Genet. Res., Camb. (1990), **55**, *pp.* 177–181 *With 2 text-figures Printed in Great Britain*

Elevated galactosyltransferase activity on *t*-bearing sperm segregates with T/t-complex distorter loci-2

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(*Received 17 January 1990*)

Summary

Sperm bearing complete *t*-haplotypes are preferentially transmitted during fertilization from heterozygous $+/t$ males, often in excess of 95% relative to their +-bearing meiotic partner. Sperm from *t*-bearing males have an approximate two- to fourfold increase in β1,4-galactosyltransferase (GalTase) activity, a cell surface protein that mediates sperm binding to the egg zona pellucida. The elevated GalTase activity strictly correlates with the preferential transmission of *t*-sperm from $+/t$ males, since eight other enzymes show normal levels of activity on *t*-sperm. Furthermore, sperm bearing proximal partial *t*-haplotypes, which are no longer favoured during fertilization, have normal levels of GalTase activity. Nevertheless, it has been unclear whether the elevated sperm GalTase activity on *t*-sperm is due to specific loci in the distal segment of the T/t-complex, or rather, is an indirect consequence of the abnormal sperm function characteristic of $+/t$ and t^x/t^y males. In this study, it is shown that the elevated sperm GalTase activity is due specifically to factors that reside within the distal segment of the T/t complex, which also contains *Tcd-2*, the strongest of the distorter loci. Since the structural locus for GalTase is located on mouse chromosome 4, these results also show that T/t-complex alleles on chromosome 17 are regulatory in nature and affect the expression of sperm surface components critical for normal fertilization. Models are presented to explain how elevated GalTase activity could contribute to sperm transmission distortion.

1. Introduction

Mouse *t*-halotypes are transmitted during fertilization at non-Mendelian ratios due to a selective advantage of the *t*-bearing sperm relative to their +-meiotic partner (Bennett, 1975; Silver, 1985). Recent genetic studies suggest that this *t*-sperm transmission ratio distortion (TRD) is due to interactions between multiple distorter loci, *Tcd-1* to *Tcd-4*, and a responder locus, *Tcr* (Lyon, 1984, 1986). Present models hypothesize that gene products of the *Tcd* loci interact in both *cis* and *trans* configurations with the wild-type *Tcr* (Tcr^+) allele, leading to functional inactivation, or 'poisoning', of the recipient +-sperm (Seitz & Bennett, 1985; Olds-Clarke & Peitz, 1985). However, mutations in the *Tcr* allele of the *t*-chromosome (Tcr^t) render this responder gene less susceptible to the deleterious effects of *Tcd* gene products, and the resulting *t*-bearing sperm is preferentially transmitted during fertilization. Of the *Tcd* loci thus far identified, *Tcd-2* has the greatest impact on TRD, since transmission ratios return to 50% in its absence, even when other *Tcd* loci are present (Silver, 1990). While other *Tcd* loci do not contribute to TRD to the same degree as does *Tcd-2*, multiple copies of *Tcd-1* and *Tcd-4* can partially compensate for the loss of *Tcd-2*, suggesting that *Tcd* gene products share some common mode of action (Silver, 1990). Nevertheless, despite our extensive genetic knowledge of TRD, the underlying biochemical mechanisms remain unknown.

Some biochemical abnormalities have been documented previously in *t*-spermatogenic cells, such as polymorphisms in testicular proteins as revealed by 2-D SDS–PAGE analysis (Silver *et al.* 1983). However, the most extensively studied biochemical abnormality associated with these sperm is a specific increase in the activity of β1,4-galactosyltransferase (GalTase) on $+/t$ and t^x/t^y sperm populations (Shur & Bennett, 1979; Shur, 1981). The increased GalTase activity on *t*-sperm populations is interesting in light of studies showing that GalTase functions as a gamete receptor

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https://doi.org/10.1017/S0016672300025507 Published online by Cambridge University Press

during fertilization by binding to glycoprotein substrates in the egg zona pellucida (Shur & Hall, 1982*a*, *b*; Lopez *et al.* 1985; Shur & Neely, 1988). Furthermore, the elevated GalTase activity on transmission-distorting *t*-sperm populations shows both biochemical and biological specificity. GalTase is the only enzyme of nine assayed, including three glycosyltransferases, three glycosidases, and three phosphatases, that shows differences in activity between normal and *t*-bearing sperm populations (Shur & Bennett, 1979). In addition, sperm bearing recombinant partial *t*-haplotypes that have lost distal *Tcd* loci, and that are no longer preferentially transmitted, have normal levels of GalTase activity (Shur, 1981). Consequently, the elevated GalTase activity strictly correlates with the preferential transmission of *t*-sperm during fertilization. However, it is unclear whether the elevated sperm GalTase activity is due to specific gene products in the distal segment of the T/t-complex, or rather, is an indirect consequence of the abnormal behaviour characteristic of sperm bearing complete *t*-haplotypes, and therefore, unrelated to any of the individual *Tcd*/*Tcr* loci. The present study showed that the elevated sperm GalTase activity is due entirely to factors that reside within the distal segment of the T/t complex; a segment which also contains *Tcd-2*, the strongest of the distorter loci. Since the structural locus for GalTase is located on mouse chromosome 4 (Shaper *et al.* 1987), the results also showed that T/t-complex alleles on chromosome 17, segregating with or identical to *Tcd-2*, are regulatory in nature and affect the expression of sperm surface components that function during fertilization.

2. Materials and methods

(i) *Mice*

Males bearing recombinant partial *t*-haplotypes were the kind gift of Dr Lee Silver (Princeton University), and are identical to mice described in Silver & Remis (1987). In this study, two proximal partial *t*-haplotypes were used, t^{3} and t^{h2}, as well as one distal partial *t*-haplotype, t^{h18}, carried in *cis* configuration with the *T* allele. [The *T* allele has no effect on sperm transmission ratios or sperm GalTase activity (Shur & Bennett, 1979).] The relative *t*-DNA associated with these partial *t*-haplotypes is illustrated in the text (see Fig. 2), as are other *t*-haplotypes previously assayed (Shur, 1981) for sperm GalTase activity. $Tt^{h18}+/t^{h2}tf$ (no-tailed, non-tufted) × $+tf/+tf$ (normal-tailed, tufted) crosses generated $Th^{h18}+/+tf$ (short-tailed, non-tufted) and $+tf/t^{h2}tf$ (normal-tailed, tufted) progeny. $Tt^{h18}+/+tf$ × $+tf/+tf$ crosses were used to generate littermates segregating for the Tt^{h18} haplotype (short-tailed, non-tufted). $+tf/t^{3}+$ (normal-tailed, non-tufted) × $+tf/+tf$ crosses produced t^{3}-bearing males that were distinguished from their normal littermates by the tufted phenotype. All $+tf/+tf$ animals were derived from the inbred C3H background.

(ii) *Galactosyltransferase assay*

Cauda epididymal sperm from 10- to 12-week-old males were isolated as described (Shur, 1981) and washed three times by centrifugation (900 **g**, 10 min, 23 °C) in sperm incubation buffer [NaCl, 7·5 g/l; KCl, 0·4 g/l; Hepes buffer, 4·76 g/l; pH 7·2 supplemented with a protease inhibitor cocktail (Shur & Neely, 1988)]. Sperm β1,4-GalTase activity towards *N*-acetylglucosamine was measured as described (Shur, 1981). Briefly, 50 μl assay mixtures contained 200 μM uridine diphosphate [^{3}H]galactose (UDPGal) (574 dpm/pmol; Dupont), 1 mM 5′-AMP (to inhibit UDPGal degradation competitively), 30 mM *N*-acetylglucosamine (Sigma Chemical Co.), 10 mM $MnCl_2$, and approximately 0·5 × 10^{6} sperm. After the indicated incubation time at 37 °C, the reaction was terminated with Na EDTA and subjected to high-voltage borate electrophoresis to separate the radiolabelled product, *N*-acetyllactosamine, from unused UDPGal and any breakdown products. The reaction components were shown in previous studies (Shur & Bennett, 1979) to be optimal for all parameters.

All sperm GalTase activities were assayed over a 2 h incubation period, and the rate of enzyme activity was used to determine the degree of [^{3}H]galactosylated product/10^{6} cells/h. In all instances, sperm GalTase activities were assayed in parallel from littermates segregating for the *t*-haplotype of interest, and *t*-specific GalTase activities were expressed relative to their wild-type littermates.

3. Results and discussion

Sperm bearing mutant *t*-alleles are characterized by a specific increase in the activity of surface GalTase (Shur & Bennett, 1979). Males bearing recombinant proximal *t*-haplotypes, which have lost the distal *Tcd* loci, show wild-type levels of GalTase activity (Shur, 1981). To determine whether the elevated GalTase activity on *t*-sperm reflects an indirect biochemical consequence of the TRD phenotype or is the direct result of specific *T*/*t* loci, surface GalTase activity was assayed under optimal conditions on sperm bearing various recombinant partial *t*-haplotypes.

A representative sperm GalTase assay from males segregating for the t^{h18} and t^{h2} alleles from a t^{h18}/t^{h2} × $+/+$ cross is shown in Fig. 1. Activity is linear for the duration of the 2 h assay, from which the rate of enzyme activity was calculated and compared between genotypes. Surface GalTase activity on $+/t^{h18}$ sperm is more than twice that found on $+/t^{h2}$ littermate sperm, suggesting that the elevated GalTase activity characteristic of sperm bearing complete *t*-haplotypes is due to factors within the distal t^{h18} haplotype, rather than the proximal t^{h2} haplotype.

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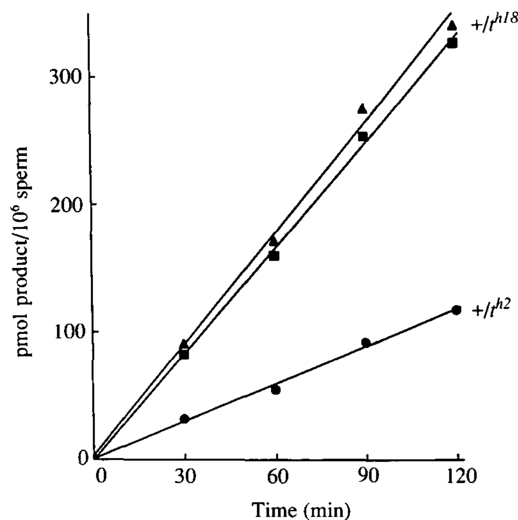

Fig. 1. Sperm surface GalTase activities from littermates resulting from a male $Tt^{h18}+/t^{h2}tf \times$ female $+tf/+tf$ cross. In this assay, $+/t^{h18}$ sperm from two males had more than twice the activity of $+/t^{h2}$ sperm.

Table 1. *GalTase activities on sperm bearing partial* t-*haplotypes*

t-haplotype	No. males/ no. litters	GalTase activity (pmol product/10^6 sperm/h)
$+/+$	23/17	$54 \cdot 5 \pm 4 \cdot 4$
$+/t^{h18}$	13/8	$111 \cdot 5 \pm 9 \cdot 0$*
$+/t^{h2}$	16/9	$52 \cdot 8 \pm 4 \cdot 5$
$+/t^{3}$	14/6	$53 \cdot 4 \pm 4 \cdot 4$

Sperm GalTase activities were assayed as described in the Materials and Methods. All sperm from *t*-bearing males were assayed relative to their $+/+$ littermate sperm, except for three litters of t^{h18}-bearing males which were assayed relative to $+/t^{h2}$, rather than $+/+$, littermate sperm (as in Fig. 1).
* $P = 0{\cdot}001$ relative to $+/+$ based on Student's *t*-test.

However, the combined t^{h2} and t^{h18} segments do not encompass all of the chromatin normally contained within a complete *t*-haplotype, since the t^{h2} recombinant still retains small proximal and central segments of wild-type chromatin (Silver & Remis, 1987). To determine whether these loci also contain GalTase regulatory elements in addition to those contained within the t^{h18} distal segment, sperm bearing the t^3 haplotype were assayed, since t^3 and t^{h18} together encompass the complete *t*-haplotype (Silver & Remis, 1987).

t^3-Bearing sperm populations contained wild-type levels of GalTase activity, showing that the elevated GalTase activity characteristic of all complete *t*-haplotypes is due entirely to factors contained within the distal t^{h18} segment. These data are presented in Table 1, along with the average sperm GalTase activities from littermates segregating for the following alleles assayed in this study: $+/t^{h18}$ and $+/t^{h2}$, $+/t^{h18}$ and $+/+$, $+/t^{h2}$ and $+/+$, $+/t^3$ and $+/+$. In all instances, multiple litters were assayed of each genotype to insure that the factors responsible for regulating sperm GalTase activity segregated with *t* alleles of interest.

The cumulative data are summarized in Fig. 2, along with the relative sperm GalTase activities associated with a number of other complete and partial *t*-haplotypes previously reported (Shur, 1981). All *t*-specific GalTase activities are normalized relative to $+/+$ littermates. The elevated GalTase activity characteristic of sperm bearing complete *t*-haplotypes, e.g. t^{12}, as well as the distal t^{h18} haplotype, is not seen on sperm bearing the proximal haplotypes t^{Orl}, t^{w82}, t^3, or t^{h2}.

The significance of these observations is threefold. First, while previous results have shown that elevated sperm GalTase activity is characteristic of all complete *t*-haplotypes, it has been unclear whether the elevated sperm GalTase activity reflects an indirect biochemical consequence of the TRD phenotype or is due to specific loci contained within the T/t-complex. While we are presently unable to define a specific genetic locus responsible for regulating sperm GalTase activity, these results do show that the distal segment of the *t*-haplotype, containing *Tcd-2*, is sufficient to account for the elevated GalTase activity found on sperm bearing complete *t*-haplotypes. Since both the *H-2* major histocompatibility complex and *Tcd-2* loci are contained within the distal segment of the T/t-complex (Silver & Remis, 1987), and may in fact be functionally related to one another, it is possible that alleles adjacent to the *H-2* complex, shown by others to affect the levels of multiple glycoprotein and glycolipid galactosyltransferase activities (Hashimoto *et al.* 1985; Furukawa *et al.* 1986; Kemp *et al.* 1987), may be identical to those responsible for regulating sperm GalTase activity.

Second, the fact that the elevated GalTase activity segregates with *Tcd-2*, rather than with any of the other defined *Tcd* loci, is interesting since *Tcd-2* has the greatest impact on the degree of TRD of the known *Tcd* loci in that recombinants lacking *Tcd-2* are transmitted at near 50% frequencies (Silver, 1990). The mode of action of *Tcd-2* is unknown, since it is defined genetically rather than molecularly, and the distal segment containing *Tcd-2* is large and likely contains many genes. It is unclear, therefore, if elevated GalTase activity is simply linked to, or a direct consequence of, *Tcd-2*. Nevertheless, since elevated GalTase activity maps to this portion of the T/t complex, which significantly affects the degree of TRD, it is possible that elevated GalTase activity is one factor that contributes to *t*-sperm TRD.

The mechanism by which elevated GalTase could contribute to TRD is presently unknown. Originally, the most obvious possibility was that the elevated GalTase activity was present on *t*-bearing, rather than +-bearing sperm, which accounted for the doubly elevated activity on t^x/t^y sperm. Presumably, the

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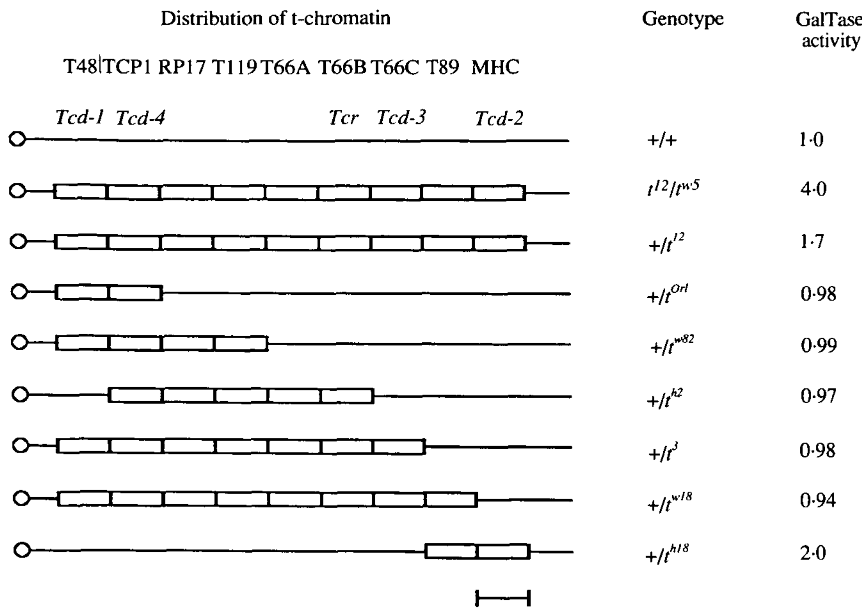

Fig. 2. Elevated sperm surface GalTase activity on *t*-bearing sperm segregates with *Tcd-2*. Portions of chromosome 17 are shown containing various *t*-haplotypes with the telomere at the extreme left. The nine subregions defined by molecular markers that constitute a complete *t*-haplotype are shown across the top of the figure and are represented by open boxes. In italics are shown the relative positions of all distorter loci identified to date (Silver & Remis, 1987). GalTase activities are shown relative to +/+ (2 significant figures). Activities from t^{h2}, t^{3}, and t^{h18}-bearing sperm are presented in this study; all others are from previous studies (Shur & Bennett, 1979; Shur, 1981). The t^{w18} haplotype referred to in this figure represents a non-transmission-distorting haplotype containing the *tf* marker, which lies within wild-type chromatin corresponding to the MHC/*Tcd-2* segment (Shur & Bennett, 1979; Silver & Remis, 1987).

elevated GalTase activity somehow contributed to the physiological superiority of the *t*-bearing sperm in +/*t* males, possibly by facilitating tighter binding to the zona pellucida. However, this notion is incompatible with the fact that t^x/t^y males are sterile. In light of recent studies showing that TRD results from dysfunctional +-sperm (Seitz & Bennett, 1985; Olds-Clarke & Peitz, 1985), rather than from superior *t*-sperm, it is more likely that the elevated GalTase activity is associated with the poisoned +-sperm. This is a particularly attractive hypothesis, since it accounts for the doubly elevated GalTase activity found on t^x/t^y sperm (Shur, 1981), all of which are thought to be poisoned as a result of homozygosity of *Tcd* loci (Lyon, 1986).

Elevated GalTase activity on +-sperm in +/*t* mice could render the +-sperm dysfunctional, or poisoned, by one of two mechanisms. The elevated GalTase activity could make the +-sperm too adhesive for glycoconjugates in the female reproductive tract, such as at the uterotubal junction, thus preventing their transport to the site of fertilization (Olds-Clarke, 1989). Alternatively, elevated GalTase activity could trigger a precocious acrosome reaction in +-sperm, rendering them unable to bind and fertilize eggs. Consistent with this, cross-linking GalTase induces the acrosome reaction (Shur, unpublished observations), raising the possibility that abnormally high levels of GalTase may increase the rate of spontaneous acrosome reactions by interacting with multivalent glycoconjugate substrates in reproductive tract fluids (Shur & Hall, 1982*a*, *b*). Furthermore, some *t*-haplotypes (Brown *et al.* 1990), especially those with the greatest TRD, though not all *t*-haplotypes (Olds-Clarke, 1989), are associated with an increased frequency of spontaneous acrosome reactions. To address these possibilities, it will be necessary to define the sperm genotype within +/*t* populations that is associated with elevated GalTase activity.

Though it is possible that elevated GalTase activity could be one factor influencing *t*-sperm TRD, elevated GalTase activity is neither necessary nor sufficient to account for TRD. As shown in this study, elevated GalTase activity segregates with the distal *Tcd-2* loci; however, multiple copies of proximal *Tcd* loci can generate some degree of TRD and sterility. Furthermore, the distal segment of the T/t complex, containing alleles that elevate sperm GalTase activity, is not in itself sufficient to produce TRD without accompanying heterozygosity at the *Tcr* locus (Lyon, 1984; 1986; Silver, 1990).

Finally, these results show that gene products contained within the T/t-complex and associated with the TRD phenotype are regulatory in nature. *In situ* hybridization studies have localized the structural locus for GalTase to mouse chromosome 4 (Shaper *et al.* 1987), while alleles within the distal segment of the T/t-complex on chromosome 17 regulate the degree of surface GalTase expression. The mechanism of this *trans* chromosomal regulation is presently unknown, but interestingly, GalTase activity becomes elevated postmeiotically during *t*-spermatogenesis at the round

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spermatid stage (Scully & Shur, 1988), coincident with the expression of two testicular proteins, Tcp-3 and Tcp-7, whose structural loci reside within the distal segment of the T/t complex (Silver *et al.* 1987). Whether in fact Tcp-3 and/or Tcp-7 are responsible for regulating sperm GalTase expression is unknown. Nevertheless, these results demonstrate that gene products within the T/t-complex are able to regulate the expression of developmentally important genes during spermatogenesis and development.

The authors are grateful to Dr Lee Silver for supplying breeding stocks of *t*-bearing mice used in this study. This work was supported by research grants from the National Institutes of Health (HD23479, HD22590) to B.D.S. and by a NRSA postdoctoral fellowship (F32 HD06590) to N.F.S.

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