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Nonprogressive sperm motility is characteristic of most complete *t* haplotypes in the mouse

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SUMMARY

Epididymal sperm from male mice carrying t^{w32} (t^{12} complementation group) exhibit a peculiar nonprogressive type of motility called 'dancing'; sperm from congenic wild-type mice do not. To determine whether this effect was unique to t^{w32} or common to all *t* haplotypes, sperm from mice carrying other *t* haplotypes were examined. A male was considered to have nonprogressive sperm if more than 20% of the motile sperm had nonprogressive trajectories. The mean percentage of nonprogressive but motile sperm for 33 wild-type and Brachyury males of various genetic backgrounds was 4. All males carrying t^{w12} (t^{w1} complementation group), t^{w5} or t^{w73}, and 56% of males carrying t^{0} or t^{Lub1} had nonprogressive motile sperm. Five per cent of males carrying *t* chromatin or a deletion in the proximal (to the centromere) half of the *t* complex had nonprogressive motile sperm, but all males carrying *t* chromatin in the distal half of the *t* complex had nonprogressive motile sperm. These observations suggest that the factor or factors causing nonprogressive sperm motility may be common to all complete *t* haplotypes, and located in the distal region of the *t* complex.

1. INTRODUCTION

The *t* complex in the mouse is a region of chromatin which affects spermatogenesis and causes transmission ratio distortion, so that *t*-bearing sperm from a *t*/+ male appear to have an advantage in gaining access to the ovum, while males carrying two complementing *t* haplotypes are sterile (Bennett, 1975; Sherman & Wudl, 1977). Factors within the *t* complex are held together by suppression of recombination, but exceptional recombinants have made it possible to separate and localize some of the components making up this complex (Silver, 1981*a*). The distorted transmission of *t* complexes through the male requires factors in the proximal (to the centromere) and in the distal regions of the *t* complex (Lyon, 1981; Styrna & Klein, 1981). The cell-surface protein, *Tcp-1*, found in highest concentration in the testis, is located in the proximal region and may correspond to a proximal sperm factor (Silver, 1981*b*). As yet no sperm-specific effects have been localized to the distal region of the *t* complex.

Recently it has been shown that sperm from males heterozygous for t^{w32}, a complete *t* haplotype (i.e. containing all the factors within the *t* complex) have a

unique pattern of movement that is easily distinguished from that of wild-type sperm (Tessler, Carey & Olds-Clarke, 1981). Epididymal sperm from $t^{w32}/+$ males, regardless of their genetic background, exhibit a nonprogressive swimming trajectory, called 'dancing' to distinguish it from other types of nonprogressive motility such as hyperactivation (Yanagimachi, 1981). To determine whether dancing was a characteristic of t^{w32}, or common to all t haplotypes, epididymal sperm from males carrying a variety of t haplotypes were examined (see Fig. 1). Representative haplotypes of six of the seven known complementation groups were included, along with partial t haplotypes carrying only proximal or distal t chromatin. At least some males of every complete t haplotype examined contained nonprogressively motile sperm, as did males carrying only distal t chromatin, suggesting that the factor or factors responsible for dancing are common to all complete t haplotypes and are located in the distal region of the t complex.

2. MATERIALS AND METHODS

Inbred strains of mice carrying t^{w32} were derived as described previously (Olds-Clarke & McCabe, 1982). The designation (B6 × C3H)F_1 includes both (♀B6 × ♂C3H)F_1 and (♀C3H × ♂B6)F_1 males. All other males were generously contributed by Drs Lee Silver, Dorothea Bennett, Lynn Shevinsky, Bruce Babiarz, Craig Hammerberg and Helena Axelrod. All mice not of standard inbred strains or an F_1 cross of standard inbred strains are designated 'outbred'.

To obtain males carrying two complementing t haplotypes on a defined genetic background, B6-$t^{w32}/+$ females were crossed with C3H-$t^0/+$ males; at weaning all male pups were tested for fertility with three fertile females for at least 3 months. All fertile males produced several litters within a month of first mating; males that produced no litters in three months were considered sterile, and therefore were t^0/t^{w32}. This assumption seemed reasonable because of previous experience with this hybrid strain: of 60 (B6 × C3H)F_1 $-+/+$ or $-t^{w32}/+$ males tested, no sterile or semisterile mice were observed (Tessler *et al.* 1981; Olds-Clarke, 1983).

Spermatozoa used for observation were removed from the cauda epididymides of individual males, and incubated at 37 °C in 5% CO_2 in air in a modified Krebs–Ringer–bicarbonate medium capable of supporting fertilization *in vitro* (Olds-Clarke & Carey, 1978). The percentage of motile sperm, their average net velocity, and the percentage of vigorously motile sperm with a nonprogressive swimming trajectory were determined within 30 min of isolation from the epididymis, and again 2–5 h later.

The initial percentage of motile sperm varied from 12 to 80% among males of different strains, but there was not a significant decrease in motility during incubation *in vitro* (see also Fig. 2 in Olds-Clarke, 1983). Populations with less than 20% motile sperm were discarded. The net velocity (swimming speed) of each sperm population was estimated by the method of Ojakian & Katz (1973), slightly modified for mouse sperm (Tessler *et al.* 1981). Sperm were classified as moving nonprogressively if they were vigorously motile but remained within a haemocytometer square 200 μm on a side for more than 4 s (Olds-Clarke, 1983).

During the first 2 h *in vitro*, net velocity decreases while nonprogressiveness increases among sperm from $t^{w32}/+$ males; thereafter no further changes in these values occur (Tessler *et al.* 1981; Olds-Clarke, 1982). Since the same time course was observed in sperm populations used in this study, only those values observed at 2–5 h of incubation *in vitro* are reported here.

Table 1. *Sperm motility: males with no* t *haplotype*

Male genotype	Male strain	No. of males tested	Mean ± S.E.M. Net sperm velocity (μ/sec)	Mean ± S.E.M. Sperm moving non-progressively (%)	No. of males with > 20% sperm moving non-progressively
+/+	C3H*	9	163 ± 16	0	0/9
	C57BL/6*	5	114 ± 4	0	0/5
	(B6 × 3H)F_1*	5	173 ± 25	1 ± 1	0/5
	129/J	4	125 ± 2	0	0/4
	C57BL/10	2	106 ± 6	0	0/2
	outbred	2	150 ± 10	0	0/2
$T/+$	C3H	1	180	0	0/1
	(B6 × C3H)F_1	1	98	6	0/1
$T^{2J}/+$	C57BL/6	2	94 ± 48	50 ± 50	1/2
	(B6 × C3H)F_1	2	112 ± 6	1 ± 1	0/2

* From Olds-Clarke (1983).

3. RESULTS

Tables 1–3 present the net velocities and percentages of nonprogressive motile sperm for males with no *t* haplotype, complete *t* haplotypes, and partial *t* haplotypes, following incubation *in vitro* for 2–5 h. The net velocities of sperm populations vary significantly among strains (e.g. C3H − +/+ *v.* B6 − +/+ in Table 1), so that net velocities can only be compared among congenic males. Males of the 129 strain carrying t^{w12} had a very low mean sperm net velocity, compared to congenic wild-type males, and sperm from C3H males carrying t^{w5} had much lower net velocities than sperm from wild-type C3H males (Tables 1, 2). The net velocity of sperm from C3H males carrying t^0 varied greatly, so that the average for this genotype was somewhat reduced, but not significantly, from the congenic wild-type. The C3H males carrying t^{w18} had sperm net velocities similar to C3H wild-type males (Table 3).

Genetic background had no apparent effect on the progressiveness of sperm motility. With one exception, sperm populations from wild-type or Brachyury mice moved progressively (Table 1). Sperm from males carrying one complete *t* haplotype behaved differently, depending on the complementation group (Table 2). Most motile sperm from all males carrying t^{w32}, t^{w12} or t^{w5} were nonprogressive. Sperm populations from males carrying t^0 or $t^{Lub\,1}$ were highly variable; for example, for five C3H-$t^0/+$ males, the percentage of nonprogressive motile sperm from each male was 2, 17, 56, 75 and 92, respectively. The two males carrying t^{w73} each had about 25% of their motile sperm moving nonprogressively. All motile

Table 2. *Sperm motility: males with complete* t *haplotypes*

Male genotype	Male strain	No. of males tested	Mean ± S.E.M. Net sperm velocity (μ/sec)	Mean ± S.E.M. Sperm moving non-progressively (%)	No. of males with > 20% sperm moving non-progressively
$t^{w32}/+$	C57BL/6*	4	28 ± 3	90 ± 3	4/4
	C3H*	4	52 ± 8	93 ± 1	4/4
	(B6 × C3H)F_1*	12	49 ± 7	84 ± 14	12/12
T/t^{w32}	(B6 × C3H)F_1*	5	48 ± 7	77 ± 14	5/5
$t^{w12}tf/+$	129	2	33 ± 11	94 ± 2	2/2
Ttf/t^{w5}	C3H	2	32 ± 4	93 ± 5	2/2
$t^0/+$	CH3	5	93 ± 23	48 ± 17	3/5
	129	1	139	0	0/1
	Outbred	5	99 ± 15	33 ± 14	3/5
$t^{w73}/+$	Outbred	2	114 ± 1	25 ± 1	2/2
$t^{Lub\,1}/+$	Outbred	5	87 ± 28	38 ± 19	3/5
t^0/t^{w32}	(B6 × C3H)F_1	4	—†	100 ± 0	4/4

* From Olds-Clarke (1983).
† Not accurately measurable; < 10 μm/sec.

Table 3. *Sperm motility: males with partial* t *haplotypes*

Male genotype	Male strain	No. of males tested	Mean ± S.E.M. Net sperm velocity (μ/sec)	Mean ± S.E.M. Sperm moving non-progressively (%)	No. of males with > 20% sperm moving non-progressively
$t^{w18}/+$	C3H	2	156 ± 61	0	0/2
$t^3/+$	Outbred	7	172 ± 12	0	0/7
t^3/t^3	Outbred	2	94 ± 44	22 ± 22	1/2
t^{w95}/t^{w95}	Outbred	1	139	0	0/1
t^{55}/t^{55}	Outbred	1	179	0	0/1
$t^{h2}/+$	Outbred	5	137 ± 7	0	0/5
t^{h2}/t^{h2}	Outbred	1	111	0	0/1
$T^{0r1}/+$	Outbred	2	166 ± 5	0	0/2
$T^{hp}/+$	Outbred	1	155	0	0/1
$Tt^{h18}/+$	Outbred	4	46 ± 8	78 ± 8	4/4
$Tt^{h18}/t^{h2}tf$	Outbred	2	39 ± 10	94 ± 2	2/2
T/t^{h17}	Outbred	1	N.D.	88	1/1
$T/t^{h45}t^{h17}$	Outbred	1	N.D.	28	1/1

N.D. = not determined.

sperm from sterile males carrying both t^{w32} and t^0 were nonprogressive immediately upon release from the epididymis.

Sperm from mice carrying a viable *t* haplotype were progressively motile, as were sperm from males carring T^{hp} or T^{0r1}, deletions in the proximal region of the *t* complex (Fig. 1). With one exception, sperm from males homozygous for viable

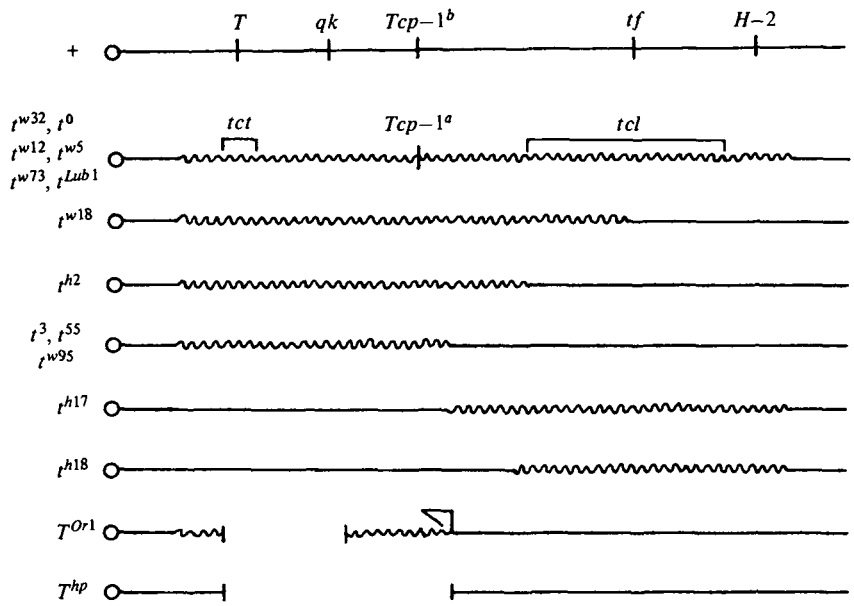

Fig. 1. The *t* complex region of chromosome 17 and the extent of various complete and partial *t* haplotypes and deletions in this region. The top line represents a wild-type (+) chromosome with known loci: *T*(T^{2J}) (Brachyury), *qk* (quaking), *Tcp-1* (*t* complex protein 1), *tf* (tufting), *H-2* (histocompatibility-2 complex). The second line represents the *t* haplotypes thought to extend over the entire length of the *t* complex, with postulated locations of genetically separable factors: *tct* (tail interaction factor) and *tcl* (lethal factors). A wavy line indicates *t* chromatin, and a straight line, normal chromatin. The *t* haplotypes indicated are representative of six complementation groups: t^{12} (t^{w32}), t^{w1} (t^{w12}), t^{w5}, t^{0}, t^{w73} and $t^{Lub\,1}$. Lines 3–9 represent partial *t* haplotypes or deletions as indicated (Lyon, 1981). T^{Or1} is a deletion which also contains a short stretch of *t* chromatin (Silver, Lukralle & Garrels, 1983). T^{hp} is a larger deletion (Erickson, Lewis & Slusser, 1978).

t haplotypes were also progressively motile. Of partial *t* haplotypes with a lethal factor, only males with t^{w18} had motile sperm populations that were entirely progressive (Table 3).

4. DISCUSSION

Sperm from mice carrying t^{w32} have a much lower mean net velocity than do sperm from congenic wild-type males (Tessler *et al.* 1981). The net velocity of a sperm is its progressive swimming speed, so that sperm with nonprogressive swimming trajectories will have a low net velocity regardless of how fast they are moving. In the case of sperm from $t^{w32}/+$ mice, direct estimates of the percentage of sperm with nonprogressive trajectories has suggested that the low net velocities are caused at least in part by a change from progressive to non-progressive motility (Olds-Clarke, 1983). The estimates of net velocities reported here for sperm from mice carrying t^{w12} or t^{w5} suggest that these *t* haplotypes cause a reduction in net velocities, while t^{w18} has no effect. Because sperm velocities vary with genetic background (Table 1 and Tessler *et al.* 1981), the net velocities of sperm from males with other *t* haplotypes have no appropriate control for comparison.

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Since virtually no wild-type males had sperm moving nonprogressively, the progressiveness of motility does not appear to vary among strains of mice, so that comparisons of mice with different genetic backgrounds are possible. A nonprogressive swimming trajectory was characteristic of sperm populations from mice carrying a *t* haplotype from three complementation groups (t^{12}, t^{w1}, or t^{w5}), and was variably present or present at lower levels in sperm populations from mice carrying t^{w73}, t^{0} or $t^{Lub\,1}$. The lack of consistency among sperm populations from males carrying t^{0}, $t^{Lub\,1}$ and t^{w73} is difficult to interpret, but reasons can be found to support the hypothesis that each of these is not a 'typical' complete *t* haplotype. While t^{w5}, t^{12} and t^{w1} haplotypes appear to have a common evolutionary origin, t^{0} and t^{w73} have independent origins (Bennett, Dunn & Artzt, 1976), and $t^{Lub\,1}$ is associated with a translocation Rb(4.17)13 Lub (Winking & Guenet, MNL 59:33, 1978, quoted in Guenet *et al.* 1980). There is evidence that t^{w73} harbours a piece of chromosome not found in other *t* haplotypes (Babiarz, Garrisi & Bennett, 1982). Another difference among the t^{0}, t^{w73}, t^{w5}, t^{12} and t^{w1} haplotypes is that they carry different H-2 haplotypes (table v in Klein *et al.* 1981). Motility of sperm from males with two complementing *t* haplotypes appears to be more severely affected than that of males carrying any single *t* haplotype.

Sperm from males heterozygous for a viable *t* haplotype exhibited progressive motility. While one of the two t^{3}/t^{3} males had sperm that were nonprogressive, the other did not, nor did three other males homozygous for viable *t* haplotypes (Table 3). Further, sperm from males carrying deletions in this region (T^{hp}, $T^{0r\,1}$) were consistently progressive. This data suggests that the factor or factors responsible for nonprogressive sperm motility are not located in the proximal portion of the *t* complex. It is interesting that elevated levels of sperm galactosyltransferase activity are also characteristic of complete *t* haplotypes, but not of viable *t* haplotypes (Shur, 1981); these two sperm characteristics may be related.

Of the males carrying just the distal portion of the *t* complex, all had significant numbers of nonprogressive motile sperm. This suggests that only the distal portion of the *t* complex is necessary for nonprogressive sperm motility, and that the factor or factors responsible for nonprogressive sperm motility may be a distal sperm factor. It is not yet clear whether nonprogressive motility is detrimental or an advantage to the sperm (Olds-Clarke, 1983), but *in vivo*, sperm from these populations are able to reach the eggs sooner than normal sperm (Tessler & Olds-Clarke, 1981). This sperm factor could be a component of the transmission ratio distortion, the sterility effect on t^{x}/t^{n} males, or both.

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