The *teflon* Gene Is Required for Maintenance of Autosomal Homolog Pairing at Meiosis I in Male *Drosophila melanogaster*

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Manuscript received July 10, 2000
Accepted for publication September 29, 2000

ABSTRACT

In recombination-proficient organisms, chiasmata appear to mediate associations between homologs at metaphase of meiosis I. It is less clear how homolog associations are maintained in organisms that lack recombination, such as male Drosophila. In lieu of chiasmata and synaptonemal complexes, there must be molecules that balance poleward forces exerted across homologous centromeres. Here we describe the genetic and cytological characterization of four EMS-induced mutations in *teflon* (*tef*), a gene involved in this process in *Drosophila melanogaster*. All four alleles are male specific and cause meiosis I-specific nondisjunction of the autosomes. They do not measurably perturb sex chromosome segregation, suggesting that there are differences in the genetic control of autosome and sex chromosome segregation in males. Meiotic transmission of univalent chromosomes is unaffected in *tef* mutants, implicating the tef product in a pairing-dependent process. The segregation of translocations between sex chromosomes and autosomes is altered in *tef* mutants in a manner that supports this hypothesis. Consistent with these genetic observations, cytological examination of meiotic chromosomes suggests a role of *tef* in regulating or mediating pairing of autosomal bivalents at meiosis I. We discuss implications of this finding in regard to the evolution of heteromorphic sex chromosomes and the mechanisms that ensure chromosome disjunction in the absence of recombination.

MAINTAINING the integrity of bivalents at meiosis I is essential for the proper orientation and subsequent disjunction of homologous chromosomes. Several different strategies have evolved to ensure maintenance of pairing between homologs prior to anaphase I. In recombination-proficient organisms, homolog associations are usually maintained by chiasmata, the physical structures assembled at sites of reciprocal exchange. These structures are the last remaining sites of physical attachment between homologs at meiosis I metaphase and appear to play a role in balancing opposing poleward forces on the bivalent.

In the absence of recombination, it has been suggested that the synaptonemal complex (SC) may assume this role in some organisms (Rasmussen 1977a; Walker and Hawley 2000). Asynaptic *Bombyx mori* females, for example, assemble SC that is structurally modified rather than disassembled in prophase and that connects homologs until their separation at anaphase (Rasmussen 1977b).

In other organisms, neither chiasmata nor SC are required for bivalent integrity. The mechanisms mediating homolog associations in these organisms are less well understood (see Wolf 1994 for review). The male fruit fly, *Drosophila melanogaster*, is the best studied of this class of organisms. The male fly lacks both meiotic recombination and detectable SC (Meyer 1960; Rasmussen 1973). The genetic controls of meiosis I chromosome behavior in the male appear largely separate from those in the recombination-proficient female sex, as most mutations that affect meiosis I-specific aspects of chromosome segregation are specific to one sex (reviewed by Orr-Weaver 1995). There are underlying similarities, however, that suggest that there may be commonalities not yet uncovered by genetic screens, which to date have not been carried out to saturation. Notably, in both males and females the establishment of homolog pairing is homology based (McKee *et al.* 1993). The more fundamental difference between male and female meiosis may be how they maintain bivalent integrity rather than how they pair. Whereas females rely on recombination-associated structures (SC and chiasmata) to hold homologs together, the evolution of an alternative conjunctive mechanism in males may have allowed the elimination of recombination. In this regard, it will be important to determine if the molecules involved in bivalent stability in males are intimately associated with those that mediate pairing or are assembled independently.

There are suggestions from observations on sex chromosome pairing that the same *cis*-acting sites may be involved in both partner identification and association. The X and Y pair and are subsequently joined at proxi-

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mal regions of the X and the short arm of the Y (Cooper 1959), the sites of the tandemly repeated rDNA repeats. Transgene studies have established that the sequences required to establish pairing correspond to 240-bp repeats that reside in the intergenic spacer between the 18S and 28S coding regions (McKee and Karpen 1990; Merrill *et al.* 1992; Ren *et al.* 1997). Cytological observations suggest that sex chromosome conjunction is limited to the vicinity of these pairing sites (McKee and Karpen 1990; Ault and Reider 1994); however, it is not known if the pairing sites *per se* are required to maintain the connections once established.

Sex chromosome pairing, however, may be unusual in that it is restricted to a particular site, whereas autosomes can pair via many different homologous sequences (McKee *et al.* 1993). In addition, ultrastructural observations of fibrillar material exclusively associated with the site of XY attachment (Ault *et al.* 1982) imply that the mechanism of sex bivalent association may be unique. A re-examination of the XY bivalent in a more recent study, however, questions this conclusion and suggests that this fibrillar material may correspond to a residual fragment of the disassembled nucleoli material rather than XY-pairing structures (Ault and Reider 1994). Whether sex chromosomes and autosomes differ in their conjunctive mechanism is an unresolved issue.

Here we have sought to identify genes involved in the processes of homolog pairing in male Drosophila by a genetic screen for mutations that affect meiotic chromosome segregation. We report on the genetic and cytological characterization of mutations in one such gene, *teflon* (*tef*). Our observations suggest that the *tef* gene plays a role in establishing and/or regulating the pairing of all autosomal bivalents at meiosis I.

MATERIALS AND METHODS

Stocks: All Drosophila stocks were grown at 25° on standard media consisting of cornmeal, molasses, yeast, and agar. The *T(1;4)h32;102, mscd1* and *T(1;4) h34;102, mscd2* chromosomes are described in Briscoe and Tomkiel (2000). The *CB25 T(Y;2h;4)* rearrangement is described in Ault and Lyttle (1988). The number associated with each *tef* allele corresponds to the stock number in a collection of lines carrying homozygous viable but heavily ethyl methanesulfonate (EMS)-mutagenized autosomes. These lines are maintained in the lab of C. Zuker. All other mutations and chromosomes are described in Lindsley and Zimm (1992) or in FlyBase (http://flybase.bio.indiana.edu/).

Isolation of *tef* alleles: The *tef* alleles were induced by EMS on second chromosomes marked with *cn* and *bw* (E. Koundakjian, R. Hardy, D. Cowen and C. Zuker, personal communication). Five alleles were isolated by screening 4349 chromosomes in males for mutations that increased the rate of fourth chromosome loss or nondisjunction. Four are described here. Preliminary examination of a fifth allele, tef^{z3455}, suggests that its genetic and cytological phenotypes are consistent with those of the other four alleles.

Mapping of *tef*: All five alleles were first mapped by recombination with respect to *cn* and *bw*. Females heterozygous for *cn bw tef* and a Canton-S wild-type chromosome were mated to *cn bw tef/SM1, Cy* males, and the Cy^+ sons then mated to females carrying the chromosome 4 compound *C(4)EN, ci ey*. Paternal fourth chromosome loss or nondisjunction events, revealed by the presence of ci ey progeny, were used to map the mutations to 2–80.0 MU. The mutations were then mapped by complementation with deficiencies and by recombination with respect to *P*-element insertions. All alleles were complemented by *Df(2R)Jp8* (52F-53A) and by *Df(2R)Pcl7B* (54E-55C), suggesting that *tef* was located in the interval between salivary chromosome bands 53A and 54E. No deletions were available in this region, perhaps because it contains a haplo-insufficient *Minute* locus, *M(2)53*. This region was subdivided by recombination mapping *cn* tef^{Z5864} *bw* with respect to 11 *P[w+]* transposon insertions, using the *w+* gene as a visible marker. These were *l(2)k02836*, *l(2)k07127*, *l(2)k12701*, *l(2)k04810*, *l(2)k15914*, *l(2)k08805*, *l(2)k09202*, *l(2)k04222*, *l(2)k07433*, *l(2)k07509*, and *l(2)k11505* and are located at salivary gland chromosome bands 53B, 53C, 53D, 53E, 53F, 54A, 54B, 54C, 54D, 54E, and 54F, respectively. Of 691 recombinants with *l(2)k08805*, 3 were *cn* tef^+ and 2 were *P[w+] tef bw*. Of 67 recombinants with *l(2)k09202*, 1 was *cn P[w+] tef*. Recombination mapping with all 9 other *P* insertions was consistent with a placement of *tef* between *l(2)k08805* and *l(2)k09202* at 53F-54A.

Successive brooding experiments: Males 1–2 days posteclosion were mated to *C(4)EN, ci ey* females in a ratio of 1:3 on day 1. Every 3 days females were removed and replaced with new females. Progeny from five successive broods were scored on days 13, 15, and 18. At least 20 males of each genotype were tested, and a minimum of 500 progeny from each brood was scored.

Cytology: Testes were dissected in Schneider's Drosophila media (GIBCO BRL, Gaithersburg, MD) from larvae, pupae, or adults. For meiotic chromosome squashes, testes were fixed in 45% acetic acid for 5 min and then transferred to 1 μM 4′6-diamidino-2-phenylindole (DAPI) and squashed under silanized coverslips. Alternatively, whole-mount preparations of intact or ruptured testes were gently flattened under coverslips without squashing. Coverslips were removed after freezing in liquid nitrogen, and testes were fixed in methanol, acetone, and acetic acid/PBS as described by Pisano *et al.* (1993). Tissue was incubated 1 hr at room temperature with rat monoclonal antitubulin antibodies (MAS-078, Harlan Sera-lab, Sussex, England) diluted 1:200 in PBS plus 0.1% Triton X-100. Tissue was then washed three times for 1 min with PBS, incubated 1 hr at room temperature in FITC-conjugated goat anti-rat secondary antibodies (FI-4001, Vector Labs, Burlingame, CA), and diluted 1:500 in PBS plus 0.1% Triton X-100. Washes were repeated, and slides were mounted in 50% glycerol in PBS and examined using a Nikon Optiphot epifluorescence or a Zeiss Laser Scanning 310 confocal microscope. Images were captured using a Sensys CD camera or the Zeiss confocal software and were cropped and arranged using Adobe Photoshop 3.0 software.

RESULTS

***tef* is a male-specific mutation that affects autosome segregation:** We identified five EMS-induced mutations in a screen for mutations that increased the frequency of progeny lacking a paternal fourth chromosome. The characterization of four of these mutations is described here. We mapped these mutations by recombination to 80.0 MU on chromosome 2, and between two *P* elements inserted at salivary gland chromosome bands 53F and 54A (see MATERIALS AND METHODS). We have examined

these mutations for their effects on meiotic and mitotic chromosome segregation and to determine if they exhibit chromosome and/or sex specificity.

Sex and fourth chromosome nondisjunction were simultaneously monitored by scoring the progeny of *Xy/y$^+$Y; tef; spa^{pol}* males mated to *y w sn^3; C(4)EN, ci ey* females. From such matings, *diplo-XY* and *nullo-XY* sperm resulting from sex chromosome nondisjunction produce y$^+$ daughters and y w sn sons, respectively. Fourth chromosome nondisjunction results in *diplo-4* sperm, which give rise to spa progeny, and *nullo-4* sperm, which produce ci ey progeny. We tested all possible combinations of the *tef* alleles by such assays. No increases in sex chromosome nondisjunction or loss were observed. In contrast, fourth chromosome exceptions were observed at frequencies between 28.1 and 43.7% (Table 1). For most of the allelic combinations, the frequency of the *nullo-4* progeny slightly exceeded that of the *diplo-4* progeny, suggesting that *tef* may cause some loss in addition to nondisjunction.

Fourth chromosome nondisjunction is underestimated by these crosses because we could not distinguish *tetra-4* progeny (*spa/spa/C(4)EN, ci ey*), which result from *diplo-4* sperm, from progeny resulting from normal *haplo-4* sperm (*spa/C(4)EN, ci ey*). From independent crosses of *C(4)EN spa* males and *C(4)EN, ci ey* females, we found that 40–50% of such *tetra-4* progeny survive to adulthood (data not shown). If we adjust our data to account for this by subtracting half the number of *diplo-4* progeny from the wild-type class, the true rate of fourth nondisjunction caused by several of the *tef* alleles approaches 50%. This suggests that fourth chromosome homologs may be segregating at random at meiosis I.

We asked if *tef* had similar effects on sex and fourth chromosome segregation in females by mating *y ; tef; spa^{pol}* females to *y w sn/y$^+$Y; C(4)EN, ci ey* males. We observed wild-type frequencies of sex and fourth chromosome nondisjunction in females homozygous for each of the four alleles (<0.5%, data not shown). Thus, all four *tef* alleles are male specific.

To determine if *tef* is specific for the fourth chromosome, or if the other autosomes are also affected, we crossed *tef* males to females bearing compound autosomes. Females carrying *C(2)EN, bw sp* or *C(3)EN, st cu e* produce eggs that are *nullo-* or *diplo-* for chromosome 2 or chromosome 3, respectively. The only progeny that survive from these females are products of sperm bearing zero or two copies of the major autosome assayed. These assays are qualitative in nature; because euploid sperm do not produce viable offspring we cannot determine absolute nondisjunction frequencies.

For each test, we mated 50 *tef* or *tef/Cy* control males to 100 compound-bearing females and counted the adult progeny produced. Males homozygous for any of the four *tef* alleles produced significantly more progeny than their heterozygous *tef/Cy* brothers (Table 2). Because

TABLE 1

Sex and fourth chromosome disjunctional data from crosses of y/y$^+$Y; tef; spa^{pol} males to y w sn ; C(4)EN ci ey/0 females

	Recovered male gametes										% 4th nd	
Paternal genotype	*Y;4*	*X;4*	*0;4*	*X/Y;4*	*Y;0*	*X;0*	*Y;4/4*	*X;4/4*	*0;0*	*0;4/4*	*X/Y;0*	*X/Y;4/4*	Total	nullo-4	diplo-4
tef^{z4169}/tef^{z4169}	416	777	0	0	210	290	173	252	0	0	0	0	2118	23.6	20.1
tef^{z4169}/SM1, Cy	395	786	1	2	0	0	1	1	0	0	0	0	1186	0.0	1.7
tef^{z5549}/tef^{z5549}	478	799	3	0	215	207	139	236	0	0	0	1	2078	20.3	18.1
tef^{z5549}/SM1, Cy	445	805	1	0	1	1	0	0	0	0	0	0	1253	0.2	0.0
tef^{z1869}/tef^{z1869}	825	1342	2	2	150	256	166	185	1	0	1	0	2930	13.9	12.0
tef^{z1869}/SM1, Cy	382	618	0	0	0	0	0	0	0	0	0	0	1000	0.0	0.0
tef^{z5864}/tef^{z5864}	359	445	1	0	116	131	125	136	0	0	0	0	1313	18.1	19.9
tef^{z5864}/SM1, Cy	610	968	0	0	0	2	0	0	0	0	0	0	1580	1.3	0.0
tef^{z4169}/tef^{z5549}	267	402	0	0	134	138	87	132	0	0	0	0	1160	23.5	18.9
tef^{z4169}/tef^{z1869}	244	438	1	0	90	143	73	136	0	0	2	1	1128	20.8	18.6
tef^{z4169}/tef^{z5864}	292	431	1	1	122	168	96	146	1	0	0	1	1259	23.1	19.3
tef^{z5549}/tef^{z1869}	327	517	1	1	128	176	86	126	0	0	0	0	1362	22.3	15.6
tef^{z5549}/tef^{z5864}	286	424	2	3	146	158	106	127	1	1	0	1	1255	24.3	18.7
tef^{z1869}/tef^{z5864}	213	265	1	4	57	84	61	56	0	0	0	0	741	19.0	15.8

nd, nondisjunction.

TABLE 2

Results of crosses of *tef* males to *C(2)EN, bw sp* or *C(3)EN, st cu e* females

Maternal genotype:	*C(2)EN, bw sp*		*C(3)EN, st cu e*	
Sperm genotype: Paternal genotype	diplo-2	nullo-2	diplo-3	nullo-3
+/+	0	2	0	5
tef^{z4169}/tef^{z4169}	38	143	101	135
$tef^{z4169}/SM1,Cy$	3	2	0	0
tef^{z5549}/tef^{z5549}	22	84	5	20
$tef^{z5549}/SM1,Cy$	0	2	0	0
tef^{z1869}/tef^{z1869}	3	6	18	56
$tef^{z1869}/SM1,Cy$	0	1	0	4
tef^{z5864}/tef^{z5864}	46	141	14	21
$tef^{z5864}/SM1,Cy$	0	5	0	1

Numbers indicate viable adult progeny produced from matings of 50 males and 100 females of the indicated genotypes.

the compound autosomes were marked with visible recessive mutations, we could distinguish progeny produced from diplo- *vs.* nullo-exceptional sperm. In all cases, there were considerably more progeny produced by nullo-exceptional sperm. This difference may reflect meiotic autosome loss or selection for nullo- over diploautosomal sperm (*e.g.*, meiotic drive).

To estimate the frequency of nondisjunction of chromosomes 2 and 3, we collected eggs from the compound-bearing females mated to tef^{z5864} males and counted the proportion that hatched. Because embryos aneuploid for either chromosome 2 or 3 do not survive to hatch (Merrill *et al.* 1988), this number allows estimation of the nondisjunction frequency. From crosses to *C(2)EN* females, 75/500 (15%) hatched, and from crosses to *C(3)EN* females, 61/500 (12.2%) hatched. These numbers are not significantly different from the 12.5% egg hatch expected if the autosomes were segregating at random. Together with the data on fourth chromosome disjunction, this suggests that all autosomes may segregate at random at meiosis I in tef^{z5864} males.

Meiosis I specificity of the *tef* defect: To address whether *tef* is required for chromosome disjunction during male germline mitotic, MI, or MII divisions, we performed two experiments. We first asked if *tef* affected mitotic germline divisions or was specific to meiosis. In testes, stem cells continuously divide throughout adulthood to replenish depleted sperm pools (Hannah-Alava 1965). Thus, if mitotic nondisjunction events were contributing significantly to the overall nondisjunction frequency, then nondisjunction should increase with age (*i.e.*, in proportion to the number of stem cell divisions preceding meiosis). We measured fourth chromosome disjunction in five successive broods from males homozygous for each of the four *tef* alleles and observed no increase in nondisjunction or loss in later broods (data not shown). This suggests that *tef* does not affect germline stem cell mitoses, but rather that the defect occurs in meiosis.

To discriminate between a meiosis I and meiosis II defect, we crossed *tef* males that were heterozygous for the fourth chromosome recessive marker spa^{bol} to tester *C(4)EN, ci ey* females. The informative progeny from these crosses are the *diplo-4* exceptions. Fourth chromosome nondisjunction at meiosis I in these males would produce *diplo-4* sperm heterozygous for the spa^{bol} mutation and thus produce spa^{+} progeny. Precocious sister chromatid separation at meiosis I or nondisjunction of sister chromatids at meiosis II could produce *diplo-4* sperm homozygous for spa^{bol}, which would result in spa progeny. Males bearing each of the four alleles of *tef* were tested, and of 2916 total progeny scored, 615 (21.1%) were ci ey, but none were spa. This suggests that meiosis II is normal in *tef* males and that the defect in chromosome segregation is specific to meiosis I. Furthermore, it argues that *tef* mutations do not affect sister chromatid cohesion.

***tef* affects a pairing-dependent aspect of meiosis I chromosome segregation:** The events of meiosis I chromosome segregation can be generally classified as pairing dependent or pairing independent. Pairing-dependent processes include partner recognition, establishment and regulation of homolog connections, and orientation of homologs to opposite spindle poles. Pairing-independent events include spindle assembly, kinetochore function, sister chromatid cohesion, and anaphase movements. To ask which general class of events might be affected by *tef* mutations, we examined the transmission of univalent chromosomes from *tef* males. Univalent chromosomes lack a pairing partner, and thus their behavior should not be affected by mutations in pairing-dependent processes.

We separately assayed the transmission of two univalent chromosomes, a mini-chromosome derivative of the X, *Dp(1;f)1187*, y^{+} and the compound-4 *C(4)EN. Dp(1;f)1187* is a 1.3-Mb chromosome that retains all sequences required for normal transmission (Karpen and Spradling 1992) but lacks XY pairing sites and assorts randomly with respect to sex chromosomes during meiosis I in males (Parry and Sandler 1974). Although we had failed to see an effect of *tef* on the segregation of intact sex chromosomes, it remained a possibility that this smaller derivative might show a size-dependent sensitivity.

Transmission of *Dp(1;f)1187*, y^{+} was measured by the percentage of $yellow^{+}$ progeny produced by *Xy/Dp(1;f) 1187,* y^{+}/Y males mated to *y* females. The results were similar for each of the four *tef* alleles tested. In total, males homozygous for *tef* produced 46.4% *Dp-bearing* progeny (of 4068 total) whereas control *X/Y/Dp; tef/ SM1,Cy* brothers produced 45.4% *Dp-bearing* progeny (of 3592 total). Thus the transmission of *Dp(1;f)1187* was unaffected by *tef* mutations.

Transmission of the compound-4 was measured by the percentage of ci ey progeny produced from matings of *tef; C(4)EN, ci ey* or control *tef/ SM1, Cy*; *C(4)EN, ci ey* males to *C(4)EN, spa^{pol}* females. (Note that the *C(4)EN/ C(4)EN* progeny also received the paternal C(4)EN chromosome, but were excluded from consideration here because their survival is highly variable from cross to cross.) In tests of all four *tef* alleles, the transmission of the *C(4)EN* chromosome from *tef* homozygous males was similar to that of their *tef/ SM1, Cy* brothers. From *tef* males, a combined total of 36.7% of the progeny were ci ey (1907 ci ey and 3292 spa F_1). Control *tef/ SMI* males produced 32.7% ci ey progeny (1615 ci ey and 3323 spa F_1). These results suggest that the *tef* gene product is involved in a process that is either required for or dependent on homolog pairing.

***tef* influences the behavior of translocations between a sex chromosome and autosome:** We reasoned that if *tef* specifically affects a process related to autosome pairing, then it might modify the meiotic behavior of translocations between a sex chromosome and autosome. In such translocations, homologous centromeres can undergo nondisjunction resulting in adjacent II segregation. Elimination of pairing between the autosomal portions of such a translocation might be expected to increase the frequency of proper disjunction of the sex chromosomes.

We tested this premise using *T(Y;2h; 4)CB25*, a translocation in which the fourth chromosome is attached to the tip of the long arm of the Y (Ault and Lyttle 1988). This chromosome regularly pairs with both the X and the free fourth chromosome. Otherwise wild-type males bearing this translocation produce 10% XY exceptions and 15–20% fourth chromosome exceptions (Ault and Lyttle 1988). This translocation appears to retain both the Y and fourth centromeres, and it has been postulated that nondisjunction results from dicentric behavior (Ault *et al.* 1982). However, in the majority of meioses examined, only a single centromere region stains with antibodies to the essential kinetochore component ZW10 (Williams *et al.* 1996). Thus, although both centromeres on this translocation may be capable of functioning, it appears that nondisjunction may be a consequence of its pairing configuration rather than the opposing activities of two centromeres.

The frequency of sex chromosome and fourth chromosome nondisjunction was monitored by matings of *T(Y;2h;4)CB25*-bearing males to *y w sn; C(4)EN, ci ey* females. We could not detect progeny produced from *diplo-4* sperm from these crosses, because the translocation was wild type for fourth chromosome visible markers. However, fourth chromosome nondisjunction could be estimated by the frequency of *nullo-4* exceptions and was elevated by *tef* as expected. In contrast, sex chromosome nondisjunction was more than fivefold lower in *tef* males compared to their *tef/ Cy* brothers (Table 3). We suggest that these results reflect a decrease in the ability of the free fourth chromosome to direct the orientation of its homolog in *T(Y;2h;4)CB25; tef* males. The orientation of the translocation on the meiotic spindle may be dictated by the relative strength of the pairing interactions between the sex chromosomes compared to that of the autosomal portions. If so, then the absence of stable interactions between the fourth chromosomes may increase the frequency of orientation of the translocation opposite the X chromosome. This interpretation implies that the *tef* gene product normally plays a role in orientation of the fourth chromosome bivalent at meiosis I either directly or indirectly through the establishment or maintenance of homolog connections.

We examined the segregation of two additional translocations in *tef* males to determine if the *tef* effect was general or specific to *CB25*. We previously isolated two *X;4* translocations in which the fourth chromosome is appended to the short right arm of the X. Both of these translocations (called *mscd1* and *mscd2* for *male sex chromosome disjunction*) cause nondisjunction of the sex chromosomes and can pair in a trivalent with the free fourth chromosome and the Y at meiosis I (Briscoe and Tomkiel 2000). In homozygous *tef^{z5864}* males, each *mscd* translocation segregated from the Y chromosome with higher fidelity than in *tef^{z5864}/ Cy* siblings (Table 4). These results are consistent with the hypothesis that *mscd* sex chromosome nondisjunction results at least in part from trivalent formation with the fourth chromosome and that *tef* mutations alter this pairing configuration or its consequences on orientation.

Cytological examination of meioses in *tef* males: We examined DAPI-stained meiotic chromosome spreads from squashes of testes from *tef* males and their heterozygous *tef/+* brothers for visible defects in chromosome behavior. Males homozygous for each of the four alleles were examined, as well as *trans*-heterozygotes for the *tef^{4169}* and *tef^{5864}* alleles. Phenotypes were similar in all *tef* allelic combinations. The earliest abnormality was detected at the S6 stage of spermatocyte growth (Cenci *et al.* 1994). At these stages in wild-type males, the two major autosomes and sex chromosome bivalents appear as three discrete DAPI-positive bodies. The fourth chromosomes can occasionally be visualized as a small spot, but because of their small size are not always visible. This stage is considered to be late meiotic prophase, although prophase in Drosophila spermatocytes differs from that of most organisms in that the progressive condensation and synapsis of homologs that can be seen in most organisms does not occur. Rather, the chromatin remains in three large clumps and a small spot. Real-time observations of chromosome condensation and movements in spermatocytes expressing the green fluorescent protein-labeled histone H2AvDGFP (Clarkson and Saint 1999) confirm that these clumps each contain a single bivalent (J. Tomkiel, unpublished results). Whether homologs are paired in these clumps or are merely associated as a vestige of mitotic somatic pairing

TABLE 3

Sex and fourth chromosome disjunctional data from *y/y+ T(Y;2h;4)CB25; tef ; spa pol* males crossed to *y w sn ; C(4)ci ey/0* females

	Recovered male gametes							Total (%)			
Paternal genotype	*T(Y;2h;4);0*	*X;4*	*0;4*	*X/T(Y;2h;4);0*	*X;0*	*0;0*	Total	*X/T(Y;2h;4);0*	*0;4*	*X;0*	*0;0*
+	1573	1122	67	88	325	0	3175	2.8	2.1	10.2	0
tef^{z4169}/tef^{z4169}	629	428	3	0	296	3	1359	0	0.2	21.8	0.2
$tef^{z4169}/SM1,Cy$	351	288	12	17	58	0	726	2.4	1.7	8.0	0
tef^{z5549}/tef^{z5549}	289	167	0	0	155	1	612	0	0	25.3	0.2
$tef^{z5549}/SM1,Cy$	383	358	25	66	128	4	964	6.8	2.6	13.3	0.4
tef^{z1869}/tef^{z1869}	935	643	9	15	299	0	1901	0.8	0.5	15.7	0
$tef^{z1869}/SM1,Cy$	244	266	16	16	27	7	576	2.8	2.8	4.7	1.2
tef^{z5864}/tef^{z5864}	348	293	2	0	168	0	811	0	0.3	20.7	0
$tef^{z5864}/SM1,Cy$	327	254	28	19	54	13	695	2.7	4.0	7.8	1.9

is unknown. The end of this stage is characterized by the disassembly of the network of microtubules surrounding the nucleus and the appearance of asters, which begin to migrate to opposite sides of the spermatocyte nucleus (Cenci *et al.* 1994). In *tef* males, the homologs of either one or both of the autosomal bivalents were separate in 10% of the S6 stage meiocytes examined (143/1400, Figure 1). This phenotype was never observed in over 1000 *tef/+* and wild-type meiocytes examined at this stage.

In squashed preparations of *wildtype* or *tef/+* metaphase I cells, the three major bivalents and the fourth chromosome bivalent often appear as three large and one small DAPI-positive body (Figure 2A). Thus, the forces placed on the cell during squashing often disperse chromosomes from the metaphase plate, but very rarely disrupt homolog associations. The only homologs that are typically separated in wild-type squashes are the fourth chromosomes. The fourth chromosomes often disjoin slightly before the other bivalents (Lin *et al.* 1981). In contrast, in *tef* meiocytes, unpaired autosomes were frequently observed, often at or near the spindle poles, whereas the sex bivalent was intact and often positioned medially between the poles (Figure 2, B–D). In addition, the precocious separation of the fourth bivalent appeared exaggerated in *tef* cells, as the fourth chromosomes had often reached the spindle poles before the sex chromosomes had visibly separated (Figure 2D). These phenotypes suggest that *tef* either weakens autosomal homolog associations or alters the timing of their dissolution.

To avoid the possibility of artifacts introduced by squashing, we also examined meiocytes in whole mounts of dissected or intact testes using confocal microscopy. Meiocytes from *tef/+* and *tef* males were fixed and stained with antitubulin antibodies and DAPI, and spindle morphology and chromosome distributions were compared. No defects in meiotic spindles could be detected in *tef* mutants. In 50 *tef/+* cells, all chromosomes were observed together in a single cluster on the metaphase plate at stages M2 and M3. These stages correspond to the end of prometaphase, in which chromosomes have nearly completed (M2) or completed (M3) congression to the metaphase plate (Cenci *et al.* 1994). In *tef/+* control males, the chromosomes were usually clustered such that boundaries of individual chromo-

TABLE 4

Sex and fourth chromosome disjunctional data from *T(1;4)y mscd* males crossed to *y w sn ; C(4)ci ey/0* females

	Recovered male gametes						Total (%)			
Paternal genotype	T(1;4);4	Y;4	T(1;4)/Y;4	0;4	Y;0	0;0	*T(1;4)/Y;4*	*0;4*	*Y;0*	*0;0*
T(1;4)y mscd1; tef^{z5864}/tef^{z5864}	219	111	0	0	70	0	0	0	17.5	0
T(1;4)y mscd1; $tef^{z5864}/SM1$	526	230	4	20	0	0	0.5	2.6	0	0
T(1;4)y mscd2; tef^{z5864}/tef^{z5864}	388	143	0	1	83	1	0	0.2	13.5	0.2
T(1;4)y mscd2; $tef^{z5864}/SM1$	1475	526	61	316	134	0	2.4	12.6	5.3	0

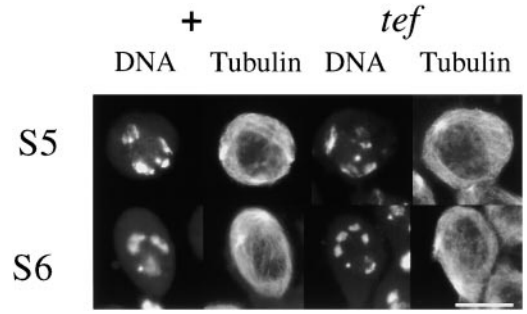

FIGURE 1.—DAPI-stained primary spermatocytes from *tef*^{z5864} and sibling *tef*^{z5864}/*Cy* males, showing normal appearance of chromatin and microtubules in *tef* males during the S5 stage of spermatocyte growth. In contrast, a separation between homologs of the autosomal bivalents is observed at late prophase, stage S6, in *tef* meiocytes.

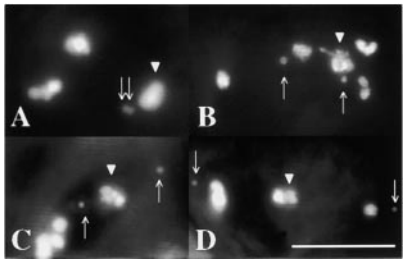

FIGURE 2.—DAPI-stained meiotic chromosome squashes. (A) *tef*^{z5864}/+. (B) *tef*^{z5864}. (C) *tef*^{z4169}. (D) *tef*^{z5864}/*tef*^{z4169}. Arrowheads point to the paired sex chromosomes, and the arrows point to the fourth chromosomes, which are unpaired in *tef* meiocytes. The major autosomes are also unpaired (B) and are often positioned unevenly on the spindle (C and D). Bar, 10 μm.

somes could not be discerned, but in some cells single bivalents were observed slightly separated from the plate (Figure 3, top). These were presumed to be in M2 and still in the process of congression. In 10/50 *tef*/+ cells one or both fourth chromosomes were observed slightly separated from the other chromosomes. In contrast, in stage M2 and M3 *tef* cells the chromosomes were dispersed along the pole-to-pole axis. In 72 metaphase *tef* cells in which the fourth chromosomes could be seen, they appeared unpaired (Figure 3, middle and bottom). In 48/96 metaphase cells, homologs of at least one of the major autosomes were also unpaired. In an additional 22 cells, the autosomal bivalents had an unusual dumbbell-like shape or were separated by a slight gap, suggesting that they were not as tightly associated as in wild type (Figure 3, middle). These bivalents were also often displaced from the metaphase plate, perhaps also indicating a problem in orientation. In contrast, in all *tef* cells in which the sex chromosomes could be identified, they were closely apposed in a configuration not discernibly different from wild type. These observations suggest that pairing and/or subsequent conjunction, specifically between autosomal bivalents, is defective in *tef* mutants and that subsequent failure of unpaired or weakly paired autosomes to orient to opposite poles results in nondisjunction.

DISCUSSION

Our genetic and cytological observations of meiotic chromosome behavior in *tef* mutants point to a role of the wild-type gene product in the establishment or maintenance of autosomal homolog pairing at meiosis I in males. None of the *tef* allelic combinations tested affect sex chromosome segregation, whereas our observations are consistent with nearly random segregation of the autosomes. Univalent chromosomes, including a mini-X derivative and a compound-4 chromosome, are transmitted normally from *tef* males, suggesting a defect in a pairing-dependent process. Consistent with this interpretation, we found no effects of the mutations on germline mitoses or meiosis II. Additionally, the behaviors of three translocations between sex chromosomes and chromosome 4 were altered in a manner suggesting that the *tef* gene product is required for orientation of the fourth chromosomes, a function dependent on bivalent formation and maintenance.

We found no effect of the *tef* mutations on female meiosis. This could mean that *tef* defines either a pathway not operable in female meiosis or one that is redundant with other segregation mechanisms. Expression and localization studies of the *tef* gene product(s) will also be informative in determining if its role is limited to male meiosis.

The phenotypes we have observed in *tef* mutants are similar to those described for two alleles of *meiS8* (*meiotic mutant of Salaria #8*). Like *tef* mutations, the *meiS8* alleles were male specific and caused meiotic nondisjunction and loss of fourth chromosomes, but did not affect sex chromosome transmission and mapped to 80 MU (SANDLER *et al.* 1968). Because both alleles of *meiS8* have been lost, we could not test for complementation by the *tef* mutations. The *tef* and *meiS8* mutations may identify the same gene or functionally related genes that are physically linked.

Our cytological observations failed to reveal any differences between *wildtype* and *tef* spermatocytes until late prophase. Therefore, we speculate that the *tef* defect is in the maintenance rather than the initiation of homolog pairing. It is not known, however, to what extent the early prophase chromosome configuration reflects meiotic *vs.* premeiotic homolog associations. In Drosophila, homologous chromosomes are paired in somatic cells (METZ 1916). It is unknown if similar homolog associations occur in mitotically dividing germline cells. To the extent that premeiotic associations might contribute to the early prophase chromosome configuration, it is difficult to rule out a role of *tef* in meiotic pairing initiation on the basis of cytology alone. Molecu-

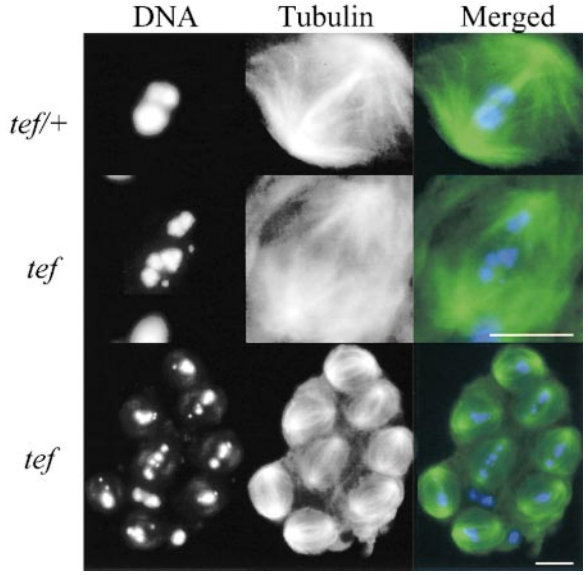

Figure 3.—Confocal images of whole-mount meiosis I metaphase cells (stage M2–3) from either $tef^{z5864}/+$ or tef^{z5549}/tef^{z5864} males, stained with DAPI (blue) and antitubulin antibodies (green). (Top and middle) Single meiocytes. (Bottom) One-half of a 16-cell cyst. Note the separation between autosomal homologs, particularly the dot-like fourth chromosomes, in *tef* cells. Bars, 10 μm.

lar characterization of the *tef* gene and the temporal and spatial distribution of its protein may help clarify this issue. In addition, it will be important to confirm that a null allele does not show earlier or more severe defects in homolog associations in meiosis and/or mitosis. All four alleles and allelic combinations show essentially random segregation of the autosomes, suggesting that the phenotypes we have observed are likely to reflect the null phenotype. Further genetic and/or molecular characterization of these mutations will be required to determine if any are true null alleles.

The observation that *tef* mutations have no detectable effects on sex chromosome segregation, whereas the behavior of all of the autosomes is severely disrupted, strongly suggests that there are regulatory and/or mechanistic differences between sex chromosome and autosome pairing. A number of observations have previously suggested that sex chromosomes and autosomes differ in their conjunctive mechanisms. First, Baker and Carpenter (1972) isolated a collection of male meiotic mutants that affected sex chromosome, but not fourth chromosome, segregation. These mutations all caused meiotic drive, a characteristic of deletions of X chromosome pairing sites (Lindsley and Sandler 1958; McKee and Lindsley 1987) suggesting that they might be specific for the establishment or maintenance of XY pairing. Second, the distribution and usage of homologous sequences for pairing differs between sex chromosomes and autosomes. Autosomes can pair using different sequences at multiple sites along the euchromatic chromosome arms (McKee *et al.* 1993). In contrast, sex chromosome pairing is restricted to a single region of homology between the X and Y (Cooper 1964), despite the presence of other homologous sequences that could potentially be used as pairing sites (reviewed in Robbins 1999). Whereas the XY pairing sites are embedded in the heterochromatin, heterochromatin is ineffective at promoting autosome pairing in males (Yamamoto 1979; Hilliker *et al.* 1982). Last, the sex bivalent is uniquely associated with a fibrillar material, which may or may not be related to a unique conjunctive mechanism (Ault *et al.* 1982; Ault and Reider 1994). Elucidating the activity of the *tef* gene product may yield insight into the significance of the differences between sex chromosome and autosome pairing.

Whether there may be a selective advantage for differences in sex chromosome and autosome conjunction is unclear. The differences may instead be a consequence of the evolution of heteromorphic sex chromosomes. The lack of male recombination is a common feature of higher Diptera (suborder Brachycera; White 1973; Gethmann 1988). This conforms to the rule that, with rare exceptions (Fang and Jagiello 1991), recombination levels are lower in the heterogametic sex of a sexually dimorphic species. It is generally accepted that heteromorphic sex chromosomes evolve via an initial sex-determining allelic difference on a homologous pair of autosomes. This is followed by selection for linkage between sex-specific favorable mutations and the progenitor sex-determining alleles (for recent discussion see Lucchesi 1999). A decrease in sex chromosome recombination would in turn select for a recombination-independent mechanism of conjunction. It is possible that the selection for decreased XY recombination might favor the fixation of mutations that generally abolish male recombination for both sex chromosomes and autosomes. In species such as Drosophila, the small chromosome number would minimize the deleterious effects of such mutations, because even random segregation of the autosomes would still allow for the production of an appreciable number of euploid gametes. If such a recombination-defective mutation occurred early in the evolution of heteromorphic sex chromosomes, one might expect the evolution of a single conjunctive mechanism for both sex chromosomes and autosomes. If the putative mutation arose after the sex chromosomes had already begun to evolve a recombination-independent mechanism of bivalent attachment, then one might expect that a conjunctive mechanism for the autosomes would evolve independently and may differ from that of sex chromosomes.

Comparative studies of the *tef* gene in various Diptera may provide a tool for probing the relationship between the elimination of male recombination, the evolution of heteromorphic sex chromosomes, and the evolution of separate mechanisms that ensure proper segregation of the sex chromosomes and autosomes.

We are especially grateful to C. Zuker and the members of his lab, E. Koundakjian, R. Hardy, and D. Cowen, for allowing us to screen their collection of EMS-mutagenized strains. We also thank L. Mayernik of the Center for Molecular and Cellular Toxicology core facility for assistance with confocal microscopy, and D. Lindsley, C. Herrera, A. Gaston, and A. Richardson for technical assistance. We appreciate the helpful comments of G. Yasuda and O. J. Miller. This work was supported by National Institutes of Health grant GM54769 to J.E.T. and National Science Foundation awards HRD-9627074 and DCB-9506927 to B.T.W.

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Communicating editor: R. S. Hawley