THOMAS, AMANDA L., M.S. A Screen for Modifiers of *teflon* Identifies Novel Components of the Meiotic Segregation Pathway in Male *Drosophila melanogaster*. (2007)
Directed by Dr. John Tomkiel. 62pp.

The *teflon (tef)* gene is required specifically for ensuring adhesion between autosomes in male meiosis. In *tef* mutants, autosomal homologs pair correctly, but separate prior to metaphase, resulting in random segregation of homologs at meiosis I .

To identify genes that interact with *tef*, we have performed a screen for dominant second site modifiers of a hypomorphic allele, tef^{P1150}. We have tested a collection of third chromosome deletions, which collectively remove ~90% of the third chromosome, as well as a collection of previously identified male meiotic mutants (Wakimoto et al. 2004).

We identified 15 regions containing Enhancers, and 6 regions containing Suppressors of *tef*. One of the enhancing deletions removes *mod(mdg4)*, which has been previously proposed to interact with tef to ensure autosomal conjugation (Thomas et al. 2005). A second enhancer was mapped to *autophagy specific gene 2 (atg2)*, which had not previously been implicated in the meiotic homolog segregation pathway in *Drosophila* males.

A SCREEN FOR MODIFIERS OF *TEFLON* IDENTIFIES NOVEL COMPONENTS OF THE MEIOTIC SEGREGATION PATHWAY IN MALE *DROSOPHILA MELANOGASTER*

By

Amanda L. Thomas

A Thesis Submitted to
The Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
2007

Approved by

Dr. John Tomkiel
Committee Chair

To

My mother, father, and sister

1 Cor 13:13

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at the University of North Carolina at Greensboro.

Committee Chair Dr. John Tomkiel

Committee Members Dr. Dennis LaJeunesse

Dr. Karen Katula

April 30th, 2007
Date of Acceptance by Committee

April 30th, 2007
Date of Final Oral Defense

ACKNOWLEDGEMENTS

There are several people without whom this work would not have been possible

I would like to thank my advisor, Dr. John Tomkiel, for his guidance and support throughout my Undergraduate and Graduate career. I am very grateful for his time and the resources spent for my intellectual and professional development.

I would like to thank my committee members, Dr. Dennis LaJeunesse and Dr. Karen Katula for their comments, guidance, and support in preparation of this thesis work. Also to Dr. LaJeunesse for providing the β-tubulin antibodies.

I am thankful to Dr. Barbara Wakimoto for providing the Zuker Collection of male meiotic mutants. Also, to Dr. Bruce McKee and Sharon Thomas for personal communications regarding *mnm* as well as other meiotic mutants.

My time in the Tomkiel Lab has been an experience invaluable to my personal, professional and intellectual growth. I would like to thank my colleagues Ms. Maiko Masuda, Mr. Krishn Sharma, Ms. Wendy Johnson, Mr. Antony Miller, Mr. Louis Bryan, Ms. Gunjan Arya, Mr. Matthew LoDico, Ms. Stephanie Owen, Mr. Stephen Gegick, Ms. Aprille Griffin, and Mr. Rohul Amin, for their support throughout my time in the lab.

Finally, I would like to thank my parents, Mr. Larry Thomas, and Mrs. Joan Thomas, and my sister, Ms. Heather Thomas for their continued love, and for their support in everything that I do.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

In order for sexual reproduction to occur successfully, a diploid organism must reduce its chromosome number by half so that a union between its gametes and those of another of the same species will produce an euploid zygote. The process by which this halving of chromosomes occurs is meiosis. Meiosis has two stages, Meiosis I in which homologous chromosomes are partitioned into two separate cells, and Meiosis II in which each homolog is divided into sister chromatids that are then segregated into two cells. The overall outcome of meiosis is that a single diploid cell goes through two stages of chromosomal division to form haploid gamete cells.

Proper segregation of homologs during meiosis I is essential for the survival of the resulting zygote as well as for its development. Erroneous homolog segregation is the cause of genetic syndromes such as Turner, Klinefelter, and Down's Syndromes, and contributes a significant percentage of miscarriages (HASSOLD and JACOBS 1984). In a broader context, if we can begin to understand the nature of the meiotic complexes in a non-recombinant or achiasmatic system, it could lead to a better understanding of how human aneuploidies occur when recombination is reduced or abolished as it is in a number of human trisomies (BUGGE *et al.* 1998; LAMB *et al.* 1997; ROBINSON *et al.* 1998).

Ensuring proper homolog segregation is an evolutionary conserved process that shares similarities across many species, although the specific complexes involved vary both between and within species. Regardless of the organism, three events must happen to ensure that homologs segregate properly at anaphase of meiosis I. First, each homolog must find its partner and pair. Secondly, there must be some form of adhesion or connection between the two homologs to ensure that they remain together until they reach the metaphase plate. Finally, at the right time pairing between homologs must be abolished, allowing partners to segregate to opposite poles of the dividing cell.

Different mechanisms have evolved to ensure or facilitate homolog pairing, and multiple studies have been done in a variety of organisms in attempt to understand this process. During leptotene of prophase I in fission yeast *Saccharomyces pombe*, chromosomes condense and their telomeres can be seen associating near the spindle pole body. This bouquet formation has been found to promote alignment of homologous sequences for pairing (CHIKASHIGE *et al.* 1994). Similar phenomenon, called a horsetail stage, have been seen in budding yeast *Saccharomyces cerevisiae* (TRELLES-STICKEN *et al.* 1999) and in *zea maize* (GOLUBOVSKAYA *et al.* 2002). In all cases these formations seem to be important in tethering the telomeres of homologous chromosomes adjacent to one another. This alignment of homologous sequences is needed to facilitate proper pairing and synapsis.

In the male fruit fly *Drosophila melanogaster*, there are genetically separable pairing pathways for autosomes and sex chromosomes. It has been shown that pairing between autosomes requires the presence of euchromatic pairing sites on homologous

chromosomes, as rearrangements or deletions of heterochromatic satellite DNA does not affect autosomal pairing (YAMAMOTO 1979). Other evidence has shown that translocations of any second chromosome euchromatin to the Y results in pairing of the translocated euchromatin with the intact second homolog (MCKEE *et al.* 1993). While autosomal pairing seems to be dependent upon euchromatic homology, there is no euchromatic homology between the X and Y chromosome. Rather, the X and Y use cis-acting heterochromatic pairing sites that reside in the intergenic spacers of the rRNA genes (MCKEE and KARPEN 1990; REN *et al.* 1997). Pairing ability has been mapped to 240 bp repeats in the promoter regions of the rDNA cistrons, which reside in clusters within the heterochromatin of both the X and Y chromosomes. As few as six of these repeats are sufficient to restore complete pairing ability to an rDNA-deleted X chromosome (MCKEE and KARPEN 1990).

Studies in *Caenorhabditis elegans* have shown that pairing is facilitated by chromosomal pairing centers that act as sites for binding of proteins necessary to stabilize pairing, and subsequently facilitate synapsis (MACQUEEN *et al.* 2005). HIM-8 is a zinc finger protein that is required for proper pairing and synapse of the X chromosome in *C. elegans*. HIM-8 is recruited to the pairing center and is involved in associating the chromosome with the nuclear envelope. A point mutation in HIM-8 that neither abolishes localization to the pairing center nor changes the association with the nuclear envelope does not properly establish homolog stabilization, suggesting that tethering to the nuclear envelope may be important, but is alone not sufficient for pairing or synapsis (PHILLIPS *et al.* 2005).

While pairing seems to be accomplished via a variety of processes, it seems that sequence homology in either the euchromatin, heterochromatin, or both is necessary for the homologs to pair properly. In most cases, once the homologs have found and paired with their partner, they must remain together throughout prophase and maintain their interaction to ensure proper alignment at metaphase. While the most common and best understood mechanism to ensure homologs remain together until anaphase involves recombination between homologs, there are other mechanisms that are less well characterized. Understanding these alternative pathways for chromosome segregation may shed light on some of the early aspects of chromosome pairing that are obscured or complicated by processes involved in recombination.

During prophase of meiosis I in recombination-proficient organisms, homologous chromosomes pair and then recombine. Homologous recombination, or crossing over, is the exchange of genetic material between maternal and paternal chromosomes that contributes to the genetic diversity that is a hallmark of sexual reproduction. Crossovers also ensure that homologs remain in close proximity to each other prior to metaphase. The process of recombination involves the utilization of double strand break and double strand break repair proteins (for review see (SZOSTAK *et al.* 1983). More importantly for the process of homologous segregation, it involves the formation of recombination sites called chiasmata. These chiasmata are essential for the maintenance of a physical interaction between homologs after pairing, and are also involved in the orientation of centromeres.

In yeast it has been shown that cohesin proteins that maintain sister chromatid cohesion play a part in stabilizing homologous chromosomes after recombination has occurred. The meiotic form of one of these yeast cohesion proteins, Rec8, is required to stabilize homologs by physically holding recombinant sister chromatids together. The Rec8-mediated physical connections between sister chromatids prevents the resolution of chiasmata, keeping homologs as well as sister chromatids together until Rec8 is cleaved by separin at the metaphase-anaphase transition. Release of sister chromatid cohesion distal to chiasmata allows their resolution and the ensuing segregation of homologs to opposite poles (BUONOMO *et al.* 2000).

In the absence of recombination, some species modify a recombination-associated structure, the synaptonemal complex (SC), to take the place of the chiasmata. The SC is a three part structure consisting of two lateral elements that physically interact with one arm of each homolog, and a central element that physically interacts with the two lateral elements (WETTSTEIN *et al.* 1984). In non recombinant organisms utilizing this complex, a modified SC is retained through late stages of meiosis, rather than disassembling at the end of pachytene as it does in recombinant-proficient species. This retained SC appears to physically bind the homologues together until they begin to segregate to opposite poles at anaphase I (RASMUSSEN 1973).

In some organisms however, both chiasmata and SC are absent. In non-recombinant organisms such as male *Drosophila melanogaster*, pairing and adhesion are accomplished in the absence of both recombination and SC by a different, albeit poorly understood pathway. Once homologs are paired, however, the behavior of bivalents in

males differs in an unexpected way. Vasquez et al. (2002) used transgenic flies expressing a GFP-Lac repressor fusion protein to label several euchromatically integrated arrays of LacO sites, and expression of a GFP fusion protein with the centromere protein CID, to visualize the centromeres. This system takes advantage of the Lac Operon system in which the LacI repressor, fused to the green fluorescent protein, will tightly bind to any Lac operator (LacO) sites on the chromosome. When excited with fluorescent light, the GFP fluoresces at the integrated LacO sites where it is bound. This allowed Vasquez et.al to analyze the distance between those GFP foci on each sister chromatid in a homologous pair. In males homozygous for a particular LacO array, a single spot was observed in nuclei of most premeiotic cells (spermatogonia) at interphase, suggesting a very tight association of both sister chromatids and homologous pairs prior to meiosis. This pairing is maintained through prophase in primary spermatocytes, implying that homologs enter meiosis already paired and that pairing persists through the early stages of prophase I (stage S2). During the early primary spermatocyte stage S3, homologous chromosomes are segregated into distinct nuclear domains along the periphery of the nuclear envelope. The nature of this physical separation and segregation of chromosomes into territories is unknown, but it has been suggested that the chromosomes may somehow be tethered to the nuclear envelope (VAZQUEZ *et al.* 2002). Regardless of the mechanism involved in the creation of nuclear territories, the GFP-Lac system shows that homologous chromosomes enter these domains already paired.

By spermatocyte stage S5 (mid prophase), four distinctively separate LacO spots are observed. The presence of four spots in this stage of meiosis suggests that

homologous pairs, as well as sister chromatids, have separated at the observed locus. At this point it seems as though the mechanism responsible for maintaining intimate pairing between homologous loci is relaxed or abolished, although homologs and sister chromatids still remain in proximity within the nuclear domains. Vasquez et al. (2002) also showed that homologs reestablish a closer physical proximity to each other upon chromosome condensation, and these associations are maintained until the metaphase-anaphase transition. Whether homologs somehow remain attached or "re-pair" at these later stages is unknown. Interactions do not appear to be maintained via euchromatic interactions nor by the centromeres, as CID-GFP fluorescence shows distinctly separate centromeres and euchromatic LacO loci never re-associate as closely as in S3 (VAZQUEZ *et al.* 2002). The "re-pairing" of homologous chromosomes within the domains before their separation at anaphase is essential for the correct segregation of the chromosomes. Since both euchromatic interactions and centromere interactions seem to be excluded from the unknown mechanism for this pairing, it has been hypothesized that associations within these domains may be limited to pericentric heterochromatin, and that initiation and maintenance of pairing may be two mechanistically different processes (VAZQUEZ *et al.* 2002). Alternatively, chromosomes may completely unpair within domains, and re-establish pairing at the end of prophase.

In male fruit flies, recent studies have begun to elucidate genetic pathways regulating the maintenance of pairing. An extensive collection of genes involved in meiosis was identified by screening for paternal fourth chromosome loss (WAKIMOTO *et al.* 2004). One of the mutants revealed in this screen, and a component of the pairing

pathway in males is *teflon (tef)*. Tef is required for proper segregation of autosomal homologues during meiosis I (TOMKIEL *et al.* 2001). The *tef* gene maps to salivary gland chromosome band 53F2 on the second chromosome. It encodes a 88 kD protein with three zinc fingers. Zinc fingers are highly conserved protein domains that coordinate with a zinc ion to form a finger-like fold. Because zinc fingers often confer DNA binding ability, it is thought that Tef may bind DNA. Furthermore, characterization of the Tef protein has shown that the three zinc finger domains are necessary for proper function, as point mutations or truncations of the zinc fingers abolish Tef function (ARYA *et al.* 2006). The *tef* gene has homologs in other species of *Drosophila* such as *D. pseudoobscura*, *D. simulans*, and *D. yakuba*. Outside *Drosophila* species there are no known homologs of *tef*, and homology to other proteins is limited to the zinc finger domains (ARYA *et al.* 2006).

Genetic characterization revealed that mutations in *tef* lead to random segregation of fourth chromosome bivalents, but have no effect on the segregation of sex chromosomes. Similar tests were performed to determine if *tef* had any effect on homolog segregation in females, and results of those genetic assays determined that *tef* has no observable effect in female meiosis. To assess if *tef* males had a meiosis II segregation defect, heterozygous *spa*/+ males were crossed to compound-4 females. Compound-4 (C(4)) females have fourth chromosome homologs that are physically fused together. Therefore, these females always produce eggs aneuploid (*nullo-4* or *diplo-4*) for the fourth chromosome. Any *spa* progeny resulting from *tef/tef; spa*/+ males mated to C(4) virgin females would represent a meiosis II defect. No *spa* progeny resulted from

those matings, leading to the conclusion that *tef* specifically affects meiosis I. Further analysis indicated that *tef* is involved in some aspect of pairing (TOMKIEL *et al.* 2001).

Cytological comparisons of *tef* versus wildtype spermatocytes shows a marked difference in the location of autosomes during late prophase (stage S6). Paired autosomes appear as distinct chromatin masses at the nuclear periphery in wildtype spermatocytes, whereas in *tef* mutants autosomal bivalents appear clearly separated from each other, although still within nuclear domains. By metaphase in wildtype spermatocytes all four sets of homologs have moved to the metaphase plate and usually appear as a single large spot. In contrast, in *tef* mutants autosomes often appear as multiple spots off the metaphase plate, suggesting that autosomal bivalents do not maintain the associations necessary to ensure their proper metaphase alignment, or fail to re-pair (TOMKIEL *et al.* 2001).

Recent molecular characterization of *tef* using the bipartite GAL4/ UAS expression system (BRAND and PERRIMON 1993) to regulate expression of a *tef* transgene have shed light on the temporal requirements for *tef*. Four different GAL4 drivers causing expression of a *UAS tef::GFP* transgene in germline cells prior to stage S4 rescue the nondisjunction phenotype in *tef* mutants, whereas a driver causing expression in germline cells after stage S4 fails to rescue. These results indicate that Tef is required prior to stage S4 (mid prophase), when homologs are paired and moving into their nuclear domains, but prior to the stage at which tight connections between homologs have been abolished (ARYA *et al.* 2006).

Current models propose that *tef* either acts in a bridging complex that maintains a physical interaction between paired autosomal homologs, or acts as a transcription factor that regulates other genes involved in pairing maintenance. The protein has thus far not been localized, thus both possibilities are still tenable. Two other proteins thought to be involved in pairing maintenance in *Drosophila* males have been described. *Modifier of mdg4 in Meiosis (mnm)* and *Stromalin in Meiosis (snm)* were shown by Thomas et.al (2005) to have meiotic phenotypes very similar to that of *tef*, with the exception that mutations in these genes also affect sex chromosome conjunction.

Mnm is a meiosis specific allele of *modifier of mdg4 (mod(mdg4)*, a gene that can be alternatively spliced and trans-spliced to produce more than thirty distinct proteins (DORN *et al.* 2001). *Mod(mdg4)* gene products have been implicated in a variety of processes, including position effect variegation, programmed cell death, and control of the gypsy insulator function (BUCHNER *et al.* 2000). The gene consists of a common region that encodes a functionally conserved BTB/POZ domain, and at the C-terminus a variable region that results from trans-splicing of either a 3' or 5' DNA strand (DORN and KRAUSS 2003). It has been proposed that the BTB domain in the common region may be necessary for dimerization of two MNM protein units, while the free carboxyl termini may directly bind homologs (SOLTANI-BEJNOOD *et al.* 2007).

The *snm* gene has not been as well characterized as *mnm*, but is similar at the primary amino acid level to proteins involved in the cohesin complex required for sister chromatid cohesion in both mitosis and meiosis. Thomas et.al. (2005) performed a phylogenetic analysis comparing SNM with its meiotic family members, REC11 and

STAG3, and to its mitotic paralogs SA, PSC3, and STAG1/STAG2. The results of the analysis concluded that SNM was more closely related to its mitotic paralog, Stromalin (SA), than to any members of the meiotic family of proteins. This suggests that *snm* arose as a gene duplication event and has since evolved a new meiotic function (THOMAS *et al.* 2005).

Unlike *tef*, both *mnm* and *snm* are required for sex chromosome as well as autosome segregation at meiosis I. Thomas et.al (2005) introduced a MNM:GFP transgene, which was used in combination with SNM antibody staining to show that MNM and SNM co-localize to the nucleolus along with nucleolar protein fibrillarin early in spermatogenesis. By mid prophase the staining of the nucleolus is replaced by a single spot on one of the major chromosomes, which was later determined via FISH analysis to be on the XY bivalent. With respect to the sex chromosomes, MNM and SNM localization is maintained through metaphase, but gone at the metaphase-anaphase transition.

The MNM-GFP fusion protein was also visualized on the autosomes as multiple spots within the chromatin territories, and consistent with localization to the X-Y bivalent all GFP foci were gone by anaphase. Both GFP fluorescence and antibody staining of SNM failed to localize the protein to the autosomes. This was suggested to be artifactual because antibodies to the common region of Mod(mdg4) also did not produce a detectable signal, therefore general accessibility to the antibody binding regions of MNM and SNM is thought to be problematic, so it is proposed that SNM does actually localize with MNM to the autosomes (THOMAS *et al.* 2005).

Nucleolar localization of MNM-GFP was absent in *snm* mutants, suggesting that MNM localization to the nucleolus is *snm*-dependent. Nucleolar SNM staining was also absent in *mnm* mutants, suggesting that the two proteins are co-dependent for their localization to the nucleolus. MNM-GFP localization was completely absent on sex as well as autosomes in *snm* mutants, and SNM localization to the XY bivalent was absent in *mnm* mutants, suggesting that localization to the sex chromosomes is co-dependent for both proteins, and *snm* is required for localization of MNM to the autosomes (THOMAS *et al.* 2005).

Thomas et.al (2005) also investigated the role of Tef in the localization patterns of MNM and SNM. In *tef* mutants, neither SNM nor MNM-GFP foci were disrupted on sex chromosomes, however MNM-GFP foci were completely absent from the autosomes in *tef* mutants. Taking these data into consideration, Thomas et.al (2005) proposed a model in which *tef* is required to recruit MNM and SNM to the autosomal bivalents, and once there the two proteins may interact to provide a connection between the homologs.

The inability to localize Tef protein makes it difficult to determine where in the pairing and conjunction pathway *tef* is found and where it is necessary. The focus of this study has been to identify other genes involved with *tef* in the pairing and segregation pathway in male *Drosophila melanogaster*.

To aid in fully understanding the nature of the Tef protein, its expression pattern, and the temporal nature of its interactions, several alleles can be utilized. There are five EMS alleles, tef^{4169}, tef^{3455}, tef^{1869}, tef^{5864}, tef^{5349}, all of which fail to complement the nondisjunction phenotype. Homozygous mutations in any of these EMS alleles leads to

completely random segregation of the autosomes at meiosis I, which results in 50% nondisjunction of autosomes in the progeny of the homozygous male.

There is also an allele that results from a P-element insertion at amino acid 102, just before the first intron (See Figure 1). That allele, tef^{P1150} is a hypomorph, such that when it is transheterozygous with a *tef* null allele (i.e. tef^{P1150}/tef) , it results in between 4-8% meiosis I nondisjunction of the fourth chromosome. A tef^{P1150}/ tef^{P1150} male is wildtype with respect to meiosis (<1% nondisjunction). In addition, the tef^{P1150} allele has a mini-white gene [w^{+}] within the P-element insertion. This marker gene produces a small amount of eye color pigmentation that creates orange eyes in a white background, which is advantageous when following the segregation of the tef^{P1150} allele.

The sensitive nature of the hypomorphic P-element allele is a powerful genetic tool that can be used to screen for other genes in the pairing and segregation pathway in *Drosophila* males. If other components of this pathway could be identified, it may shed some light on the nature of pairing and segregation in general, as well as perhaps providing valuable information on the nature of the Tef protein.

Figure 1. **Structure of the *tef* gene showing P-element insertion site**

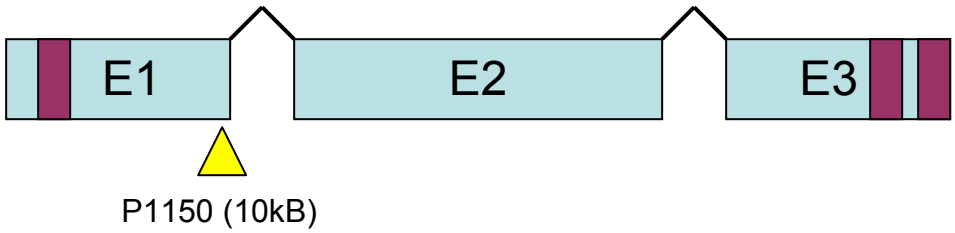

Figure 1. Structure of the *tef* gene showing site of P-element insertion just before the first exon/intron boundary, at the codon specifying amino acid 102 in the full length protein (649 amino acids). Approximate location of regions encoding zinc fingers are indicated as purple boxes.

CHAPTER II

MATERIALS AND METHODS

Drosophila culture and stocks

Deficiency stocks were obtained from the Bloomington Stock Center (www.flybase.org). A collection of EMS-induced male meiotic mutants was kindly provided by B. Wakimoto and C. Zuker (KOUNDAKJIAN *et al.* 2004; WAKIMOTO *et al.* 2004). The *tef* alleles used, an EMS null alleles (*z5864*) and a hypomorphic P insertion allele (*P1150*) have been previously described (ARYA *et al.* 2006). All crosses were maintained on standard cornmeal, molasses, yeast, agar medium at 25˚C.

Modifier screen for *En(tef)* or *Su(tef)*

A collection of third chromosome Deficiency-bearing chromosomes known as the Bloomington Deficiency kit were tested for dominant effects on the incidence of *tef*-induced fourth chromosome nondisjunction. These deficiencies collectively delete 90% of the third chromosome euchromatic genes. Test males and sibling control brothers were generated that were transheterozygous for tef^{z5864} and tef^{P1150} , bearing either a third chromosome deficiency (test) or a Balancer chromosome (control)(Figure 2). Individual test and control males were mated to 3-5 *ywsn;C(4)ciey* virgin females, and progeny were scored for fourth chromosome nondisjunction on days 13, 15 and 18 (Figure 2).

The paternal fourth chromosome homologs are homozygous for *spa*, and contain a wildtype copy of both *ci* and *ey* genes. The maternal compound fourth chromosomes

are homozygous for both *ci* and *ey*, but contain a wildtype copy of the *spa* gene. Therefore paternal nondisjunction can be inferred from the phenotype of the progeny, where normal segregation of the paternal fourth chromosome results in wildtype progeny, and a nondisjunction event will produce sparkling (diplo-4), or cubitus interruptus, eyeless (nullo-4) exceptional progeny (Figure 3).

Figure 2 Crosses used to generate test and control males for dominant second site modifier screen for *En(tef)* and *Su(tef)*

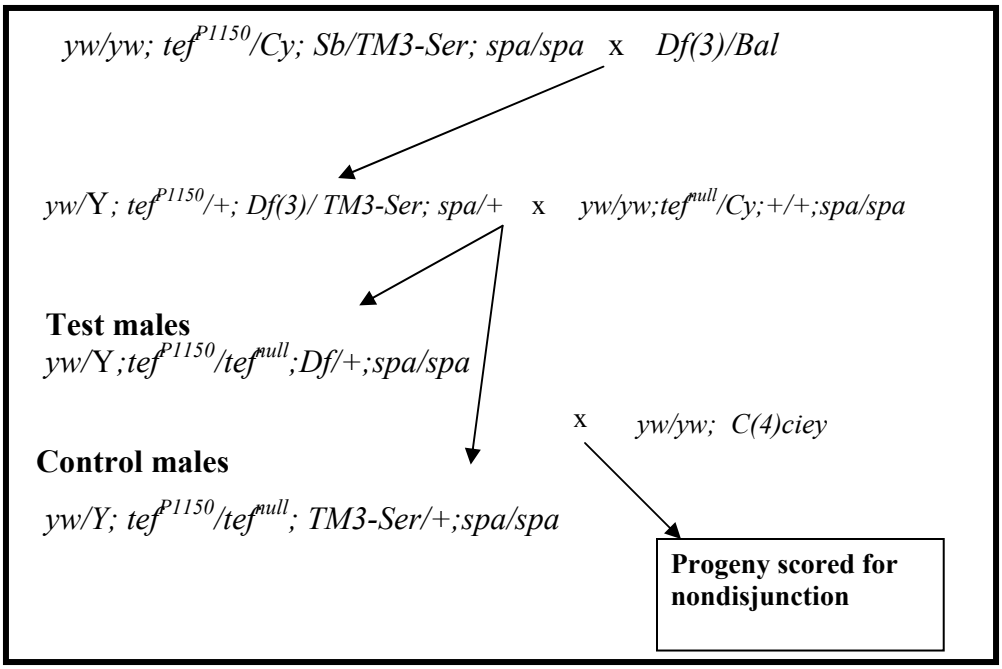

Figure 2. Crosses used to generate test and control males for dominant second site modifier screen for *En(tef)* and *Su(tef)*

Figure 3 Possible progeny of test and control males in a screen for *Modifiers of tef*

♂ \ ♀	*0*	*C(4)ciey*
spa (normal)	*spa* **(Minute)Dead**	*C(4)ciey/spa* **Wildtype**
spa/spa (diplo-4)	*spa/spa* **Spa**	*C(4)ciey/spa/spa* **(Minute)Dead**
0 (nullo-4)	**Dead**	*C(4)ciey* **ci ey**

Figure 3. Possible progeny of test and control males. Nullo and diplo-4 gametes will produce *ci ey* or *spa* progeny respectively (shaded green). Normal segregation will produce progeny that are wildtype (shaded yellow).

Chi-square analysis was performed to determine statistical significance of data. Deficiencies in test males that resulted in a frequency of nondisjunction at least 1.8x greater than that of their control siblings, and that were statistically significant to ($p>0.05$) were classified as Enhancers, whereas deficiencies that resulted in a frequency of nondisjunction at least 1.8x lower than that of the control males, and that were statistically significant were classified as Suppressors. A minimum of 200 progeny from each test and control genotype was scored.

Deficiency mapping of regions containing *En(tef)* or *Su(tef)*.

Smaller deficiencies were obtained which overlapped the two strongest enhancers of *tef*. For *Df(3L)BSC23* (62E8-63B6), overlapping deficiency *Df(3L)Exel6091* (62E8-62F5) was tested, as were individual P-element mutations in genes *CG1240* (*PBac{w[+mC]=WH}Mrtf[f00366]* stock#18321), *CG12093* (*PBac{w[+mC]=WH}CG12093[f07141]* stock#19050), *atg2* (*P{w[+mC]=EP}Atg2[EP3697]* stock#17156, and *P{GawB}NP7457* stock#Kyoto105468), and *pgant6* (*PBac{w[+mC]=RB}pgant6[e00279]* stock#17836).

For overlapping deficiencies *Df(3L)vin5* (68A2-69A3) and *Df(3L)vin7* (68C8-69B5), smaller deficiencies *Df(3L)ED4470* (68A6;68E1), *Df(3L)ED4475* (68C13;69B4), *Df(3L)BK9* (68E;69A1), *Df(3L)Exel6115* (68E1;68F1), and *Df(3L)Exel6116* (68F2;69A2) were obtained and tested through the same screen in which the original overlapping deficiencies were identified as enhancers.

Overlapping deficiencies *Df(3R)e-F1* (93B6-93E2) and *Df(3R)e-N19* (93B-94), both of which contain the *mod(mdg4)* locus were mapped by directly testing two different EMS null alleles of *mod(mdg4)*, *z3-3298* and *z3-5578*.

Screen of third chromosome male meiotic mutants for *En(tef)* and *Su(tef)*

EMS male meiotic mutations were screened for dominant modification of *tef*-induced fourth chromosome nondisjunction in the same manner as described for the deficiency screen. (See Figure 2 for comparable scheme).

Test for sex chromosome nondisjunction in *tef^{P1150}/tef;atg2/+* males

To test for an effect on sex chromosome segregation, males of genotype *w/y+Y; tef^{P1150}/tef;atg2^{Kyoto105468}/+;spa* were produced. To generate these test males, *yw/Y^{y+};tef/Cy;spa* males were mated to *w; atg2^{Kyoto105468}* virgin females, and progeny of genotype *w/ Y^{y+};tef/+;atg2^{Kyoto105468}/+;spa/+* were collected on days 13-18 and crossed to *yw;tef^{P1150}/Cy;TM3-Ser/Sb;spa* virgin females. The *atg2^{Kyoto105468}* insertion is marked with a wild-type copy of the *white* gene which gives eyes a red pigmentation indistinguishable from the wild-type eye color in a *white* background. Therefore, it is not possible to distinguish the genotype of *yw/Y^{y+}; tef/tef^{P1150}; atg2^{Kyoto105468}/TM3-Ser or Sb;spa* vs. *yw/Y^{y+}; tef^{P1150}/+; atg2^{Kyoto105468}/TM3-Ser or Sb;spa* progeny resulting from the parental cross of *w/Y^{y+}; tef/+; atg2^{Kyoto105468}/+;spa* to *yw;tef^{P1150}/Cy;TM3-Ser/Sb;spa* parents until the F2 generation, when progeny are scored for nondisjunction of the fourth chromosome. Flies with straight wings, red sparkling eyes, which were Serate or Stubble (genotypes *yw/Y^{y+}; tef/tef^{P1150}; atg2^{Kyoto105468}/TM3-Ser or Sb;spa* or *yw/Y^{y+}; tef^{P1150}/+; atg2^{Kyoto105468}/TM3-Ser or Sb;spa*) were collected, as well as control males of genotype *yw/Y^{y+}; tef/tef^{P1150}; +/TM3-Ser or Sb;spa* and crossed to *yw;C(4)ciey* virgin females (See figure 4). Progeny were collected and scored on days 14, 16, 18 for nondisjunction of sex and fourth chromosomes (Figure 5), and parental genotypes deduced based on the presence or absence of fourth chromosome nondisjunction associated with a *tef^{P1150}/tef* genotype.

Figure 4 Generation of test males for sex and 4th chromosome nondisjunction test

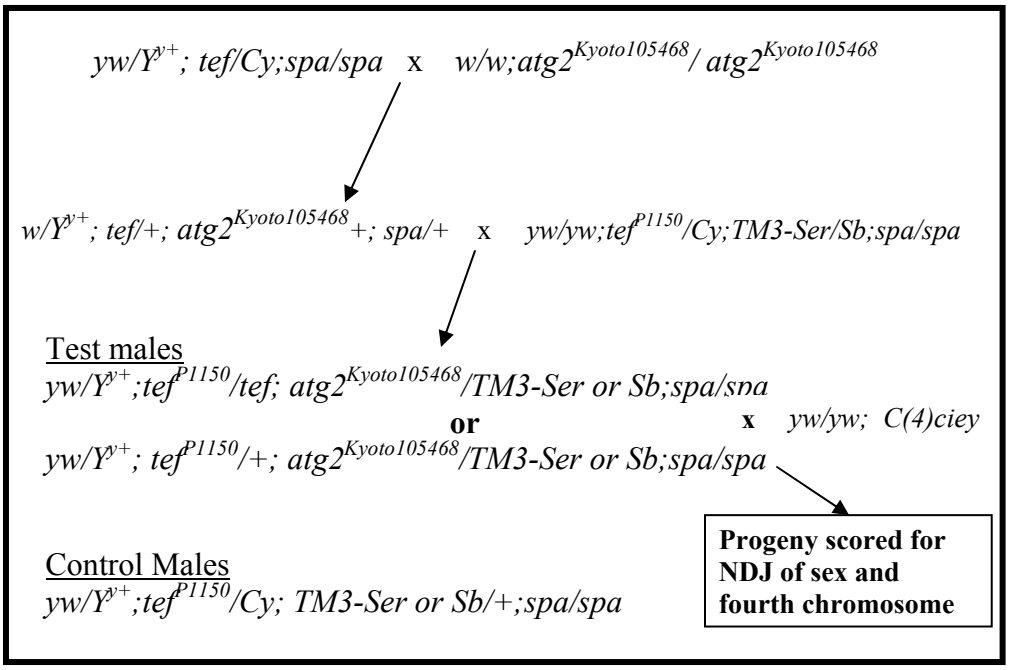

Figure 4. Scheme used to generate test and control males needed to assess percentage of sex chromosome nondisjunction in *yw/Y^{y+};tef^{P1150}/tef; atg2^{Kyoto105468}/TM3-Ser or Sb;spa/spa* males.

Figure 5 Possible progeny genotypes for sex and 4th chromosome nondisjunction test

♂ \ ♀	X^{ywsn}; 0	X^{ywsn}; C(4)ciey
X^{yw}; spa (normal sex, normal 4)	X^{ywsn} X^{yw};spa **Dead**	X^{ywsn} X^{yw};C(4)ciey/spa **yw female**
X^{yw}; spa/spa (normal sex; diplo-4)	X^{ywsn} X^{yw};spa/spa **yw spa female**	X^{ywsn} X^{yw};C(4)ciey/spa/spa **Dead**
X^{yw}; 0 (normal sex; nullo-4)	X^{ywsn} X^{yw};0 **Dead**	X^{ywsn} X^{yw};C(4)ciey **yw ciey female**
Y^{y+};spa (normal sex, normal 4)	X^{ywsn} Y^{y+};spa **Minute Male (Dead)**	X^{ywsn} Y^{y+};C(4)ciey/spa **w sn male**
Y^{y+};spa/spa (normal sex, diplo-4)	X^{ywsn} Y^{y+};spa/spa **w sn spa male**	X^{ywsn} Y^{y+};C(4)ciey/spa/spa **Dead**
Y^{y+};0 (normal sex, nullo-4)	X^{ywsn} Y^{y+};0 **Dead**	X^{ywsn} Y^{y+};C(4)ciey **w sn ci ey male**
X^{yw} Y^{y+};spa (diplo-XY, normal 4)	X^{ywsn} X^{yw} Y^{y+};spa **w spa female Minute (Dead)**	X^{ywsn} X^{yw} Y^{y+};C(4)ciey/spa **w female**
X^{yw} Y^{y+};spa/spa (diplo-XY, diplo-4)	X^{ywsn} X^{yw} Y^{y+};spa/spa **w spa female**	X^{ywsn} X^{yw} Y^{y+};C(4)ciey/ spa/spa **Dead**
X^{yw} Y^{y+};0 (diplo-XY, nullo-4)	X^{ywsn} X^{yw} Y^{y+};0 **Dead**	X^{ywsn} X^{yw} Y^{y+};C(4)ciey **w ci ey female**
0, spa (nullo-XY, normal 4)	X^{ywsn};spa **y w sn spa Minute male (Dead)**	X^{ywsn};C(4)ciey/spa **y w sn male**
0, spa/spa (nullo-XY, diplo-4)	X^{ywsn};spa/spa **y w sn spa male**	X^{ywsn};C(4)ciey spa/spa **Dead**
0, 0 (nullo-XY, nullo-4)	X^{ywsn}; 0 **Dead**	X^{ywsn}; C(4)ciey **y w sn ci ey male**

Figure 5. Possible outcomes of *yw/Y^{y+};tef^{P1150}/tef; $atg2^{Kyoto105468}$/Bal;spa* x *ywsn C(4)ciey*

Cytological characterization of *atg-2* mutants

To assess the meiotic phenotype of *atg-2* mutants, *{w[+mC]=EP}Atg2[EP3697]* (stock#17156) was obtained from Bloomington Stock Center. This P-element line has been described as larval stage lethal (www.flybase.org), although under our rearing conditions $atg2^{P17156}$ homozygotes maintained at 18˚C survive to early pupae. Homozygous *yw/Y;* $atg2^{P17156}$ mutant larvae were dissected in Schneider's Drosophila medium (GIBCO BRL, Gaithersburg, MD), and larval testes viewed under phase microscopy.

To visualize chromosome morphology and possible segregation defects, whole larval testes were dissected in Schneider's Drosophila medium, transferred to silinized coverslips and gently flattened. Slides were then frozen in liquid nitrogen, the coverslips were rapidly removed, then slides were placed in cold methanol for 10 minutes. After fixation, slides were washed 3 times in 1x Phosphate Buffered Saline (PBS; 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4) for five minutes each. After washes, tissues were incubated overnight at 4˚C with mouse monoclonal anti-tubulin antibodies (kindly provided by Dr. Dennis LaJeunesse) diluted 1:200 in PBS + 3% BSA. Three five minute washes in 1x PBS were repeated, and tissues were incubated for 1 hr with goat anti-mouse Cy3 secondary antibodies (Jackson ImmunoResearch Laboratories) diluted 1:1000 in 3% BSA. Two five minute washes in 1x PBS followed by one minute in 0.1µM 4'6-diamidino-2-phenylindole (DAPI), and then mounted in 50% glycerol in PBS.

Alternatively, whole larval testes were dissected in Schneider's Drosophila medium, transferred to a drop of 45% acetic acid on a silinized coverslip and fixed for 5

minutes. Tissue was then squashed, the coverslips removed by freezing in liquid nitrogen, and slides incubated for five minutes in 0.1 μM DAPI in PBS, and mounted in 50% glycerol in PBS.

To examine mitotic divisions, larval neuroblast squashes of *atg2* mutants were made. Brains were dissected from $atg2^{P17156}$ third instar mutant larvae in Schneiders Drosophila medium. Tissue was transferred to a silinized coverslip and incubated for 10 minutes in 10% sodium citrate, followed by 5 minutes in 45% acetic acid. Brains were then squashed, the coverslips removed by freezing in liquid nitrogen. Two five minute washes in PBS were followed by incubation in 1 μM DAPI in PBS, mounted in 50% glycerol in PBS. All slides were examined with an Olympus Fluoview FV500 confocal laser scanning microscope. Images were cropped using Adobe Photoshop (Adobe Systems. San Jose, California).

Verification of *atg2* as an *En(tef)*

To verify that *atg2* was the *En(tef)* identified in the modifier screen, precise excisions were generated of the causative P element in the $atg2^{P17156}$ allele via introducing a source of transposase. Males containing the transposase source, *+/Y; cnbw;Δ2-3 Sb* were mated to *w;* $atg2^{P17156}$*/Tb* virgin females. Progeny of genotype *w/Y;cnbw/+;Δ2-3 Sb/*$atg2^{P17156}$ were collected on days 13 through 18 and individuals were mated to 3-5 *yw;*tef^{P1150}*/Cy;Sb/TM3-Ser;spa* virgins. Progeny of this cross produced males in which the P-element in the *atg2* gene had been excised. These progeny were of genotype *yw/Y; +/Cy;* P-revertant*/TM3-Ser; spa*, and were identified by the loss of the mini-white gene associated with the P-element $atg2^{P17156}$. Putative

revertant males were collected and crossed individually to *yw; atg2^{P17156}/Tb* virgin females to identify revertants that were viable *in trans* with the original *atg2^{P17156}* allele. Sibling *yw/w;+/Cy;P-rev/TM3-Ser;spa* flies were used to generate stocks. Viable *yw/Y;+/Cy; P-rev/ atg2^{P17156}*males were tested for fourth chromosome nondisjunction as above.

CHAPTER III

RESULTS

A screen of male meiotic mutants confirms that *mod(mdg4)* interacts genetically with *tef*.

There are two EMS mutations in the *mod(mdg4)* gene, *z3-3298* and *z3-5578*, and both have been previously characterized as alleles of *mnm*, an isoform of *mod(mdg4)* specifically involved in male meiosis (THOMAS *et al.* 2005). Both of these alleles were identified in a screen for mutations that resulted in paternal fourth chromosome loss (KOUNDAKJIAN *et al.* 2004; WAKIMOTO *et al.* 2004). Since MNM localization has been shown to be dependent on *tef*, we hypothesized that other male meiotic mutants may also be dependent on, or required for proper function of *tef*. To test this hypothesis, we utilized the hypomorphic allele of *tef*, tef^{P1150}, that when heterozygous with the null allele produces roughly 4% nondisjunction in the progeny of the tef^{P1150}/tef male. The combination of the hypomorphic and null allele creates a genetic background that will be sensitive to a reduction in the amount of any other gene product that is involved in the same pathway with *tef*. Examples of these dose sensitive interactions are proteins that form a complex with Tef, or proteins that are transcriptionally regulated by Tef. Therefore, these dominant second site modifiers of *tef* will either increase or decrease the rate of nondisjunction in the progeny of a test male, dependent upon the nature of the

interaction. This background can be used to screen for other components involved with Tef in pairing and segregation in attempt to elucidate the role of *tef* in that pathway.

In addition to directly testing the two EMS alleles of *mnm*, we also screened all third chromosome male meiotic mutants (Table 1, Figure 6). Both alleles of *mnm z3-3298* and *z3-5578*, enhanced the rate of nondisjunction, producing a 1.88 and 1.81 fold increase in nondisjunction in progeny of experimental vs. control males, respectively. This supports the model proposed by Thomas, et al. (2005) that *mnm* and *tef* are involved in the meiotic pairing complex, and also supports the observation that *tef* is required to localize MNM to the autosomes. Interestingly, we found no genetic interaction between *tef* and six different alleles of *snm* (*z3-0317*, *z3-2086*, *z3-2094, z3-3320, z3-3426*, and *z3-4141*) also a component of the meiotic pairing complex and required for localization of MNM to the sex chromosomes.

Only one other male meiotic mutant, *z3-5860*, had a significant effect on the rate of nondisjunction in a hypomorphic *tef* background. This mutant produced progeny that had lower amounts of nondisjunction than their control siblings, therefore the mutant is considered a *Su(tef)*. The *z3-5860* mutation has not been previously described and is not mapped to a specific locus. Complementation testing is now underway to determine which gene contains the mutation that is responsible for the suppression of *tef*.

Initial results produced by screening male meiotic mutants for *Modifiers of tef* were informative as they conclusively supported a genetic interaction between *tef* and *mnm*, as well as supported our hypothesis that a second site modifier screen would identify other components involved in the same pathway as *tef*.

Table 1 Results of a Male Meiotic Mutant Screen for *Modifiers of tef*

	Exp.		Control			
Mutant Line	**%NDJ[1]**	**N**	**%NDJ[1]**	**N**	**E/C**	**En/Su**
Z3-0317^{++}	13.9	599	16.9	412	.82	--
Z3-0375	12.1	589	16.2	423	.74	--
Z3-0589	12.1	561	13.0	429	.93	--
Z3-0684	5.9	396	7.1	460	.84	--
Z3-0777	8.7	439	11.0	282	.79	--
Z3-1483	15.2	811	12.2	675	1.24	--
Z3-1492	17.2	816	16.0	574	1.08	--
Z3-1550	9.3	549	8.7	480	1.06	--
Z3-1641	4.2	589	5.1	296	.82	--
Z3-1898	8.2	1018	11.0	702	.74	--
Z3-1956	8.0	904	7.2	571	1.12	--
Z3-2086^{++}	8.6	265	9.4	310	.92	--
Z3-2094^{++}	8.7	800	9.2	590	.94	--
Z3-2566	9.8	650	13.6	521	.72	--
Z3-2585	12.2	488	9.8	468	1.24	--
Z3-2761	7.1	1905	6.8	1090	1.04	--
Z3-3298^{+}	20.3	748	10.8	838	1.88	En**
Z3-3320^{++}	7.2	454	7.2	359	.99	--
Z3-3370	19.0	774	21.9	686	.87	--
Z3-3426^{++}	8.7	283	7.7	227	1.13	--
Z3-3808	10.6	1067	9.1	788	1.17	--
Z3-3822	10.2	777	9.1	630	1.12	--
Z3-3946	9.4	571	12.4	550	.75	--
Z3-4141^{++}	15.3	605	17.0	375	.90	--
Z3-5121	12.1	658	14.7	529	.83	--
Z3-5468	13.6	413	10.1	240	1.34	--
Z3-5502	6.9	429	6.7	321	1.04	--
Z3-5578^{+}	20.2	858	11.2	573	1.81	En**
Z3-5839	6.5	402	5.2	313	1.26	--
Z3-5860	7.1	416	10.6	404	.67	Su*
Z3-6257	9.3	446	11.0	443	.85	--

Table 1. Results of a male meiotic mutant screen for enhancers or suppressors of *tef*. All results were analyzed via chi square analysis where *p<.05 (χ^2 3.841),**p<.01 (χ^2 6.635),***p<.001 (χ^2 10.83). [1] where %NDJ refers to the number of *spa* or *ciey* progeny/100. $^{+}$ indicates an allele of *mnm*. $^{++}$ indicates an allele of *snm*.

Figure 6 **Results of a Male Meiotic Mutant screen for *Modifiers of teflon***

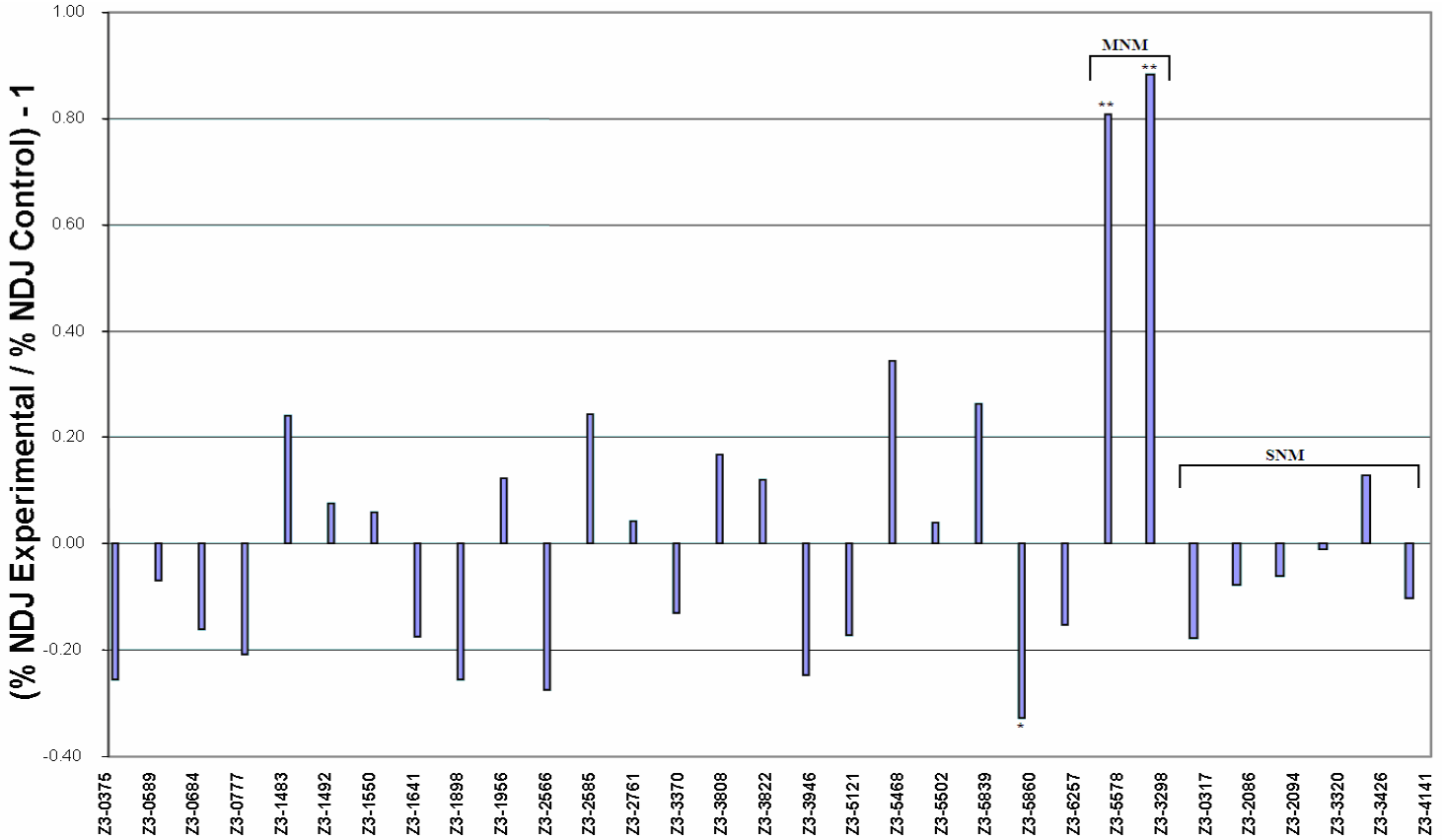

Figure 6. Results of a male meiotic mutant screen for enhancers and suppressors of tef. *p<0.01 and **p<0.001.

Each meiotic mutant lines is identified by its arbitrarily assigned z3 (3rd chromosome Zuker collection) number

A Screen for *En(tef)* and *Su(tef)* reveals regions on the third chromosome containing dominant second site modifiers of *tef*.

Our screen of male meiotic mutants for second site modifiers of *tef* produced results that supported our experimental design and showed that such a screen for modifiers would give us the ability to identify other components of *tef* pathway. To continue our search for Enhancers or Suppressors of *tef*, we performed a large scale screen for deficiencies on the third chromosome that would increase or decrease the frequency of nondisjunction in tef^{P1150}*/tef; Df/+* experimental vs. tef^{P1150}*/tef; Bal/+* control males. Results of the modifier screen are displayed in Table 2 and as a graph in Figure 7.

Fifteen regions were identified that contain *En(tef)s*, and seven of those regions were partially mapped by overlapping deficiencies that also contained *En(tef)s*. The three regions that contain the strongest enhancers are 62E8-63B6 (which is entirely contained within deficiency *Df(3L)BSC23*), 68C8-69A1 (this deficiency is contained within overlapping deficiencies *Df(3L)vin5* and *Df(3L)vin7*), and 92B3-D6 (contained within overlapping deficiencies *Df(3R)Dl-BX12* and *Df(3R)H-B79*).

One of the deficiencies that produced a significant enhancement of tef is *Df(3R)e-n19*, which produced a 2.36 fold increase in nondisjunction over the control. This finding was verified with an smaller deficiency *Df(3R)e-F1*, which produced a 1.9 fold increase of nondisjunction in the progeny of the experimental over control males. Within *Df(3R)e-F1* is the *mod(mdg4)* locus, and test males carrying the deficiency for *mod(mdg4)* produced nearly identical rates of nondisjunction as did both EMS alleles.

These results are further support for both the validity of the modifier screen, as well as for the genetic interaction between *mnm* and *tef*. Furthermore, given that the allele tef^{P1150} does produce full length Tef protein, albeit in presumably lower amounts, our data suggests a dosage dependent interaction between *tef* and *mnm* (ARYA et al. 2006).

There are six regions of the third chromosome that contain *Su(tef)s*, the strongest of these is contained within *Df(3R)BSC24* whose breakpoints are 85C4-D14. This *Modifier* is further mapped by overlapping deficiencies that do not suppress the nondisjunction phenotype to between breakpoints 85D1 and 85D11.

Three deficiencies produced test males that were sterile. Further analysis revealed that the first of these deficiencies *Df(3R)Antp17* (84A5;84D9) produces males with non-motile sperm. No defects were found in spermatocyte morphology, and nondisjunction was not evident, therefore the interaction is postulated to be a *tef*-independent synthetic sterile (data not shown).

The second of these sterile combinations was found with *Df(3L)BSC20* (76A7-B1;76B4-5). These test males produce spermatocytes that have a distinct and interesting mitochondrial phenotype in post-meiotic cells. This phenotype however, seems to be unrelated to chromosome segregation during meiosis as no obvious nondisjunction phenotype was noted. In addition, spermatocyte morphology looked normal, and there were no obvious defects in sperm motility or morphology. Due to the lack of a nondisjunction phenotype in the post-meiotic cells, the sterility of these test males is likely *tef*-independent, although further studies should be done to conclusively determine the nature of this sterility.

The final sterile deficiency is *Df(3R)M-Kx1* (86C1-87B5). Test males heterozygous for this deficiency had no obvious defects in sperm morphology, sperm motility, spermatocyte morphology, nor any defects in meiotic spindles or post meiotic cells. Furthermore three other deficiencies completely overlapping this sterile (*Df(3R)cu*, *Df(3R)ED5514*, and *Df(3R)T-32*) were fertile and produced viable progeny, although two of the three overlapping deficiencies contained suppressors or enhancers. The causative agent of the sterility associated with this deficiency could be *tef*-independent, and related to the genetic background of the test males, or the size of the deficiency, or alternatively the deficiency chromosome could contain two modifiers of *tef* that when double heterozygous could produce a sterile interaction. Further studies should be done to elucidate the mechanism of this sterility.

Table 2 Results of a 3rd Chromosome Deficiency Screen for *Modifiers of tef*

Df	Deficiency Breakpoints	Exp. %NDJ[1]	Exp. *N*	Control %NDJ[1]	Control *N*	E/C	En/Su
Df(3L)emc-E12	61A-61D3	7.4	628	9.5	762	.78	--
Df(3L)Ar14-8	61C5-8;62A8	2.8	627	10.1	829	.28	Su***
Df(3L)Aprt-1	62A10-62D2	6.6	549	6.3	608	1.05	--
Df(3L)R-G7	62B9-62E7	6.4	529	3.1	564	2.06	En***
Df(3L)BSC23	62E8;63B5-6	34.4	381	11.6	555	2.96	En***
Df(3L)M21	62F-63B10	5.5	312	11.6	388	.47	Su**
Df(3L)ED4293	63C1;63C1	8.7	252	9.5	314	.91	--
Df(3L)HR119	63C2;63F7	8.2	247	8.9	339	.92	--
Df(3L)GN24	63F6-64C15	20.2	205	12.0	234	1.68	En*
Df(3L)ZN47	64C-65C	13.3	242	4.5	399	2.92	En***
Df(3L)XDI98	65A2-65E1	10.4	485	8.5	540	1.22	--
Df(3L)BSC27	65D4-5;65E4-6	9.2	619	5.7	417	1.63	En*
Df(3L)RM5-2	65E1-66B2	3.8	407	2.7	431	1.4	--
Df(3L)ZP1	66A17-66C5	5.2	618	6.5	650	.81	--
Df(3L)BSC13	66B12-C1;66D2-4	10.3	665	8.1	801	1.26	--
Df(3L)h-i22	66D10-66E2	5.8	322	7.1	944	.83	--
Df(3L)Scf-R6	66E1-6;66F1-6	13.3	790	6.6	619	2.0	En***
Df(3L)BSC35	66F1-67B3	8.0	503	6.3	531	1.27	--
Df(3L)AC1	67A2;67D7-13	16.0	279	8.4	761	1.9	En***
Df(3L)BSC14	67E3-7;68A2-6	6.5	431	6.3	434	1.04	--
Df(3L)vin5	68A2-3;69A1-03	19.5	507	7.8	1557	2.52	En***
Df(3L)vin7	68C8-11;69B4-5	23.9	1612	6.6	1449	3.61	En***
Df(3L)eyg[C1]	69A4-69D6	5.2	750	6.9	611	.76	--
Df(3L)BSC10	69D4-5;69F5-7	5.9	448	7.9	339	.75	--
In(3LR)C190	69F3-70A2	9.1	330	5.8	344	1.58	--
Df(3L)fz-GF3b	70C1-2;70D4-5,66E	4.3	778	4.5	665	.97	--
Df(3L)fz-M21	70D2-71E5	12.7	562	7.9	716	1.62	En**
Df(3L)XG5	71C2-3;72B1-C1	14.9	921	10.2	976	1.46	En**
Df(3L)st-f13	72C1-D1;73A3-4	8.4	578	6.0	1112	1.40	--
Df(3L)81k19	73A3-74F4	10.2	168	10.1	498	1.01	--
Df(3L)ED4685	73D5;74E4	14.3	276	10.7	192	1.34	--
Df(3L)BSC8	74BC-75A1;75B2-5	9.0	445	7.3	559	1.23	--
Df(3L)W10	75A6-7;75C1-2	6.1	153	8.8	83	.70	--
Df(3L)Cat	75C1-F1	6.6	835	6.1	868	1.09	--
Df(3L)ED4782	75F2-76A1	5.0	553	4.5	442	1.10	--
Df(3L)fz2	75F10-11;76A1-5	6.5	749	6.4	677	1.02	--
Df(3L)BSC20	76A7-B1;76B4-5	--	--	--	--	--	sterile
Df(3L)XS533	76 B4; 77 B1	9.3	456	7.0	386	1.34	--
Df(3L)rdgC-co2	77A1-77D1	10.5	555	9.9	327	1.06	--
Df(3L)ri-79c	77B-C;77F-78A	4.9	682	7.6	720	.34	Su*

Df	Deficiency Breakpoints	Exp. %NDJ[1]	*N*	Control %NDJ[1]	*N*	E/C	En/Su
Df(3L)31A	78A;78E, 78D;79B	6.0	404	8.6	329	.64	--
Df(3L)ED4978	78D5-79A2	16.3	206	11.4	287	1.42	--
Df(3L)Ten-AL29	79C-79E8	13.7	221	13.1	286	1.05	--
Df(3L)HD1	79D3-E1;79F3-6	14.2	886	14.9	565	.95	--
Df(3L)BSC21	79E5-F1;80A2-3	14.5	650	15.0	589	.96	--
Df(3L)ED5017	80A4;80C2	8.9	226	10.3	279	.86	--
Df(3L)6-61	80F-80F	6.0	641	7.0	452	.86	--
Df(3R)ME15	81F3-6;82F7	7.6	242	5.1	259	1.49	--
Df(3R)3-4	82F3-4;82F10-11	8.5	898	5.7	954	1.48	--
Df(3R)e1025-14	82F8-83A3	7.3	701	6.5	618	1.12	--
Df(3R)Exel6144	83A6-B6	9.4	281	4.7	428	2.00	En*
Df(3R)BSC47	83B7-C1;83C6-D1	19.2	467	10.0	677	1.93	En***
Df(3R)WIN11	83E1--84B1	11.5	255	9.6	834	1.19	--
Df(3R)Scr	84A1-2;84B1-2	12.9	149	10.7	268	1.21	--
Df(3R)Antp17	84A5;84D9	--	--	--	--	--	sterile
Df(3R)dsx2M	84C1-3;84E1	4.7	448	2.0	486	2.32	En*
Df(3R)dsx10D	84D11-85A3	5.8	403	7.5	630	.78	--
Df(3R)p-XT103	85A2-85C2	9.4	1110	8.5	747	1.11	--
Df(3R)ED5330	85A5;85D1	13.4	213	8.4	523	1.60	En*
Df(3R)BSC24	85C4-9;85D12-14	1.1	640	6.0	671	.18	Su***
Df(3R)by62	85D11-14;85F6	6.7	321	6.5	488	1.03	--
Df(3R)BSC38	85F1-2;86C7-8	2.9	830	2.4	695	1.22	--
Df(3R)cu	86C1-2;86D8	4.0	501	8.4	447	.48	Su**
Df(3R)ED5514	86C7;86E11	12.9	222	7.4	412	1.75	En*
Df(3R)T-32	86E2-4;87C6-7	5.1	242	3.5	218	1.44	--
Df(3R)ry615	87B12-87E8	3.2	825	4.4	1068	.72	--
Tp(3;Y)ry506	87D1-88E6	9.3	543	13.2	473	.71	--
Df(3R)ea	88E7-13;89A1	8.4	513	6.8	615	1.23	--
Df(3R)sbd105	88F9-89A1;89B9	5.6	368	4.8	219	1.18	--
Df(3R)sbd104	89B5;89C2-7	7.2	609	8.9	525	.81	--
Df(3R)P115	89B7-89E7	7.3	537	7.1	602	1.02	--
Df(3R)DG2	89E1-F4;91B1-B2	3.0	197	4.4	216	.56	--
Df(3R)Cha7	90F1-4;91F5	8.0	1624	7.4	1556	1.09	--
Df(3R)Dl-BX12	91F1-2;92D3-6	12.1	917	5.9	1423	2.03	En***
Df(3R)H-B79	92B3;92F13	22.2	245	6.8	423	3.25	En***
Df(3R)BSC43	92F7-A1;93B3-6	3.3	1259	6.4	686	.52	Su**
Df(3R)e-F1	93B6-7;93E1-2	15.1	203	7.9	372	1.9	En**
Df(3R)e-N19	93B;94	7.2	310	3.1	286	2.36	En*
Df(3R)hh	93F11-14;94D10-13	9.3	224	8.4	271	1.10	--
Df(3R)BSC56	94E1-94F2	9.8	643	8.6	556	1.15	--
Df(3R)Exel6194	94F1;95A4	9.6	1279	5.7	1198	1.67	En***
Df(3R)mbc-30	95A5-7;95C10-11	11.7	545	7.9	456	1.48	--

Df	Deficiency Breakpoints	Exp. %NDJ[1]	*N*	Control %NDJ[1]	*N*	E/C	En/Su
Df(3R)mbc-R1	95A5-7;95D6-11	12.2	488	9.8	468	1.24	--
Df(3R)crb-F89-4	95D7-D11;95F15	6.4	393	5.7	199	1.13	--
Df(3R)crb87-5	95F7;96A17-18	4.0	737	3.7	236	1.10	--
Df(3R)slo8	96A2-7;96D2-4	8.3	363	3.8	480	2.19	En**
Df(3R)Exel6202	96D1-96E2	7.0	864	8.8	819	.80	--
Df(3R)Exel6203	96E2-96E6	7.8	768	7.7	574	1.01	--
Df(3R)Espl3	96F1;97B1	8.2	586	7.7	588	1.06	--
Df(3R)Tl-P	97A-98A2	9.8	780	7.8	1186	1.25	--
Df(3R)D605	97E3;98A5	5.2	744	4.8	578	1.09	--
Df(3R)BSC42	98B1-2;98B3-5	6.5	743	7.7	417	.84	--
Df(3R)Exel6259	98C4;98D6	10.2	247	8.2	405	1.25	--
Df(3R)Exel6209	98D6;98E1	7.0	533	6.8	369	1.02	--
Df(3R)3450	98E3;99A6-8	7.6	472	4.8	574	1.59	--
Df(3R)Dr-rv1	99A1-2;99B6-11	4.7	367	3.8	201	1.22	--
Df(3R)L127	99B5--99F1	8.8	331	8.9	287	.99	--
Df(3R)B81	99D3;3Rt	7.2	538	4.4	195	1.64	--

Table 2. Results of a 3rd chromosome deficiency screen for enhancers or suppressors of tef. All results were analyzed via chi square analysis where *p<0.05 (χ^2 3.841),**p<0.01 (χ^2 6.635), ***p<0.001 (χ^2 10.83). [1] where %NDJ refers to the number of *spa* or *ciey* progeny / 100

Figure 7 Results of a 3rd chromosome deficiency screen for *Modifiers of tef*

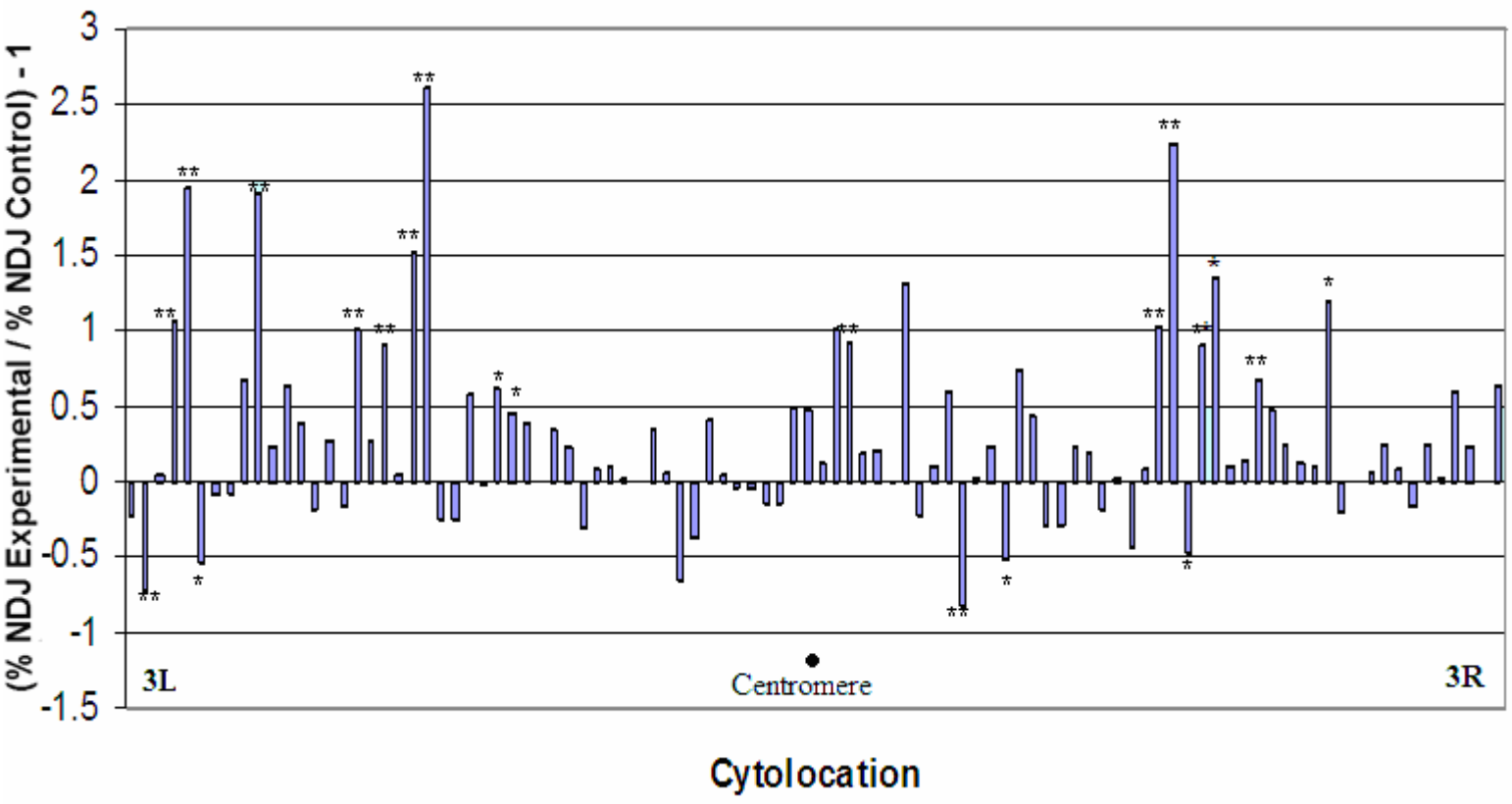

Figure 7. Results of a modifier screen for enhancers or suppressors of *tef*. Deficiencies are plotted in order of location on the chromosome *p<0.01 and **p<0.001

Mapping of region 62E8-63B6

One of the strongest enhancers of *tef* is contained within *Df(3L)BSC23* (62E8-63B6). A smaller deficiency that partially overlaps this region, *Df(3L)Exel6091* (62E8-F5), was tested in the same manner as other deficiencies in the modifier screen. *Df(3L)Exel6091* also produced a rate of nondisjunction in the progeny of the test male nearly two fold that of the control male (see Table 3).

There were no smaller deficiencies available that would assist in mapping the gene of interest, but there were several P-element disruptions within the region of interest (Figure 8). We obtained P-element insertions for genes *G1240* (*PBac{w[+mC]=WH}Mrtf[f00366]* stock#18321), *CG12093* (*PBac{w[+mC]=WH}CG12093[f07141]* stock#19050), *atg2* (*P{w[+mC]=EP}Atg2[EP3697]* stock#17156, and P{GawB}NP7457 stock#Kyoto105468)). Each of these P-element insertions were put through the modifier screen and results analyzed via chi square to determine which of them had statistically significant differences in nondisjunction between experimental and control groups. Only the P-elements that disrupted *atg2* had a significant increase in nondisjunction in progeny of experimental males, although the two alleles resulted in different degrees of *tef* enhancement. This is most likely due to the nature of the P-element construct and the resulting inserted material, and/or the insertion site relative to the *atg2* gene. The $atg2^{17156}$ P-element is just outside of the putative promoter of *atg2* and contains an upstream activating sequence (UAS). The $atg2^{Kyoto105468}$ allele, on the other hand, contains a GAL4 driver associated with it and is inserted within the putative promoter of

atg2 (Figure 8). The *atg2*^{Kyoto105468} allele is homozygous viable, which suggests that there is some full length functional protein present, and is most likely a hypomorphic allele.

Figure 8 **Mapping of *atg2***

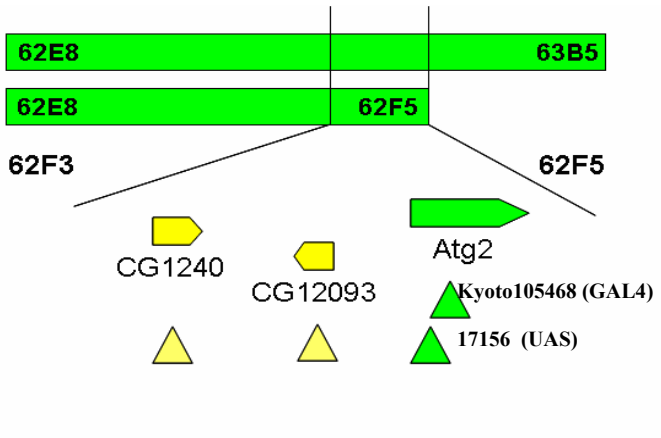

Figure 8. A deficiency map of salivary gland chromosome bands 62E8-63B5. Deleted segments are indicated by green bars. Transcripts of genes are indicated by arrows. Green indicates that the deficiency or gene enhanced *tef;* yellow indicates a negative test. Triangles indicate sites of P element insertions, boxes are deficiencies (see Table 3 for genetic data.

Table 3 Results of Mapping for 62E8-63B6

Df or P-element disrupted gene	% NDJ[1] Exp.	%NDJ[1] Control	E/C
Df(3L)Exel6091	11.2	6.0	1.86**
G1240	8.1	9.8	.83
CG12093	11.0	10.7	1.03
$atg2^{17156}$	15.6	11.1	1.41*
$atg2^{Kyoto105468}$	14.0	5.3	2.66***

Table 3. Results of the genetic crosses used to map region 62E8-63B6. *p<0.05 (χ^2 3.841), **p<0.01 (χ^2 6.635), ***p<0.001 (χ^2 10.83). [1] where %NDJ refers to the number of *spa* or *ciey* progeny / 100

***atg2* does not affect sex chromosome segregation**

Mutations in *tef* produce autosome-specific nondisjunction, but have no effect on sex chromosomes. One possibility this suggests is that there are two different mechanism ensuring proper autosome and sex chromosome segregation. The other, and perhaps more likely explanation is that there is a redundant system for sex chromosomes that is still able to maintain proper conjunction and segregation in the absence of *tef*. We were interested to see if *atg2* had any affect on sex chromosome segregation.

To assess sex chromosome segregation, we generated *yw/Y^{y+};tef^{P1150}/tef; atg2^{Kyoto105468}/TM3-Ser or Sb;spa/spa* test males and crossed them to *yellow, white, singed, compound-4* females (See Figure 4). In addition, we also tested *yw/Y^{y+}; tef^{P1150}/+; atg2^{Kyoto105468}/TM3-Ser or Sb;spa/spa* and *yw/Y^{y+}; tef^{P1150}/tef; +/TM3-Ser or Sb;spa/spa* and *yw/Y^{y+}; tef^{P1150}/+; +/TM3-Ser or Sb;spa/spa* and *yw/Y^{y+}; tef/Cy; atg2^{Kyoto105468}/TM3-Ser or Sb;spa/spa* males as controls. The marked Y and fourth chromosomes allows us to simultaneously monitor both sex and fourth chromosome nondisjunction (See Figure 5).

There were two progeny (out of 985) that resulted from paternal sex chromosome nondisjunction, one of these exceptions was from a test male of genotype *tef^{P1150}/tef;atg2/TM3-Ser or Sb* and the other exceptional progeny was from a paternal control genotype *tef^{P1150}/+; atg2/ TM3-Ser or Sb*, demonstrating that while rare, exceptional cases of nondisjunction in sex chromosomes do occur. The frequency of sex chromosome nondisjunction in the experimental males was not elevated over the low

background frequency seen in controls; therefore we conclude that *atg2* does not affect a putative redundant system for sex chromosome segregation (Table 4).

As expected, we did see an elevation in fourth chromosome nondisjunction in the *tef* hypomorphic, *atg2* heterozygous test males, although it failed to produce the two fold increase in nondisjunction as we have previously seen with this *atg2* allele. This is explained because of our inability to differentiate between *yw/Y^{y+};tef^{P1150}/tef; +/TM3-Ser or Sb;spa/spa* males and *yw/Y^{y+};tef^{P1150}/+; +//TM3-Ser or Sb;spa/spa* males. Both of these classes of males are phenotypically identical, therefore we genotyped them based solely on the presence of nondisjunction associated with the hypomorphic *tef^{P1150}/tef* background. Undoubtedly some of the test males that were *tef^{P1150}/tef* were genotyped as *tef^{P1150}/+* because exceptional progeny were not scored due to reduced viability of aneuploid progeny or low number of total progeny per male. The result of mis-genotyping has most likely artificially increased the rate of fourth chromosome nondisjunction for the *tef^{P1150}/tef*; *+/Bal* genotype.

All other paternal genotypes were wildtype with respect to nondisjunction of fourth chromosomes (<1%) (Table 4).

Table 4 **Sex and 4th chromosome nondisjunction in *atg2* mutants in a hypomorphic *tef* background**

	Genotype												% NDJ	
Paternal Genotype	X;4	Y;4	X;0	Y;0	X;44	Y;44	XY;4	XY;0	XY;44	0;4	0;0	0;44	4	XY
tef^{P1150}/tef;atg2/Bal	115	99	3	11	5	3	0	0	0	1	0	0	9.3	0.4
tef^{P1150}/tef; Bal/+	58	32	0	2	3	1	0	0	0	0	0	0	6.3	0
tef^{P1150}/+; Bal/+	103	66	0	0	0	0	0	0	0	0	0	0	0	0
tef^{P1150}/+; atg2/Bal	230	151	0	0	1	0	0	0	0	1	0	0	0.3	0.3
tef/Cy; atg2/+	53	47	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Results of a test for sex and 4th chromosome nondisjunction in *tef^{P1150}/tef;atg2/Bal* males compared to their control siblings.

Cytological characterization of *atg2*

Since *atg2* mutations were found to be an *En(tef)s*, we were interested to see if they would produce a meiotic phenotype in a *tef*+ background. The $atg2^{17156,}$ is a late pupal recessive lethal. It is maintained over a third chromosome *Tubby* (*Tb*) balancer. The Tb marker, which produces a dominant shortening of the body, allowed identification of homozygous *atg2* individuals in the larval and pupal stages. To see if *atg2* homozygous flies produced a *tef* independent meiotic phenotype, we performed testis dissections of $atg2^{17156}/ atg2^{17156}$ (phenotypically Tb^{+}) third instar larvae and early stage pupae. We viewed the spermatocytes under a phase microscope for nondisjunction which is evident in post meiotic cells by various sized nuclei. We found that $atg2^{17156}$ homozygous larvae exhibited a nondisjunction in postmeiotic cells, with nuclear sizes ranging from micronuclei to almost 2x the average size (Figure 9).

In addition we examined the cytology of $atg2^{Kyoto105468}$ and $atg2^{Exeld03351}$ homozygotes**.** Unlike $atg2^{17156}$, alleles $atg2^{Exeld03351}$ and $atg2^{Kyoto105468}$ are homozygous viable. We dissected larval and pupal testis to examine post meiotic cells for nondisjunction. All post-meiotic cells examined for the $atg2^{Kyoto105468}$ allele appeared to be wildtype, and no cases of variable size nuclei were noted (Figure 9).

Stocks of $atg2^{Exeld0335}$ are also maintained over a balancer containing a Tb marker, so Tb^{+} larvae and pupae were selected and testis dissected. Postmeiotic cells of $atg2^{Exeld03351}$ larvae exhibited several examples of nondisjunction, although the majority of cells were wildtype with respect to uniform nuclei size (Figure 9).

Figure 9 **Nondisjunction in various alleles of *atg2***

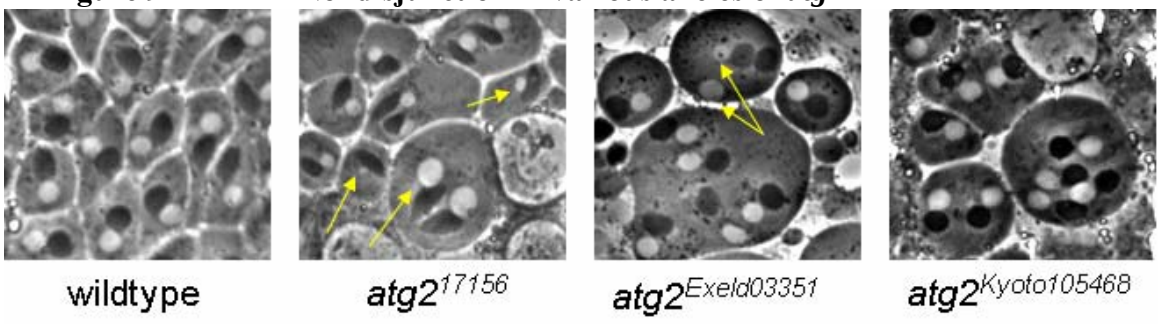

Figure 9. Nondisjunction evident as different size nuclei of postmeiotic onion stage spermatids of homozygous *atg2* mutants of the indicated genotypes. Yellow arrows indicate examples of nondisjunction

To further evaluate pairing and segregation in *atg2* mutants, we examined larval testis after fixation in 45% acetic acid, which condenses chromatin and allows identification of individual chromosomes. We performed this assay on $atg2^{17156}$ larvae since this allele provided the best cytological phenotype and was balanced over a Tb marker for easy and accurate genotyping.

Prophase and metaphase of meiosis I appeared largely normal in these mutants. Occasionally we noticed distance between the 4th chromosome homologs during prophase and prometaphase, but this observation constituted a very small proportion of the cells we examined. The majority of the prophase, prometaphase, and metaphase cells appeared wildtype in chromosome morphology, homolog pairing, and movement to the metaphase plate.

Most of the segregation defects we observed were seen during anaphase and telophase of meiosis I. During these stages we often observed "lagging" or nondisjoining fourth chromosomes that were separated from the mass of normally segregating chromosomes, and often in these cases fourth chromosome homologs were separated a significant distance from one another (Figure 10). Although we did not observe this in every cell at this stage, it did occur in roughly 5-10% of metaphase I and telophase I cells. Future studies will be necessary to determine the frequency of cells in which fourth chromosomes are lagging or nondisjoined in *atg2* mutants.

There was no observed pairing or segregation defects in sex chromosomes or major autosomes in *atg2* homozygous mutants. It is possible that the defect in *atg2* mutants is fourth chromosome-specific, although not likely considering its genetic interaction with *tef*, which is specific for all autosomes. A genetic test of $tef^{P1150}/tef;$ *atg2/+* males crossed to *compound-2* females could be done to assess if the genetic interaction between *atg2* and *tef* affects the major chromosomes as well as fourth chromosomes.

Meiosis II appeared normal at each stage in $atg2^{17156}$ homozygous larvae. There were no observed defects in segregation of sister chromatids in any of the cells observed. All sister chromatids segregated normally and there were no observed examples of lagging four sisters or fourth chromosome nondisjunction (Figure 11). Larval brain squashes were examined to determine if mitosis occurred normally in *atg2* mutants. All observed mitotic neuroblasts appeared normal, therefore we conclude that *atg2* mutants to not have a defect in mitosis (data not shown).

Figure 10 4^{th} chromosome segregation defects in *atg2*^{17156} mutants

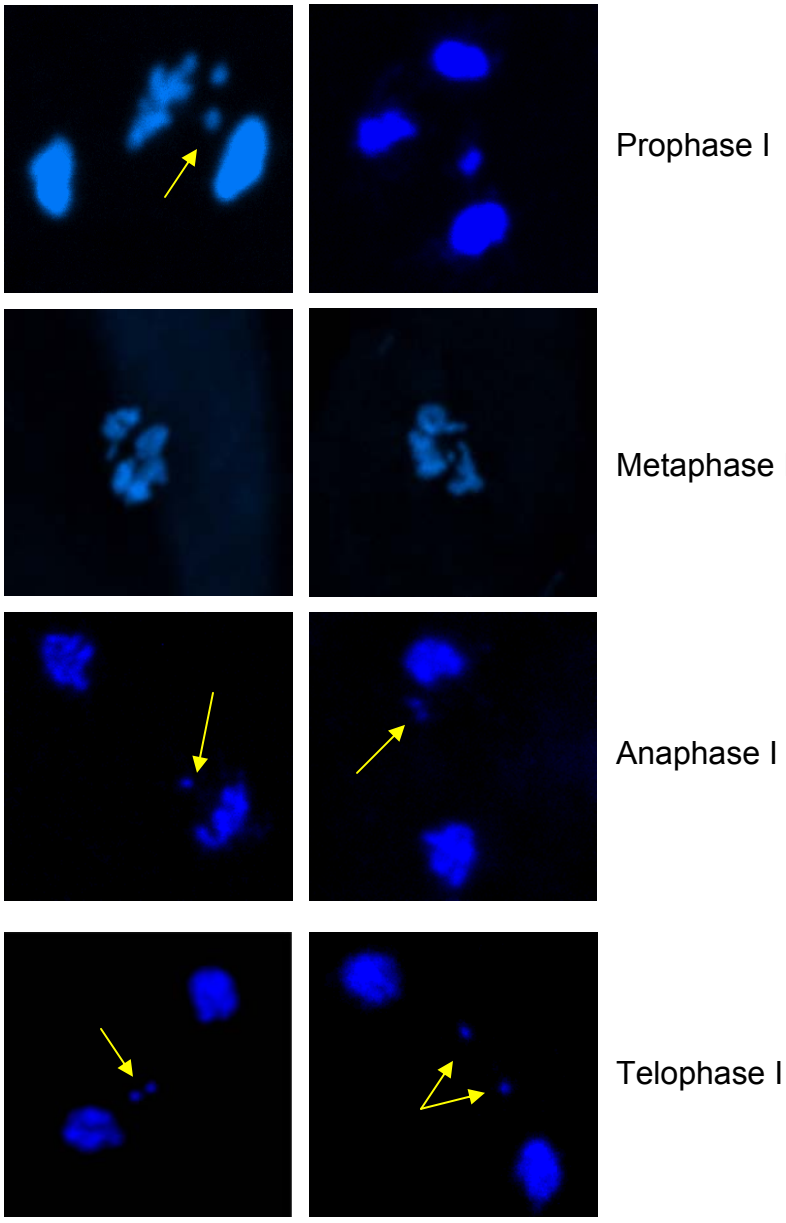

Figure 10. Acetic acid fixation followed by DAPI staining in *atg2*^{17156} homozygous larvae. Note nondisjunction of fourth chromosomes in anaphase and telophase as well as separated fourth chromosomes in prophase (as indicated by yellow arrows).

Figure 11 Meiosis II segregation occurs normally in *atg2*^{17156} mutants

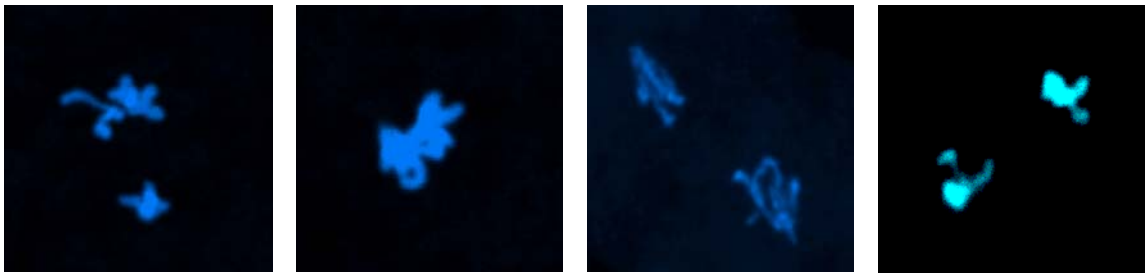

Figure 11. Meiosis II appears normal at each stage in *atg2*^{17156} homozygous larvae.

To verify that the segregation defect observed in anaphase and telophase of meiosis I in *atg2*^{17156} was not an artifact of squashing or the acetic acid fixation, we dissected *atg2*^{17156} homozygous larvae or pupae and immunostained with β–tubulin antibodies. Results of this immunostaining supported our previous findings of fourth chromosome nondisjunction at anaphase and telophase of meiosis I, and this occurred at approximately the same rate as was seen in our acetic acid squashes (Figure 12a and b). As with our previous assays, we found visible defects prior to anaphase of meiosis I, and all stages of meiosis II were phenotypically wildtype with respect to sister chromatid segregation (data not shown).

Additionally, with the β–tubulin staining we were able to clearly identify cysts of spermatids. All of the spermatid cysts we looked at contained sperm heads of various sizes, which is indicative of nondisjunction (Figure 12c and d). The integrity of all spermatid cysts was maintained, so all cells within each cyst should be at the same stage

in development and sperm heads should all be of uniform size. This was clearly not the case in *atg2*^{17156} homozygous pupae where the size difference between individual sperm heads suggests major chromosome nondisjunction.

Figure 12 β-tubulin immunostaining of *atg2*^{17156} homozygous larvae

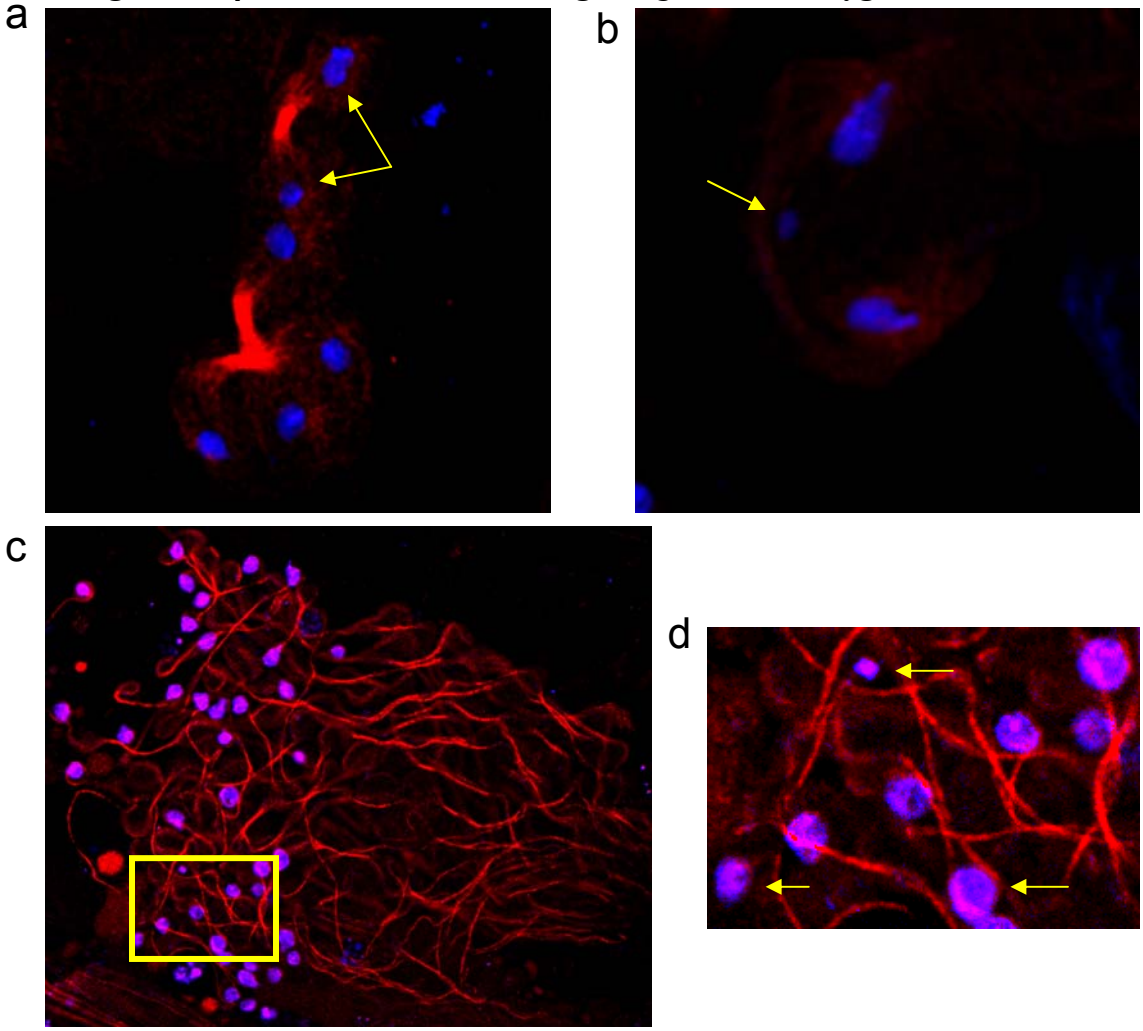

Figure 12. β-tubulin immunostaining of *atg2*^{17156} homozygous larvae**.** β-tubulin (red) and DAPI (blue) a) nondisjunction at telophase I, indicated by arrow. b) Fourth chromosome loss at anaphase of meiosis I, indicated by arrow. c) spermatids. Sperm heads (blue) of various sizes indicate nondisjunction during meiosis. d) Enlarged section of spermatids in C, showing sperm heads of various sizes indicative of meiotic nondisjunction.

CHAPTER IV

DISCUSSION

To elucidate the role of *tef* in the achiasmatic pairing and segregation pathway in *Drosophila melanogaster* males, we performed a modifier screen to identify dominant second site enhancers or suppressors of *tef*. Initial screening of the EMS induced male meiotic mutant collection produces results that shed new light on the role of *tef* in pairing and segregation of autosomes in meiosis I

This candidate approach of screening known male meiotic mutants for *Modifiers of tef* was limited in several respects. Genes that have redundant functions in meiosis may not show a meiotic defect when mutated, and thus would not be represented in this collection. This collection was also limited to mutations that were homozygous viable, and thus many genes that have additional essential functions would not likely be present in this collection. Finally, genes involved in multiple aspects of germline development (such as spermatogonial division) would likely be sterile, and were also absent from this collection. To perform a more comprehensive screen for dose-specific modifiers, we tested a collection of third chromosome deficiencies for dominant modification of *tef*. These deficiencies collectively remove approximately 90% of the genes on the third chromosome.

Only two mutants were identified in our male meiotic mutant screen as *En(tef)*. These two mutants were both alleles of *mod(mdg4)*. The *mod(mdg4)* gene is a complex

locus, producing 31 different gene products as a result of alternative and *trans* splicing (DORN and KRAUSS 2003). Both alleles that enhance *tef* affect a specific isoform of the *mod(mdg4)* gene called *modg(mdg4) in meiosis* (*mnm*).

To verify the finding that *mnm* is an *En(tef)*, we tested a deficiency that removed one copy of the *mod(mdg4)* locus and repeated our fourth chromosome nondisjunction test. The results of the deficiency were remarkably similar to the results of the EMS alleles of *mnm* with regards to the degree of nondisjunction in the progeny of experimental over control males.

Our observation of enhancement of *tef* by *mnm* alleles is consistent with observations from Thomas et al. (2005) who showed that localization of the MNM protein to autosomal bivalents at meiosis I is abolished in *tef* mutants. Our study extends their findings, showing a dose-sensitive synergistic interaction between the two genes. Reducing the amount of MNM by half in an otherwise wildtype fly has no effect on chromosome segregation, however a significant disruption in autosomal segregation occurs when Tef is simultaneously reduced.

This information leads to new insights on the function of these two proteins in pairing and segregation of autosomal homologs. A MNM-GFP fusion protein first appears localized to the nucleolus during early G2 prior to chromosome condensation, then as a single focus on the X-Y bivalent. Autosomal localization is very different, appearing early in prophase (stage S2) as multiple smaller and fainter spots seen within the autosomal domains. This pattern is changed to a few denser spots usually appearing

symmetrically on the autosomes during mid-prophase I that remain until the metaphase/anaphase transition (THOMAS *et al.* 2005).

The Tef protein has not yet been localized, but transgene rescue studies have shown that *tef* germline expression is required prior to S4 to effect rescue (ARYA *et al.* 2006). Thus Tef is required at or before the time when MNM protein first appears on autosomes.

One model to explain dosage sensitivity could be that Tef is involved in establishing prerequisite connections between condensing homologs, and MNM is one of several proteins required to physically interact with Tef to stabilize and strengthen those connections prior to prometaphase. This type of physical interaction between Tef and MNM would explain how reducing the amount of both proteins lowers the efficiency of the entire system, as well as give some insight into possible inaccessibility of Tef epitopes for antibody binding and subsequent inability to visualize Tef on the chromosomes. This model would also explain how MNM localization is abolished in tef mutants, and a similar model in which MNM is recruited to the pairing sites by Tef has been proposed by Thomas et al. (2005). It is also possible, given the temporal requirements of *tef* early in spermatocyte development, that it could be a transcription factor for some other gene that is required to interact with *mnm* for proper pairing of autosomes.

To distinguish between these possibilities an RT-PCR should be performed to assess the transcription level of *mnm* in homozygous *tef* mutants relative to *tef* heterozygous and/or wildtype controls. If transcription of *mnm* is altered it would

suggest that *tef* has a role in either directly or indirectly regulating that transcription. Alternatively, an immunoprecipitation could be performed to see if Tef and MNM share a physical connection. MNM localization should be analyzed in the hypomorphic *tef* background, although a MNM:GFP transgene would need to be provided or created for these purposes, as the antibodies to the common region of Mod(mdg4) have thusfar failed to localize the protein (THOMAS *et al.* 2005).

Unlike *mnm*, alleles of *stromalin in meiosis* (*snm*) failed to enhance *tef*. Both SNM and MNM alleles have a similar phenotype of causing premature separation of all bivalents during late prophase I (THOMAS *et al.* 2005). MNM localization to the X-Y bivalent and autosomes has been shown to be SNM dependent, and SNM has been proposed to be part of the meiotic pairing complex (THOMAS *et al.* 2005). The lack of a genetic interaction between *tef* and *snm* suggests a difference in the requirement for SNM versus MNM in the proposed pairing complex. Our results may merely reflect an excess of SNM protein, such that lowering the dose of SNM has little or no effect on the amount of functional pairing complex formed. Alternatively, they may reflect functional differences in the pairing complexes involving SNM versus MNM.

SNM has not been localized to autosomes, but it has been argued that it is likely there based on the disruption of autosomal pairing in *snm* mutants (THOMAS *et al.* 2005). Our results raise the possibility that this simple model of a single complex mediating both sex and autosome adhesion may be incorrect. They are, however consistent with a model in which sex chromosome and autosome conjunction occur by two different mechanisms (or by protenacious complexes of different composition). It is possible that SNM

substitutes for Tef in the adhesion complex on the X-Y bivalent, or that there is a redundant pathway for sex chromosome segregation. Our findings also suggest that the inability to see SNM on the autosomes may not be a technical artifact, but may reflect that *snm* does not have a role in autosomal pairing or segregation, at least not in a *tef*-dependent manner.

Genetic analysis of *tef/+; snm/mnm* and tef^{P1150}*/tef; snm/mnm* should be performed for sex and fourth chromosome nondisjunction, as well as SNM and MNM localization in these mutants. Such assays may shed further light on the interaction between the three proteins, and conclusively determine if *tef* and *snm* interact. Our efforts to generate these triple mutant flies have thus far been unsuccessful, most likely due to a reduction in viability attributed to the combination of balancer chromosomes.

From this deficiency screen, multiple regions containing modifiers of *tef* were identified. We mapped one of the strongest enhancers to a single gene, *autophagy specific gene 2 (atg2).* Two P-element insertion alleles of *atg2* showed an enhancement of *tef* similar to the encompassing deletion. To confirm a role of *atg2* in meiotic chromosome segregation, we examined these two alleles for *tef*-independent meiotic defects. The first of these alleles, $atg2^{17156}$ (which contains a P-element insertion with a UAS sequence), is a pupal lethal. However, we were able to examine meiotic tissue in early pupae and larvae. Males homozygous for $atg2^{17156}$ had a clear cytological phenotype indicative of meiotic nondisjunction, There were clear differences in nuclear volume during early post-meiotic stages of spermatid differentiation (known as the onion stage (TOKUYASU 1975)). The other allele, $atg2^{Kyoto105468}$ contains a P-element with a

GAL4 driver. This allele produces the largest increase in fourth chromosome nondisjunction among progeny in the *tef* modification screen, although produces no detectable cytological phenotype. The $atg2^{Kyoto105468}$ allele is homozygous viable, and therefore may produce more wildtype protein than the $atg2^{17156}$ allele. A third allele has not yet been tested for *tef* enhancement. This allele, $atg2^{Exeld03351}$, contains an inserted gypsy insulator element that sets up an insulator in the promoter of the *atg2* gene. Its cytological phenotype is intermediate between $atg2^{17156}$ and $atg2^{Kyoto105468}$, with some spermatid nuclei of variable size, but not nearly as many nor as dramatic size differences as seen in $atg2^{17156}$. The different cytological and genetic phenotypes of these three alleles most likely reflect the extant and perhaps tissue specificity of the disruption of the *atg2* gene. Regardless of the nature of the alleles, all three either enhance *tef* and/or cause a *tef*-independent meiotic phenotype, suggesting that *atg2* is the responsible gene that maps within the enhancing deficiency. Further verification that the meiotic defects can be attributed to *atg2* will involve reverting the P element alleles and confirming restoration of wildtype meiosis.

Further cytological evaluation of $atg2^{17156}$ homozygous larvae identified a meiotic phenotype distinguishable by fourth chromosome nondisjunction and lagging fourth chromosomes at anaphase and telophase of meiosis I. While we did not notice any obvious defects in pairing, chromosome morphology, or segregation of major autosomes in $atg2^{17156}$ homozygous males, that is not to say that such problems do not exist in a hypomorphic *tef* background. All of the problems we visualized in *atg2* homozygotes were defects in fourth chromosome segregation, however similar fourth chromosome

segregation defects have been seen in *tef*^{P1150} homozygous males who are phenotypically wildtype in regards to nondisjunction of major autosomes, suggesting that the fourth chromosome homologs are more sensitive to defects in the pairing and segregation pathway (ARYA *et al.* 2006). This could be due to the large amount of heterochromatin on the fourth chromosomes, the small size of the chromosome, or some other unknown factor.

Atg2 has not been very well characterized, and a role for it in meiosis is a novel finding. In general, autophagy is the term given to describe the process of degrading organelles or proteins through an apoptotic-like mechanism of sequestering targeted material through membrane rearrangement, and then delivering the sequestered materials to the lysosome for degradation (For review see (LEVINE and KLIONSKY 2004). While it was named presumably for homology to other autophagy specific genes found in yeast, in *Drosophila, atg2* has been shown to be involved in autophagy, programmed cell death, and has also been implicated in other processes such as synaptogenesis (KRAUT *et al.* 2001; LEE *et al.* 2003; SCOTT *et al.* 2004). Other studies have provided a link between autophagy and dividing mitotic cells. In cell culture, Eskelinen et al (2001) was able to show that autophagy is inhibited by failure to accumulate autophagic vacuoles, at the beginning of mitosis and is not reactivated until late telophase (ESKELINEN *et al.* 2002). The rationale behind such studies is reasonable, as dissolution of the nuclear membrane in prophase leaves the mitotic, and thus meiotic, machinery vulnerable to activated autophagic vesicles. It is possible then that genes involved in autophagy may have

additional roles in mitosis and meiosis that are unrelated to autophagy, and that signals to inhibit the formation of autophagic vesicles activate gene variants.

Determining the role of *atg2* in meiosis, and the nature of its interaction with *tef* is an important step in understanding the process of pairing and segregation. At this point it is difficult to speculate on the role of *atg2* in meiosis and how it is affecting the ability of *tef* to function properly. Our research has shown that *atg2* is a modifier of *tef*, and also that *atg2* has a previously uncharacterized *tef*-independent meiotic phenotype. We have shown that *atg2* homozygotes have cytological phenotypes that include fourth chromosome nondisjunction, separated fourth chromosome homologs, and lagging fourth chromosomes at meiosis I, and that it has no apparent meiosis II cytological phenotype. The mechanism of this segregation defect has not yet been identified, and further studies should be done to elucidate the spatial and temporal requirements for *atg2* for proper homolog segregation, and further characterize its interaction with *tef*. In the future, antibodies should be created for Atg2 and immunoassays performed to assess the localization of Atg2, any physical interaction between *atg2* and *tef*, or *atg2* and *mnm*. In addition the other regions containing enhancers or suppressors of *tef* that were identified in the deficiency screen should be mapped and characterized in an effort to elucidate the pathway responsible for pairing, adhesion, and segregation of homologous chromosomes during meiosis in male *Drosophila melanogaster*.

REFERENCES

ARYA, G. H., M. J. LODICO, O. I. AHMAD, R. AMIN and J. E. TOMKIEL, 2006 Molecular characterization of teflon, a gene required for meiotic autosome segregation in male Drosophila melanogaster. Genetics **174:** 125-134.

BRAND, A. H., and N. PERRIMON, 1993 Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development **118:** 401-415.

BUCHNER, K., P. ROTH, G. SCHOTTA, V. KRAUSS, H. SAUMWEBER *et al.*, 2000 Genetic and molecular complexity of the position effect variegation modifier mod(mdg4) in Drosophila. Genetics **155:** 141-157.

BUGGE, M., A. COLLINS, M. B. PETERSEN, J. FISHER, C. BRANDT *et al.*, 1998 Non-disjunction of chromosome 18. Hum Mol Genet **7:** 661-669.

BUONOMO, S. B., R. K. CLYNE, J. FUCHS, J. LOIDL, F. UHLMANN *et al.*, 2000 Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell **103:** 387-398.

CHIKASHIGE, Y., D. Q. DING, H. FUNABIKI, T. HARAGUCHI, S. MASHIKO *et al.*, 1994 Telomere-led premeiotic chromosome movement in fission yeast. Science **264:** 270-273.

DORN, R., and V. KRAUSS, 2003 The modifier of mdg4 locus in Drosophila: functional complexity is resolved by trans splicing. Genetica **117:** 165-177.

DORN, R., G. REUTER and A. LOEWENDORF, 2001 Transgene analysis proves mRNA trans-splicing at the complex mod(mdg4) locus in Drosophila. Proc Natl Acad Sci U S A **98:** 9724-9729.

ESKELINEN, E. L., A. R. PRESCOTT, J. COOPER, S. M. BRACHMANN, L. WANG *et al.*, 2002 Inhibition of autophagy in mitotic animal cells. Traffic **3:** 878-893.

GOLUBOVSKAYA, I. N., L. C. HARPER, W. P. PAWLOWSKI, D. SCHICHNES and W. Z. CANDE, 2002 The pam1 gene is required for meiotic bouquet formation and efficient homologous synapsis in maize (Zea mays L.). Genetics **162:** 1979-1993.

HASSOLD, T. J., and P. A. JACOBS, 1984 Trisomy in man. Annu Rev Genet **18:** 69-97.

KOUNDAKJIAN, E. J., D. M. COWAN, R. W. HARDY and A. H. BECKER, 2004 The Zuker collection: a resource for the analysis of autosomal gene function in Drosophila melanogaster. Genetics **167:** 203-206.

KRAUT, R., K. MENON and K. ZINN, 2001 A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in Drosophila. Curr Biol **11:** 417-430.

LAMB, N. E., E. FEINGOLD, A. SAVAGE, D. AVRAMOPOULOS, S. FREEMAN *et al.*, 1997 Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. Hum Mol Genet **6:** 1391-1399.

LEE, C. Y., E. A. CLOUGH, P. YELLON, T. M. TESLOVICH, D. A. STEPHAN *et al.*, 2003 Genome-wide analyses of steroid- and radiation-triggered programmed cell death in Drosophila. Curr Biol **13:** 350-357.

LEVINE, B., and D. J. KLIONSKY, 2004 Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell **6:** 463-477.

MACQUEEN, A. J., C. M. PHILLIPS, N. BHALLA, P. WEISER, A. M. VILLENEUVE *et al.*, 2005 Chromosome sites play dual roles to establish homologous synapsis during meiosis in C. elegans. Cell **123:** 1037-1050.

MCKEE, B. D., and G. H. KARPEN, 1990 Drosophila ribosomal RNA genes function as an X-Y pairing site during male meiosis. Cell **61:** 61-72.

MCKEE, B. D., S. E. LUMSDEN and S. DAS, 1993 The distribution of male meiotic pairing sites on chromosome 2 of Drosophila melanogaster: meiotic pairing and segregation of 2-Y transpositions. Chromosoma **102:** 180-194.

PHILLIPS, C. M., C. WONG, N. BHALLA, P. M. CARLTON, P. WEISER *et al.*, 2005 HIM-8 binds to the X chromosome pairing center and mediates chromosome-specific meiotic synapsis. Cell **123:** 1051-1063.

RASMUSSEN, S. W., 1973 Ultrastructural studies of spermatogenesis in Drosophila melanogaster Meigen. Z Zellforsch Mikrosk Anat **140:** 125-144.

REN, X., L. EISENHOUR, C. HONG, Y. LEE and B. D. MCKEE, 1997 Roles of rDNA spacer and transcription unit-sequences in X-Y meiotic chromosome pairing in Drosophila melanogaster males. Chromosoma **106:** 29-36.

ROBINSON, W. P., B. D. KUCHINKA, F. BERNASCONI, M. B. PETERSEN, A. SCHULZE *et al.*, 1998 Maternal meiosis I non-disjunction of chromosome 15: dependence of the maternal age effect on level of recombination. Hum Mol Genet **7:** 1011-1019.

SCOTT, R. C., O. SCHULDINER and T. P. NEUFELD, 2004 Role and regulation of starvation-induced autophagy in the Drosophila fat body. Dev Cell **7:** 167-178.

SOLTANI-BEJNOOD, M., S. E. THOMAS, L. VILLENEUVE, K. T. SCHWARZ, C. S. HONG *et al.*, 2007 Role of the mod(mdg4)common region in homolog segregation in Drosophila male meiosis. Genetics.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-break repair model for recombination. Cell **33:** 25-35.

THOMAS, S. E., M. SOLTANI-BEJNOOD, P. ROTH, R. DORN, J. M. LOGSDON, JR. *et al.*, 2005 Identification of two proteins required for conjunction and regular segregation of achiasmate homologs in Drosophila male meiosis. Cell **123:** 555-568.

TOKUYASU, K. T., 1975 Dynamics of spermiogenesis in Drosophila melanogaster. VI. Significance of "onion" nebenkern formation. J Ultrastruct Res **53:** 93-112.

TOMKIEL, J. E., B. T. WAKIMOTO and A. BRISCOE, JR., 2001 The teflon gene is required for maintenance of autosomal homolog pairing at meiosis I in male Drosophila melanogaster. Genetics **157:** 273-281.

TRELLES-STICKEN, E., J. LOIDL and H. SCHERTHAN, 1999 Bouquet formation in budding yeast: initiation of recombination is not required for meiotic telomere clustering. J Cell Sci **112 (Pt 5):** 651-658.

VAZQUEZ, J., A. S. BELMONT and J. W. SEDAT, 2002 The dynamics of homologous chromosome pairing during male Drosophila meiosis. Curr Biol **12:** 1473-1483.

WAKIMOTO, B. T., D. L. LINDSLEY and C. HERRERA, 2004 Toward a comprehensive genetic analysis of male fertility in Drosophila melanogaster. Genetics **167:** 207-216.

WETTSTEIN, D. V., S. RASMUSSEN and P. HOLM, 1984 The Synaptonemal Complex in Genetic Segregation. Annual Reviews of Genetics **18:** 331-413.

YAMAMOTO, M., 1979 Cytological studies of heterochromatin function in the Drosophila melanogaster male: autosomal meiotic paring. Chromosoma **72:** 293-328.