Differences in or near the responder region of complete and partial mouse *t*-haplotypes

MARY F. LYON AND JOANNA ZENTHON

M.R.C. Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, U.K.

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Summary

The transmission ratio distortion seen in males heterozygous for a mouse *t*-complex has been explained on the basis of trans-acting distorter genes, having a harmful effect on a responder gene. The *t*-complex form of the responder is relatively resistant to these harmful effects and hence is preferentially transmitted. Animals homozygous for the *t*-complex responder would be expected to show equal transmission of the two homologous chromosomes, but this is not always so. Studies described in this paper have shown differences among complete *t*'s in their transmission when opposite a constant responder carrying partial *t*-haplotype. In addition, the proximal partial haplotypes t^{h49} and t^{w18}, both derived from t^{w5} but of different lengths, behave differently when opposite a responder. The three central partial haplotypes, t^{lowH}, t^{low2H} and t^{low3H}, also differ, in that t^{low3H} shows lower transmission than t^{lowH} or t^{low2H} when opposite either wild-type, or another responder, or distorter genes. These results can be explained either on the basis of differences in the responder region of various haplotypes, including the possibility of varying numbers of copies of the relevant sequences, or on the basis of differences in cis-acting (as opposed to trans-acting) distorter genes.

1. Introduction

The genetic basis of the transmission ratio distortion and male sterility caused by the mouse *t*-complex, on chromosome 17, has recently been explained in terms of distorter genes acting on a responder (Lyon, 1984, 1986). However, some details remained to be elucidated. In particular, the work of Hammerberg (1982) showed that the phenomena occurring in animals carrying the *t* form of the responder on both homologues of chr 17 required further explanation.

According to the model put forward by Lyon (1984, 1986) there are three or more trans-acting distorters that act in a harmful way on the wild-type form of the responder (*Tcr+*). The sterility genes (*tcs*-1, etc., or S1, etc.) are believed to be identical with the distorters. The *t*-form of the responder (*Tcr*ᵗ) is resistant to the harmful action of the distorters, and hence in heterozygous animals, *Tcr+/Tcr*ᵗ, the chromosome carrying *Tcr*ᵗ is preferentially transmitted. In animals homozygous for *Tcr*ᵗ, equal transmission of both homologues would be expected, and in some cases this is indeed so (e.g. t^6/t^{h49} in Lyon, 1984). However, Hammerberg (1982) found various deviations from this expectation. He studied a partial haplotype, t^{w100}, derived from t^{w18} (and hence in turn from the complete

naturally occurring haplotype t^{w5}), which according to Lyon's terminology carried the proximal distorter (*Tcd*-1 or D1) and *Tcr*ᵗ or R. He bred animals having t^{w100} opposite other partial haplotypes carrying R, or opposite complete haplotypes. In some cases he obtained equal transmission of t^{w100} and the test haplotype, but in others not. In particular, there were differences among complete haplotypes, in that t^{w100}/t^0 gave equal transmission whereas t^{w100}/t^{w5} gave 65% t^{w100}. Furthermore, there were differences among partial haplotypes and the haplotypes from which they arose, in that t^{w100}/t^{low} and t^{w100}/t^{h2} (where t^{low} and t^{h2} are both derived from t^6) gave significantly different results from t^{w100}/t^6. As pointed out by Hammerberg, such effects could be explained either by polymorphism among *t*-haplotypes in factors affecting ratio distortion, or by there being cis-acting as well as the already demonstrated trans-acting effects of the distorters.

Hammerberg's work was carried out before the present model of ratio distortion and male sterility in the *t*-complex was proposed, and also before it was possible to study DNA markers carried by different partial and complete haplotypes (Röhme *et al.* 1984; Fox *et al.* 1985; Herrmann *et al.* 1986). It was therefore valuable to carry out further work like that of

Hammerberg, using haplotypes already studied by Lyon, with two aims. The first was to obtain more detailed insight into the genetics of ratio distortion and male sterility. The second was to search for polymorphisms or other genetic differences which could be correlated with the DNA markers present in the various *t*-haplotypes, with the long-term aim of cloning the various distorter and responder genes.

2. Materials and methods

The structure of the various partial *t*-haplotypes used is indicated in diagrammatic form in Fig. 1. This diagram aims to indicate the relative lengths and positions (proximal, distal or central) of the haplotypes, the distortion, sterility and responder factors they are thought to carry, and a few of the relevant DNA markers. No attempt has been made to indicate the various inversions, duplications and deletions that are known to be present (Artzt, Shin & Bennett, 1982; Shin *et al.* 1983; Fox *et al.* 1985; Herrmann *et al.* 1986; Sarvetnick *et al.* 1986; Schimenti *et al.* 1987).

The partial haplotypes vary not only in length, but also in the origin of their various regions (Table 1). Some have been derived by recombination of a *t*-haplotype with a wild-type chromosome, and others by recombination between a partial *t*-haplotype and a different complete *t*-haplotype. Thus, the haplotype of origin of the various *t*-factors may be known, e.g. t^{h2}, t^{h17}, t^{w18}, or may be doubtful, e.g. t^{h49}, t^{s6}, where the point of crossing-over between the two haplotypes of origin is unknown. The haplotype t^{hr1}, although complete, is included in Table 1, because it is of composite origin, having arisen as a result of crossing-over between the two partial haplotypes t^{w18} and t^{h18} (Fox *et al.* 1985).

For tests of transmission ratio, male mice were placed with one or two females of the inbred strain TFH/H, and allowed to breed. Females were inspected for pregnancy weekly, and pregnant females were examined for births on days Monday to Friday. Young were classified for tail-length at birth and for tufted (*tf*) at 4–5 weeks. In crosses involving the haplotype t^{s6}, which carries the weak allele of *tf* present in the $t^{w12}tf$ haplotype, discrimination could be made between animals carrying the weak (t^{w12}) allele (called tf^1) and the standard mutant allele at this age, since animals of genotype tf^t/tf do not begin to lose fur until about 7 weeks. The aim was to raise 40 young from each of 5 males in each test. In a few cases, females other than TFH/H were used, but there was no indication that this affected the results.

3. Results

(1) *Double heterozygotes for a partial and a complete haplotype*

In order to test for differences among complete haplotypes in their behaviour in relation to a
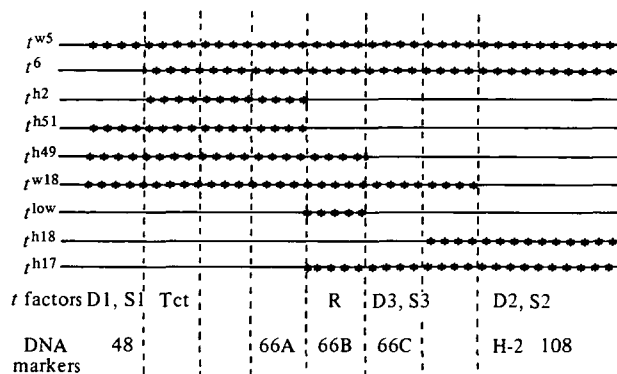

Fig. 1. Diagrammatic representation of the lengths, relative positions and ratio distortion factors (t-factors) carried by the various *t*-haplotypes used. *t*-chromatin is denoted by asterisks and wild type by a plain line. D1, S1, etc., denote *Tcd*-1, *tcs*-1, etc., and R denotes *Tcr*. The *tcs* genes are the male sterility factors believed to be identical with the distorter genes (Lyon, 1986).

responder on the homologous chromosome, double heterozygotes were bred for different complete *t*-haplotypes and a constant partial haplotype, specifically t^{lowH}. As shown in Fig. 1 and Table 1, t^{lowH} carries the central region only of the *t*-complex, derived from t^6.

The first complete haplotype tested against t^{lowH} was t^{w5} (Table 2). The transmission of the two haplotypes differed clearly from equality, with a statistically significant excess of the chromosome carrying t^{lowH}. In order to test whether other complete haplotypes behaved differently, and also to find whether the effect obtained with t^{w5} was repeatable, t^{lowH} was then crossed with t^{w1}, and with t^{hr1}. t^{w1} was chosen as being a haplotype that, according to the evolutionary tree of *t*-haplotypes proposed by Nižetić *et al.* (1984), is widely separated from t^{w5} and close to t^6. Thus, it was likely to behave differently from t^{w5}.

Table 1. *Origin of partial haplotypes and allelic form of the various regions*

Partial haplotype	Haplotype of origin	Allelic form of region			
		D1	R	D3	D2
t^6	t^{Lmb}	+	6	6	6
t^{h20}	t^6	+	6	6	6
t^{h2}	t^6	+	6	+	+
t^{lowH}	t^{h2}/t^{h17}	+	6	+	+
t^{low2H}	t^{h2}/t^{h17}	+	6	+	+
t^{low3H}	t^{h2}/t^{h17}	+	6	+	+
t^{h17}	t^6	+	6	6	6
t^{s6}	$t^{h17}/t^{w12}tf$	+	?*	?*	w12
t^{w18}	t^{w5}	w5	w5	w5	+
t^{h49}	t^{w5}/t^{lowH}	w5	?†	+	+
t^{h18}	t^6	+	+	+	6
t^{hr1}	t^{w18}/t^{h18}	w5	w5	w5	6

+ Wild type; 6, w5 and w12 denote allelic form present in t^6, t^{w5} and t^{w12} respectively.
* Could be 6 or w12. † Could be 6 or w5.

Table 2. *Transmission of* t^{lowH} *relative to complete* t-*haplotypes*

| Complete t | No. males | Offspring | | | $\chi^2_{1:1}$ |
		T	t	t^{low} (%)	
t^{w5}	5	130	69	65·3 (56·4–84·2)	18·7
t^{hr1}	5	177	107	62·3 (55·8–68·7)	17·3
t^{w1}	6	156	158	49·7 (40·4–55·3)	0·01

Test males $Tt^{lowH}tf/t^x+$.
Range of values of % t^{low} for individual males given in parentheses.

On the other hand, t^{hr1} was derived from t^{w5} (Table 1) in all but its distal region, and therefore would be expected to give results like those of t^{w5}. These expectations were fulfilled. In $Tt^{lowH}tf/t^{w1}+$ hetero-zygotes, the transmission of t^{lowH} and t^{w1} to the offspring was very close to 50%, whereas $Tt^{lowH}tf/t^{hr1}+$ heterozygotes gave a significant excess of t^{lowH}, the ratio being similar to that obtained with t^{w5}. The results from t^{w1} were significantly different from those obtained with both t^{w5} and t^{hr1} (t^{w5}, $\chi^2_1 = 12·09$; t^{hr1}, $\chi^2_1 = 9·66$). Thus, these results are consistent with differences of some kind among complete t-haplotypes.

(ii) *Double heterozygotes of different proximal and distal partial haplotypes with the responder*

Lyon (1984) used animals of genotype $t^6 + /t^{h49}tf$ as controls, to show that, when both homologues of chromosome 17 carried the responder, equal trans-mission occurred, and indeed such animals gave transmission of the two haplotypes very close to 50% (Table 3). However, when animals of genotype $t^6 + /t^{w18}tf$ were tested, the transmission was signifi-cantly different from 50%, with an excess of t^6. In order to test the repeatability of this effect, t^{h49} and t^{w18} were crossed to other haplotypes resembling t^6 in the set of distorters and responders they carried. Both t^{h49} and t^{w18} were crossed to t^{s6}, which resembles t^6 in lacking the proximal distorter, but differs in that t-chromatin begins somewhat more distally; t^{w18} was also crossed to t^{h20}, which is identical with t^6 except for a small deletion in the distal region.

In the combinations involving t^{w18}, the fertility of the males was impaired (Lyon, 1986) and hence only relatively small numbers of offspring were obtained. However, the results showed good repeatability. Males of genotype $Tt^{s6}/t^{h49}tf$ showed 50% transmis-sion of the two haplotypes, like t^6/t^{h49}. Conversely, the heterozygotes involving t^{w18}, $t^{h20}tf/t^{w18}+$ and $Tt^{s6}/t^{w18}tf$ showed abnormal transmission, with an excess of the chromosome not carrying t^{w18}. The differences between t^6/t^{h49} and t^6/t^{w18} and between Tt^{s6}/t^{h49} and Tt^{s6}/t^{w18} were both statistically signifi-cant ($\chi^2_1 = 6·43$ and $\chi^2_1 = 23·12$, respectively). Within the limits of the small numbers of young obtained, the ratios appeared similar in the three crosses involving t^{w18}. Thus, the results with t^{h49} and t^{w18} are repeatable, and it appears that these two haplotypes differ in their behaviour relative to a second haplotype carrying the

Table 3. *Transmission of* t^{w18} *and* t^{h49} *against* t-*haplotypes with responder*

| Test male type | No. males | Offspring | | | $\chi^2_{1:1}$ |
		t^x	t^{h49}	t^x (%)	
t^6/t^{h49}	5	79	81	49·4 (38·1–65·7)	
Tt^{s6}/t^{h49}	4	114	119	48·9 (41·1–55·5)	
		t^x	t^{w18}		
t^6/t^{w18}	4	23	8	74·2 (50, 80, 100, 100)	7·26
t^{h20}/t^{w18}	2	22	11	66·7 (63·2, 71·4)	3·66
Tt^{s6}/t^{w18}	5	76	22	77·5 (69·6–100)	29·8

responder. t^{w18} and t^{h49} are known to differ in length of t-chromatin present (Fox *et al.* 1985; Herrmann *et al.* 1986; Schimenti *et al.* 1987), and in that t^{w18} carries the distorter D3 (Lyon 1984, 1986). The proximal regions of both are derived from t^{w5} (Table 1). However, there is doubt concerning the responder region of t^{h49}, since it arose by recombination in a $Tt^{lowH}tf/t^{w5}+$ heterozygote, and hence the responder region could be derived either from t^{lowH} (and thus from t^6) or from t^{w5}.

(iii) *Differing behaviour of partial haplotypes of the* t^{low} *type*

The previous work on ratio distortion had suggested a difference between the two t^6-derived partial haplotypes t^{lowH} and t^{low3H}. These two haplotypes are both of the central type, carrying the responder and unknown lengths of t-chromatin on either side. When opposite a wild-type chromosome both were transmitted at far less than 50% frequency, but the transmission of t^{lowH} was somewhat higher than t^{low3H} (17% *v.* 11%). Furthermore, when placed opposite the two distorter genes D1 and D2 (carried in the haplotypes t^{h51} and t^{h18}), t^{lowH} was again transmitted at a higher frequency than t^{low3H} (86%, *v.* 62%).

In order to study further the differences among t^{low} haplotypes, more data were collected on the transmission of t^{lowH}, t^{low2H} and t^{low3H} against a wild-type chromosome. In addition, each of these haplotypes was put opposite another haplotype with a responder also derived from t^6, namely t^{h2}. This was to test the relative behaviour of two responders both of the same origin. Thirdly, for completeness, data were collected on the transmission of t^{low2H} in males of genotype $Tt^{low2H}/t^{h51}t^{h18}$.

The additional data on transmission of the t^{low}'s opposite a normal chromosome confirmed the previous data, in that t^{low3H} maintained a lower frequency than t^{lowH}. The transmission of t^{low2H} resembled that of t^{lowH}; although t^{low2H} appeared to give a somewhat higher value than t^{lowH}, the difference was not statistically significant (Table 4). The value for t^{low3H} was significantly lower than both t^{lowH} and t^{low2H}. In double heterozygotes with t^{h2}, t^{low3H} again behaved differently from t^{lowH} and t^{low2H}. Males of genotype $Tt^{lowH}tf/t^{h2}tf$ and $Tt^{low2H}tf/t^{h2}tf$ transmitted the two partial haplotypes at frequencies close to 50%. In the case of t^{low3H}, however, $Tt^{low3H}tf/t^{h2}tf$ males gave only 33% of offspring carrying t^{low3H}. The value differed significantly from 50% ($\chi^2_1 = 24 \cdot 0$), and from the ratios obtained with t^{lowH} and t^{low2H} (Table 4). There were 3 males of genotype $Tt^{low2H}tf/t^{h51}t^{h18}+$ and these produced 143 short-tailed and 9 normal-tailed offspring, giving a transmission of t^{low2H} of 94·1%. This is slightly and in fact significantly higher than the values obtained earlier for $Tt^{lowH}/t^{h51}t^{h18}$ (86·3%; $\chi^2 = 6 \cdot 07$), and considerably higher than that for $Tt^{low3H}/t^{h51}t^{h18}$ (61·9%) (Lyon, 1984). Thus the combined evidence from heterozygotes with normal, t^{h2} and $t^{h51}t^{h18}$ is consistent in indicating that t^{low3H} differs in some way from t^{lowH} and t^{low2H}. There is a rather lower possibility that t^{low2H} may in turn differ from t^{lowH}.

4. Discussion

The data concerning the transmission of t^{w5}, t^{w1} and t^{hr1} provide evidence that complete t's differ. The different complete t's were not on a constant genetic background, so it might be argued that any difference was in fact not in the t-haplotypes themselves but in the genetic background. Evidence against this is that

Table 4. *Transmission of different* t^{low} *haplotypes against wild-type or against the responder-carrying haplotype* t^{h2}

Test chrom	$+tf$				$t^{h2}tf$			
	No. males	T	$+$	t^{low} (%)	No. males	T	$+$	t^{low} (%)
t^{lowH}	4*	34	163	17·3	4*	112	123	47·7
t^{low2H}	7†	108	341	24·1	6	199	195	50·5
t^{low3H}	10‡	60	470	11·3	4*	72	144	33·3

	$+tf$		$t^{h2}tf$	
	χ^2_1	P	χ^2_1	P
t^{lowH} *v.* t^{low2H}	3·69	< 0·1	0·48	> 0·5
t^{lowH} *v.* t^{low3H}	4·50	< 0·05	9·07	< 0·01
t^{low2H} *v.* t^{low3H}	27·72	< 0·001	16·67	< 0·001

Test males $Tt^{low}/+tf$ or $Tt^{low}/t^{h2}tf$.
* One male was mated to non-standard females.
† Four males were mated to non-standard females.
‡ Three males were mated to non-standard females.

t^{hr1}, which is derived from t^{w5} in all but its distal region, behaved like t^{w5} although on a different background. Further evidence will be needed to resolve this problem completely. However, from the results so far it does appear that there is some difference among t-haplotypes in ratio distortion properties when the homologous chromosome carries a responder. The present results indicate two types of t-haplotypes and agree well with those of Hammerberg (1982). In both sets of results t^{w5} was transmitted at significantly less than 50% frequency when opposite a responder, as also was t^{hr1}, which, as mentioned above, is derived from t^{w5} except for its distal region. On the other hand t^{w1} (our work) and t^0 and t^6 (Hammerberg) showed no significant departure from 50% transmission when opposite a responder. Among this group, t^{w1} and t^6 are related in that both carry the same H-2 haplotype. t^0, although it carries the same lethal as t^6, is not otherwise thought to be closely related to the other haplotypes mentioned. The relevant differences could be in the responder region itself, or there could be cis-acting effects of the distorters, in addition to their trans-acting effect. This would imply some difference between t's either in the distorters that they carry or in the mode of action of these distorters.

The difference in behaviour of t^{h49} and t^{w18} seemed clearly repeatable, and thus a real effect, but was more difficult to interpret. One difficulty lies in the origin of t^{h49}. It arose by recombination in a doubly heterozygous animal, $Tt^{lowH}tf/t^{w5}+$. Thus it is possible that the crossover occurred in the short region of t-chromatin in the responder region which is common to t^{lowH} and t^{w5}. However, it is also possible that there was a recombination, outside this region, between t-chromatin and wild type. In the former case some at least of the responder region would be derived from t^6 rather than t^{w5}, and this might explain the difference between t^{h49} and t^{w18}. In the latter case the whole of the t-chromatin of t^{h49} would be derived from t^{w5}. The difference between t^{h49} and t^{w18} would then be ascribable to differing lengths of t-chromatin. This might result in differing numbers of copies of a relevant sequence in the responder region or, as with the complete haplotypes, might lead to differing cis-acting effects of distorters. Since the proximal ends of t^{h49} and t^{w5} are presumed identical by descent, these differing cis-acting effects would have to be due to factors in the region of t^{w18} distal to the responder region. Thus, as with the variation between complete t-haplotypes, there are again the two possible explanations of differences in the responder region, or of cis-acting effects of distorters; however, the possible location of any cis-acting agents is narrowed to a region between the responder and the distal end of t^{w18}.

In the case of the partial haplotypes of the t^{low} type, such t-chromatin as they carry is known to be derived from t^6. Thus, any differences between them are unlikely to be due to qualitative differences in DNA sequences, but rather to differing lengths of t-chromatin. The results showed clearly that t^{low3H} differed from t^{lowH} and t^{low2H}, in its transmission against wild-type chromatin, t-complex distorter genes, and the proximal partial haplotype t^{h2}, which also was derived from t^6. In addition there was a suggestion of a difference between t^{lowH} and t^{low2H} in their behaviour against wild-type, and against t-complex distorters. The three t^{low}'s were all derived by recombination in doubly heterozygous t^{h2}/t^{h17} animals, and hence, as with t^{h49}, the exact position of the crossover is unknown. Both t^{lowH} and t^{low3H} are known not to carry the t-complex DNA markers T66A or T66C (Fox *et al.* 1985), but to carry T66B, hence both carry only a short length of t-chromatin. However, Herrmann (pers. comm.), using a cosmid probe for the T66 region, has shown that t^{lowH} extends further distally than t^{low3H}. Thus, the DNA data support the genetic data in indicating a difference between these two haplotypes. As with the complete haplotypes, and with t^{h49} and t^{w18}, however, there are still differing possible genetic explanations of the effects. One possible interpretation is that the responder involves multiple copies of some sequence, and that t^{lowH} and t^{low3H} have different numbers of these copies, thus resulting in differing sensitivities of their responder regions. Another possible explanation, as before, is that of cis-acting effects on the responder. In this case the postulated cis-acting elements would need to be located close to the responder. Lyon (1984) suggested that t^{lowH} extended further distally than t^{low3H}, as has indeed been found, and that t^{lowH} might carry the distorter D3. This latter point now seems doubtful. D3 is postulated to be a trans-acting factor, which impairs fertility when homozygous. There is no evidence that t^{lowH} carries a factor for impaired fertility, and whatever the difference between t^{lowH} and t^{low3H}, the factor concerned must act in cis, to explain the effects when against t^{h2}.

Thus, to summarize, all the results, with complete t-haplotypes, with the partial haplotypes t^{h49} and t^{w18}, and with the t^{low} haplotypes, can be explained in either of two ways. There may be differences in the responder region, or there may be cis-acting effects of distorter genes. If there are differences in the responder, they may involve varying numbers of copies, since they can occur between haplotypes with a common origin. If there are cis-acting effects, they may be due to factors very close to the responder. It seems unlikely that these possible explanations will be resolved by breeding tests alone. Evidence will in addition be needed from DNA, including perhaps gene cloning. It is encouraging that differences in the DNA of t^{lowH} and t^{low3H} have already been found.

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