nadeau & Eicher, 1982). It is well-established that Robertsonian translocations suppress recombination on the centromeric portions of certain chromosomes (Cattanach, 1978). Recombination suppression imposed by *Rb2* is controversial, however, because one of the genes used in the original study (Cattanach, 1978), *asp*, is no longer believed to be located on Chr 4 (Seyfried & Glaser, 1981). The purpose of this note is to estimate the length of the segment marked by *Galt* and *Aco-1* and to determine whether the *Rb2* translocation suppresses recombination on the proximal portion of Chr 4.

2. Materials and methods

(i) *Mice*

Mice were obtained from the research and production colonies of the Jackson Laboratory.

(ii) Galt *and* Aco-1 *assays*

Methods described by Nadeau & Eicher (1982) were used for typing *Galt* and *Aco-1*.

3. Results and discussion

To measure the recombination frequency between *Galt*, *Aco-1*, and brown (*b*) in a linkage cross not involving a Robertson translocation, C57BL/6J (*Galt*^{b} *Aco-1*^{a} *B*) females were crossed to ST/bJ

Table 1. *Recombination between* Galt, Aco-1, *and* b*: allelic combination inherited from the* F_1 *parent.* × *indicates the position of a crossover.*

Allelic combination					
Galt		*Aco-1*		*b*	No. observed
b		a		B	65
a		c		b	93
b	×	c		b	2
a	×	a		B	2
b		a	×	b	19
a		c	×	B	18

Recombination frequency

Galt–Aco-1: 4/199 = 0·020 ± 0·010
Aco-1–b: 37/199 = 0·186 ± 0·028
Galt–b: 41/199 = 0·206 ± 0·029

(*Galt*^{a} *Aco-1*^{c} *b*) males and F_1 hybrid males were backcrossed to DBA/2J (*Galt*^{b} *Aco-1*^{a} *b*) females. Progeny were typed for *Galt*, *Aco-1*, and brown (*b*) whose location on Chr 4 is well established (Cattanach, 1978; Nadeau & Eicher, 1982; Nadeau *et al.* 1986). A total of 199 progeny were typed (Table 1). GALT and ACO1 were retyped in each putative recombinant to verify the initial typing. Double crossovers were not observed. The most likely gene order and recombination frequencies were *Galt*–0·020 ± 0·010–*Aco-1*–0·186 ± 0·029–*b*.

Recombination data can be used to estimate the length of the chromosomal segments that have been conserved since divergence of lineages leading to mouse and man. It is usually assumed that these segments are not interrupted by unrelated genes. Genes on Chromosome 9 in man may represent an exception. Although many of the murine homologues of genes on human Chromosome 9 are located on mouse Chromosome 4, the homologues of several others, e.g. *Abl*, *Ak-1* and *Fpgs*, are located on mouse Chromosome 2. Although there is no direct evidence, it is likely that some or all of these genes with homologues on mouse Chromosome 2 are located between GALT (and ACO1) and ORM1 (Smith & Spence, 1985). Thus *Orm-1* is not considered to be part of the conserved segment marked by *Aco-1* and *Galt*. *Lv*, however, is included as part of the conserved segment because there is no contrary circumstantial or direct evidence.

De Maeyer & Dandoy (1987) recently showed that *Ifa* is proximal to misty (*m*) on Chromosome 4 and closer to *b* than previously estimated. Unfortunately, one of the strains used in their mapping studies, HW13, has a small inversion involving the *b* locus (DeMaeyer and Dandoy, 1987; D. W. Bailey, personal communication). This inversion probably accounts for the reduced recombination frequency between *Ifa* and *b*. In addition, they did not include *Orm-1* in their study. Thus it remains likely that *Ifa* and *Ifb* are located distal to *Orm-1* and are not part of the conserved segment marked by *Galt*, *Aco-1* and *Lv* (Nadeau *et al.*, 1986).

In the mouse, the weighted average recombination frequency between *Galt* and *Aco-1* is 0·03 ± 0·01 (9/305), combining data from Nadeau *et al.* (1986) and this study, and between *Aco-1* and *Lv* 0·12 (Nadeau *et al.* 1986). Therefore the recombination frequency between *Galt* and *Lv*, the outermost genes marking the conserved segment, is 0·15. By using equation 2 of Nadeau & Taylor (1984), the length of the conserved chromosomal segment marked by these three genes is estimated to be 24 cM.

Ovarian teratoma mapping provides a reliable way to estimate centromere–gene recombination frequencies (Eicher, 1978; Eppig & Eicher, 1983; Ott *et al.* 1976). We used this method to estimate the centromere–*Galt* recombination frequency and, by comparison with recombination frequencies in previous crosses involving a Robertsonian translocation, determine whether *Rb2* suppresses recombination on the proximal portion of mouse Chr 4. These studies required construction of an LT/Sv congenic strain with a variant allele of *Galt*. To construct this strain (LT/Sv [*Galt*^{b}] × ST/bJ [*Galt*^{a}])F_1 hybrid females were backcrossed to LT/Sv males. Blood samples were collected from backcross progeny and typed for GALT. Male or female *Galt*^{a}/*Galt*^{b} heterozygotes were selected and backcrossed to LT/Sv. This process was repeated for 4 backcross generations. Both male and female *Galt*^{a}/*Galt*^{b} heterozygotes were then crossed to the recombinant inbred strain LTXBO (*Galt*^{a}). Of these F_1 hybrids, 80–90 % have spontaneous ovarian teratomas (J. H. Nadeau and D. S. Varnum, unpublished observations). Virgin F_1 hybrid females that were heterozygous for *Galt* were autopsied when they were 70–90 days old. Teratomas were removed and typed for *Galt*. A kidney from each female in which a teratoma was found was also typed to verify maternal heterozygosity for *Galt*. The frequency of heterozygous teratomas was 0·48 (Table 2). Ott *et al.* (1976) showed that the recombination frequency between the centromere and a gene is immeasurable if the frequency of heterozygous teratomas is greater than 0·33. In addition, they showed that the frequency of heterozygous teratomas approaches 2/3 as the number of crossovers between the centromere and the gene increases. Because the observed frequency of heterozygous teratomas (0·48) exceeds 0·33, the recombination frequency between the centromere and *Galt*

Table 2. *GALT phenotypes of ovarian teratomas that arose spontaneously in heterozygous females*

GALT phenotype	No. observed
GALT-AB	15
GALT-A	8
GALT-B	8

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is immeasurable. The distance between the centromere and *Galt* is probably large, however, because the frequency of heterozygous teratomas exceeds 0·33.

A comparison of the recombination frequency between the centromere of Chr 4 and *Galt* in standard linkage testing crosses in which *Rb2* segregated and in ovarian teratoma mapping experiments that did not involve a Robertsonian translocation demonstrated that *Rb2* suppressed recombination on the proximal portion of mouse Chr 4. The recombination frequency between the centromere and *Galt* was $0{\cdot}035 \pm 0{\cdot}020$ in a linkage cross involving *Rb2* (Nadeau & Eicher, 1982), whereas *Galt* appeared to be unlinked to the centromere in the present ovarian teratoma mapping experiment (Table 2). This difference in recombination frequency for the interval proximal to *Galt* was highly significant ($\chi_1^2 = 23{\cdot}00$; $P < (0{\cdot}005)$, assuming that the high frequency of heterozygous teratomas corresponds to a recombination frequency of at least 0·165 (c.f. Ott *et al.* 1976). The χ^2 value is an underestimate because the recombination frequency between the centromere and *Galt* was probably greater than 0·165. By contrast, recombination distal to *Galt* was not suppressed. The recombination frequency between *Galt* and *Aco-1* was $0{\cdot}047 \pm 0{\cdot}021$ in a linkage cross involving *Rb2* (Nadeau & Eicher, 1982), where the frequency was $0{\cdot}020 \pm 0{\cdot}010$ in the present linkage testing cross that did not involve a Robertsonian translocation (Table 1). This difference in recombination frequencies for the interval distal to *Galt* was not significant ($\chi_1^2 = 2{\cdot}06$; $P > 0{\cdot}05$). *Rb2* therefore suppressed recombination proximal but not distal to *Galt*.

Previously, Cattanach (1978) argued that *Rb2* suppressed recombination between the centromere and *b*. Location of the gene audiogenic seizure prone *asp*) near the centromere of mouse Chr 4 (Collins, 1970) was essential to Cattanach's argument for suppression. The gene *asp* is now believed to be located on Chr 12 (Seyfried & Glaser, 1981; Lusis *et al.* 1987). Therefore, Cattanach's results do not provide evidence that *Rb2* suppresses recombination. Results of the present study, however, clearly demonstrate that *Rb2* suppresses recombination between the centromere and *Galt*.

Genomic DNAs have been preserved from most of the animals in the linkage cross. Individuals interested in using these DNAs for mapping molecular markers should contact J. H. N.

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