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Deletion mapping of the *t* complex of chromosome 17 of the mouse

THE dominant T mutation in the t complex on chromosome 17 of the mouse causes a shortening of the tail in heterozygotes and embryonic lethality in homozygotes[1]. Recessive t alleles are defined by their interaction with T to cause tail-lessness in double heterozygotes (T/t) and are often associated with three other properties: (1) homozygous lethality at several different embryonic stages, (2) effects on male reproduction including sterility and transmission ratio distortion, and (3) crossover suppression[2,3]. Although the multiplicity of these effects suggests that several genes are involved, crossover suppression has made classical genetic analysis of the region difficult. As T/t animals are viable, the lethal factors associated with recessive t homozygosity cannot be allelic to those associated with T homozygosity. Lyon *et al.* studied exceptional crossovers and found evidence that the homozygous lethality of t alleles and their property of interacting with T to cause tail-lessness were specified at different genetic loci[4,5]. We have studied a presumed deletion, T^{Orl}, in crosses with recessive t alleles as well as with alleles at closely linked loci to map further the multiple effects associated with the t complex. We report here that factors associated with t lethality are distal to the T^{Orl} deletion and separable from those responsible for at least one kind ot t allele-associated male sterility apparently included in the deletion.

Moutier suggested in a preliminary report[6] that T^{Orl} was a deletion and extended as far as quaking, as the latter, recessive mutation was expressed in a heterozygote (*trans*) with T^{Orl}, due to hemizygosity. We have confirmed this (Fig. 1): all the short-tailed offspring (T^{Orl}/qk) from $qk/qk \times T^{Orl}/+$ crosses expressed the quaking phenotype but none of the normal-tailed mice showed the quaking phenotype. Furthermore, T^{Orl}/qk males were sterile and their vasa deferentia contained no sperm, a characteristic of male qk homozygotes.

The fused-kink locus and the tufted locus are, respectively, two and three centimorgans distal to qk (Fig. 1). Bennett has reported that the deletion of T^{Orl} does not extend to the recessive marker tf (ref. 7). We have crossed heterozygotes for T^{Orl} with heterozygotes for Fu^{ki}, which is also lethal when homozygous[8]. Of 76 offspring, 25 had tails characteristic for T^{Orl}, 19 had tails characteristic for Fu^{ki} (there is some overlap between the phenotypes, so these classifications are not absolute), 12 had a tail-less phenotype, and 20 had normal tails. The 3:1 segregation of abnormal tails to normal tails suggests that Fu^{ki}/T^{Orl} compound heterozygotes are viable and have abnormal tails. From the apparent viability of double heterozygotes we conclude that the deletion represented by T^{Orl} does not extend as far as Fu^{ki} and terminates somewhere between quaking and fused-kink.

In the other direction on chromosome 17, the results of crosses of T with T^{Orl} strongly suggest that T^{Orl} acts as an allele of T with regard to developmental lethality. Examination of embryos from $+/T^{Orl} \times +/T^{Orl}$ matings revealed that the

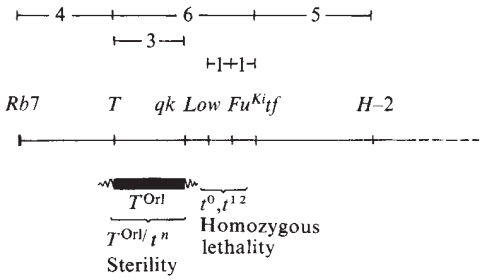
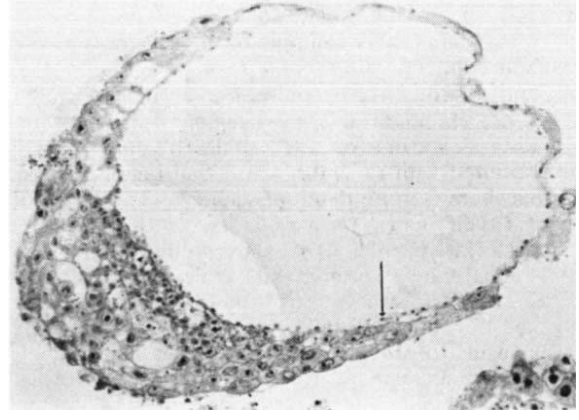


Fig. 1 The proximal portion of chromosome 17 of the mouse and the location of T^{Orl}-mapped t factors. Above, linkage distances (from refs 14–16); centre, the chromosomal map; below, the location of the T^{Orl} deletion and new assignments (bracketed) of t allele effects from this paper. qk, quaking, Fu^{ki}, fused-kink, tf, tufted.

T^{Orl}/T^{Orl} progeny are similar in many ways to lethal triploid[9] and T^{Orl} homozygous[7] embryos, although T^{Orl}/T^{Orl} embryos are more retarded in growth and seem to have a somewhat earlier lethal period than either of these (Fig. 2). These T^{Orl}/T^{Orl} homozygotes die before 10 d of development and bear no resemblance to T/T. However, in studies of embryos from $+/T^{Orl} \times +/T$ matings we found that the double heterozygote T/T^{Orl} resembled T/T lethal embryos. These data indicate that the T^{Orl} deletion extends to T.

The interaction of T^{Orl} with recessive lethal t mutations belonging to two different complementation groups was also studied. t^{12} and t^0, when homozygous, result in developmental arrest at the morulae and egg cylinder stages, respectively[2]. Crosses of $+/t^{12}$ as well as $+/t^0$ with $T^{Orl}/+$ resulted in viable tail-less offspring. These must be T^{Orl}/t^{12} and T^{Orl}/t^0 rather than highly expressed (tail-less) $T^{Orl}/+$ because (1) crosses of putative $T^{Orl}/t^{12} \times T^{Orl}/t^{12}$ mice only resulted in tail-less offspring, in very small numbers (that is, the intercross creates a balanced lethal line where only double heterozygotes survive), and (2) the tail-less males are quasi-sterile but $T^{Orl}/+$ males are not (see below). Furthermore, we have cultured 41 $3\frac{1}{2}$-day-old embryos from $+/t^{12} \times T^{Orl}$ matings for one day and found only 2% of the embryos arrested developmentally as morulae. This is significantly less than expected if the T^{Orl}/t^{12} progeny died at this stage ($\chi^2 = 8.4$, $P < 0.005$). Thus, development of t^{12}/T^{Orl} embryos cannot cease at the morula stage. As t^n lethality is recessive, hemizygosity for t^n would be expected to be lethal or nearly so. Thus, factors determining t^0 and t^{12}

Fig. 2 Histological appearance of T^{Orl}/T^{Orl} embryos at 8·5 d of development showing features similar to those seen in triploid embryos. These lack any embryonic development and consist only of an inner parietal endoderm layer (arrow) surrounded by trophoblastic giant cells. Longitudinal, 7-μm section stained with haematoxylin and eosin. Magnification ×1,500.



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embryonic lethality are likely to be located distal to the termination of the T^{Orl} deletion as depicted in Fig. 1. These results agree with those of Lyon and Mason[10] which indicate that factors associated with recessive *t* alleles map near *tf*, which is also outside the T^{Orl} deletion.

Lethal *t* alleles affecting different stages of embryonic lethality complement each other at least partially, although complementing, doubly heterozygous males are sterile. To determine if a portion of the genome responsible for male sterility is included in the chromosomal region deleted in T^{Orl}, we tested the fertility of several T^{Orl}/t^{12} and T^{Orl}/t^{0} males and found it to be markedly reduced in males of both genotypes (Table 1). Although a few offspring were born to such males, their fertility was less than 5% of normal and they are thus quasi-sterile. Thus, T^{Orl} complements t^{12} and t^{0} for lethality but not for full fertility. Lyon and Mason[10] defined one class of sterility caused by genes in the *t* complex as the interaction of *t* alleles derived from t^{6} with t^{w5} to cause sterility. This sterility effect was inseparable by recombination from *t* allele lethality. In contrast, our data show a dissociation of at least one kind of sterility from lethality in the *t* complex. In addition, it is possible that factors affecting male *t* allele transmission ratio distortion are also located in the chromosomal region deleted by T^{Orl}. T^{Orl} shows a slight segregation distortion in males, 220/180, $\chi^2 = 4.0$, $P < 0.05$.

Table 1 Fertility of T^{Orl} in combination with various alleles compared to controls

Male genotype	No. tested	No. female* weeks per male	Newborns per female per week†
$T^{Orl}/+$	3	15	5.61
T^{Orl}/t^{12}	4	15	0.08
T/t^{12}	2	18.5	5.65
T^{Orl}/t^{0}	3	14	0·28
T/t^{0}	5	27	7·85

* The indicated males were caged with mature, virgin, random-bred CD_1 females of unproven fertility which were replaced at the end of each 7-d period.

† The separated females were examined twice a week for newborns which were counted and removed.

In summary, we suggest that T^{Orl} is a deletion extending from *T* to some point distal to *qk*, but not as far as Fu^{ki}. Furthermore, the developmental lethal effects of t^{12} and t^{0} apparently map distal to the end of the deletion. Lyon and Bechtol[11] have presented evidence that a t^{6}-derived *t* allele, t^{h20}, is a deletion in the region of *tf*. As this deletion allows the expression of other *t* allele recessive lethal factors, a location near tufted is likely.

Thus, quaking is mapped well within the *t* complex. Quaking is a neurological mutant which is male sterile. Morphological studies have shown marked similarities in the distortion of sperm head shape found at the late spermatid stage in *qk/qk* (ref. 12) and in mice homozygous for a semi-lethal, male sterile *t* allele.[13] Furthermore, at least one exceptional crossover from t^{6} was neurologically abnormal[5]. Thus, the phenotypic expression of *qk* may be another example of the spectrum of effects that are induced by mutations in the *t* complex.

Lyon and Mason[10] have presented arguments for the separation of an 'abnormal ratio' region from a 'sterility' (defined only as sterility of t^{n}/t^{w5} males) region which was closely associated with an embryonic lethal region. Our data suggest that there is more than one cause of sterility and that sterility of T^{Orl}/t^{n} males is separable from t^{n}/t^{n} lethality and could be associated with *qk/qk* sterility. Furthermore, this sterility is apparently associated with male transmission ratio distortion. There is also more than one region in this part of chromosome 17 controlling embryonic development. Thus, the *t* complex seems to provide an example of a 'supergene'—a large chromosomal segment with multiple genes involved in similar, or closely related functions. The nearby *H-2* region is, of course, another example of this phenomenon and the proximity of the two supergenes has often raised the question of whether the multiple functions of these two regions could be related.

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Received 13 March; accepted 5 May 1978.

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Simian virus 40 T antigen binds to host cell chromosomes

T ANTIGEN of simian virus 40 (SV40) is the product of the *A* gene of the virus[1] and has been shown to have a role in the establishment and maintenance of transformation[2–5]. T antigen has also been shown to be required for the initiation of each round of viral DNA synthesis[6] and for induction of host DNA synthesis[7–11]. Furthermore, it has been suggested that T antigen may initiate DNA synthesis in transformed cells and thereby help maintain those cells in the transformed state[6,12–14]. The SV40 genome has three preferential binding sites for T antigen, one of which is at or near the site at which viral DNA synthesis is initiated[15]. The binding of T antigen to eukaryote DNA is less well defined but shows a preference for double-stranded over single-stranded DNA[16]. Similar findings have been reported for tumour-specific transplantation antigen[17]. SV40 nucleohistone has also been reported to bind T antigen in amounts threefold greater than does native SV40 DNA[18]. To investigate the role of T antigen in the regulation of the host cell, we have asked whether host chromosomes bind detectable amounts of T antigen; and, if so, whether the binding is restricted to specific chromosomes or to specific chromosomal loci. Although earlier studies have suggested that T antigen is associated with nuclear chromatin of infected cells[19], this study is the first to report the binding of SV40 T antigen to mammalian chromosomes.

We have used a fibroblastic cell strain derived from a male Indian deer, *Muntiacus muntjak*[20]. This cell strain can be infected by SV40 virus as shown by the nuclear accumulation of T antigen, and the induction of host DNA synthesis; neither capsid protein nor infectious progeny are produced (E.L.G., unpublished). Our choice of cell strain was dictated by the fact that the male muntjac has only seven chromosomes, which can be unequivocally identified. Karyotype analysis is easily performed in such cells and the T antigen binding sites readily identified.

Cultures were grown in Eagle's medium supplemented with foetal bovine serum 10% (Gibco), potassium penicillin G (500,000 units l^{-1}), streptomycin sulfate (100 mg l^{-1}), and mycostatin (25,000 units l^{-1}). Cultures were maintained at a density of 3×10^{3} to 8×10^{4} cells cm^{-2} by weekly passage in plastic roller bottles (Corning) or 75 cm^{2} flasks (Falcon). All experiments were performed with cells in their 20th to 35th generation. Cells seemed to be free of mycoplasma by fluorescence staining with Hoechst 33258[21] and by scanning electron

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