Fig. 4 Schematic summary of the organization of the HLA–DR α-chain sequence. The summary is based on the data of Figs 1–3 and follows their numbering and conventions. α_1, α_2, and TMC refer to the N-terminal, membrane-proximal, transmembrane-cytoplasmic exons or domains respectively, and 3'-UT refers to the 3' untranslated region exon. The rectangle identifies translated sequence. The solid region is the signal sequence, the shaded regions are those with greatest homology to other domains, and the stippled region is the hydrophobic transmembrane segment.

A schematic summary of the organization of the HLA–DR α-chain sequence based on the data of Figs 1, 2 and 3 is given in Fig. 4. The most obvious features revealed by the sequence analysis are the organization of the HLA–DR α-chain into exons that correspond surprisingly well to those defined for HLA–ABC chains, β_2 microglobulin and, of course, immunoglobulins, and the demonstration of a remarkable correspondence between exon organization and the presumptive protein domains. Another striking feature of the HLA–DR α-chain sequence data is the homology of the external domain next to the membrane with other comparable domains of HLA products, with β_2-microglobulin and, to a lesser extent, with immunoglobulin constant domains. The fact that this homology applies to comparisons between specific domains, such as those close to the membrane, underlines the relative independence of the evolution of different domains, or exons. Though the homologies between the HLA products (ABC, DR α and DR β-chains) are weak compared with homologies between HLA–ABC and H-2KDL, or between the latter and the H-2 region coded TL and Q gene products, they are sufficient to support the suggestion that the whole, or at least a major part, of the HLA region may ultimately have a common origin by a series of duplication events[25]. The implied separation of the different classes of HLA products by duplication must, however, have occurred up to 500 million years ago, and certainly well before the evolutionary divergence of the mammals[26].

The suggestion that there might be homology between HLA products and immunoglobulins was made on the basis of a functional analogy, namely the possibility that HLA products might have recognition functions analogous to those of the variable regions of immunoglobulins[11,12]. Subsequently, however, it became clear that while HLA products do play a part in cellular interactions and recognition, at least in the immune system, they do not have an analogue of the immunoglobulin variable region. Thus, it is not surprising that homologies between HLA and immunoglobulins revealed by sequence analysis involve particularly the constant region domains, as might be expected if HLA products are involved in interactions similar to those that occur between immunoglobulins and complement[25], Fc receptors, monocyte receptors and secretory components[27] as well as between complement and cellular component receptors. Another relevant analogy might be that between the C_H3 domain of IgG and the appropriate receptor on specific antibody dependent killer cells[28].

We thank P. Friedland, R. Staden, A. Williams, J. Rogers, A. Mellor, T. Lund, R. Flavell, D. Larhammar, A. Korman and C. Furse for their help.

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Electron microscopy of
t-allele synaptonemal
# complexes discloses no inversions

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The t complex of mice has been an enigma for over half a century[1]. The recessive mutations (t^n) on chromosome 17 of the mouse have been characterized by four properties: interaction with the dominant mutation T (Brachyury) to cause taillessness, transmission ratio distortion in males, a series of homozygous lethal mutations, and cross-over suppression[2–4]. The last property has led to many studies of meiosis in t-allele-bearing males. Forejt found fewer chiasmata, the cytological correlate of crossing-over, in the region of t haplotypes[5,6]. Lyon et al.[7] confirmed these results and suggested that chiasma suppression determined by t alleles is probably desynaptic but that electron microscopy would probably be needed to settle this point. We have now extended these observations by using a centromeric translocation and analysed synaptonemal complexes by electron microscopy to study t-haplotype pairing. We have found normal synaptonemal complexes without any evidence of inversions but detected early disjunction of the chromosome 17 homologues, supporting the idea of desynaptic chiasma suppression.

The cross-over suppression of t haplotypes masks the true genetic nature of the region. It has previously been convenient to consider the t alleles as point mutations mapping near T.
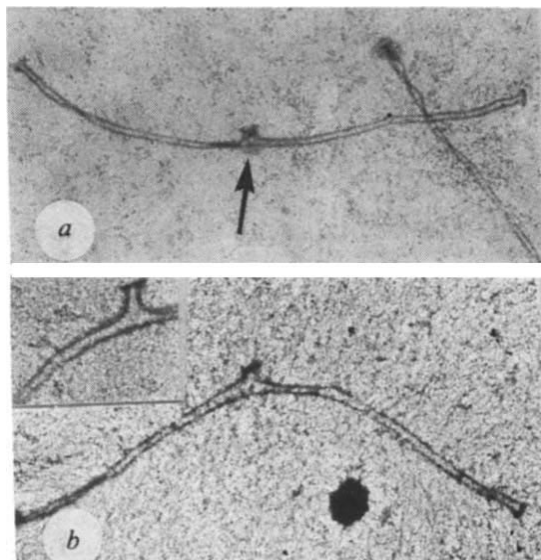
Fig. 1   Whole-mount electron microscopic preparations of mouse pachytene spermatocytes. Testicular cells were dissociated and suspended in phosphate-buffered saline as described previously[17]. Nuclear contents were dispersed in a detergent (NP40) solution and transferred to a plastic trough containing electron microscope copper grids and a solution of 0.1 M sucrose with 10% formaldehyde. The sample was centrifuged at 3,000 r.p.m. for 5 min at 4 °C. Grids were rinsed in 0.5% Kodak Photo-flo, air-dried and stained with 1% phosphotungstic acid in 95% ethanol. A full description of this technique has been reported elsewhere[17]. The criteria for identification of meiotic prophase stages in whole-mount electron microscopic preparations have been described in ref. 18. a, Whole-mount electron micrograph of an early pachytene mouse spermatocyte displaying a synaptonemal complex between robertsonian translocation 7 and chromosomes 16 and 17 ($Rb7/t^{w5}$). The arrow indicates the paracentromeric chromatin region. × 4,600. b, Pairing of chromosomes 16 and 17 mediated by a synaptonemal complex in a mouse spermatocyte carrying $Rb7/T$. × 6,200. Inset: detail of the telomeric pairing of chromosomes 16 and 17 in the presence of $Rb7/T$. × 11,000.

Lyon's thorough analysis of this chromosomal region using rare cross-overs established the non-allelism of several t-complex properties[8,9], and the apparent size of the t complex was estimated by deletion mapping to be at least 6 centimorgans[10,11]. More recently, Artzt, Bennett and co-workers have used increased cross-over frequencies, which occur in heterozygotes containing two different haplotypes, to suggest that the t complex may be 15 centimorgans or more long[12] and involves a reverse order of H-2 and tufted[13]. A chromosomal inversion would readily explain the t-complex cross-over suppression. There have been previous reports of chromosomal aberrations (interpreted as deficiencies)[14] in this region of chromosome 17 but inversions were not found[15].

We have used a robertsonian translocation of chromosomes 16 and 17 (Rb7, ref. 16) as a marker for studies of the synaptonemal complex in the t region by silver staining of light microscopic preparations and by electron microscopy. Rb7, previously designated T7Bnr, was obtained from Dr Charles J. Epstein in homozygous condition on the C57BL/10J background. F_1 mice from the cross of the balance lethal T/t^{w5} (originally obtained from Dr Hugh McDevitt) and T/t^{12} (originally obtained from Dr Salome Glucksohn-Waelsch) lines were known to be $Rb7/t^{w5}$ (or $Rb7/t^{12}$) if they had normal tails while $T/Rb7$ males had short tails. Spermatocytes were prepared by detergent solubilization of cell membranes and dispersion of nuclear contents which were then collected on microscope slides or electron microscopy grids[17,18]. The whole-mount electron microscopic preparations were examined in a JEM 100 B transmission electron microscope operated at an accelerating voltage of 60 kV. Chromosome 16 and 17 monovalents formed normal synaptonemal complexes with the
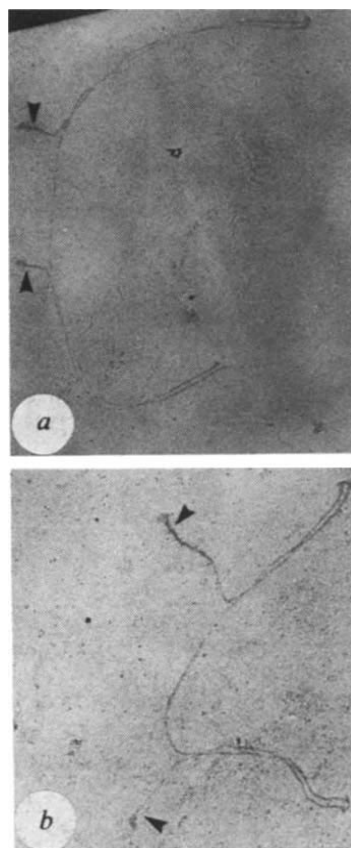

Fig. 2   Whole-mount electron microscopic preparations of mouse spermatocytes (middle pachytene) from $Rb7/t^{w5}$ males. See Fig. 1 legend for preparation details. Early disjunction of chromosomes 16 and 17 is observed at the centromeric regions, including the chromosomal segment corresponding to the t complex. Arrowheads indicate early disjunction. a, × 4,000; b, × 5,000.

Rb7 bivalent; the centromeric region seemed to pair and project on one side of the complex (Fig. 1a). Very similar configurations have been described for silver-stained trivalents in the male cotton rat where a balanced robertsonian polymorphism occurs[19]. These synaptonemal complexes in t-haplotype-bearing mice were identical to those observed in the sibling $T/Rb7$ controls (Fig. 1b). In middle pachytene spermatocytes, early disjunction of the centromeric regions of chromosomes 16 and 17 were observed in the presence of t haplotypes (Fig. 2a, b). This early disjunction of both chromosomes 16 and 17 in the trivalent was seen equally with t^{12} (Fig. 3). They were also noted in silver-stained preparations of the pachytene spermatocytes (Fig. 4).

Thus, normal synaptonemal complexes occur between t-haplotype chromosomes 17 and their normal homologues. However, early disjunction of the paracentromeric regions of
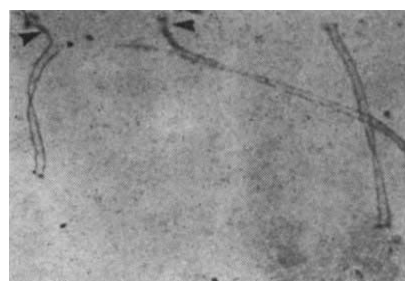
Fig. 3   Middle pachytene spermatocyte, prepared as described in Fig. 1 legend. Early disjunction of $Rb7/t^{12}$ at the paracentromeric region is indicated by arrowheads. × 4,800.
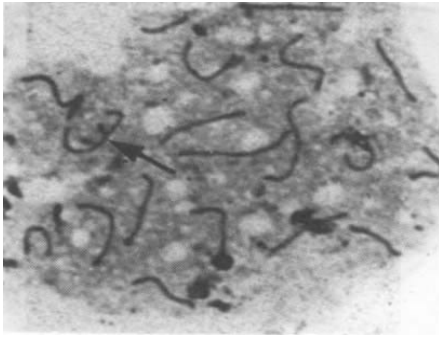
Fig. 4 Silver-stained light microscopic preparation of a pachytene mouse spermatocyte ($Rb7/t^{w5}$) displaying synaptonemal complexes of different lengths. Arrow indicates early disjunction of chromosomes 16 and 17 at the paracentromeric region. The preparation was according to ref. 17. ×1,430.

chromosomes 16 and 17 occurs in the presence of the *t* haplotype. This phenomenon may be related to the *t* haplotype and not to *Rb7*, because early disjunction does not occur in the *T/Rb7* sibling controls. Our electron microscopic studies also support the assumption of Lyon et al.[7] that chiasma suppression in *t* haplotypes results from normal synapsis without chiasma formation (desynapsis).

Artzt, Shin and Bennett have found an anomalous position of *H-2* with respect to *tufted* and *t*-haplotype lethality in t^{w5}, one of the *t* haplotypes used in this study[13]. The position could be most readily explained by an inversion of chromosome 17. Such inversions have not previously been found. Our electron microscopic and silver-staining studies of synaptonemal complexes have shown no evidence of inversions in either t^{w5} or t^{12}. As the *t* complex may extend for about 15 centimorgans, inversion loops would be expected if they were of this size—inversion loops of synaptonemal complexes were found in 100% of inversion carriers at early stages of meiosis[20]. Alternatively, the apparent change of position of *H-2* in these *t* alleles could represent expression of previously unexpressed type 1 histocompatibility antigen loci—recent evidence suggests much greater dispersion of type 1 loci, for at least several centimorgans, than had previously been expected[21]. Despite the central nce of crossing-over suppression for an understanding of the *t* complex, our electron microscopic analysis of synaptonemal complexes has not shown any clear-cut abnormalities. Thus, the final resolution of the enigma of the *t* complex must await the availability of DNA clones of the region.

Although our studies do not reveal the mechanism of *t*-allele-mediated cross-over suppression, further chromosomal studies are warranted as cross-over suppression and transmission ratio distortion in males, not embryonic lethality, are the truly unique properties of the *t* complex. The frequency of developmental lethals is probably no greater than expected in 15 centimorgans of DNA—the cross-over suppression maintains a block of DNA which can accumulate lethals and the unique population-selection properties of the *t* complex outbalance the loss due to homozygous lethality[22]. Recent discoveries of novel *t* alleles in novel complementation groups[23] suggest that previous developmental descriptions, based on a small number of complementation groups, have given a mistaken notion of molecular relatedness among these developmental lethals. Thus, all *t* lethals, and not just t^{w73} (ref. 24), may be 'parasitic'.

This work was supported by NIH grants HD 11738 and HD 11884.

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Cooperative motion and hydrogen exchange stability in protein β-sheets

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Protein molecules are dynamical structures due to the continual exchange of thermal energy between them and the solvent environment[1,2]. This dynamic behaviour is manifest in hydrogen exchange experiments, which reflect transient solvent accessibility of groups usually buried in the protein interior[3,4]. However, studies of hydrogen exchange kinetics in pancreatic trypsin inhibitor (PTI) reveal a small subset of amide protons which exchange very slowly[5,6]. Four of these groups form successive interchain hydrogen bonds in the central region of an antiparallel β-sheet[7] (Fig. 1). Here I suggest that the unusual exchange stability of these β-sheet protons reflects the structure's intrinsic flexibility. This property allows transient energy fluctuations to be accommodated as cooperative motions which do not locally strain the interchain hydrogen bonds.

Observed hydrogen exchange rates for proteins typically span several orders of magnitude[3], which may reflect both intrinsic and environmental differences among the individual exchanging groups[8,9], as well as their static or dynamic accessibility to solvent molecules[4]. Nevertheless, examination of either the geometries[7,10] or solvent accessibilities[11] of the PTI sheet hydrogen bonds suggests little apparent reason why protons in the centre of the sheet should exchange more slowly than other similarly inaccessible groups (Fig. 1). Indeed, the data suggest that whatever the amplitude of dynamical motions in the fluctuating molecule[1,2], the central-sheet hydrogen bonds rarely break, as required to make them both accessible to, and reactive with, an exchanging water molecule[3,4,9].

Features of the antiparallel β-sheet which give rise to its flexibility are illustrated in Fig. 2, which shows a double-stranded sheet with linear N—H—O—C interchain hydrogen-bonds[12] (conformation 1 on the ϕ, ψ plot of Fig. 3). The structure can be viewed as a set of interconnected large and small hydrogen-bonded rings, each of which possesses a local dyad symmetry axis owing to the antiparallel sense of the polypeptide chains. This 'classical' structure can be continuously compressed in accordion-like manner so that it retains its symmetry properties[13]. This involves equivalent alteration in all the backbone ϕ, ψ angles and isoenergetic bending of the hydrogen-bonds[12,13] at the carbonyl oxygens (that is, conformations 1–4 in Fig. 3). Similarly, each flat sheet conformation can twist to

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