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Mapping studies of the distal imprinting region of mouse Chromosome 2

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Summary

The known limits of the distal imprinting region of mouse Chromosome (Chr) 2 are defined by the breakpoints of the translocations T(2;8)2Wa, (T2Wa), and T(2;16)28H, (T28H), in distal H3, and proximal H4 respectively. We have shown that T2Wa and T(2;4)1Go, (T1Go), which has a breakpoint in central H3 map close to *a*, non-agouti. *Ada*, adenosine deaminase, lies very near the proximal boundary and *Ra*, ragged, maps very close to the distal boundary, and is less than 0·2 cM from wasted, *wst*. From the current data *Ada* can be taken as the proximal, and *Ra* as the distal gene marker of the imprinting region on the linkage map. From consensus maps twenty three other markers, including fourteen genes, lie between *Ada* and *Ra*, some of which may be useful in investigations of imprinting. Of the markers included in the study reported here, four, *Ada*, *ls*, lethal spotting, *Ra* and *wst* lie or probably lie within the region but none display any evidence of imprinting. We suggest that recombination frequency is elevated in distal Chr 2, because in none of the crosses could the most closely linked marker be ordered in relation to the translocation breakpoint due to the high frequency of double crossovers.

1. Introduction

Parental imprinting is the phenomenon whereby maternally and paternally derived alleles are differentially expressed during development (Cattanach & Jones, 1994). At present 15 imprinting effects involving ten regions on five chromosomes have been described (Beechey & Cattanach, 1994; Cattanach & Jones, 1994). Maternal duplication/paternal deficiency and paternal duplication/maternal deficiency for a distal region of Chromosome (Chr) 2 have been found to cause different neonatal lethalities with opposite anomalous phenotypes and behaviour (Cattanach & Kirk, 1985; Cattanach, 1986; Cattanach & Beechey, 1990). Thus, maternal duplication/paternal deficiency leads to mice with long flat sided bodies with arched backs that become almost totally inactive within a few hours of birth, with very few surviving more than 24 h. The reciprocal type, maternal deficiency/paternal duplication shows an effectively opposite phenotype. These mice have short square bodies with broad flat backs and are notably hyperkinetic, and survive for several days.

The region was originally defined as lying between the breakpoints of two reciprocal translocations T(2;11)30H and T(2;16)28H with their Chr 2 breakpoints in bands H1 and H4 of the G-banded map, respectively (Cattanach & Kirk, 1985) but the results of more recent work with T(2;4)1Go which has a more distal Chr 2 breakpoint in H3 have narrowed the size of the imprinting region to little more than a single band (Cattanach *et al.* 1991). The size of the region has now been reduced even further and is defined proximally by the Chr 2 breakpoint of T(2;8)2Wa which is even more distal in H3 than T(2;4)1Go (Cattanach *et al.* 1992) (Fig. 1).

Although the positions of these translocations have been well defined on the cytogenetic map, neither T(2;4)1Go nor T(2;8)2Wa have been placed on the linkage map. We have commenced linkage studies with T(2;4)1Go, T(2;8)2Wa, and T(2;16)28H against the genetic markers a^t, black and tan, *Ada*, adenosine deaminase, *ls*, lethal spotting, and *Ra*, ragged, on Chr 2 in order to establish the limits of the imprinting region on the linkage map (Fig. 1). We have also investigated linkage between *Ra*, and *wst*, wasted, whose loci were thought to be close to, or within, the imprinting region. The data from these experiments together with information from the linkage map were

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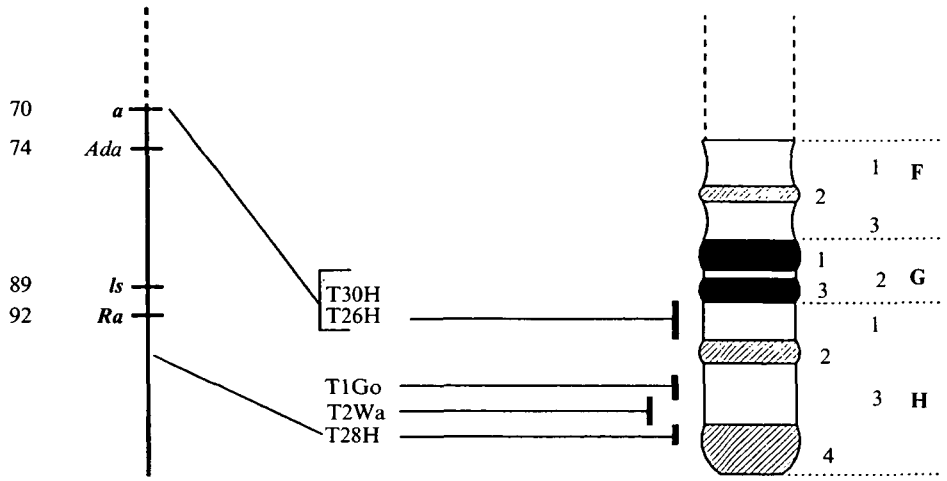

Fig. 1. Genetic and G-banded maps of distal Chromosome 2 showing the positions of translocation breakpoints that define, or are close to the imprinting region, and loci used in the study. Anchor loci are shown in bold; genetic distances in cM from the centromere (Hillyard *et al.* 1993) are shown to the left of the genetic map. The maps are derived from Beechey (1994).

then used to predict the genes that probably lie within the imprinting region.

2. Methods

The reciprocal translocation T(2;4)1Go (Beerman *et al.* 1987) was obtained from I. Hansmann, University of Göttingen, Germany; T(2;8)2Wa (Searle, 1989) from P. de Boer, Wageningen, The Netherlands; T(2;16)28H (Searle, 1989), the ADA-B stock which carries an electrophoretic variant of adenosine deaminase (Abbott *et al.* 1991), and the WST stock which carries *Ra* and *wst* in repulsion came from the colony maintained at the MRC Radiobiology Unit, Chilton. Hereafter the translocations will be referred to as T1Go, T2Wa and T28H respectively.

These translocations have recently been reanalysed by fine G-banding and the Chr 2 breakpoints are now as follows: central H3 for T1Go (Cattanach *et al.* 1991), distal H3 for T2Wa (Cattanach *et al.* 1992), and proximal H4 for T28H (Beechey *et al.* 1992) (Fig. 1). In linkage test crosses, semi-sterility (and therefore presumptive translocation heterozygosity) of offspring was determined by crosses to wild-type mice and subsequent examination of uterine contents at 12·5–16·5 days gestation using the criteria of Carter *et al.* (1955). However, with T28H embryonic survival is often high in such outcrosses, embryonic death being frequently post-implantation and associated with exencephaly, and these latter criteria were used in typing T28H carriers.

Chromosome preparations were obtained directly from bone marrow and G-banded following the method of Evans (1987).

ADA isozymes were scored in haemolysates using the method of Abbott *et al.* (1986).

3. Results

(i) *Crosses with T1Go*

To determine the position of the T1Go breakpoint on Chr 2 two crosses were set up. In the first (Table 1), *a* T1Go+/a^t+*ls* males were backcrossed to homozygous a^t *ls* females and the offspring were test mated to distinguish between a^t/a^t and a^t/a genotypes, and also T1Go/+ and +/+. The phenotypes were: 11 T1Go, 16 a^t *ls*, 1 *ls*, 0 a^t T1Go, 4 T1Go *ls*, 2 a^t, 1+, 0 a^t T1Go *ls*, total 35. Thus the recombination frequency (RF) between a^t and T1Go is 5·7±3·9%, and between T1Go and *ls* is 20·0±6·8%, while that between a^t and *ls* is 20·0±6·8%, similar to the value given on the locus map of the mouse (Hillyard *et al.* 1993). The order could be a^t-T1Go-*ls* or T1Go-a^t-*ls* for with either there is a single double recombinant.

Table 1. *Offspring from crosses of T(2;4)1Go with Chromosome 2 markers*

Parental mating *a* T1Go+/a^t+*ls* × a^t+*ls*/a^t+*ls*	
Offspring	Number
Non recombinant	
a T1Go+	11
a^t+*ls*	16
Single recombinant	
a+*ls*	1
a^tT1Go+	0
a T1 Go*ls*	4
a^t++	2
Double recombinant	
a++	1
a^tT1Go *ls*	0

Recombination frequencies (%)		
a^t-T1Go	2/35	5·7±3·9
T1Go-*ls*	7/35	20·0±6·8
a^t-*ls*	7/35	20·0±6·8

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Table 2. *Offspring from crosses of T(2;4)1Go with Chromosome 2 markers*

Parental mating $Ada^b + Ra/Ada^a$T1Go+ × Ada^a + +/Ada^a + +	
Offspring	Number
Non recombinant	
$Ada^b + Ra$	29
Ada^aT1Go+	46
Single recombinant	
Ada^bT1Go+	0
$Ada^a + Ra$	1
Ada^b + +	12
Ada^aT1Go*Ra*	12
Double recombinant	
Ada^a + +	0
Ada^bT1Go*Ra*	1

Recombination frequencies (%)		
Ada-T1Go	2/101	2·0±1·4
T1Go-*Ra*	25/101	24·8±4·3
Ada-Ra	25/101	24·8±4·3

Table 3. *Offspring from crosses of T(2;8)2Wa with Chromosome 2 markers*

Parental mating + T2Wa + /$a^t + ls$ × $a^t + ls/a^t + ls$	
Offspring	Number
Non recombinant	
+ T2Wa +	71
$a^t + ls$	48
Single recombinant	
+ + *ls*	3
a^tT2Wa +	0
+ T2Wa *ls*	19
a^t + +	15
Double recombinant	
+ + +	1
a^tT2Wa *ls*	2

Recombination frequencies (%)		
a^t-T2Wa	6/159	3·8±1·5
T2Wa-*ls*	37/159	23·3±3·4
a^t-*ls*	37/159	23·3±3·3

However, it is known that the G band breakpoints of two reciprocal translocations at the *a* locus, T(2;8)26H and T(2;11)30H are in H1 whereas the Chr 2 breakpoint in T1Go is in H3 (de Boer & van Gijsen, 1974; Washburn & Eicher, 1977; Cattanach *et al.* 1991). Thus, the likely order is a^t-T1Go-*ls*.

In the second cross (Table 2), $Ada^b + Ra/Ada^a$ T1Go+ males were mated to homozygous Ada^a females and offspring were classified for T1Go by testing for semi-sterility, and blood samples were taken to determine their ADA phenotype. The following phenotypes were found: 29 ADA-AB *Ra*, 46 ADA-A T1Go, 0 ADA-AB T1Go, 1 ADA-A *Ra*, 12 ADA-AB, 12 ADA-A T1Go *Ra*, 0 ADA-A, 1 ADA-AB T1Go *Ra*. The RFs are: *Ada* to T1Go 2·0±1·4%, T1Go to *Ra* 24·8±4·3%, *Ada* to *Ra* 24·8±4·3%, total 101. The order, as judged by the rarity of double recombinants, could be *Ada*-T1Go-*Ra* or T1Go-*Ada-Ra* for with either there is a single double recombinant.

(ii) *Cross with T2Wa*

To determine the position of the T2Wa breakpoint on Chr 2, + T2Wa + /$a^t + ls$ males and females were backcrossed to homozygous a^t *ls* and offspring were classified for T2Wa by testing for semi-sterility. The following offspring were found (Table 3): 71 T2Wa, 48 a^t *ls*, 3 *ls*, 0 a^t T2Wa, 19 T2Wa *ls*, 15 a^t, 1 +, 2 a^t T2Wa *ls*, total 159. Thus, the RF between a^t and T2Wa is 3·8±1·5% with 23·3±3·4% between T2Wa and *ls*, and between a^t and *ls*. Similar recombination frequencies occurred in both male and female heterozygous parents. The order could be a^t-T2Wa-*ls* or T2Wa-a^t-*ls* for with either there are three double recombinants. However, the G-band breakpoint of T2Wa is more distal in H3 than that of T1Go and thus the more likely order is a^t-T2Wa-*ls*.

Table 4. *Offspring from crosses of T(2;16)28H with Chromosome 2 markers*

Parental mating $Ada^b Ra + /Ada^a$ + T28H × Ada^a + +/Ada^a + +	
Offspring	Number
Non recombinant	
$Ada^b Ra$ +	103
Ada^a + T28H	77
Single recombinant	
Ada^b + T28H	10
$Ada^a Ra$ +	13
$Ada^b Ra$T28H	1
Ada^a + +	2
Double recombinant	
Ada^b + +	2
$Ada^a Ra$T28H	0

Recombination frequencies (%)		
Ada-Ra	25/208	12·0±2·3
Ra-T28H	5/208	2·4±1·1
Ada-T28H	26/208	12·5±2·3

(iii) *Cross with T28H*

To determine the position of the T28H breakpoint on Chr 2 Ada^b $Ra+/Ada^a$ + T28H males and females were backcrossed to homozygous Ada^a and offspring were classified for semi-sterility and blood samples were taken to determine their ADA phenotype. The following phenotypes were found (Table 4): 103 ADA-AB *Ra*, 77 ADA-A T28H, 10 ADA-AB T28H, 13 ADA-A *Ra*, 1 ADA-AB *Ra* T28H, 2 ADA-A, 2 ADA-AB, 0 ADA-A *Ra* T28H, total 208. Thus, the recombination frequency between *Ada* and *Ra* is 12·0±2·2%, and that between *Ra* and T28H is 2·4±1·1%. Similar recombination frequencies were found in both male and female heterozygous parents. The recombination frequency between *Ada* and *Ra* is in good agreement with the estimate of 13·8±2·3%

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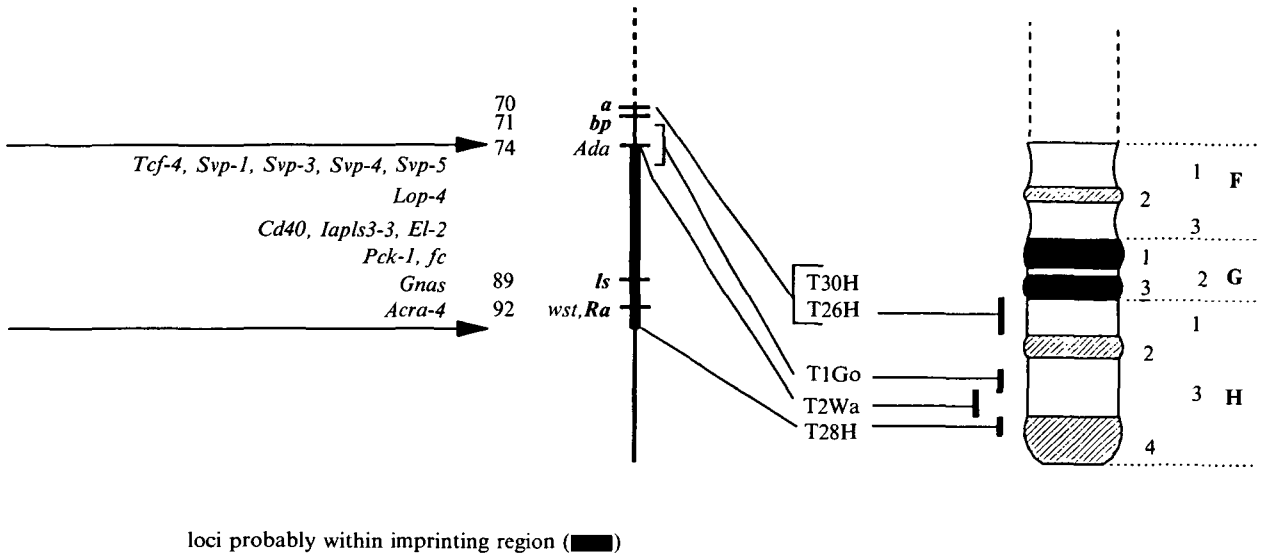

Fig. 2. Genetic and cytogenetic maps of distal Chromosome 2 showing the locations of translocation breakpoints that define, or are close to, the imprinting region and loci that are known or predicted to lie within the region. The maps are constructed from data presented in this paper, as well as Beechey (1994), Hillyard *et al.* (1993) and Siracusa & Abbott (1993).

reported by Abbott *et al.* (1991) but slightly lower than the value of 17·4±2·6% found by Le Tissier *et al.* (1993). The tentative order is *Ada-Ra*-T28H for there are two double recombinants with this order but three if the order is *Ada*-T28H-*Ra*.

(iv) *Linkage of* Ra *and* wst

To examine the linkage of *Ra* and *wst*, $Ra+^{wst}/+^{Ra}wst$ were intercrossed and offspring were classified at weaning for *Ra* and at up to 35 days of age for *wst*. The following offspring were found: 115 *Ra Ra*, 1337 $Ra+^{wst}$, 727 $+^{Ra}wst$ and 5 $+^{Ra}+^{wst}$, total 2184. By fitting a multinomial distribution to these data, it was estimated that only 16·7±1·6% *Ra Ra* mice survive to weaning. Assuming that *Ra wst*/$+^{Ra}wst$ have similar viability to *wst/wst* the recombination fraction between *Ra* and *wst* is 0·179±0·165%, indicating that these genes are very closely linked. Previous estimates, based on much lower sample sizes have been 0·93±1·30% (Sweet, 1984), 0-7·87% (Abbott *et al.* 1991), and 0-3·24% (Le Tissier *et al.* 1993).

4. Discussion

The closest gene markers to the proximal boundary of the imprinting region (defined by T1Go by Cattanach *et al.* 1991, and refined to T2Wa by Cattanach *et al.* 1992) were *a* and *Ada*. From earlier work on the cytogenic localisations of *a*, T1Go and T2Wa (de Boer & van Gijsen, 1974; Washburn & Eicher, 1977; Cattanach *et al.* 1991) it is clear that *a* lies outside the region. *Ada* must lie very close to the proximal boundary of the imprinting region, for we found it to be 2·4 cM from T1Go; it is known to map about 4 cM distal to *a* (Siracusa *et al.* 1989, 1990; Le Tissier *et al.* 1993) and it has been shown to be in H3 by *in situ hybridization* (Abbott *et al.* 1991). Recent data (Williamson *et al.* submitted) have demonstrated that *Ada* is inside the imprinting region for it has been found to be distal to T2Wa from recombinational analysis of an interspecific backcross.

From our data the distal boundary of the imprinting region, T28H, lies close to *Ra* for these two markers were found to be only 2·4 cM apart. However, the position of *Ra* is uncertain, although we tentatively suggest that it is proximal to T28H. Support for this conclusion comes from earlier findings that *Ra* is 3 cM distal to *ls* (Phillips, 1966) and *ls* is 7 cM proximal to T28H (Searle & Beechey, 1970); however, these data were obtained in different crosses and genetic distance can vary from cross to cross.

ls must lie within the imprinting region for it is clearly distal to both T1Go and T2Wa and is known to be proximal to T28H (Searle & Beechey, 1970).

Four of the five gene markers included in the study, *ls*, *wst*, *Ra* and *Ada* lie or probably lie within the imprinting region but none displays any evidence of imprinting. If either *ls* or *wst* were imprinted they would be expected to show dominance when inherited from one parent but recessivity when inherited from the other, but both show recessive inheritance regardless of parental origin. Similarly, if *Ra* was imprinted, heterozygotes *Ra*/+ would be expected to have the phenotype characteristic of the *Ra/Ra* homozygote when the mutant allele was inherited from one parent and the *Ra*/+ phenotype when inherited from the other, but *Ra*/+ mice have similar phenotypes regardless of the derivation of the *Ra* allele. Investigations of *Ada* expression in conceptuses at 10·5 days p.c. showed that both maternally and paternally derived alleles are expressed (Peters & Ball, 1989).

From the current data *Ada* can be taken as the

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proximal, and *Ra* as the distal gene marker of the imprinting region on the linkage map. Twenty-three other markers, including nine simple sequence repeats, between *Ada* and *Ra* are shown on a map of Chr 2 (Siracusa & Abbott, 1993). Thus there are 14 loci which may determine expressed genes and some of these may be useful for investigations of imprinting (Fig. 2).

Both *a* and *Ra* are anchor loci and are known to be 20–24 cM apart (Davisson *et al.* 1989) and two studies have shown that the distance between *a* and *Ada* is about 4 cM (Siracusa *et al.* 1989; Le Tissier *et al.* 1993). Thus *Ada* and *Ra* might be expected to be separated by 16–20 cM and this would represent the genetic length of the imprinting region. On consensus maps (Hillyard *et al.* 1993; Lyon & Kirby, 1993; Siracusa & Abbott, 1993) *Ada* and *Ra* are shown to be between 11–15 cM apart. Direct estimates of the genetic distance between *Ada* and *Ra* range from 12 cM in the T28H cross to 25 cM in the cross with T1Go. The reasons for this difference are not clear. Earlier estimates in crosses which do not involve translocations are 14 cM (Abbott *et al.* 1986) and 17 cM (Le Tissier *et al.* 1993).

In none of the crosses reported here involving translocation breakpoints has it been possible to order the closest marker in respect of the breakpoint from recombinational analysis. This is because of the occurrence of double crossovers, suggesting that recombination may be enhanced in distal Chr 2. Several other reports support this suggestion. Lyon (1976) observed that loci are clustered in the central region of Chr 2 and more widely dispersed at either end of the chromosome. The chromosome is much more densely mapped now than it was in 1976 but current composite maps (Hillyard *et al.* 1993; Lyon & Kirby, 1993; Siracusa & Abbott, 1993) still show clustering of genes in the middle region with wider spacing at the centromeric and telomeric ends. Lyon (1976) suggested that if initiation of chiasmata occurs equally from either end of Chr 2, where the mean number of chiasmata approaches 2·0, then it is to be expected that there would be clustering of genes in the middle of the chromosome and wide spacing at either end. A very pronounced peak of chiasmata has been found in bands H3/H4 and a lesser one around the centromere (Gorlov *et al.* 1986; Hulten & Lawrie, in Povey *et al.* 1992; Evans (unpublished)).

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