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A UK-centric history of studies on the mouse *t*-complex

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Early work on discovery of the *t*-complex and its peculiar properties

Early work on the mouse *t*-complex was well reviewed by Grüneberg (1943), then reader in genetics at University College, London. The Brachyury or T-locus was discovered by Dobrovolskaia-Zavadskaia in Paris in 1927. Animals heterozygous for *T, T*/+, have a short tail and homozygotes, *T/T,* die prenatally. In 1932, however, Dobrovolskaia-Zavadskaia reported on three lines of tailless mice which had been obtained by crossing *T*/+ animals with mice derived from the wild (Dobrovolskaia-Zavadskaia, 1927). Each of the tailless lines bred true, and in 1935 it was suggested that each line constituted a balanced lethal system in which *T/t* animals were tailless and both *T/T* and *t/t* died prenatally. Later, two of the three lines were proved to carry the same recessive lethal, called t^0, and the other a different lethal, called t^1. Genetically t^0/t^1 animals were viable and normal-tailed. However, t^0/t^1 males were sterile. In early breeding studies, T/t^0 and T/t^1 females transmitted *T* and *t* normally in equal ratios to their offspring. However, both T/t^0 and T/t^1 males gave a large excess of young carrying t^0 or t^1 over the expected Mendelian value of 50%. The abnormal ratios were not due to misclassification of *T* and *t,* nor to reduced viability of *T*-carrying young. Thus, *T/t* males transmit the *t*-allele with an abnormally high ratio. Reviewing the work at this stage, Grüneberg made the prescient suggestion that the properties of *t*-alleles (as they were then known) could be due to inversions or to other structural changes. Further properties of the *t*-complex were discovered soon after. Dunn and Caspari found crossover-suppression between *T* and the nearby locus of *Fu* in mice heterozygous for *t* (Dunn and Caspari, 1945), and in balanced lethal *T/t* stocks Dunn found repeat "mutations" to other *t*s (Dunn and Gluecksohn-Schoenheimer, 1950), either lacking lethality or with a new complementing lethal. *t*-alleles were found commonly in wild mouse populations. Most naturally occurring *t*s were recessive lethals but some were viable when homozygous; however, homozygous males were sterile. Dunn and his co-workers suggested that *t*s were maintained in the wild by their high transmission from heterozygous males.

Elucidation of genetics of transmission ratio distortion and male sterility

The involvement of British geneticists in studies of the *t*-complex began when Carter and Phillips found a *t*, termed t^6, in a laboratory

Abbreviations used in this paper: T, Brachyury; t^o, t^1, etc, *t*-haplotype chromosomes; *Fu*, fused; *tf*, tufted; A-factor, abnormal ratio (later named responder/Tcr[1]); *Tcd*, *t*-complex distorter; MHC, major histocompatibility complex; RFLV, restriction fragment length variant; Mbp, megabase pairs; *Tcp-1*, mouse *t*-complex polypeptide-1 gene; TCP-1, human t-complex polypeptide-1 gene or protein.

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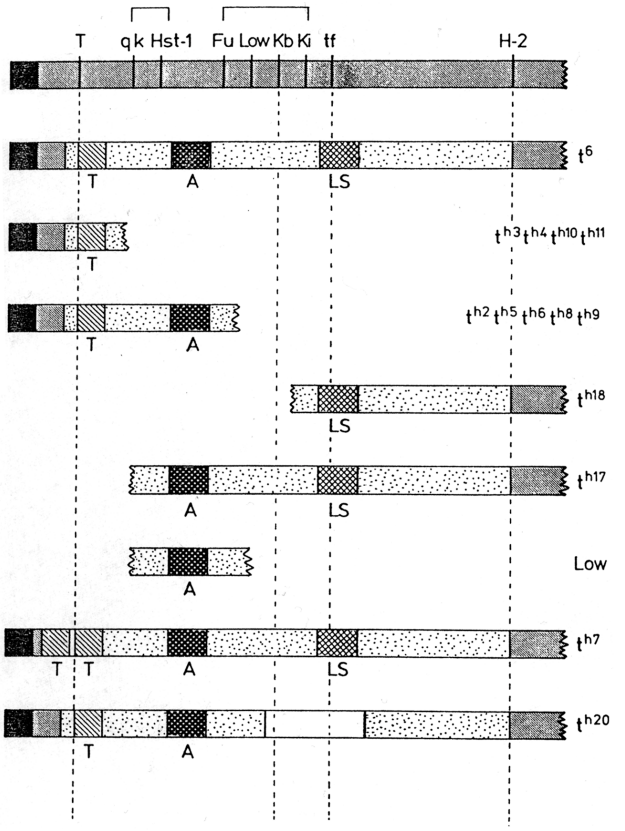

Fig. 1. Lyon and Mason's interpretation of t-complex ratio factors. *The interpretation is based on the haplotype t^6 and partial haplotypes derived from it. t-chromatin is shown by a dotted area. The hatched areas marked T, A and LS denote the approximate positions of the factors concerned in T-interaction, abnormal ratio, and lethality-sterility, respectively (Lyon and Mason, 1977).*

stock of mice (Carter and Phillips, 1950). Soon after, Lyon (1956) found a spontaneous recessive mutation, tufted, *tf*, which affected the appearance of the coat and mapped near the Brachyury locus. It thus provided a visible marker of crossing-over in the neighbourhood of *T*. Lyon and Phillips used the tufted mutant to show that the crossover-suppression due to t^6 extended at least to the locus of *tf* (Lyon and Phillips, 1959). They were able to show for the first time that "mutation" of *t*-alleles was due to crossing-over in the suppressed region, since they obtained a "mutant" lacking the lethality of t^6 and carrying the *tf* marker. Lyon and Meredith carried this work further by studying a number of "mutant" or recombinant haplotypes derived from t^6 in a stock of *Ttf*/t^6 + x *Ttf*/t^6 + (Lyon and Meredith, 1964). There were two main types: those which retained the property of interaction with *T* to give taillessness, but had lost the lethality and were carrying the *tf* marker; and the complementary type which had lost the *T*-interaction property but retained the lethality and did not carry *tf*. The non-lethal type could be further subdivided into those with a normal transmission ratio and those with an abnormally low (less than 50%) ratio. Until this time the view had been that the various recessive lethals exhibited by *t*-haplotypes were allelic with Brachyury. This work showed for the first time that *t*-haplotypes occupied a length of chromatin and that the various properties mapped to different points. The *T*-interaction factor mapped close to Brachyury, whereas the lethal factor of t^6 mapped close to *tf*. Since all the mutant haplotypes had lost the high transmission ratio in males, this suggested that this ratio depended on factors at more than one point.

Lyon and Mason obtained further insight into transmission ratio and male sterility by making various combinations, in cis and trans, of the recombinant alleles (Lyon and Mason, 1977). They postulated a factor, which they called the A-factor (for abnormal ratio), which lay between the *T*-interaction and lethal factors, and which was required for an abnormal ratio. When present alone, the A-factor resulted in an abnormally low ratio. Combinations of haplotypes carrying the A-factor with various other haplotypes could lead to an increased ratio. Lyon and Mason also postulated a factor for male sterility carried in haplotypes which also carried the lethal factor (Fig. 1). Lyon later obtained further recombinant haplotypes from t^6, including some central haplotypes, having the putative A-factor only, and also studied other recombinant haplotypes derived from them (Lyon, 1984). Studies of numerous combinations of these haplotypes led to further insights into ratio distortion and male fertility. It was suggested that ratio distortion was produced by the action of distorter genes on a responder. The responder in the central part of the *t*-complex corresponded to the previous A-factor, and when present alone was transmitted at a low frequency. At least three distorter genes, *Tcd-1*, *-2* and *–3*, in the proximal, distal and central regions respectively, acted additively, either in cis or in trans, to raise the transmission ratio of the responder. Heterozygosity for the responder was necessary for an abnormal ratio, and if the distorters were present without the *t*-form of the responder *Tcr*^{t}, the transmission ratio was normal. When the responder was heterozygous and all three distorters were heterozygously present, in either cis or trans configuration, whichever chromosome carried the *Tcr*^{t} was transmitted at high ratio. Further evidence suggested that male sterility was due to homozygosity of the distorter genes (Lyon, 1986). Sterility did not require the presence of the *Tcr*^{t}. Indeed, in the absence of the responder, distorters could impair fertility even when only heterozygous rather than homozygous (Lyon, 1987). The interpretation made was that the distorters acted in concert to produce a harmful effect on sperm carrying the wild-type allele of the responder, and that sperm carrying the *t*-allele of the responder *Tcr*^{t} were relatively resistant to this harmful action. When the distorters were homozygous, the harmful effect was so severe that the resistance of the *Tcr*^{t} was overcome and sperm carrying it too were harmed. Thus, when the distorters and responder were heterozygous, sperm with *Tcr*^{t} preferentially took part in fertilization, giving high transmission of *Tcr*^{t}. When the distorters were homozygous, few or no sperm could fertilise and sterility resulted.

This interpretation fits with the work of others on the reproductive physiology of the effects of the *t*-complex. Seitz and Bennett (Seitz and Bennett, 1985) and Olds-Clarke and Peitz (Olds-Clarke and Peitz, 1985) showed by different methods that the high transmission ratio was not due to superiority of *t*-bearing sperm but to a deleterious effect on wild-type meiotic partners of *t*-sperm. The numbers of *t*- and wild-type sperm are equal at the time of mating and at deposition of sperm in the uterus (Silver and Olds-Clarke, 1984). Thus, the abnormality lies in the function rather than in the formation of sperm bearing the *t*-chromosome.

Implicit in Lyon's explanation of the effects on transmission ratio and sterility was the concept that different partial *t*-haplotypes had different lengths and positions of *t*-chromatin. Early work on the *t*-

complex was hampered by lack of sufficient genetic markers to test these matters. This changed with the advent of molecular cloning and the isolation of DNA markers. Apart from those in the MHC region which is located within the *t*-complex, some of the first available markers of the *t*-complex region were developed by microdissection of the chromosome (Röhme *et al.*, 1984). Study of RFLVs between *t* and wild-type for some of these markers confirmed Lyon's postulates of the lengths of the various partial haplotypes (Fox *et al.*, 1985).

Physical structure of *t*-haplotypes

The properties of *t*-haplotypes were studied for two decades at the Sloan-Kettering Memorial Cancer Hospital in New York, USA, in the groups of Bennett and Artzt, themselves scientific progeny of L.C. Dunn. Silver and Artzt (1981) showed, by use of a *t*-haplotype with a mutant *tf* marker (t^{w12} *tf*), that crossing-over occurs freely in homozygous *t*-chromatin, indicating chromosome rearrangements in *t*-complex chromosomes as compared to wild-type chromosome 17's of laboratory mice. These US investigators first defined the distal inversion in the MHC complex (Shin *et al.*, 1983), and a UK group identified a second, proximal inversion (Herrmann *et al.*, 1986). Later, Silver and colleagues characterised two other inversions (Hammer *et al.*, 1989); thus four inversions comprise the full extent of the *t*-complex, two large and two small (early work reviewed in Silver, 1985) (Fig. 2).

Using DNA probes from the two large proximal and distal inversions, our groups showed, by *in situ* hybridisation, the location of the *t*-complex with respect to the Giemsa banding pattern of chromosome 17 (Lyon *et al.*, 1986) and visualised the inversions on *t*-chromosomes marked by translocations (Lyon *et al.*, 1988). Thus, by the end of the 1980's it was known that the *t*-complex occupied around 30Mbps of chromosome 17, covering several chromosomal bands that had undergone various independent rearrangement events during its evolution. Furthermore, it had become clear that all the isolates of *t*-chromosomes, from wild mouse populations all over Europe, Asia and the continental US, and including both subspecies *M. m. domesticus* and *M.m. musculus*, had such similar physical structures that they all derived from a single founding population.

Rogers, at the MRC Laboratory of Molecular Biology, Cambridge, studied extensively the MHC complex of *t*-haplotypes using many DNA markers which became available in the early 1980's (Rogers *et al.,* 1985). This DNA-sequence-based approach also suggested that *t*-haplotypes were much more similar to one another than had been previously surmised from serological analysis of the MHC. This work addresses the important but still unsolved question of whether particular gene families may evolve at different rates depending upon their chromosomal positions.

Relation of properties of *t*-complex to each other

For many years the nature of the embryonic lethality phenotypes of *t*-haplotypes divided investigators into those who proposed that the *t*-complex encoded a large developmental control region, and others (Silver, 1985) including ourselves, who thought the accumulation of different lethal genes was a by-product of transmission ratio distortion. Briefly, the argument supporting this second view runs as follows: *t*-haplotypes are maintained in

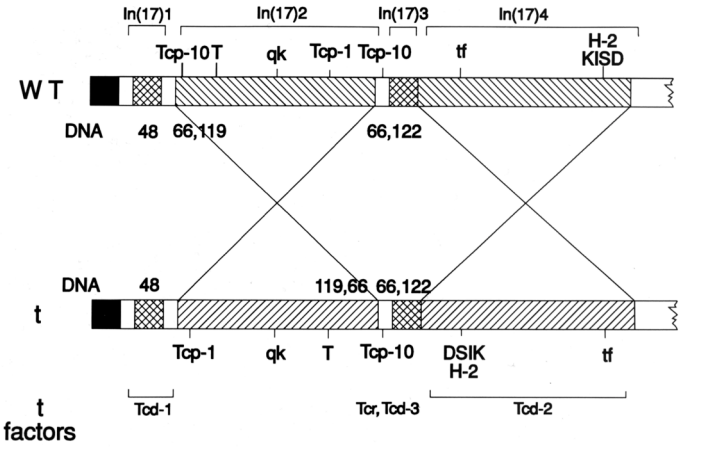

Fig. 2. Chromosome structure of the *t*-complex. *The four inversions are shown as hatched boxes, with the wild-type orientation above and t-complex below, and the centromere (black) to the left. The relative positions of some known genes and DNA markers are indicated, and the approximate positions of the t-complex ratio factors, Tcd-1 to –3 and Tcr. Tcr is thought to lie between In(17)2 and In(17)3; Tcd-1 is to the left of In(17)2; Tcd-3 is in In(17)3 or In(17)4; and Tcd-2 in In(17)4; (Lyon, 1992).*

populations of wild-mice by high transmission from heterozygous males. This requires at least four factors distributed throughout the 30Mbps of the *t*-complex and thus the inversions confer a selective advantage in holding these factors together, since recombination is suppressed. However, the same factors which give high transmission lead to male sterility in homozygotes; therefore, lethals which have arisen at random during *t*-chromosome evolution are strongly selected for, since prenatal lethality of homozygotes is less deleterious than presence of live sterile males in breeding populations. Now 16 different lethals are known which are distributed throughout the *t*-complex. Although even to this day none has been molecularly cloned and characterised, it would seem most unlikely that they could share any common functions or regulatory interactions. Moreover, detailed analysis of other mouse chromosome regions indicates that finding 16 recessive lethal loci within a 30Mbp stretch is well within the bounds of expectation.

Structure of partial *t*- haplotypes

The availability of many DNA markers, combined with the understanding of the four inversions, enabled the elucidation of the structure of partial *t*-haplotypes and their mode of origin. The majority are formed by homologous recombination in the small regions between the inversions, particularly between the proximal inversion In(17)2 and the middle inversion In(17)3. However, some are formed by crossing-over within an inversion. Owing to the disparate orientation of the DNA between *t* and wild-type this results in duplication and/or deletion of small stretches of chromatin (reviewed in Silver, 1985; Artzt *et al.*, 1991). Groups in the UK have particularly studied the proximal partial haplotypes of the t^9 group (Bucan *et al.*, 1987; Crossley and Little, 1991; Barclay *et al.*, 1996). These haplotypes, comprising t^4, t^9, t^{w18} and t^{ks1}, have arisen by crossing-over between a complete *t*-haplotype and wild-type in the distal inversion. All members of the group are recessive prenatal lethals which impede gastrulation and do not complement

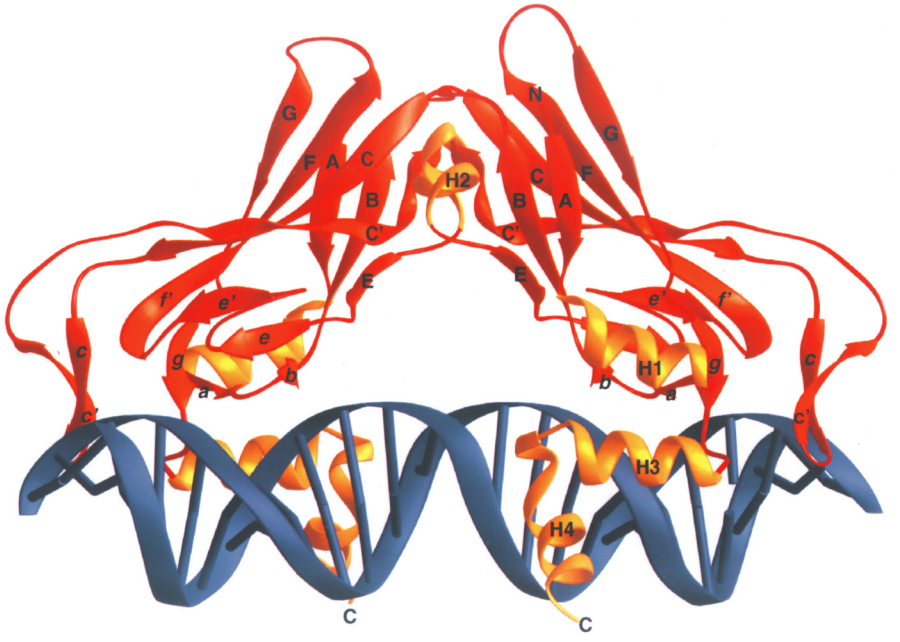

Fig. 3. Brachyury bound to DNA. *Ribbon diagram of the T-domain dimer bound to DNA. Depicted are residues 39-221 of both monomers (strands and loops: red; helices: yellow) and the 24-mer DNA duplex (blue). Labels mark strands (A-G) of the central core of the monomers, which is a seven-stranded β-barrel related to an immunoglobulin fold. View perpendicular to the DNA axis with the dyad vertical. Reprinted from Nature with permission.*

each other. The crossovers which gave rise to them occurred near the proximal breakpoint of In(17)4. The DNA up to the start of In(17)4 is derived from the *t*-complex, the proximal part of In(17)4 is deleted and the distal part is duplicated, there being one *t*-complex and one wild-type copy. The deleted region is at least 3.3Mbp in length (Barclay *et al.*, 1996) and includes a number of zinc finger sequences (Crossley and Little, 1991). Although the deletion is presumed to be the cause of the prenatal lethality, the exact gene(s) responsible have not been identified. Other haplotypes have been formed by crossing-over within inversion In(17)2. Wild-type chromatin carries an inverted duplication in this region, with one duplicated copy in the same orientation as the t-complex copy (Herrmann *et al.*, 1987). Some partial haplotypes have arisen through crossing-over in this region. Two particularly interesting haplotypes are *Tt*^{Orl} and *Tt*^{Lub2}. These have both arisen by crossing-over within the proximal inversion, and each has duplicated and deleted sequences which are complementary in the two haplotypes (Sarvetnick *et al.*, 1986).

Spermatogenesis

The enigma of the effects of *t*-complex genes on spermatogenesis proved a great impetus to the discovery and characterisation of genes expressed in the germ cells of the testis (Willison and Ashworth, 1987). Since transmission ratio distortion is most likely to be a consequence of perturbations in the function of genes expressed post-meiotically, laboratories became interested in the control of haploid gene expression. Silver and colleagues had discovered, by 2-D gel electrophoresis, several polypeptide spots expressed in adult testis germ cells which were mapped to the *t*-complex: TCP-1 to TCP-9. TCP-1 exists as a more acidically migrating polypeptide species in *t*-haplotypes, TCP-1A, compared to wild-type chromosomes, TCP-1B (Silver *et al.*, 1979). In 1979, cDNA cloning techniques were developing to the extent that it seemed feasible to isolate tissue-expressed sequences by probing libraries with ^{32}P-labelled probes made from total or size-fractionated mRNA populations and Willison's laboratory attempted and eventually succeeded in cloning *Tcp-1* cDNA (Willison *et al.*, 1986). A set of several hundred testis-expressed genes were initially recovered, some of which were expressed in haploid spermatids (Dudley *et al.*, 1984). A number of these haploid-expressed genes were mapped onto mouse chromosomes. Using probes enriched for *Tcp-1* mRNA, we found a cDNA clone, pB1.4, which mapped to the *t*-complex and was shown to encode TCP-1B. We sequenced and characterised the *Tcp-1*^{a} and *Tcp-1*^{b} genes in detail (Willison *et al.*, 1986). Willison's group at the Institute of Cancer Research (ICR) showed that Tcp-1 mRNA (Haffner, 1988) and protein was expressed in all spermatids of *T*^{hp}/+ testes and, since *T*^{hp} is deleted for *Tcp-1*, the group postulated that the products of haploid expressed genes are shared between meiotic partners (Haffner, 1988; Willison *et al.*, 1988). Braun showed, by driving human growth hormone expression in spermatids with the mouse protamine-1 gene promoter, that spermatids shared gene products by diffusion of molecules throughout the syncytium of spermatogenic cells (Braun *et al.*, 1989). These experiments provided molecular proof of the concept of shared meiotic gene expression derived from genetic and physiological experiments with *t*-haplotypes. Many laboratories characterised *t*-complex encoded genes expressed during spermatogenesis; *117c3* (Rappold *et al.*, 1987), the *Tcp-10* gene family (Schimenti *et al.*, 1988), the *Tctex* series (Huw *et al.*, 1995) and *Tcp-11* (Hosseini *et al.*, 1994), but their functions remain undetermined. Despite the increased understanding of the genetic basis of *t*-complex transmission ratio distortion at the phenotypic level, the molecular identity of the genes involved remains unknown and provides a challenge for the future. By detailed study of DNA markers in partial *t*-haplotypes with and without the *t*-complex responder *Tcr*^{t}, the position of the responder has been accurately mapped to an interval of ~40kb (reviewed by Snyder and Silver, 1991). In this region there is a gene termed *Tcr10*^{b} which is meiotically expressed and is differentially spliced in *t*-complex DNA (Cebra-Thomas *et al.*, 1991). This gene seemed a strong candidate for the responder. However, both *Tcr10*^{b} transgenes (Snyder and Silver, 1991) and targeted mutagenesis of *Tcr10*^{b} failed to provide evidence for such a function and the responder remains unidentified (Ewulonu *et al.*, 1996).

The positions of the distorter genes are less accurately known, making the identification of possible candidate genes among the numerous testis-expressed genes in the *t*-complex region much more difficult. Mazarakis reported a testis-expressed gene termed *Tcp11* which maps within the distal inversion and shows sequence differences between *t* and wild-type (Mazarakis *et al.*, 1991). It is

thus a possible candidate for *Tcd2*. However, all that is known of the location of *Tcd2* is that it lies in the distal inversion, and since this is relatively large there may be several other candidate genes to be found. A candidate for *Tcd1* in the proximal region of the complex showed overexpression of mRNA in *t*-haplotypes, but a normal amount of protein with some amino-acid changes (O'Neill and Artzt, 1995). Lyon found that a deletion of wild-type chromatin in the *Tcd1* region had an effect like that of $Tcd1^t$, suggesting that $Tcd1^t$ was an amorph or hypomorph (Lyon, 1992). This makes O'Neill and Artzt's candidate gene unlikely but does not exclude it, as the protein could be non-functional. On the other hand, a candidate for *Tcd3*, named *Tcte2*, found by Braidotti and Barlow (1997) is indeed deleted in *t*-haplotypes. This makes it a somewhat stronger candidate for *Tcd3* than a gene previously found by Rappold *et al.* (1987). Interestingly, *Tcte2* is also a candidate for a hybrid-sterility gene. Three such genes, *Hst1, Hst4,* and *Hst6,* map in the *t*-complex region (Pilder *et al.*, 1991, 1993). Hurst has suggested that the distorted transmission of *t*-haplotypes has arisen by the suppression of hybrid-sterility barriers (Hurst, 1993). As the *t*- form of *Tcte2* involves a deletion, this could mean that it is deleting a hybrid-sterility gene. Thus, the identification of the *t*-complex responder and distorter genes remains an outstanding problem which could potentially throw light not only on the mechanism of transmission ratio distortion itself, but also on sperm function and on hybrid-sterility.

Evolution of *t*-complex

Although there is no compelling evidence yet linking TCP-1 or, indeed, any of the TCP proteins (now TCP-1- TCP-11) directly to *Tcr* or *Tcd* function, further study on *Tcp-1* has illuminated many areas of genetics and biochemistry. Sequencing *Tcp-1* genes provided important insight into the evolution of the *t*-chromosomes. The comparison of RFLVs and the DNA sequences of *Tcp-1* from laboratory strains, *t*-haplotypes and wild-caught mice showed that all the *t*-haplotypes carried identical $Tcp\text{-}1^a$ genes, leading Willison (Willison *et al.*, 1986) to suggest that all *t*-haplotypes had radiated from a common population of mice very recently, possibly only 7000 years' ago, concomitant with human migration from the fertile crescent. Later, DNA sequencing of introns from the *Tcp-1* gene supported this idea that *t*-chromosomes originated very recently, and introgressed into *Mus musculus* and *Mus domesticus* (Morita *et al.*, 1992). Again, characterisation of the human TCP-1 gene and related sequences provided insight into the syntenic relationship between mouse chromosome 17 and human chromosome 6. A long-standing controversy over the existence or otherwise of a human equivalent of the mouse *t*-complex was settled by the demonstration that human TCP-1 mapped to the long-arm of chromosome 6 very distant from, and furthermore across the centromere from, the human MHC and other linked markers; the 30Mbp *t*-complex region is fragmented in humans (Willison *et al.*, 1987).

Developmental genetics

The most important contribution of *t*-complex studies to developmental genetics is indisputably the molecular cloning of the Brachyury gene, *T*, by Herrmann and colleagues (Herrmann *et al.*, 1990) at the National Institute for Medical Research (NIMR) laboratory, in London. Brachyury is a DNA binding transcription factor which specifies mesoderm formation not only in mouse, but in all vertebrates. Brachyury is the first member of a gene family, T-box genes, which are master-controlling genes in mesoderm induction and organ rudiment specification. The crystallographic structure of the *T* domain-DNA complex has revealed a new way in which a protein can recognize DNA (Fig. 3) (Muller and Herrmann, 1997). Reduction in Brachyury function is the cause of the short-tail phenotype in *T/t*-animals, but it still remains to be understood how *T* interacts with products of *t*-haplotype factors to cause absence of tails altogether.

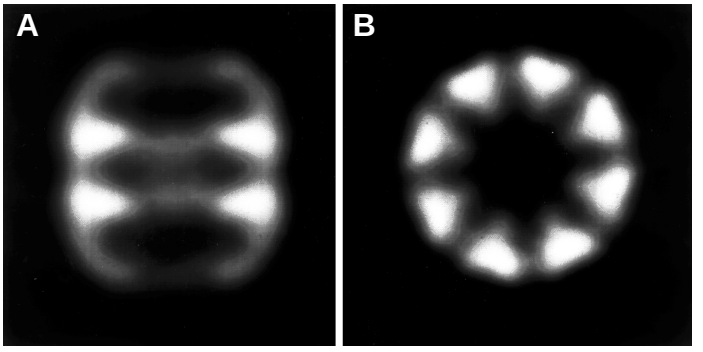

Fig. 4. Average images of nucleotide-free murine CCT. (A) *Side view;* **(B)** *Front view. The side view shows the characteristic 4-stripe appearance of chaperonins, although CCT shows differences to GroEL in mass distribution. The front view reveals the 8 subunits of the CCT ring, one of which is TCP-1. Figure adapted from Llorca et al. (1998).*

An allele of Brachyury, T^{hp}, was known to carry a second mutation called *Tme*, '*T maternal effect*'. For many years this mutation was merely a curiosity, but subsequent research has shown it to be an early example of an imprinted genetic locus. Imprinted genes are those whose expression properties are dependent upon whether they are maternally or paternally inherited. The fine mapping of the *t*-complex and knowledge of the syntenic relationship between mouse chromosome 17 and human chromosome 6 led Barlow *et al.* (1991) to identify the IgfII receptor (*Igf2r*) as the imprinted gene lying within the T^{hp} deletion and responsible for the *Tme* effect (Barlow *et al.*, 1991).

Protein folding

Remarkably, the study of TCP-1 has opened up a new area of research in eukaryotic cell and molecular biology. TCP-1 has turned out to be one of the subunits of the eukaryotic molecular chaperone named the Chaperonin Containing TCP-1, CCT, which is present in the cytosol of all eukaryotes. CCT is active in the folding pathways of newly synthesised actins and tubulins through nucleotide binding and ATP-dependent release of folding intermediates of these substrates after their synthesis on the ribosome. Willison used monoclonal antibodies raised against TCP-1 (Willison *et al.*, 1989) to purify TCP-1 biochemically initially from testis. Willison's group at the Institute of Cancer Research (ICR) discovered TCP-1 to be one of the subunits of a large heteromeric protein complex (Lewis *et al.*, 1992) which had many biochemical properties in common with the chaperonin of *E. coli*, GroEL. TCP-1 is also weakly related in sequence to GroEL reflecting, in the main, the conservation of the nucleotide binding sites (Lewis *et al.*, 1992; Kim

et al., 1994). This group went on to isolate the mouse genes for the 8 subunits of CCT (Kubota *et al.*, 1994; Kubota and Willison, 1997) which are all related in sequence to *Tcp-1*. These 8 mouse genes have been named *Ccta-Cctq* and are each orthologous to the corresponding yeast gene, CCT1-CCT8 (Kubota and Willison, 1997). Because CCT is a very large protein complex with a molecular weight of 950 kDa, it can be visualised under the electron microscope (Fig. 4) (Llorca *et al.*, 1998).

It can be seen that CCT is composed of two back-to-back rings having 8 subunits in each ring and Willison's group has developed a model for the order of arrangement of the subunits within a single ring (Liou *et al.*, 1998). Work is in progress to determine the function of each different CCT subunit in binding and folding of the intermediates of actin and tubulin. The mechanism of protein folding is one of the outstanding problems in modern biology, and further studies on CCT will illuminate further how proteins fold in eukaryotic cytosol (Willison, 1999).

Summary

The *t*-complex of the mouse was discovered over 70 years' ago and British geneticists have been involved in studies on this fascinating region of the mouse genome for 50 years. As a classical genetic system, *t*-complex studies have illuminated the nature of inversion polymorphism and recombination suppression, the behaviour of non-Mendelian factors and transmission ratio distortion, and genetic imprinting. This work helped to establish the *t*-complex region on chromosome 17 as one of the best mapped and characterised mouse chromosomes, and provided the framework for pioneering positional cloning and genome analysis approaches. Brachyury, *T*, was the first mouse gene isolated by positional cloning and this was a direct consequence of the genetic and structural analysis of the *t*-complex. Tcp-1 was discovered and subsequently molecularly cloned by the application of novel 2D-PAGE technology and differential cDNA cloning in conjunction with genetic analysis of partial *t*-haplotypes. Characterisation of the DNA- binding transcription factor *T* has been a vital and unique contribution to vertebrate developmental genetics. TCP-1 is a component of the chaperonin containing TCP-1, CCT, an absolutely conserved protein folding machine present in the cytosol of all eukaryotes. We look forward to the complete DNA sequencing of the 30MBp *t*-complex within the next few years. Then perhaps the major outstanding problem of the components and mechanism of male transmission ratio distortion will be elucidated.

KEY WORDS: *t-complex, spermatogenesis, mouse genetics, protein folding*

Note added in proof: Herrmann and colleagues have recently shown that the *t*-complex responder is a novel protein kinase probably involved in regulating sperm motility [(see Herrmann, B.G. *et al.,* (1999); *Nature 402:* 141-146 and accompanying News and Views article by Willison, K.R. (1999) *Nature 402*: 131-132]. This discovery will now allow the study of the molecular cell biological processes which cause transmission ratio distortion

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