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Molecular analysis of mouse spermatogenesis: isolation of the *t*-complex polypeptide-1 gene and related sequences

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PRESENT VIEW OF THE *t*-COMPLEX

The mouse *t*-complex is a region of chromosome 17, found in wild mouse populations, which is grossly rearranged when compared to those of normal laboratory strains. So far, two large, independent inversions have been demonstrated. The distal inversion includes the entire Major Histocompatibility Complex (MHC) (Artzt, Shin & Bennett, 1982; Shin *et al.* 1983; Pla & Condamine, 1984) and the recently discovered proximal inversion (Herrmann *et al.* 1986) also contains many genes, including the *t*-complex polypeptide-1 gene (*Tcp-1*) discussed in this article. Using *in situ* hybridization, the MHC (Lader *et al.* 1985) and *Tcp-1* (Lyon *et al.* 1986) genes have been positioned on chromosome 17 and the *t*-complex would appear to occupy Giemsa bands 17B and 17A3, representing roughly 15 % of the chromosome. Presumably, in addition to those already mapped, many hundreds of genes are located in this region.

The *t*-complex has been extensively studied because independent *t*-haplotype chromosomes carry recessive embryonic lethal genes that result in embryonic death at different stages of development (Bennett, 1975). The different lethal genes map throughout the *t*-complex (Artzt, McCormick & Bennett, 1982; Artzt, 1984) and it is suggested that they are related in function in the sense that they are sequentially required during early embryonic development (Shin, McCormick, Artzt & Bennett, 1983). This genetic model has not been proven, since none of the lethal genes, and there are at least sixteen independent recessive lethals carried by various different *t*-chromosomes isolated from wild mouse populations around the world (Klein, Sipos & Figueroa, 1984), have been molecularly cloned. In one instance (t^{w73}), a recessive lethal has been identified as combined lipase deficiency

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(*cld*) (Paterniti, Brown, Ginsberg & Artzt, 1983), but this haplotype carries another independent, earlier acting, embryonic recessive lethal in an unknown gene (Artzt, 1984). We feel it is unlikely that all these lethal genes, distributed over 15 centimorgans of DNA, are related to one another and concur with the idea that the lethal genes have been randomly accumulated during the evolution of *t*-chromosomes to counteract the otherwise deleterious effects of the male sterility genes that are also carried by these chromosomes (Lewontin, 1968; Lyon, 1986). Indeed, it seems the genes that cause male transmission ratio distortion and sterility, and they are probably the same (Lyon, 1986), are *prima facie* those responsible for the existence of *t*-haplotypes. We discuss transmission ratio distortion in the following section and direct readers to a lengthy and excellent review by Silver (1985) for more detail of the history, genetics and embryological properties of mouse *t*-haplotypes and also to the recent paper by Lyon (1986) on the sterility factors for the view of the *t*-haplotypes as a lethal trap.

TRANSMISSION RATIO DISTORTION (TRD)

TRD occurs in heterozygous males carrying any complete *t*-chromosome (t^x) and a wild-type chromosome 17. When such males ($t^x/+$) are mated with wild-type females, up to 99% of the eggs are fertilized by the sperm bearing the *t*-chromosome. The $t^x/+$ males produce roughly equal quantities of t^x and wild-type sperm as shown by probing their sperm DNA with polymorphic microclone probes and measuring the quantity of t and wild-type DNA by densitometry (Silver & Olds-Clarke, 1984). Recent experiments with $t^x/+ \rightarrow +/+$ chimaeras (Seitz & Bennett, 1985) and mixed inseminations of females with $t^x/+$ and $+/+$ sperm (Olds-Clarke & Peitz, 1986) show that the wild-type sperm that differentiate in a $t^x/+$ testis are dysfunctional. Presumably the damage to the developing wild-type sperm happens during the haploid phase of spermatogenesis after the 2nd reduction division of meiosis. That it could occur at all is the consequence of the fact that cytokinesis is incomplete in spermatogenesis and a syncytium of developing cells is formed; haploid spermatids are cojoined and presumably share molecules although this important point remains to be proven. Thus it would seem that *t*-spermatids 'poison' their wild-type partners. The genetics of TRD is complex (Lyon, 1984). Briefly, there are four genetic factors distributed in both inversions, hence the evolutionary requirement for the recombination suppression to keep the factors linked. Three factors, *t*-complex distorter-1, -2 and -3 (*Tcd*s) act additively in cis or trans upon a single factor *t*-complex responder (*Tcr*) to give complete TRD. The chromosome carrying the *Tcr* is transmitted at a high ratio so long as the stock is heterozygous for *Tcr*. In natural populations this will be the *t*-chromosome but in the laboratory artificial chromosomes can be constructed (the t^{low} series) which just carry the *Tcr*, and such chromosomes distort when the *Tcd*s are provided in trans. A summary diagram of Lyon's TRD model is in fig. 2 of Lyon (1986).

t-COMPLEX POLYPEPTIDES

The molecular basis of TRD and sterility is unknown. However, Silver, Uman, Danska & Garrels (1983) have described a group of polypeptides, five of which are certainly encoded by genes within the *t*-complex and are expressed in mouse testis. Some of these *t*-complex polypeptides, such as TCP-1 (Silver, 1981, 1985; Silver *et al.* 1983), may be correlated with transmission ratio distortion factors; others, TCP-3 and TCP-7, may be correlated with the distal distortion factor, *Tcd-2* (Silver *et al.* 1983). The molecular cloning of these genes might assist in assigning particular genes to particular phenotypes and we have made a start by cloning and sequencing the *Tcp-1* gene (Willison, Dudley & Potter, 1986). TCP-1 is the best studied of the set of *t*-complex polypeptides and is suggested to be nonglycosylated external membrane protein (Silver & White, 1982). It exists in two electrophoretic forms, TCP-1B and TCP-1A. Analysis so far shows that TCP-1A is encoded by all complete *t*-haplotype chromosomes and all inbred strains tested encode TCP-1B. TCP-1A has the same molecular weight as TCP-1B but a more acidic isoelectric point (Silver, Artzt & Bennett, 1979). Attempts to raise antisera to TCP-1 have been unsuccessful and no biochemical purification of the protein has been achieved.

ISOLATION OF THE *Tcp-1* GENE

Because some of the *t*-complex polypeptides had been shown to be abundant in the testes and because the various aspects of the *t* mutant phenotype described above manifest themselves in the testes, we began a molecular analysis of spermatogenesis. We isolated and started to characterize cDNAs corresponding to mRNAs that are differentially expressed in the cell types comprising the seminiferous epithelium of the testis. We described a set of cDNA clones that derives from poly(A)$^+$ RNAs that are accumulated in meiotic and postmeiotic cells (Dudley, Potter, Lyon & Willison, 1984). Two lines of evidence showed that one of the cDNA clones, pB1.4, represented a partial copy of the *Tcp-1*^{b} mRNA (Willison *et al.* 1986). First, pB1.4 detected restriction fragment length polymorphisms when comparing *t*-haplotype and wild-type DNA and the resultant genetic mapping showed complete concordance with the TCP-1A polypeptide mapping of Silver *et al.* (1983). Second, mRNA selection experiments showed that mRNA could be isolated from testis that would direct the synthesis of a 57×10^3 (M_r) polypeptide *in vitro*. When the testis RNA was isolated from various *t*-haplotypes, a more acidic form of the 57×10^3 (M_r) polypeptide could be detected on two-dimensional O'Farrell gels consistent with the known properties of TCP-1A and TCP-1B.

The wild-type (*Tcp-1*^{b}) and *t*-haplotype (*Tcp-1*^{a}) genes show multiple coding sequence base changes resulting in at least six amino acid substitutions. Presently we have only sequenced portions of two wild-type alleles, CBA/Ca and C3H. We are sequencing other wild-type alleles, such as C57BL/6, in order to discover which amino acid substitutions are *t*-specific.There are sequences in the genome

that are related to *Tcp-1* by hybridization (Fig. 1). One, *Tcp-1x*, is linked to *Tcp-1* and the other, *Tcp-1y*, has not yet been mapped.

The function of *Tcp-1* is unknown and the DNA sequence has no substantial homologies to known polypeptides or DNA sequences.

EXPRESSION OF THE *Tcp-1* GENE

Tcp-1 is a haploid expressed gene (Dudley *et al.* 1984) and is most abundantly expressed in the germ cells of adult testis but it is expressed at much lower levels in most other tissues and cell types. We have recently discovered *Tcp-1* cross-hybridizing transcripts in 8·5- and 9·5-day embryos (Dudley, Watson & Willison, unpublished results) and are sequencing cDNA copies of them to see if they represent alternative splicing products of the *Tcp-1* gene or if they are products of the *Tcp-1x* or *Tcp-1y* sequences.

FUTURE STUDIES WITH THE *Tcp-1* GENE

It might appear as though the *t*-complex is 'solved' in the sense that we broadly understand its structure and why it exists. It is not a developmental locus of the sort one finds in *Drosophila melanogaster* and we suspect that when the *t*-complex recessive lethals are identified they will turn out to be in all sorts of different types of gene. In our opinion, the outstanding biological problem is the molecular basis of TRD and sterility.

The TCPs are the best candidates for the molecules that may be responsible for TRD, but it should be stressed that these ideas are based only upon correlations of genetic and polypeptide mapping. TCP-1A, for example, would appear not to be a Tcd factor according to Lyon's (1984) present model since *Tcd-1*, *-2* and *-3* do not co-map with TCP-1A. However, Silver (unpublished results but see Silver, 1985) suggests that TCP-1A may be a distorter, *Tcd-4*, based upon experiments with t^{wLub2} and t^{h45}.

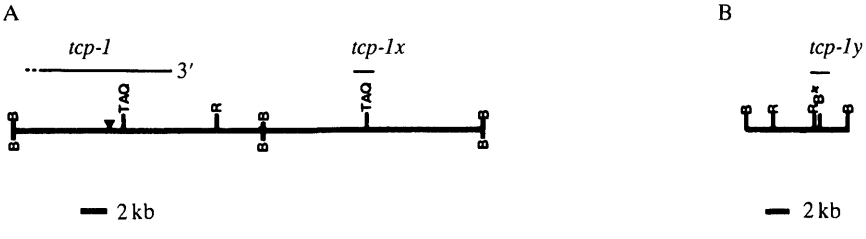

Fig. 1. Restriction maps of the *Tcp-1* genes. (A) Composite map of the *Tcp-1* and *Tcp-1x* genes in wild-type and *t*-haplotype DNA. The position and orientation of the $Tcp\text{-}1^{b}$ and $Tcp\text{-}1^{a}$ gene are shown and the additional Taq-1 site in the $Tcp\text{-}1^{a}$ coding sequence and *Tcp-1x* sequence is highlighted. (B) BamHI/EcoRI map of the *Tcp-1y* gene/pseudogene derived from Southern analysis of genomic DNA. The asterisked BamHI site is present in strain 129/Sv but absent from C57BL/6 and C3H. There is no chromosomal assignment for this gene/pseudogene.

Regardless of the possible relationship between TCP-1 and *Tcd*s, the fact that the *Tcp-1* gene is located in the best mapped and defined region of any mouse chromosome is useful. There are duplications of the *Tcp-1* gene cluster (t^{h45} and Tt^{Orl}) and deletions on wild-type (T^{hp}) and *t*-haplotype (t^{wLub2}) chromosomes. These deletion mutants raise the possibility of performing marker rescue experiments if transgenic mouse strains can be constructed that have active *Tcp-1* genes on chromosomes other than 17.

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