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Identification of a cryptic lethal mutation in the mouse t^{w73} haplotype

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Summary

t haplotypes are naturally occurring, variant forms of the *t* complex on mouse chromosome 17, characterized by the presence of four inversions with respect to wild-type. They harbour mutations causing male sterility, male transmission ratio distortion (TRD) and embryonic lethality. Mice carrying *t* haplotypes have been found throughout the world, and genetic studies of the lethal mutations have identified at least 16 complementation groups. The embryonic lethal phenotypes of many *t* haplotypes have been characterized in detail, and are thought to be the consequence of homozygosity for single gene mutations. However, the existence of additional mutations in genes that function at later stages of development would be obscured. Here we investigated the possibility of multiple mutations in *t* haplotypes by screening the t^{w73} haplotype for the presence of novel mutations. Since genetic analysis of *t* haplotype mutations is hindered by recombination suppression due to the inversions, deletion complexes covering the proximal two-thirds of the *t* complex were used to uncover the presence of any new lethal alleles. This analysis revealed a novel mutation between *D17Jcs41* and *D17Mit100*, causing mice carrying both t^{w73} and selected deletions to die at birth, prior to feeding. The finding of a new, cryptic lethal mutation in *t* haplotypes is an indication that these recombinationally isolated chromosomes, which already contain at least one lethal mutation that prevents homozygosity, may serve as sinks for the accumulation of additional recessive mutations.

1. Introduction

The *t* complex is located on the proximal portion of mouse chromosome 17 and spans approximately 20 cM, or 1·5% of the mouse genome (Silver, 1993). Mouse *t* haplotypes are variant forms of the *t* complex, characterized by four features: (1) they harbour four inversions with respect to wild-type, which act to repress recombination; (2) they contain mutations causing transmission ratio distortion (TRD) whereby males pass on their *t* haplotype chromosome greater than 90% of the time; (3) most *t* haplotypes carry recessive embryonic lethal mutations; (4) males homozyogous for non-lethal *t* haplotypes or doubly heterozygous for two complementing *t* haplotypes are either sterile or semi-sterile (Bennett, 1975; Silver, 1981).

Mice containing *t* haplotypes exist around the world and have been shown to contain different embryonic lethal mutations. Lines established from trapped animals have been placed in 16 groups based on complementation analysis (Klein *et al.*, 1984). For several decades in the mid- to late-1900s, *t* alleles provided the richest and largest collection of recessive lethal mutations in mice. Consequently, the embryonic lethal phenotypes of many *t* haplotypes have been analysed. For instance, embryos homozygous for t^{12} fail prior to blastocyst formation and implantation, whereas embryos homozygous for t^{0} implant but fail to develop normal ectodermal and endodermal layers (Bennett, 1975).

The t^{w73} haplotype was discovered in Denmark in 1969 and it was shown to contain an early-acting embryonic lethal mutation (Bennett, 1975).

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Blastocysts develop normally and begin the process of implantation but the positioning of trophectoderm and uterine decidua is aberrant and the embryos die 1–2 days later. The t^{w73} mutation was originally positioned within the *t* complex using complementation analysis with the T^{hp} deletion (Babiarz *et al*., 1982). Further genetic and YAC transgenic experiments refined the position of this mutation to one of two intervals flanking the *Igf2r* gene (Zwart *et al*., 2001).

Since lethal *t* haplotypes never exist in the homozygous state, and rarely recombine with wild-type chromosomes due to the presence of four inversions with respect to wild-type, it has been suggested that they could harbour multiple recessive embryonic lethal mutations (Babiarz *et al*., 1982). In theory, *t* haplotypes could act as sinks for other mutations given the combination of TRD and recombination suppression. The rare recombination events that do occur tend to be near the boundaries of the inversions, and allow rough mapping of the lethal loci (Silver, 1993). For example, the existence of a later-acting mutation in the t^{w73} haplotype was hypothesized based on breeding studies that failed to recover any recombinant 'partial' *t* haplotypes (Babiarz *et al*., 1982).

The existence of a second, recessive mutation in the t^{w73} haplotype was revealed when Paterniti and colleagues recovered a rare recombinant between t^{w73} and a wild-type chromosome that removed the classical early-acting t^{w73} mutation (Paterniti *et al*., 1983). Mice homozygous for this recombinant partial *t* haplotype (referred to as *cld*) developed normally to birth, but developed severe hyperchylomicronaemia (combined lipase deficiency) within 2 days post-partum as a consequence of nursing. *cld* maps very close to the recessive tufted (*tf*) mutation, on the distal side in *t* haplotypes and the proximal side in wild-type mice.

Genetic mapping and positional cloning of the *t* haplotype lethals has been hindered by the inversions that render *t* haplotypes refractory to standard genetic analyses. Indeed, none have been identified despite decades of research. Recombinational mapping is possible only in females doubly heterozygous for complementing *t* haplotypes. However, the relatively low level of polymorphism between *t* haplotypes has made this approach difficult. The use of deletions to position *t* haplotype mutations is an alternative that circumvents these difficulties. Deletions associated with the Brachyury (*T*) locus (identified by a short-tailed haploinsufficient phenotype) were used to identify and localize the T maternal effect (*Tme*), T associated sex reversal (*Tas*), and t^{w73} loci associated with *t* or + forms of the *t* complex (Babiarz *et al*., 1982; Winking & Silver, 1984; Washburn & Eicher, 1989). Recently, extensive sets of overlapping deletion complexes have been generated across the proximal two-thirds of the mouse *t* complex by the technique of embryonic stem cell irradiation (summarized in Bergstrom *et al*., 2003). These have been utilized to conduct high-resolution mapping of the head tilt (*het*) and *Tcd1* mutations (Planchart *et al*., 2000; Paffenholz *et al*., 2004). These new deletion sets represent a powerful resource for mapping or identification of other recessive *t* haplotype mutations, including the classical embryonic lethals.

Here we describe studies using these deletion complexes to identify and initially characterize a novel embryonic lethal mutation within the t^{w73} haplotype, termed t^{w73lab} (*t*, wild 73 lethality at birth). Along with the original t^{w73} mutation and the *cld* mutation, this new locus represents the third lethal mutation contained in this *t* haplotype. Since lethal-bearing *t* haplotypes are recombinationally isolated from wild-type chromosomes, there remains no barrier to accumulating additional recessive lethals, as long as heterozygotes are not compromised. These results suggest that *t* haplotypes are mutation sinks that allow faster evolution than wild-type chromosomes, and raises the possibility that other *t* haplotypes may also have additional lethal mutations whose phenotypes are masked by the consequences of that which acts earliest in development.

2. Materials and Methods

(i) *Mouse stocks*

Deletions used in this study were described in Bergstrom *et al*. (2003), and maintained at The Jackson Laboratory in the heterozygous state in mixed genetic backgrounds (including C57BL/6J and C3HeB/FeJ). t^{w73} (a generous gift from Karen Artzt) was generally maintained in repulsion to the Brachyury mutation (*T*). *T*/+ mice have short tails and *T*/*t* mice are tailless.

(ii) *Complementation matings and genotyping*

Mice carrying the deletions (induced on a 129S4/SvJae chromosome) $Nkx2.5^{df1J}$, $Nkx2.5^{df2J}$, $Nkx2.5^{df4J}$, $Nkx2.5^{df5J}$, were mated to mice carrying the t^{w73} haplotype. For $Nkx2.5^{df4J}$, t^{w73}/+ females were mated to $Nkx2.5^{df4J}$/+ males. For $Nkx2.5^{df5J}$, T/t^{w73} males were mated to $Nkx2.5^{df5J}$/+ females. For $Nkx2.5^{df2J}$, t^{w73}/+ males were mated to $Nkx2.5^{df2J}$/+ females. For $Nkx2.5^{df1J}$, t^{w73}/+ males were mated to $Nkx2.5^{df1J}$/+ females. Tail pieces were taken at weaning and DNA prepared for PCR genotyping as described (Truett *et al*., 2000; Bergstrom *et al*., 2003). Deletions and the t^{w73} chromosome were identified using a combination of *D17Mit197* and *Hba-ps4* (Schimenti, 1990), which is deleted in $Nkx2.5^{df1J}$, $Nkx2.5^{df4J}$ and $Nkx2.5^{df5J}$ (data not shown). Products

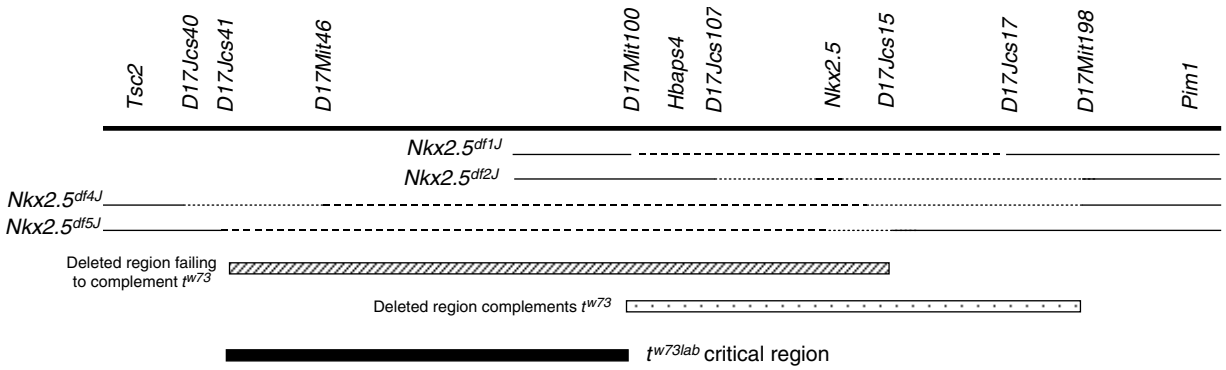

Fig. 1. Diagram to indicate the extent of the deletions and the position of the t^{w73lab} critical region. Part of chromosome 17 is depicted by a thin horizontal bar, with key markers shown above. Deletions are indicated below as horizontal lines, labelled accordingly. The dashed portions of these lines are deleted regions, dotted portions are regions in which deletion breakpoints lie, and solid portions are regions not deleted. The critical region for t^{w73lab} (thick black bar) is based upon the fact that mice carrying both t^{w73} and either $Nkx2.5^{df4J}$ or $Nkx2.5^{df5J}$ were never recovered (region indicated by striped bar) whereas mice carrying both t^{w73} and either $Nkx2.5^{df1J}$ or $Nkx2.5^{df2J}$ were recovered (dotted bar).

were resolved on 4% MetaPhor gels (Cambrex, Rockland, ME).

(iii) *Phenotype analysis*

Matings were set up between mice carrying the appropriate chromosomes, and females were checked each morning. The presence of a vaginal plug indicated embryonic day 0·5 (E0.5). Embryos from a variety of developmental stages between E8.5 and E18.5 along with newborn mice were recovered. Uterine horns were removed from pregnant females and placed in prechilled 1× phosphate-buffered saline (PBS). Decidua were removed from the uterus and embryos dissected free. The yolk sac of each embryo was removed for PCR genotyping. E8.5–E15.5 embryos were rinsed in 1× PBS and placed in Bouin's fixative overnight, rinsed in ddH_2O, and stored in 70% ethanol. E18.5 embryos were dissected free from the decidua and observed for up to 30 min, to monitor blood flow and breathing, prior to decapitation and preservation in Bouin's fixative. Litters of newborn mice were observed with their mother for up to 3 days post-partum (dpp). Notice was taken of the newborn's ability to breathe, feed and coexist with littermates. Tail pieces from live mice and mice that died spontaneously were taken for PCR analysis, prior to decapitation and preservation in Bouin's.

3. Results

Previous analysis has shown that t^{w73} contains two recessive lethal mutations: the classical t^{w73} mutation which causes lethality during implantation (Bennett, 1975) and combined lipase deficiency (*cld*) in which lethality occurs 1–2 days after birth, as a consequence of feeding (Paterniti *et al.*, 1983). In an attempt to identify novel mutations within t^{w73}, and more accurately position the previously known mutations, mice heterozygous for t^{w73} were mated to mice heterozygous for a series of deletions in the *t* complex. Litters were genotyped at weaning by PCR and it was assumed that the failure to recover mice with t^{w73} in *trans* to a deletion ('test class') offspring was an indication of the presence of a lethal mutation in t^{w73} within the extent of the deletion. In one case, when t^{w73} heterozygotes were mated to $Nkx2.5^{df4J}$ heterozygotes, no test class offspring were observed out of 44 analysed (Fig. 1, Table 1).

In order to refine the position of the lethal mutation, t^{w73} heterozygotes were mated to mice heterozygous for other overlapping deletions: $Nkx2.5^{df5J}$, $Nkx2.5^{df2J}$ and $Nkx2.5^{df1J}$ (Fig. 1). The results of the complementation analyses are summarized in Table 1. Test class animals were not recovered when t^{w73} heterozygotes were mated to $Nkx2.5^{df5J}$ heterozygotes, but were recovered when t^{w73} heterozygotes were mated to $Nkx2.5^{df1J}$ and $Nkx2.5^{df2J}$ heterozygotes. Based on the extent of the deletions, this analysis positions the lethal mutation between the markers *D17Jcs41* and *D17Mit100*. There are 57 genes in this interval, of which 34 have a reference sequence identifier (based on NCBI build 32; see http://www.ensembl.org/Mus_musculus).

To address whether the lethal mutation located in the region of *Nkx2.5* is known or novel, a series of timed matings was carried out to characterize the nature of the lethality. The results are summarized in Table 2. Test class embryos ($t^{w73}/Nkx2.5^{df4J}$) were observed at each of the midgestation E9.5, E10.5 and E11.5 time points. Given that the classic t^{w73} mutation is located approximately 20 Mb proximal to the proximal breakpoint of *Nkx2.5* (Zwart *et al.*, 2001), and causes lethality during the implantation stage, these results confirm that the lethal mutation in the *Nkx2.5* region is distinct from the classic t^{w73} mutation. Further timed matings were carried out up to E18.5 (Table 2) and visibly normal test class

Table 1. *Summary of matings between* t^{w73} *and* Nkx2.5 *deletions*

Nkx2.5^{df4J}					
Mating	*Nkx2.5^{df4J}/t^{w73}*	*Nkx2.5^{df4J}/+*	+/+	+/*t^{w73}*	Total
t^{w73}/+ × Nkx2.5^{df4J}/+	0	8	19	17	44
Nkx2.5^{df5J}					
Mating	*Nkx2.5^{df5J}/t^{w73}*	*Nkx2.5^{df5J}*/T	+/T	+/*t^{w73}*	Total
Nkx2.5^{df5J}/+ × T/t^{w73}	0	2	3	21	26
Nkx2.5^{df2J}					
Mating	*Nkx2.5^{df2J}/t^{w73}*	*Nkx2.5^{df2J}/+*	+/+	+/*t^{w73}*	Total
Nkx2.5^{df2J}/+ × t^{w73}/+	2	0	2	2	6
Nkx2.5^{df1J}					
Mating	*Nkx2.5^{df1J}/t^{w73}*	*Nkx2.5^{df1J}/+*	+/+	+/*t^{w73}*	Total
t^{w73}/+ × Nkx2.5^{df1J}/+	6	0	0	6	12

Table 2. *Summary of timed matings to characterize* $t^{w73/ab}$

Mating	dpc	*t^{w73}/Nkx2.5^{df4J}*	*t^{w73}/+*	*+/Nkx2.5^{df4J}*	+/+	Total
Nkx2.5^{df4J}/+ × t^{w73}/+	E9.5	3	2	2	2	9
Nkx2.5^{df4J}/+ × t^{w73}/+	E10.5	2	1	3	2	8
t^{w73}/+ × Nkx2.5^{df4J}/+	E11.5	1	2	2	0	5

Mating	dpc	*t^{w73}/Nkx2.5^{df4J}*	*t^{w73}/+*	*+/Nkx2.5^{df4J}*	+/+ or Nkx2.5^{df4J}	Total
t^{w73}/+ × Nkx2.5^{df4J}/+	E12.5	2	7	–	2	11
t^{w73}/+ × Nkx2.5^{df4J}/+	E18.5	1	5	0	6	12

Mating	dpc	*t^{w73}*/Nkx2.5^{df4J}	*t^{w73}*/Nkx2.5^{df4J}	T/Nkx2.5^{df4J}	T/+	Total
T/t^{w73} × Nkx2.5^{df4J}/+	1 ddp	0	4	4	5	13

Mating	dpc	*t^{w73}*/Nkx2.5^{df4J}	*t^{w73}/+*	*Nkx2.5^{df4J}/+*	+/+	Total
t^{w73}/+ × Nkx2.5^{df4J}/+	Birth	3	3	0	0	6

embryos were observed. Finally, two newborn litters were observed for a period of 8 hours after delivery. In each case, pups later genotyped as *t^{w73}/Nkx2.5^{df4J}* died prior to feeding. These pups appeared to breathe normally and turned pink, indicating heart and lungs were initially able to function normally. Given that the *cld* phenotype is characterized by death after feeding (Paterniti *et al.*, 1983; Schultz *et al.*, 2000), these results indicate that the lethal mutation is distinct from *cld*, is located between *D17Jcs41* and *D17Mit100* and is a previously unidentified, cryptic lethal mutation, now termed *t^{w73lab}* (*lab* = lethal at birth), that acts within hours of birth.

Previous data have positioned *cld* to the distal end of *t^{w73}*, closely linked to *tf* (Paterniti *et al.*, 1983). This analysis was based on the identification of a recombinant partial *t^{w73}* haplotype which lost proximal *t* haplotype chromatin including the original early-acting *t^{w73}* mutation, but retained a distal portion of the *t^{w73}* haplotype. The result reported here would suggest that this distal partial *t* haplotype must also have lost the *t^{w73lab}* mutation in order for homozygotes to survive to the stage where they manifest the post-feeding *cld* phenotype, thereby indicating that *cld* lies distal to *t^{w73lab}* in *t^{w73}*. Taking into account the inversions with respect to wild-type (Silver, 1993), this places the gene(s) responsible for *cld* proximal to *t^{w73lab}* in wild-type chromosomes but distal to the proximal breakpoint of the fourth inversion, In(17)4 (Fig. 2).

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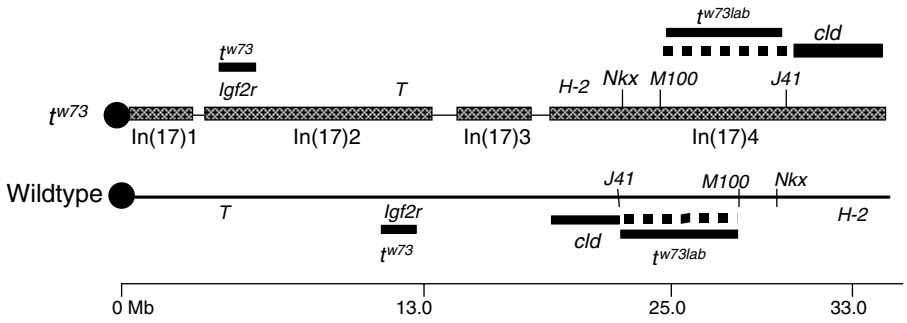

Fig. 2. Comparison of the t^{w73} haplotype with the wild-type chromosome 17. The wild-type chromosome is shown as a continuous line and the t^{w73} haplotype chromosome is shown as four linked hatched boxes indicating the four inversions with respect to wild-type (termed In(17)1, In(17)2, In(17)3 and In(17)4). Relative positions of markers and genes are indicted. *D17Mit100*, *D17Jcs41* and *Nkx2.5* have been abbreviated to *M100*, *J41* and *Nkx* respectively. This diagram is not drawn to scale, but the megabase positions of the genes and markers, as given by mouse genome assembly build *n*32, are indicated. Filled boxes indicate the positions of the three lethal mutations identified in t^{w73} (t^{w73}, *cld* and t^{w73lab}), and their relative positions on the wild-type chromosome. The filled box representing the *cld* critical region is dotted to indicate the boundary is dependent upon the exact position of the t^{w73lab} mutation.

4. Discussion

Here we have described the identification of a third lethal mutation within the t^{w73} haplotype. Mice that carry t^{w73} and either *Nkx2.5*^{df4J} or *Nkx2.5*^{df5J} develop to birth but die soon after birth, prior to feeding. Externally, test class mice appear normal, with no difference in size from normal littermates. Mice breathe and survive for hours but have never been seen to feed. Complementation analyses with a series of nested deletions, the breakpoints of which were previously mapped, position the mutation to a 1·15 Mb region between *D17Jcs41* and *D17Mit100*. The region is predicted to contain as many as 57 genes (according to Ensembl), but given that the physiological cause of death is not known it is difficult to speculate on candidate genes.

The large number of genes in the critical region means the identification of the causative mutation will probably require higher-resolution genetic localization. Given the difficulties with standard recombinational mapping in *t* haplotypes, methods to further localize t^{w73lab} would include the generation and analysis of deletions with breakpoints in the critical region or transgenic methods such as BAC rescue. Refining the phenotype may also facilitate selection of candidate genes for mutation screening.

The identification of the cryptic t^{w73lab} mutation adds further evidence to the hypothesis that *t* haplotypes are prone to accumulating mutations throughout their evolution. In addition to, or perhaps overlapping with, the mutant alleles conferring TRD, *t* haplotypes contain at least three loci causing male sterility (Lyon, 1986). It was hypothesized that recessive lethals were selectively acquired to prevent the existence of sterile *t* haplotype males, which would decrease or eliminate the productivity of a deme (Lyon, 1986). Though one recessive lethal should be sufficient for this purpose, t^{w73} contains at least three. Furthermore, we (Planchart & Schimenti, unpublished observations) and others have observed evidence for a high level of mutation or abnormal expression of *t* haplotype genes expressed during spermatogenesis (Ha *et al*., 1991; Braidotti & Barlow, 1997).

Evidence from different sources indicates that certain *t* haplotype features, particularly the inversions, contribute to the accumulation of additional mutations that do not confer a selective advantage. In *Drosophila melanogaster*, lethal mutations have been shown to be present in regions aligned with inversions on chromosome III (Albornoz & Dominguez, 1994). The Muller's ratchet hypothesis posits that the presence of inversions in small populations is responsible for the accumulation of detrimental mutations (Muller, 1964). Similarly, comparing data between two closely related species is also providing evidence of the link between inversions and the accumulation of mutations. Comparisons between human and chimp genes has shown that genes that reside on rearranged chromosomes have diverged at a faster rate than those residing on collinear chromosomes. It is suggested that this phenomenon is a result of recombination suppression on rearranged chromosomes during speciation (Navarro & Barton, 2003).

The earliest *t* complex inversion, In(17)2, marks the beginning of the evolution of the *t* haplotypes and is thought to have occurred in what became the wild-type strains around 3 million years ago (Hammer *et al*., 1989). Three subsequent inversions – in(17)1, in(17)3 and in(17)4 – occurred in the common ancestor of all *t* haplotype strains and arose over a million years ago (Silver, 1993). However, analyses of polymorphisms between *t* haplotypes suggest the common ancestor of all *t* haplotypes existed only 100 000 years ago (Hammer & Silver, 1993). The point(s) at which

the TRD and male infertility alleles arose is not known, but must have been present before the 'divergence' of the present-day *t* haplotypes, since they all share these alleles. This raises the question of when the recessive lethal mutations first occurred to counteract homozygous male sterility. The existence of many complementation groups implies that they arose separately in different populations. Nevertheless, the occurrence of additional recessive mutations should have a neutral effect, so long as such mutations are truly recessive in that the fitness of heterozygotes is unaffected. Barring any selective advantage, they would become fixed by chance in small populations and remain protected from recombination-mediated loss by the inversions.

The classical *t* lethal mutations have been refractory to cloning despite decades of investigation (Artzt *et al*., 1982*a*,*b*; Artzt, 1984; Shin *et al*., 1984). The series of newly generated deletion complexes in the proximal two-thirds of the *t* complex will be useful for the mapping of these classical mutations. As we have shown here, these deletions will also provide a powerful resource to investigate the existence of cryptic mutations in other *t* haplotypes. Such analyses would also yield information on the evolution and dispersion of *t* haplotypes around the world. Estimates suggest between 15% and 25% of mice around the world could contain a *t* haplotype and, to date, these have been placed into 16 complementation groups (Klein, 1984; Ardlie & Silver, 1996). A more recent study to isolate *t* haplotypes within wild mouse populations identified 130 *t* haploype-carrying mice in a total of 1068 mice trapped. The *t* haplotypes resemble each other more than a panel of known *t* haplotypes. It was also apparent that new *t* haplotypes were evolving by recombination events between complementing *t* haplotypes (Artzt *et al*., 1979; Dod *et al*., 2003). It is likely that additional efforts to trap and breed new *t* haplotypes would continue to expand the number.

The *t* complex is approximately 40 Mb in size, which constitutes roughly 1·5% of the mouse genome. It is predicted to contain in the order of 500 genes (according to Ensembl) or 1·8% of the current prediction of 27 000 genes in the mouse genome. Interestingly, a study to identify and map genes expressed in extraembryonic tissues of early mouse embryos found that the *t* complex region is disproportionately enriched in these genes (Ko *et al*., 1998). Given the number of *t* haplotypes known to exist around the world and that these chromosomes have had over 100 000 years to accumulate embryonic lethal mutations, continued experimentation to identify mutations within *t* haplotypes could provide a significant contribution to the elucidation of the function of genes residing in the mouse *t* complex.

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References

Albornoz, J. & Dominguez, A. (1994). Inversion polymorphism and accumulation of lethals in selected lines of *Drosophila melanogaster*. *Heredity* **73**, 92–97.

Ardlie, K. G. & Silver, L. M. (1996). Low frequency of mouse *t* haplotypes in wild populations is not explained by modifiers of meiotic drive. *Genetics* **144**, 1787–1797.

Artzt, K. (1984). Gene mapping within the *T*/*t* complex of the mouse. III. *t*-Lethal genes are arranged in three clusters on chromosome 17. *Cell* **39**, 565–572.

Artzt, K., Babiarz, B. & Bennett D. (1979). A *t*-haplotype (t^{w75}) overlapping two complementation groups. *Genetical Research* **33**, 279–285.

Artzt, K., McCormick, P. & Bennett, D. (1982*a*). Gene mapping within the *T*/*t* complex of the mouse. I. *t*-Lethal genes are nonallelic. *Cell* **28**, 463–470.

Artzt, K., Shin, H. S. & Bennett, D. (1982*b*). Gene mapping within the *T*/*t* complex of the mouse. II. Anomalous position of the H-2 complex in *t* haplotypes. *Cell* **28**, 471–476.

Babiarz, B., Garrisi, G. J. & Bennett, D. (1982). Genetic analysis of the t^{w73} haplotype of the mouse using deletion mutations: evidence for a parasitic lethal mutation. *Genetical Research* **39**, 111–120.

Bennett, D. (1975). The *T*-locus of the mouse. *Cell* **6**, 441–454.

Bergstrom, D. E., Bergstrom, R. A., Munroe, R. J., Lee, B. K., Browning, V. L., You, Y., Eicher, E. M. & Schimenti, J. C. (2003). Overlapping deletions spanning the proximal two-thirds of the mouse *t* complex. *Mammalian Genome* **14**, 817–829.

Braidotti, G. & Barlow, D. P. (1997). Identification of a male meiosis-specific gene, *Tcte2*, which is differentially spliced in species that form sterile hybrids with laboratory mice and deleted in *t* chromosomes showing meiotic drive. *Developmental Biology* **186**, 85–99.

Dod, B., Litel, C., Makoundou, P., Orth, A. & Boursot, P. (2003). Identification and characterization of *t* haplotypes in wild mice populations using molecular markers. *Genetical Research* **81**, 103–114.

Ha, H., Howard, C. A., Yeom, Y. I., Abe, K., Uehara, H., Artzt, K. & Bennett, D. (1991). Several testis-expressed genes in the mouse *t*-complex have expression differences between wild-type and *t*-mutant mice. *Developmental Genetics* **12**, 318–332.

Hammer, M. F. & Silver, L. M. (1993). Phylogenetic analysis of the alpha-globin pseudogene-4 (*Hba-ps4*) locus in the house mouse species complex reveals a stepwise evolution of *t* haplotypes. *Molecular Biology and Evolution* **10**, 971–1001.

Hammer, M. F., Schimenti, J. C. & Silver, L. M. (1989). Evolution of the mouse chromosome 17 and the origin of inversions associated with *t* haplotypes. *Proceedings of the National Academy of Sciences of the USA* **86**, 3261–3265.

Klein, J., Sipos, P. & Figueroa, F. (1984). Polymorphism of *t*-complex genes in European wild mice. *Genetical Research* **44**, 39–46.

Ko, M. S., Threat, T. A., Wang, X., Horton, J. H., Cui, Y., *et al*. (1998). Genome-wide mapping of unselected

transcripts from extraembryonic tissue of 7·5-day mouse embryos reveals enrichment in the *t*-complex and underrepresentation on the X chromosome. *Human Molecular Genetics* **7**, 1967–1978.

Lyon, M. F. (1986). Male sterility of the mouse t-complex is due to homozygosity of the distorter genes. *Cell* **44**, 357–363.

Muller, H. J. (1964). The relation of recombination to mutational advance. *Mutation Research* **106**, 2–9.

Navarro, A. & Barton, N. H. (2003). Chromosomal speciation and molecular divergence: accelerated evolution in rearranged chromosomes. *Science* **300**, 321–324.

Paffenholz, R., Bergstrom, R. A., Pasutto, F., Wabnitz, P., Munroe, R. J., Jagla, W., Heinzmann, U., Marquardt, A., Bareiss, A., Laufs, J., Russ, A., Stumm, G., Schimenti, J. C. & Bergstrom, D. E. (2004). Vestibular defects in head-tilt mice result from mutations in *Nox3*, encoding an NADPH oxidase. *Genes and Development* **18**, 486–491.

Paterniti, J. R., Jr, Brown, W. V., Ginsberg, H. N. & Artzt, K. (1983). Combined lipase deficiency (*cld*): a lethal mutation on chromosome 17 of the mouse. *Science* **221**, 167–169.

Planchart, A., You, Y. & Schimenti, J. C. (2000). Physical mapping of male fertility and meiotic drive quantitative trait loci in the mouse *t* complex using chromosome deficiencies. *Genetics* **155**, 803–812.

Schimenti, J. C. (1990). Rapid identification of mouse *t* haplotypes by PCR polymorphism (PCRP). *Mouse Genome* **87**, 108.

Schultz, C. J., Blanchette-Mackie, E. J. & Scow, R. O. (2000). Adrenal and liver in normal and *cld*/*cld* mice synthesize and secrete hepatic lipase, but the lipase is inactive in *cld*/*cld* mice. *Journal of Lipid Research* **41**, 214–225.

Shin, H. S., Bennett, D. & Artzt, K. (1984). Gene mapping within the *T*/*t* complex of the mouse. IV. The inverted MHC is intermingled with several *t*-lethal genes. *Cell* **39**, 573–578.

Silver, L. M. (1981). Genetic organization of the mouse *t* complex. *Cell* **27**, 239–240.

Silver, L. M. (1993). The peculiar journey of a selfish chromosome: mouse *t* haplotypes and meiotic drive. *Trends in Genetics* **9**, 250–254.

Truett, G. E., Heeger, P., Mynatt, R. L., Truett, A. A., Walker, J. A. & Warman, M. L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). *Biotechniques* **29**, 52–54.

Washburn, L. L. & Eicher, E. M. (1989). Normal testis determination in the mouse depends on genetic interaction of a locus on chromosome 17 and the Y chromosome. *Genetics* **123**, 173–179.

Winking, H. & Silver, L. M. (1984). Characterization of a recombinant mouse *T* haplotype that expresses a dominant lethal maternal effect. *Genetics* **108**, 1013–1020.

Zwart, R., Verhaagh, S., de Jong, J., Lyon, M. & Barlow, D. P. (2001). Genetic analysis of the organic cation transporter genes *Orct2*/*Slc22a2* and *Orct3*/*Slc22a3* reduces the critical region for the *t* haplotype mutant t^{w73} to 200 kb. *Mammalian Genome* **12**, 734–740.