12-2007

# Exploring the Mechanism of Meiosis in *Drosophila melanogaster*: Meiotic Functions of a Novel Cohesion Protein SOLO and a Translation Initiation Factor VASA

Rihui Yan
*University of Tennessee - Knoxville*

To the Graduate Council:

I am submitting herewith a dissertation written by Rihui Yan entitled "Exploring the Mechanism of Meiosis in *Drosophila melanogaster*: Meiotic Functions of a Novel Cohesion Protein SOLO and a Translation Initiation Factor VASA." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Biochemistry and Cellular and Molecular Biology.

Bruce D. McKee, Major Professor

We have read this dissertation and recommend its acceptance:

Ranjan Ganguly, Jae Park, Mariano Labrador, Yisong Wang

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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# EXPLORING THE MECHANISM OF MEIOSIS IN *DROSOPHILA MELANOGASTER*: MEIOTIC FUNCTIONS OF A NOVEL COHESION PROTEIN SOLO AND A TRANSLATION INITIATION FACTOR VASA

**A Dissertation**
**Presented for the**
**Doctor of Philosophy Degree**
**The University of Tennessee, Knoxville**

**Rihui Yan**
**December 2007**

## DEDICATION

To my wife, Hongmei Jia, for your endless love, support, and encouragement; my parents, Shuliang Yan and Zhengmei Zhang for your faithful support; my precious daughter Andrea Yan.

## ACKNOWLEDGEMENTS

This dissertation would have been impossible without the generous help and support from many people. I would like to express my gratitude to all those who gave me the possibility to complete this work.

First of all, I am deeply indebted to my advisor, Dr. Bruce McKee. Without his excellent guidance, this work would not be possible. Above all and most needed, he provided me constant encouragement, help, and support in various ways. His advice and patience are deeply appreciated. His exceptional ability to think deeply and creatively and his enthusiasm in science have greatly influenced me and inspired me to grow not only as a student, but also a researcher and a scientist.

I am particularly grateful to the members of my dissertation committee, Dr. Ranjan Ganguly, Dr. Jae Park, Dr. Mariano Labrador and Dr. Yisong Wang for the valuable suggestions, time, and support in my progression.

I wish to thank both past and present members of Dr. McKee's laboratory for their friendship and help: Dr. Sharon Thomas, Dr. Morvarid Bejnood, Hueiwen Yeh, Jue-He Tsai, Jian Ma, and Jennifer Vandiver.

Finally, I would like to express my deepest gratitude for the constant support, understanding and love from my wife Hongmei, my daughter Andrea, and my parents during the past years.

## ABSTRACT

Sister chromatid cohesion is essential for proper chromosome segregation during meiosis. However, the mechanism of meiotic cohesion in Drosophila is unclear.

We describe a novel protein, SOLO (Sisters On the LOose) that is essential for meiotic cohesion in *Drosophila melanogaster*. *solo* mutations cause high nondisjunction of sister and homologous chromatids of sex chromosomes and autosomes in both sexes. In *solo* males, sister chromatids separate prematurely and segregate randomly during meiosis II. Although bivalents appear intact throughout meiosis I, sister centromeres lose cohesion prior to prometaphase I and orient nearly randomly on the meiosis I spindle. Centromeric foci of SMC1 are absent in *solo* males at all meiotic stages. SOLO and the cohesin protein SMC1 co-localize to meiotic centromeres from early prophase I until anaphase II in wild-type males but both proteins are removed prematurely from centromeres at anaphase I in *mei-S332* mutants, coincident with premature loss of cohesion in those mutants.

*solo* mutations in females cause reduced frequency of homologous recombination between X chromosomes and autosomes, partially due to the loss of inhibition of sister chromatid exchange. Synaptonemal complex assembly is severely disrupted in early meiotic stage in *solo* females. SOLO colocalizes with SMC1 and C(3)G in meiosis. Additionally, SOLO is required for stabilizing chiasmata generated from residual recombination events.

The data about the phenotypes of *solo* males and females and

colocalization patterns of SOLO strongly suggest SOLO is a component of potential cohesin in Drosophila meiosis.

Drosophila males undergo meiosis without recombination. However, the underlying mechanism is not known. Mutations of *vasa* cause high frequency of X-Y exchange in meiosis. Chromatin bridges at anaphase I and II, due to dicentric recombination events, were observed in *vasa* males. *vas* and *solo* double mutant showed precocious segregation of homologs at metaphase I besides chromatin bridge at anaphase I and II. Our data thus for the first time demonstrate that inhibition of meiotic recombination during male meiosis requires *vas* function and interactions between *vas* and *solo* regulate chromosome dynamics in male meiosis.

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# CHAPTER 1 – GENERAL INTRODUCTION

**Overview: meiosis is a specialized cell division**

Accurate segregation of chromosomes during meiosis is required for the proper transmission of genetic material during sexual reproduction. Errors in meiotic chromosome segregation result in aneuploidy, an aberrant number of chromosomes. Aneuploidy is the primary cause of miscarriage in human beings. Approximately 35% of all miscarriages result from aneuploidy. Moreover, aneuploidy is the leading genetic cause of developmental and mental disorders, such as Down syndrome, which is caused by an extra copy of chromosome 21 (Hassold and Hunt, 2001). The study of the mechanism of meiosis thus has potential clinical relevance to human beings.

Meiosis is a specialized type of cell division that generates haploid gametes from diploid precursors. Fusion of two gametes during sexual reproduction restores diploidy in the zygote; thereby giving rise to a new individual with complete genetic information (Petronczki et al., 2003). Meiosis consists of two divisions, the first meiotic division (meiosis I) and the second meiotic division (meiosis II), preceded by a single round of DNA replication. During meiosis I, homologous chromosomes (homologs) pair and segregate but sister chromatids (two identical copies of a single chromosome that are connected at centromere) remain together, leading to the reduction of chromosome number. Thereby, it is also called the reductional division. Meiosis II is a mitosis-like division during which sister chromatids segregate equally to opposite spindle poles, thus it is called the equational division (Petronczki et al., 2003; Marston and Amon, 2004) (Fig. 1-1).

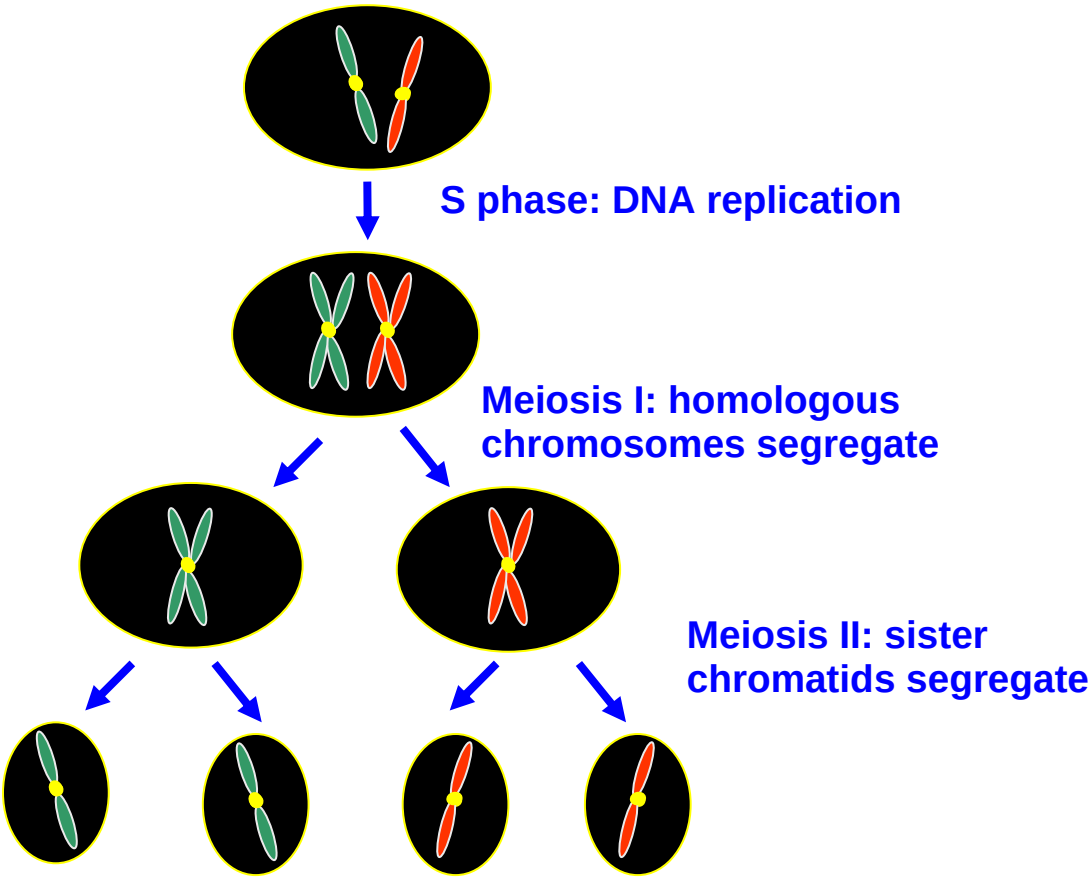



Figure 1-1. Stages of meiosis.

Through meiosis, one diploid parental cell divides into four haploid daughter cells. See the text for details.

The reductional division is divided into five stages: prophase I, prometaphase I, metaphase I, anaphase I, telophase I. Prophase I is the first stage in meiosis I when diploid cells enter meiosis. During prophase I homologs pair and form synaptonemal complexes (SCs, the proteinaceous structures that form between homologs during prophase I), recombine and form crossovers in most organisms. The crossovers lead to the formation of chiasmata, which connect homologous chromosomes when SCs are gone. The paired chromosomes are called bivalents, each consisting of two chromosomes each with two sister chromatids. Chromosomes are condensed during prophase I, allowing them to be seen under the microscope. During prometaphase I the nuclear membrane breaks down and homologous centromeres attach to the microtubules emanating from the spindle poles. The bivalents congress and align at the equatorial plate at metaphase I. At anaphase I chiasmata are resolved and homologous chromosomes, each with two sister chromatids, segregate to opposite spindle poles. At telophase I, the nuclear membrane may reform and DNA may be decondensed to some extent or the cells quickly enter into meiosis II. At this point, meiosis I ends and each daughter cell has half the number of chromosomes compared to that of the parental cell. Before entering meiosis II, some organisms may undergo a special stage called cytokinesis during which two daughter cells completely form.

Meiosis II is also divided into five stages: prophase II, prometaphase II, metaphase II, anaphase II, telophase II. During prophase II, sister chromatids condense again, showing shortening and thickening of chromosomes. Nuclear

membrane breaks down and disappears again at prometaphase II; thereby microtubules can interact with chromosomes and chromosomes congress again. Chromosomes align at equatorial plate at metaphase II. At anaphase II, the centromeres of the two sister chromatids separate and sister chromatids segregate and move to opposite spindle poles. Meiosis ends with telophase II during which chromosomes uncoil and lengthen into chromatin as microtubules disappear and nuclear envelopes reform. Through a complete meiosis, one parental cell produces four daughter cells with one copy of every unique chromosome (there are two copies of each chromosome in parental cell).

Although chromosomes properly separate into the four haploid daughter cells in almost all cases, they occasionally fail to do so. This is called nondisjunction (NDJ), the failure of chromosomes to properly disjoin during meiosis. NDJ leads to the generation of aneuploid zygotes that have one copy (monosomic) or three copies (trisomic) of the affected chromosome. NDJ is the major cause of human aneuploidy. NDJ can happen during either meiosis I or II. There are three types of NDJ: homolog NDJ in which homologs go to the same spindle pole, sister chromatid NDJ in which sister chromatids move to the same spindle pole, and precocious sister chromatid separation (PSCS) in which sister chromatids precociously separate before anaphase (Fig. 1-2). Although spontaneous NDJ occurs rarely in wild-type Drosophila, the frequency of NDJ can be very high in some of meiotic mutants. Our studies aim to understand the mechanism of meiosis through studying the factors involved in this process by genetic, molecular, and cytological methods and tools currently available.

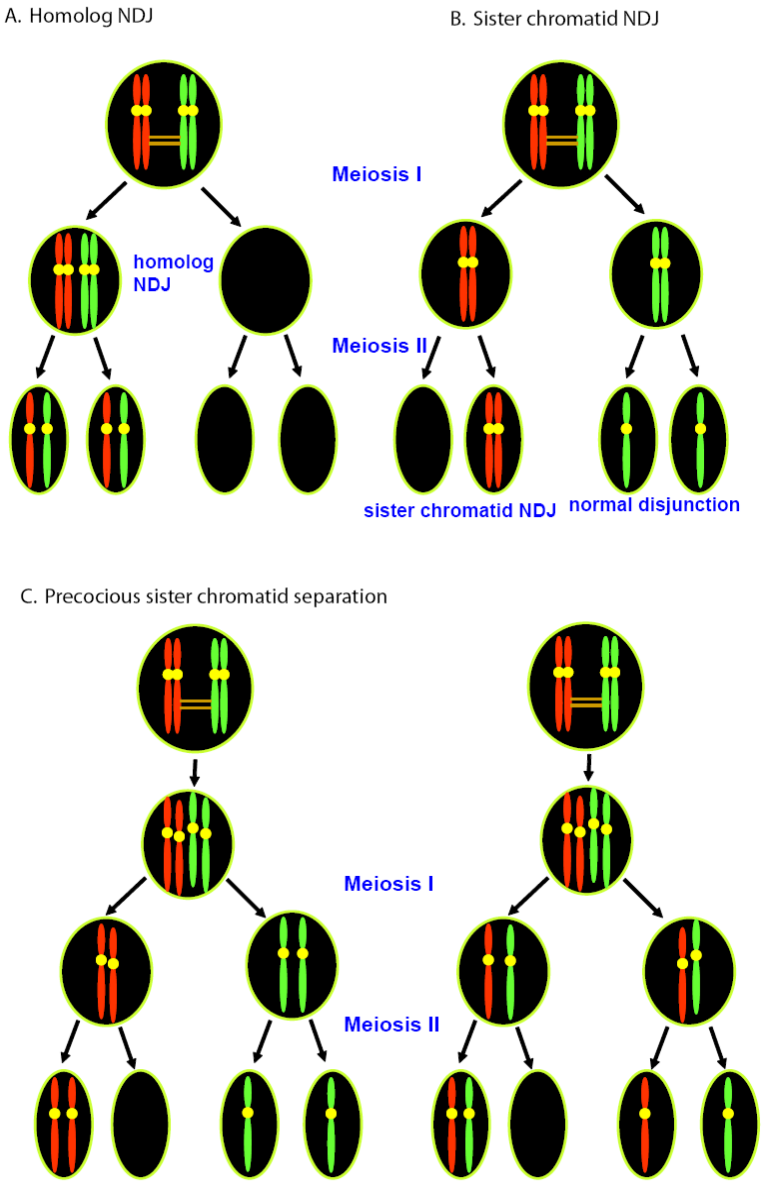



Figure 1-2. Nondisjunction of meiotic chromosomes.

Homolog NDJ occurs when homologs go to the same spindle pole during meiosis I. Sister chromatid NDJ occurs when sister chromatids do not segregate and move to the same spindle pole during meiosis II. Precocious sister chromatid separation occurs when sister chromatids separate prematurely.

**Meiosis I is unique**

Meiosis I is a unique type of chromosome segregation pattern as it is the homologous chromosomes that segregate from each other to opposite poles, rather than sister chromatids that segregate at meiosis II or mitosis. For this unique segregation to occur properly, three crucial events have to take place properly during meiosis I. First, homologous chromosomes must pair and be stably linked. The stable linkers in most organisms are chiasmata that are the cytological manifestation of crossovers between homologs. Together with sister chromatid cohesion distal to chiasmata, homologs are physically linked thereby tension can be generated when microtubules pull them from opposite directions. Secondly, both sister kinetochores within a chromosome have to attach to microtubules emanating from same spindle poles (co-orientation) to ensure sister chromatids can segregate together to the same poles during meiosis I. Thirdly, arm cohesion between sister chromatids must be destroyed in order to segregate homologous chromosomes during meiosis I. However, the cohesion at centromeric regions must be maintained beyond meiosis I to prevent precocious segregation of sister chromatids during meiosis I and ensure bipolar attachment and segregation of sister kinetochores during meiosis II.

**Homologous chromosomes must pair and be linked through chiasmata**

Homologous chromosome pairing, synapsis and segregation during meiosis I is probably the most characteristic aspect of meiosis. In general, before homologs are finally connected by chiasmata, homologs undergo rough

alignment based on DNA sequence, and synapsis, a very intimate association that is stabilized by the synaptonemal complex (SC) that lies between homologs and connects them along their entire length. In most organisms, including Drosophila females (Drosophila males use a different mechanism to hold homologs, which will be discussed in more detail later), chiasmata that hold homologs are generated by meiotic recombination. Although the mechanism of how meiotic recombination is processed to form chiasmata is not completely understood and controversial to some extent, DNA double strand breaks (DSBs) have been found to be required for this process.

**DSB generation**

In most organisms, DSBs are required for homolog pairing during early prophase I. In *Saccharomyces cerevisiae* DSBs are generated by Spo11 protein, which is related to a type II-like topoisomerase from archaebacteria (Keeney et al., 1997; Bergerat et al., 1997). In Drosophila females, *mei-W68* encodes the ortholog of Spo11 (McKim and Hayashi-Hagihara, 1998). Similarly, Spo11 orthologs have been identified in many organisms, including *Schizoacchromyces pombe* (Steiner et al., 2002), *Caenorhabditis elegans* (Dernburg et al., 1998), *Arabidopsis thaliana* (Grelon et al., 2001), mouse (Keeney et al., 1999), and human beings (Romanienko and Camerini-Otero, 1999). In all of these organisms, mutations in *spo11* lead to the failure of DSB formation, absence of meiotic recombination, and random chromosome segregation during meiosis I. When DSBs were generated by other means, like

X-irradiation, in *spo11* mutants lacking Spo11-induced DSBs in *S, cerevisiae* (Thorne and Byers, 1993), *C. elegans* (Dernburg et al., 1998), and mouse (Romanienko and Camerini-Otero, 2000), meiotic recombination and homolog synapsis were restored to some extent. These studies showed that DSBs generated by Spo11 are required for meiotic recombination in most or all organisms.

**DSB repair and generation of crossover**

The DSBs generated by Spo11 can be repaired to form two types of products, either a crossover or a non-crossover. Crossovers result from the reciprocal exchange between homologous chromosomes when the DSBs are repaired using one of sister chromatids from the homologous chromosome as the repairing DNA template. In contrast, non-crossovers are the repair products when DSBs are repaired without reciprocal exchange between homologous chromosomes.

The production of crossovers is a tightly regulated process as shown by the frequency and non-random distribution of crossovers. Under normal conditions, at least one crossover per pair of homologs is generated. In budding yeast, the crossover level is maintained at wild-type level at the expense of non-crossover in the genome during meiosis when DSBs were reduced, a phenomenon termed crossover homeostasis (Martini et al., 2006). In addition, multiple crossovers are rarely close to each other when more than one crossovers are produced, a phenomenon called interference (Muller, 1916).

Crossover interference has been extensively studied in Drosophila. Many meiotic proteins, including, SC components, *C(3)G* (*crossover suppressor on 3 of Gowen*) (Page and Hawley, 2001) and *C(2)M* (*crossover suppressor on 2 of Manheim*) (Manheim and McKim, 2003), cohesion protein *ord* (*orientation disruptor*) (Miyazaki and Orr-Weaver, 1992; Bickel et al., 2002), *mei-W68* (spo11 ortholog in Drosophila) (McKim and Hayashi-Hajihara, 1998), are required for crossover interference. In yeast, a component of synaptonemal complex, ZIP1, is essential for crossover interference (Sym and Roeder, 1994). Furthermore, in Drosophila females, sister chromatid cohesion has been shown to limit the exchange between sister chromatids in a chromosome but stimulate the exchange between homologs (Webber et al., 2004). Similar homolog bias was observed in budding yeast. *red1* and *dmc1* were required for inter-homolog meiotic recombination and the homolog bias was probably established prior to or during DSB formation (Schwacha and Klener, 1997).

**Crossover formation in Drosophila is SC-dependent**

In most organisms, homolog pairing is stabilized by a tighter association called synapsis that is defined by the formation of SC. Generally, the physical structure of SC is conserved among diverse organisms although their protein sequence similarity is very low. It consists of two lateral elements that run along the entire length of each chromosome within homologs, a central element that is midway between the lateral elements, and transverse filaments that connect the lateral elements to the central element (Page and Hawley, 2004).

In many organisms SC formation is dependent on DSBs, e.g., in *S. cerevisiae* (Keeney et al., 1997), *A. thaliana* (Grelon, et al., 2001), and mouse (Romanienko and Camerini-Otero, 2000). In *S. cerevisiae* the crossover frequency is reduced to half of the wild type level when SC does not form due to mutations of zip1, a component of the transverse filament of SC (Sym et al., 1993). In contrast, in *Drosophila* females, SC forms normally in the absence of DSBs. Mutations in *mei-W68* and *mei-P22*, which eliminate both meiotic crossovers and gene conversion, have no effect on SC formation (McKim et al., 1998). However, null mutations in C(3)G, the putative transverse filament protein in Drosophila, eliminate SC formation, meiotic crossing over (Hall, 1972; Rasmussen, 1975; Page and Hawley, 2001), intragenic exchange and gene conversion (Carlson, 1972). Moreover, defects of C(2)M, a putative component of lateral element of SC, cause significant decrease of meiotic crossover (Manheim and McKim, 2003). These studies suggest that crossovers in Drosophila females are processed by SC-dependent pathway.

**Chiasmata hold homologs together with sister chromatid cohesion**

After chiasmata are generated through meiotic recombination, they hold each pair of homologous chromosomes together. However, only chiasmata are insufficient to hold homologous chromosomes, sister chromatid cohesion distal to chiasmata is required to stabilize the interactions between homologs mediated by chiasmata. The loss of arm cohesion between sister chromatids thus can allow homologs to segregate at anaphase I when chiasmata are dissolved (Petronczki

et al., 2003). Mutations of cohesion proteins, like ORD in Drosophila and Rec8 in yeast, are required for maintaining chiasmata (Bickel et al., 2002; Buonomo et al., 2000).

**Cohesion is provided by a multi-protein complex called cohesin**

Sister chromatid cohesion is mediated by a multisubunit complex called cohesin in mitosis and meiosis. Cohesin consists of four proteins: SMC1, SMC3, SCC1/Mcd1/RAD21, and SCC3/SA (Nasmyth, 2001; Losada and Hirano, 2005). SMC1 and SMC3 belong to structural maintenance of chromosomes (SMC) superfamily that is widely conserved. The N- and C-terminal halves of each SMC1 and SMC3 fold back on themselves to form 50nm-long antiparallel coiled-coils. The N and C termini of SMC1 or SMC3 together form an ATP-binding cassette (ABC)-like “head” domain at one end of the coiled-coil, while their central sequences form a “hinge” domain at the other end. SMC1 and SMC3 associate with each other through their hinge domains, generating a V-shaped heterodimer (Melby et al., 1998; Anderson et al., 2002; Haering et al., 2002). SCC1 is a member of the α-kleisin superfamily (Schleiffer et al., 2003). The N- and C- terminal domains of SCC1 bind to the heads of SMC3 and SMC1 (Uhlmann et al., 2000), respectively, thus closing SMC1 and SMC3 heterodimer to form a tripartite ring (Haering et al., 2002), which functions by topologically encircling either a single chromatid, prior to S phase, or a pair of sister chromatids following replication (Uhlmann, 2004). Significantly, the central domain of SCC1 that connects its N- and C- termini contains a site for cleavage

by Separase, a cysteine protease conserved in many organisms. The connection of SMC1 and SMC3 heads provided by SCC1 is essential for sister chromatid cohesion. Proteolytic cleavage of SCC1 by Separase at the onset of mitotic anaphase destroys cohesion between sister chromatids, allowing sister chromatids to disjoin to opposite spindle poles (Nasmyth, 2001). Recent studies show that cohesin's hinge domains are essential not only for dimerization but also for cohesin's association with chromosomes. Transient dissociation of SMC1 and SMC3 hinge domains is required for entry of DNA into cohesin ring (Gruber et al., 2006).

Meiotic cohesins often contain novel subunits that are paralogs of the mitotic subunits (Table 1-1). SMC1β is a meiosis-specific homolog of SMC1 in mammals (Revenkova et al., 2001) and is essential for recombination, synapsis, and sister chromatid cohesion (Revenkova et al., 2004). A meiosis-specific α-kleisin, REC8, replaces SCC1/RAD21 in most meiotic cohesin complexes in many eukaryotes and is necessary for the delayed release of centromeric cohesion as well as for other meiosis-specific cohesive functions. In yeast, cleavage of REC8 by Separase occurs at both AI, in chromosome arms, and at AII, at centromeres. Mutations in the *rec8* genes of budding yeast, fission yeast, C. elegans, Arabidopsis and mice exhibit similar pleiotropic phenotypes, including failure of synapsis, reduced homologous recombination, absence of chiasmata and either premature sister chromatid separation or equational segregation during meiosis I (Klein et al., 1999; Watanable and Nurse, 1999; Eijpe et al., 2003; Cai et al., 2003; Chelysheva et al., 2005).

Table 1-1. Cohesin complex across species in mitosis and meiosis.

| Cell cycle | *S. cerevisiae* | *S. pombe* | *C. elegans* | *D. melanogaster* | *A. thaliana* | *X. laevis* | mammals |
|---|---|---|---|---|---|---|---|
| Mitosis | Smc1 | Psm1 | SMC-1/HIM-1 | DSMC1 | AtSMC1 | XSMC1 | SMC1α |
| | Smc3 | Psm3 | SMC-3 | DSMC3 | AtSMC3 | XSMC3 | SMC3 |
| | Scc1/Mcd1 | Rad21 | SCC-1/COH-2 | DRAD21 | SYN2-4[c] | XRAD21 | RAD21 |
| | Scc3 | Psc3 | SCC-3 | DSA1 | CAB45374 | XSA1, XSA2 | SA1/STAG1, SA2/STAG2 |
| Meiosis | Smc1 | Psm1 | SMC-1/HIM-1 | | | | SMC1β* |
| | Smc3 | Psm3 | SMC-3 | | | | SMC3 |
| | Rec8* | Rec8* | REC-8* | C(2)M*[a] | SYN1/DIF1* | AAH87346* | Rec8* |
| | Scc3 | Psc3, Rec11* | SCC-3 | SNM/DSA2*[b] | | | SA3/STAG3* |

*Meiosis-specific proteins.

[a]There is no obvious Rec8 ortholog in D. melanogaster. C(2)M encodes a distant α–kleisin family member that interacts with DSMC3 and is required for SC formation. The mutations of its putative Separase cleavage sites do not interfere with meiotic chromosome segregation and it has no obvious role in sister chromatid cohesion (Manheim et al., 2003; Heidmann et al., 2004).

[b]*D. melanogaster* genome contains two Scc3 paralogs, DSA1 and SNM (DSA2). SNM is a meiosis-specific protein and has been identified only for homolog paring during meiosis I in Drosophila male meiosis. It has no role in meiosis II and female meiosis and is not required for sister chromatid cohesion (Thomas et al., 2005).

[c]*A. thaliana* genome contains four SCC1/REC8 paralogs. Only SYN1 has been shown to be required for cohesion during meiosis while the other three's role remains to be determined (Cai et al., 2003).

**Cohesion is lost only at chromosome arms in meiosis I**

Sister chromatids are held together by cohesion that is provided by the complex cohesin. Cohesin is generated during DNA replication stage and forms a ring to encircle the pair of sister chromatids (Nasmyth, 2001). During mitosis, proteolytic cleavage of one of the cohesin subunits Scc1/Rad21 by Separase eliminates the cohesion between sister chromatids. Separase activity is inhibited by its inhibitor chaperone securin until the onset of anaphase. Separase is activated when its inhibitor securin is degraded by proteasome mediated by the anaphase-promoting complex/cyclosome (APC/C) together with Cdc20 (Cohen-Fix et al., 1996; Funabiki et al., 1996). The loss of sister chromatid cohesion allow them to separate to opposite spindle poles.

Since meiosis consists of two consecutive rounds of chromosome segregation, cohesion between sister chromatids is required to be lost in a stepwise manner. Loss of cohesion on chromosome arm can abolish the association between homologs and allow them to separate to opposite spindle poles in meiosis I whereas cohesion at centromeric region is maintained to ensure sister chromatids are not separated at meiosis I but can segregate to opposite spindle poles at anaphase II. Studies in many organisms have shown that cohesion loss is performed exactly in the stepwise manner as reasoned above. Rec8 is lost from chromosome arms at anaphase I but maintained at centromeres until meiosis II in *S. cerevisiae* (Klein et al, 1999), *S. pombe* (Watanabe and Nurse, 1999), *C. elegans* (Pasierbek et al., 2001), and mouse (Lee et al., 2003, Lee et al., 2006). Cohesion is released at chromosome arms

during meiosis I through degradation of Rec8, a meiotic-specific paralog of Scc1, by Separase through a similar mechanism in mitosis discussed above (Buonomo et al., 2000; Siomos et al., 2001, Kitajima et al., 2003a).

The regulation of the only loss of arm cohesion at meiosis I is an interesting issue. One can imagine at two mechanisms: the composition of cohesion along arm and at centromeric region is different, or centromeric cohesion is protected whereas arm cohesion is not.

Fission yeast has two Scc3 homologue proteins: Psc3 that is required for sister chromatid cohesion by forming a complex with Rad21, and Rec11 that is meiosis-specific and reduce recombination when it is mutated. Watanabe and his collaborators found that Rec8 forms a cohesion complex with Psc3 at centromeres but with Rec11 on chromosome arms (Kitajima et al., 2003b). This spatially distinct organization of cohesion complex on chromosome may contribute to the temporally-regulated loss of cohesion during meiosis. Similar observations were made in mammals. In diplotene stage STAG2 associates with decondensed chromatin but not with chromosome axis which STAG3 localizes to while Rad21 associates with decondensed chromatin and chromosome axis of desynapsed SCs. Furthermore, Rad21 localizes to the desynapsed chromosome region in which STAG3 shows weak or little signals (Prieto et al., 2002).

**Centromeric cohesion is protected from cleavage during meiosis I**

Studies have shown that in many organisms centromeric cohesion is retained during meiosis I and until anaphase II and that its protection during meiosis I is essential for bi-orientation and segregation of sister chromatids during meiosis II (Watanabe, 2005). How is centromeric cohesion protected during meiosis I? Several factors have been shown to be required for maintaining centromeric cohesion during meiosis I. Rec8 is essential for the meiosis-specific cohesin at centromeres escaping the cleavage at anaphase I. The replacement of Rec8 with Scc1/Rad21 results in the loss of cohesins along the entire chromosomes at anaphase I in budding and fission yeast (Toth et al., 2000; Yokobayashi et al., 2003) whereas centromeric cohesin is normally protected at anaphase I. Spo13, a meiosis-specific protein without conserved motif in current database, is required for maintaining centromeric cohesion during meiosis I in budding yeast (Shonn et al, 2002). The ability to maintain Rec8 at centromeric region during meiosis I is impaired in *spo13* cells and sister chromatid cohesion at centromeres is not protected effectively (Klein et al., 1999; Shonn et al., 2002; Katis et al., 2004b; Lee et al., 2004). Recent studies have shed lights into the mechanisms of protection of centromeric cohesion: a shugoshin protein family play a major role in protecting meiosis-specific cohesin during meiosis I.

The protector of centromeric cohesion was identified in an elegantly designed genomic screen by Kitajima et al. (2004). The authors reasoned that if Rec8 was forcibly co-expressed with a centromeric cohesion protector in mitosis,

it might be toxic to cells since sister chromatids could not segregate efficiently. They identified such a gene called Sgo1 (Shugoshin) that is a distant relative of Mei-S332 in Drosophila, which has long been thought to be a candidate for a protector of centromeric cohesion during meiosis I based on its localization, timing and phenotype (Kerrebrock et al., 1992; Kerrebrock et al., 1995). In *sgo1Δ* cells, Rec8 is not retained at centromeric regions from late anaphase I and sister chromatids segregate precociously. Moreover, Sgo1's localization to centromeres is regulated by a conserved centromere-associated kinase Bub1 that is involved spindle checkpoint for delaying activation of the APC/C until all chromosomes are under tension on metaphase plate and is required for protecting centromeric cohesion (Bernard et al., 2001). Bub1 deletion leads to disappearance of punctuate foci of Sgo1 at centromeres, suggesting the protection of centromeric cohesion by Bub1 is achieved by recruiting Sgo1 to centromeres. Three other screens carried out in fission yeast and budding yeast yielded similar results (Katis et al., 2004a; Marston et al., 2004; Rabitsch et al., 2004). A minor difference is that Sgo1 seems only exist at meiosis I in fission yeast while Sgo1 exists until metaphase II in budding yeast (Kitajima et al., 2004; Rabitsch et al., 2004). Studies show that cohesion mediated by Rec8 is properly established in *sgo1* deletion mutants and the precocious loss of cohesion at centromeric region in *sgo1* mutants is due to the failure to protect Rec8 from cleavage by Separase (Rabitsch et al., 2004). Interestingly, Spo13 functions independently of Sgo1 to protect centromeric cohesion since its depletion has little or no effect on localization of Sgo1 to centromeres (Katis et al., 2004; Lee et

al., 2004). Recent studies show that PP2A, a serine/threonine protein phosphatase 2A, co-operates with Sgo1 to protect centromeric cohesion in both fission and budding yeast (Kitajima et al., 2006; Riedel et al., 2006; Tang et al, 2006). In Drosophila, Polo kinase is required for dissociation of MEI-S332 from centromeres (Clarke et al., 2005) while Aurora B kinase is necessary for loading MEI-S332 to centromeres (Resnick et al., 2006), suggesting that MEI-S332/Sgo1's activity is regulated by phosphorylation. However, how these phosphorylation and/or dephosphorylation events are regulated to make MEI-S332/Shugoshin turn on/off at different time windows is not known yet.

**Co-orientation of sister kinetochores**

In meiosis II or mitosis, sister kinetochores (kinetochores of sister chromatids) are attached to microtubules from opposite poles (bi-orientation). However, sister kinetochores must attach to microtubules from the same pole in meiosis I to ensure that sister chromatids move together, a phenomenon called co-orientation (or mono-orientation).

The observation that homologs taken from grasshopper spermatocytes in meiosis I can segregate in a reduction-like manner (co-orientation of sister chromatids) when they were transported into a meiosis-II like spindle (Paliulis and Nicklas, 2000), suggests that kinetochores in meiosis I are modified to ensure co-orientation of sister centromeres (or sister chromatids). In *Drosophila melanogaster*, sister kinetochores are fused at early prometaphase I when microtubules begin to attach, but become two distinct kinetochores before the

onset of anaphase I (Goldstein, 1981).

It seems that chiasmata do not play a role in co-orientation of sister kinetochores. In budding and fission yeast, deletions of the spo11 or rec12 genes that generate DSB breaks do not interfere with co-orientation of sister kinetochores (Klein et al., 1999; Kitajima et al., 2003b). In *Drosophila melanogaster* females, mutations of the gene c(2)M that is required for SC formation result in failure of synapsis (thus leading to defects in chiasma formation) but show little significant defects in sister chromatid segregation (Manheim and McKim, 2003).

Sister chromatid cohesion has been shown to play an important role in co-orientation of sister kinetochores in both budding yeast and fission yeast. Loss of cohesion due to *rec8* mutation*s* leads to failure of sister kinetochore co-orientation and random sister chromatid segregation during meiosis I in budding yeast (Klein et al., 1999). In fission yeast, loss of rec8 functions causes predominantly equational orientation of sister kinetochores at meiosis I (Watanabe and Nurse, 1999; Yokobayashi and Watanabe, 2005).

In addition to cohesin, other specific complexes have been shown to be involved in co-orientation of sister chromatids in budding yeast and fission yeast. The Monopolin complex containing Mam1 (monopolar microtubule attachment during meiosis I), Lrs4 (loss of rDNA silencing-4), and Csm1 (chromosome segregation in meiosis I), is required for co-orientation of sister kinetochores in budding yeast. Mam1 is a meiosis-specific protein that resides at kinetochores from pachytene to metaphase I (Toth et al., 2000) while Lrs4 and Csm1 are

expressed during both meiosis and mitosis. Lrs4 and Csm1 localize in the nucleolus until G2 when they are released by the polo kinase Cdc5 and then form a monopolin complex with Mam1 and bind to kinetochores (Clyne et al., 2003; Rabitsch et al., 2003). Spo13 is also required for co-orientation of sister kinetochores. The monopolin complex initially associates with kinetochores but cannot be maintained in *spo13Δ* cells (Katis et al., 2004b; Lee et al., 2004). Recently, Hrr25, a highly conserved casein kinase was found binding to Mam1 to facilitate co-orientation of sister kinetochores (Petronczki et al., 2006). However, how the monopolin complex can promote co-orientation of sister kinetochores is still elusive.

Pcs1, the homolog of monopolin component Csm1, has been identified in fission yeast. Surprisingly, Pcs1 is not required for co-orientation of sister kinetochores during meiosis I but is essential for proper chromosome segregation in meiosis II and mitosis (Rabitsch et al., 2003). Recent study has found that Moa1 (monopolar attachment) is essential for co-orientation of sister kinetochores in fission yeast (Yokobayashi and Watanabe, 2005). Moa1 is a meiosis-specific protein that localizes to the central core of centromeres, which the cohesin containing Rec8 binds to. In haploid meiosis sister chromatids usually segregate reductionally but in *moa1Δ* haploids, sister chromatids segregate equationally. Interestingly, Moa1 interacts with Rec8 *in vitro* and *in vivo*, suggesting that their cooperation promotes co-orientation (Yokobayashi and Watanabe, 2005).

How does the monopolin complex or Moa1 promote sister kinetochore co-

orientation? A recent study by Amon and her collaborators found that Aurora B kinase plays a key role in this process (Monje-Casas et al., 2007). Homologous chromosomes segregate to the same spindle pole in meiosis I cells in which Ipl1 (homolog of Aurora B kinase in budding yeast) is depleted while homologous chromosomes normally segregate to opposite poles in wild type cells. Depletion of Ipl1 in *mam1Δ* cells causes sister chromatids to segregate to one spindle pole while sister chromatids segregate to opposite poles in the first division in single *mam1Δ* diploid cells, suggesting Ipl1 depletion suppresses the co-orientation defects of cells. These studies suggest that Ipl1 probably severs microtubule-kinetochore attachments that are not under tension by phosphorylating kinetochore components, as it does in mitosis (Cheeseman et al., 2002; Tanaka et al., 2002, Dewar et al., 2004). By contrast with mitosis, the presence of monopolin alters sister kinetochores such that they are under tension only when homologous chromosomes are bi-oriented.

**Meiosis II is an equational meiotic division**

Chromosome segregation in meiosis II is similar with that in mitosis. Sister kinetochores are bi-oriented, in contrast to their co-orientation in meiosis I. Centromeric cohesion, which is protected from cleavage by Shugoshin during meiosis I, can resist the pulling forces from opposite poles before anaphase II, thereby prevent sister chromatids from precocious separation. At the onset of anaphase II centromeric cohesion is degraded by Separase due to absence of Shugoshin, allowing sister chromatids segregate to opposite poles.

## Meiosis in Drosophila melanogaster

The fruit fly Drosophila melanogaster is an excellent model organism to study meiosis during spermatogenesis and oogenesis. Drosophila has a short life cycle. As early as 11 days after mating the next generation of adult offspring can emerge under standard culture conditions. *Drosophila melanogaster* has only four pairs of chromosomes, which can be easily seen during male meiosis at cytological level. The abundant collections of stocks, constructs, and clones of *Drosophila melanogaster* are invaluable to Drosophila researchers who work on various fields. Drosophila females have the “standard” meiotic system with formation of SC, recombination, and chiasmata and is used to study common meiotic events while Drosophila males undergo meiosis without SC, recombination, or chiasmata, and thus become a good model to study achiasmate meiosis. This is valuable for researchers to learn how meiosis processes when normal SC formation, recombination, and chiasmata are absent. Furthermore, Drosophila females have a distributive (“back-up”) system for pairing and segregation of chromosomes that do not exchange, like the fourth chromosomes that never recombine. Moreover, the lineages of spermatogenesis and oogenesis are beneficial to the study of control of cell cycle and other developmental mechanism.

## Meiosis in male Drosophila

### Cytological aspects of spermatogenesis

Spermatogenesis in Drosophila males has been well characterized at

cytological level by staining DNA and spindle structure. At the apex of the testis, primary spermatogonia are generated by stem cells through asymmetric divisions. They are surrounded by somatic cyst cells derived from progenitor cyst cells. The primary spermatogonia undergo four rapid mitotic divisions and generate cysts containing 2, 4, or 8 secondary spermatogonia and 16 primary spermatocytes. Secondary spermatogonia are easily recognized by the cell number in one cyst and the nuclei that are almost entirely occupied by chromatin. The primary spermatocytes undergo pre-meiotic S phase immediately after the last gonial mitotic division, followed by a 4-day growth period featuring high levels of transcription and a series of characteristic changes in nuclear morphology that lead to 25-fold increase in primary spermatocyte size (Cenci et al., 1994; McKee, 2004).

A detailed cytological analysis of spermatocyte growth and meiotic stages has been carried out by Cenci et al. (1994). Chromosomes are initially clustered as a compact chromatin mass in the center of the nucleus during S1-S2a stages (considered as early prophase I). Chromosomes resolve into three different nuclear regions known as “territories”, which are associated with the inside of nuclear membrane by stage S3. These separate territories are evident throughout mid prophase I (stages S3 and S4) and late prophase I (stages S5 and S6) (Cenci et al., 1994). Chromosomes begin condensing in stage S6 and the territories persist until prometaphase I when nuclear membrane breaks down. At prometaphase I, three big DNA clumps that represent bivalents of sex chromosomes, chromosome 2 and 3 are often seen and sometimes a fourth tiny

DNA clumps that represents the chromosome 4 bivalent. Two microtubule asters are evident from prometaphase I. At metaphase I one big DNA clump is usually seen at the center of the nucleus since bivalents are aligned at metaphase plate. At anaphase I each of two DNA clumps can be seen at opposite poles due to segregated bivalents. At telophase I chromatin is relatively decondensed and microtubules appear to decrease. At this point one intact cyst contains 32 secondary spermatocytes. The chromatin and microtubules undergo similar dynamics during meiosis II and 64 spermatids are produced (Cenci et al., 1994).

**Achiasmate meiosis in Drosophila males**

Most organisms undergo meiosis with recombination, synapsis and ensuing chiasmata. However, a non-typical meiosis, achiasmate meiosis, i.e. meiosis without chiasmata, exists in numerous eukaryotes, such as lepidopteran females and dipteran males, although homologous pairing is also essential for their meiosis. In Drosophila males, which provide the best-studied model for achiasmate meiosis, recombination is completely absent and no SC and chiasmata form, but chromosome pair and segregate regularly to opposite spindle poles (Hawley, 2002; McKee, 2004).

How do the homologs pair in Drosophila males? Although homologs do pair at prophase I without recombination and chiasmata formation, the limitations of available cytological methods have hindered study of the mechanism of pairing. Use of GFP-Lac repressor/Lac operator (LacI/O) system in which GFP-LacI can target to LacO sequences inserted on euchromatic regions of

chromosomes, allowed Vazquez and his collaborators to characterize the dynamics of pairing and to track the movements of chromosome arms (Vazquez et al., 2002. In males homozygous for a single LacO insertion, the presence of a single GFP-LacI spot indicates pairing whereas the presence of two separate spots indicates that the marked loci are unpaired. During G2, one to four separate spots can be seen if sister chromatid cohesion is absent. In live spermatogonia and spermatocytes Vazquez et al. observed that about half of pre-meiotic spermatogonia were paired (one spot) and half of them were unpaired (two spots). By contrast, more than 95% of young primary spermatocytes (stages S1 and S2) exhibited pairing as an evident fluorescent spot, suggesting a tight connection along chromosome arms. This tight pairing disappears and four separate spots become evident at stage S3, the beginning of mid prophase I when distinct territories begin to form. This result indicates that sister chromatid arms as well as homologs are separate. Interestingly, homologous and sister foci remain with a common territory throughout the late prophase I (stage S5 and S6). However, the possibility that homologs are still paired at specific regions, like rDNA region, cannot be ruled out. Further investigation is needed to elucidate this issue.

In contrast to the loss of tight pairing of sister chromosome arms separate at S3, Vazquez et al. (2002) found that sister centromeres are tightly paired throughout the prophase I and actually majority of sister centromeres are clustered at early prophase I.

The observation that homologs are paired in young spermatocytes

provides direct evidence that Drosophila males enter meiosis with already paired homologs, which is consist with the previous observation that homolog pairing occurs in pre-meiotic cells, as early as anaphase of the last mitotic gonial division (Metz, 1926). However, whether homolog pairing in meiosis originates directly from the pairing at mitotic stage is not determined. Homolog pairing established in mitosis may be lost since there is S phase between the last mitotic division and meiosis and then is re-established in the first beginning of meiosis. The fact that only half of spermatogonia are paired support the idea that at least homolog pairing in some spermatocytes is not established in the last mitosis division.

**rDNA as pairing sites for X-Y chromosomes**

Studies have shown that some specific sites or regions may facilitate pairing. The best characterized such site is the X-Y pairing site that has been mapped to the rDNA loci, which consist of 200-250 tandem copies of the genes for the 18S, 5.8S and 28S ribosomal RNAs (rRNAs). The rDNA arrays are located in the middle of the proximal heterochromatin of X chromosome and near the centromere on the short arm of Y chromosome, respectively. Deletions of X chromosome heterochromatin that remove all rDNA cause failure of X-Y pairing and X-Y nondisjunction during meiosis I, whereas incomplete deletions that leave as few as 6-8 rDNA repeats do not affect pairing and segregation of X and Y chromosomes (McKee and Lindsley, 1987). Moreover, transgenes with a single ribosomal RNA gene can partially restore X-Y pairing and disjunction when inserted into a rDNA-deficient X chromosome (McKee and Karpen, 1990).

Elegant experiments have shown that the 240bp repeat sequence in the intergenic spacer between ribosomal RNA genes, which may present in 1000-2000 copies in total, is the primary site for pairing and segregation of X and Y chromosomes (McKee et al., 1992; Merrill et al., 1992; McKee, 1996) (Fig. 1-3). In their experiments, seven tandem 240bp repeats can effectively stimulate X-Y pairing and segregation even if the rRNA transcription unit is completely removed. In contrast, the rRNA transcription units without 240bp repeats fail to stimulate X-Y pairing and disjunction.

A recent study identified two proteins that appear to act at the X-Y pairing sites of Drosophila males: Stromalin in Meiosis (SNM), and Mod(mdg4) in Meiosis (MNM) (Thomas et al., 2005). SNM shares homology with SCC3/SA, which is a component of sister chromatid cohesion complex. MNM is a protein with BTB domain, which is involved in many protein-protein interactions. During prophase I both SNM and MNM localize to multiple foci in the nucleolus, representing the rDNA region that contains the 240bp repeats. SNM and MNM colocalize and form a dense focus that is associated the 240bp repeat during prometaphase I and metaphase I but disappear at anaphase I, suggesting that in achiasmate meiosis SNM and MNM may substitute for chiasmata to hold homologs. Using heterochromatic mini-X chromosomes that lack of native rDNA but carry transgenic 240bp repeat arrays, Thomas and McKee (2007) found that mini-X chromosomes segregate primarily from normal sex chromosomes and from each other and the mini-X chromosome pairs associate with the X-Y bivalent to form trivalents and quadrivalents but do not form an

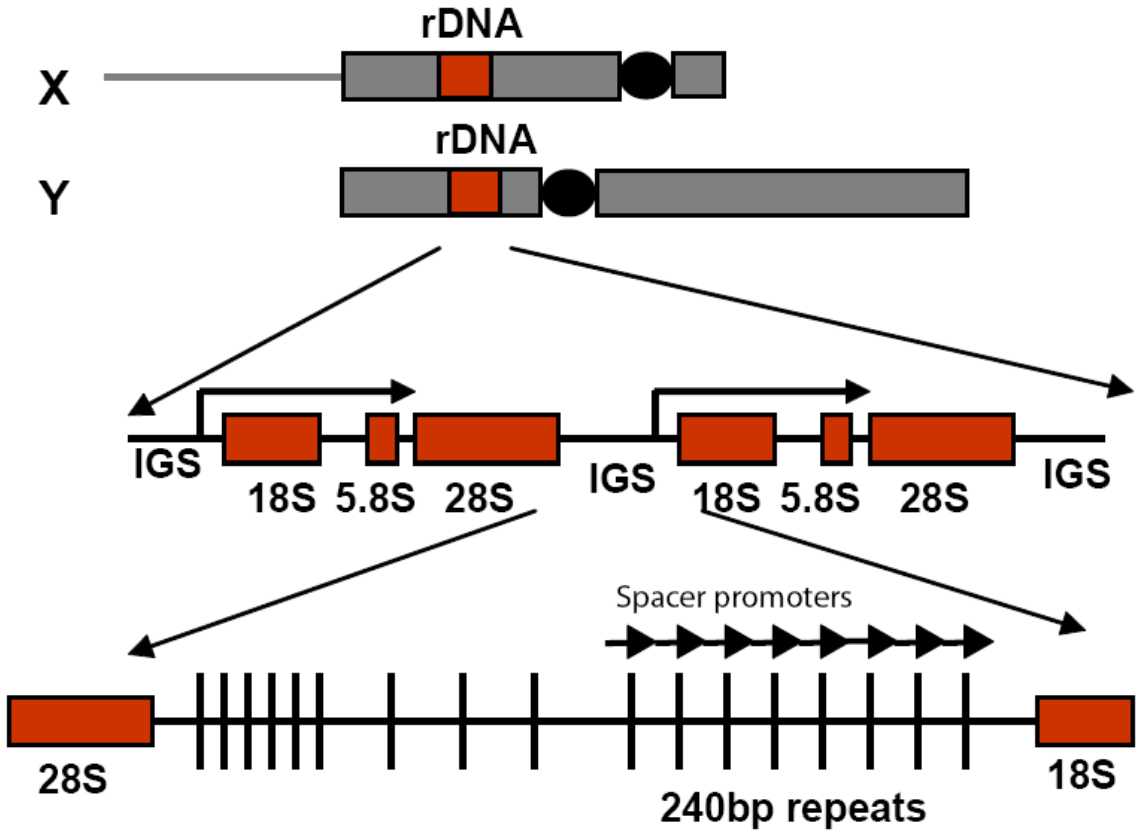



Figure 1-3. The structure of rDNA region on X and Y chromosomes.

See text for details.

additional pair of chromosomes. Furthermore they found that both SNM and MNM are required for disjunction of mini-X chromosome pairs and multivalent formation. This study strongly suggests 240bp repeat is the biding site for SNM and MNM to mediate the association among sex chromosomes.

**Oogenesis and meiosis in female Drosophila**

Drosophila females, in contrast to males, undergo classical meiosis in which crossovers between homologs are required for homolog segregation at meiosis I, like most of other sexually reproducing organisms. Oogenesis in female Drosophila is an excellent system to study meiosis because germ cells are arrayed in a linear and chronological way with respect to the order of developmental stages. Drosophila females have two ovaries that consist of 15-30 ovarioles (Fig. 1-4). At the tip of each ovariole is the germarium while the remainder is called the vitellarium.

The germarium is divided into four regions, region1, region 2a, region 2b, and region3. Stem cells at region 1 undergo 4 incomplete mitotic divisions (without cytokinesis) and generate 16-cell cysts (Spradling, 1993). In region 2a, 16-cell cysts undergo a pre-meiotic S phase and initiate meiosis. Two of 16 cells become pro-oocytes and one of the two pro-oocytes will finally develop into the oocyte while the other 15 cells will develop into nurse cells (Spradling, 1993). Two important events occur at region 2a. SC formation occurs in two pro-oocytes and may initiate in up to four cells per cyst, which can shown by antibodies against C(3)G, a transverse filament of SC, or C(2)M, a lateral

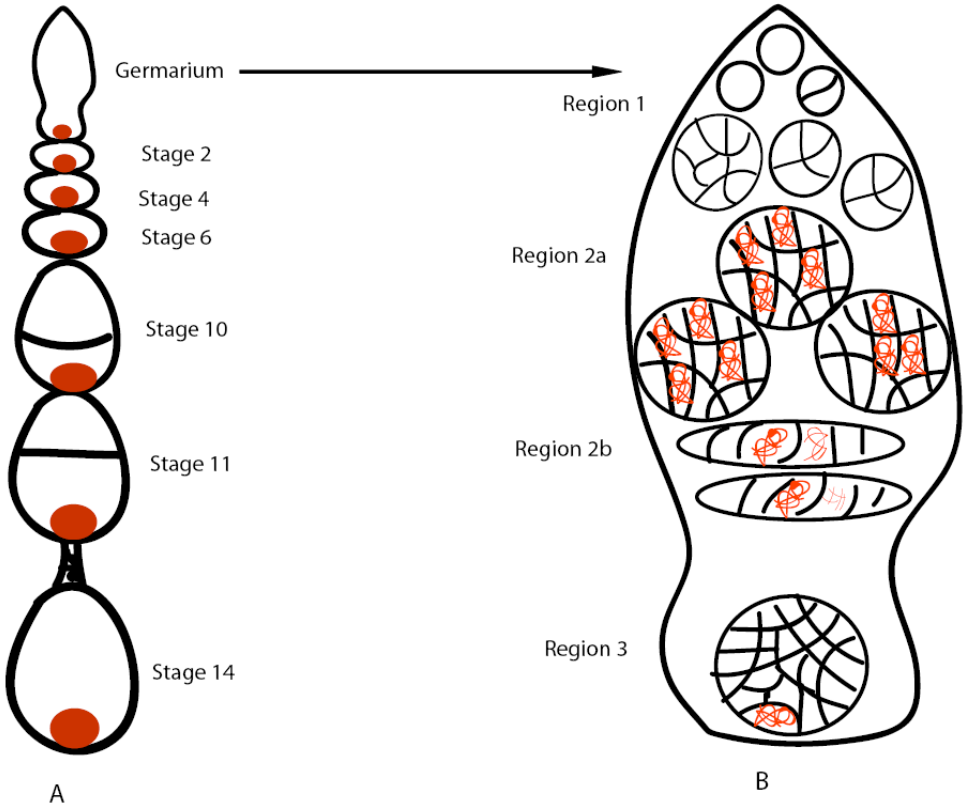



Figure 1-4. Schematic drawings of Drosophila ovariole and germarium.

Ovary consists of 15-30 ovarioles (A). Red ovals represent oocytes. Each ovarioles consists of a germarium (B) connected to series of a developing egg chambers. Mitotic divisions occur at region 1. In region 2a, meiosis initiates, synaptonemal complex (SC) assembly begins and meiotic recombination begins. In region 3 SC is restricted to the oocytes and recombination is completed. Red thread-like structures represent SC. See text for details.

element of SC. In addition, meiotic recombination is initiated in region 2a since DSBs appear and can be detected by using antibodies against phosphorylated H2Av (γ-H2Av). In region 2b, the cysts become flattened out. SCs still exist in two pro-oocytes. However, γ-H2Av foci disappear, suggesting meiotic recombination is finished or almost finished. As early as region 2bb but not later than region 3, the fates of the two pro-oocytes are determined and complete SC is restricted to the oocyte, which is located at the end of germarium (McKim et al., 2002). As cysts continue to mature, they move toward the posterior part of the ovariole. The oocyte remains in pachytene with full-length SC until stage 6. After 14-stage development, the cyst arrives at the end of the ovariole and arrests at metaphase I (stage 14). The other 15 nurse cells in each cyst undergo multiple round of S phase DNA synthesis but lacking of mitosis, leading to polyploid DNA in the cells (Dej and Spradling, 1999).

**Meiotic cohesion in Drosophila is not clear**

The knowledge about cohesion in higher eukaryotes is still limited and controversial to some extent although the mechanisms of cohesion in budding and fission yeast have been well characterized. The mechanism of meiotic cohesion in Drosophila is particularly elusive since REC8-containing cohesin is not identified and only limited mutants of cohesion genes are available.

The genome of Drosophila melanogaster has one single copy of SMC1 and SMC3, two members of SCC1 family (RAD21 and C(2)M), two members of SCC3 family (SA and SNM), but no functional REC8 ortholog (Adams et al.,

2000). No viable SMC1 and SMC3 mutants have been available for studying meiosis until now. RAD21's role in mitosis has been studied while whether it is involved in meiosis is not known (Warren et al., 2000a; Warren et al., 200b; Vass et al., 2003). Similar to RAD21, SA's role in mitosis is characterized while its role in meiosis is not known (Valdeolmillos et al., 2004). C(2)M promotes SC formation at prophase I but it shows no or little role in sister chromatid cohesion in female meiosis and it is not required for male meiosis (Manheim and McKim, 2003; Heidmann et al., 2004; Khetani and Bickel, 2007). A recent study by McKee lab has identified SNM, a SCC3/SA paralog, that is required for maintaining homolog pairing but is not required for sister chromatid cohesion in male meiosis. In addition, SNM has no role in female meiosis (Thomas et al., 2005). The studies have not provided any clue to meiotic cohesin in Drosophila: is there a novel cohesin? or is a classical cohesin just not identified?

Until now, only a few meiotic proteins required for maintaining but not establishing cohesion in both males and females have been characterized. One of them is *mei-S332*, a member of the Shugoshin family. MEI-S332 is required to prevent centromeric cohesion from degradation at meiosis I (Kerrebrock et al., 1992; Kerrebrock et al., 1995; Katis et al., 2004a; Rabitsch et al., 2004). *ord* is required for maintaining centromeric cohesion in male meiosis and is essential for maintaining SC and meiotic recombination in female meiosis (Miyazaki and Orr-Weaver et al., 1992; Bickel et al., 1997; Webber et al., 2004). INCENP (inner centromere protein), a component of chromosomal passenger complex that is required in mitosis for chromosome condensation, spindle attachment, and

cytokinesis (Adams et al., 2001; Carmena and Earnshaw, 2003; Vagnarelli and Earnshaw, 2004), is essential for successful meiosis. Mutations of *incenp* lead to premature loss of sister chromatid cohesion in meiosis (Resnick et al., 2006). A recent study has shown that BubR1, a protein required for the spindle checkpoint during mitosis, is also essential for maintaining sister chromatid cohesion at centromeres at anaphase I (Malmanche et al., 2007). However, whether BubR1 in Drosophila has a similar role with Bub1 in fission yeast to recruit MEI-S332/Shugoshin has not been determined. Other than that, no cohesion protein is identified and severely hindering the study of meiotic cohesion in Drosophila.

# CHAPTER 2 - SOLO IS A NOVEL PROTEIN REQUIRED FOR SISTER CHROMATID COHESION, SISTER CENTROMERE CO-ORIENTATION, AND CENTROMERIC LOCALIZATION OF SMC1 IN DROSOPHILA MEIOSIS

This part is modified from the manuscript that has been submitted to *Current Biology* and is under revision now.

Rihui Yan's primary contributions: identified the gene *solo*, analyzed some genetic phenotypes of *solo*, analyzed *solo* phenotypes at cytological level, cloned *solo* gene, analyzed its localization pattern in wild type and cohesion mutants ,and wrote the manuscript draft.

**Abstract**

Sister chromatid cohesion plays several essential roles in meiotic chromosome segregation, including maintenance of stable connections between homologs and sister chromatids, and establishment of correct sister centromere orientation patterns on the meiosis I and II spindles. Cohesin has been proposed as the key factor; however, its mechanism in higher eukaryotes is still elusive. We describe a novel protein, SOLO (Sisters On the LOose) that is essential for meiotic cohesion in *Drosophila melanogaster*. *solo* mutations cause high nondisjunction of sister and homologous chromatids of sex chromosomes and autosomes. In *solo* males, sister chromatids separate prematurely and segregate randomly during meiosis II. Although bivalents remain intact throughout meiosis I, sister centromeres lose cohesion prior to prometaphase I and orient nearly randomly on the meiosis I spindle. SOLO and the cohesin protein SMC1 co-localize to meiotic centromeres from early prophase I until anaphase II in wild-type males but both proteins are removed prematurely from centromeres at anaphase I in *mei-S332* mutants, coincident with premature loss of cohesion in those mutants. In addition, centromeric foci of SMC1 are absent in *solo* mutants at all stages of meiosis. The mutant phenotypes and localization patterns of SOLO and SMC1 indicate that they function together to maintain sister chromatid cohesion in *Drosophila* meiosis. Our data also show that MEI-S332 protects cohesin from premature removal at anaphase I, similar to its ortholog Shugoshin's functions in yeast.

# INTRODUCTION

Meiosis consists of two divisions, a reductional division at meiosis I in which homologous chromosomes (homologs) pair and segregate to opposite spindle poles, and an equational division at meiosis II in which sister chromatids segregate. Cohesion between sister chromatids is essential for proper chromosome segregation at both meiotic divisions (Page and Hawley, 2003; Petronczki et al., 2003; McKee, 2004). Several roles of sister chromatid cohesion in meiosis I have been defined in yeast. First, during prophase I, cohesion between sister chromatid arms is essential for formation of lateral elements of synaptonemal complexes; consequently cohesion mutations disrupt synapsis. Second, arm cohesion distal to crossover sites is required to stabilize chiasmata during late prophase I and metaphase I. Third, cohesion between sister centromeres is required for their “co-orientation” to the same pole on the meiosis I spindle, which is a prerequisite for bi-orientation of homologous centromeres. Sister chromatid cohesion is also required to prevent sister chromatids from separating prematurely prior to anaphase I, and to enable sister centromeres to orient to opposite poles (bi-orient) on the meiosis II spindle. The multiple functions of cohesion in meiosis require it to be released in a stepwise manner. Arm cohesion is released at anaphase I, destabilizing chiasmata and allowing segregation of homologs, whereas centromere cohesion is released until anaphase II, allowing segregation of sister chromatids (Lee and Orr-Weaver, 2001; Nasmyth, 2001; Petronczki et al., 2003; Page and Hawley, 2004; Hauf and

Watanabe, 2004; Watanabe, 2005).

In both mitosis and meiosis, cohesion is mediated by a complex called cohesin that consists of four proteins, two SMC (Structural Maintenance of Chromosomes) subunits, SMC1 and SMC3, and two non-SMC subunits, SCC1/RAD21 and SCC3/SA (Nasmyth, 2001; Schleiffer et al., 2003; Losada and Hirano, 2005). In mitosis, SMC1 and SMC3, and SCC1/RAD21, a member of the kleisin superfamily (Schleiffer et al., 2003), form a tripartite ring that topologically encircles either a single chromatid, prior to S phase, or a pair of sister chromatids following replication. Proteolytic cleavage of SCC1 by Separase at the onset of mitotic anaphase destroys cohesion between sister chromatids, allowing sister chromatids to disjoin to opposite spindle poles (Uhlmann, 2004).

Meiotic cohesins contain novel subunits that are paralogs of mitotic subunits (Petronczki et al., 2003; Losada and Hirano, 2005). REC8 replaces SCC1/RAD21 in most meiotic cohesin complexes and is necessary for the delayed release of centromeric cohesion as well as for other meiosis-specific cohesive functions. In yeast, cleavage of REC8 by Separase occurs at both anaphase I, on chromosome arms, and at anaphase II, at centromeres (Petronczki et al., 2003).

Cohesin genes are conserved throughout the eukaryotes (Losada and Hirano, 2005; Schleiffer et al., 2003). Although the role of cohesin in meiosis is less well-defined in higher eukaryotes, there is considerable evidence for functions related to those in yeast (Pasierbek et al., 2001; Siomos et al., 2001; Cai et al., 2003; Chelysheva et al., 2005; Xu et al., 2005; Kudo et al., 2006).

Recently, centromere proteins called Shugoshins that protect centromeric REC8 cohesin from cleavage at meiosis I have been described (Katis et al., 2004; Kitajima et al., 2004; Rabitsch et al., 2004).

However, the universality of cohesin as a mediator of meiotic cohesion has not been established in higher eukaryotes. Although immunocytological and genetic analyses have demonstrated a major role of cohesins in SC formation and chiasma function in several higher eukaryotes (Pasierbek et al., 2001; Prieto et al., 2001; Siomos et al., 2001; Cai et al., 2003; Chelysheva et al., 2005; Xu et al., 2005; Kudo et al., 2006), the mechanism underlying meiotic centromere cohesion and centromere orientation during meiosis I are poorly understood (Toth et al., 2000; Parra et al., 2004; Chelysheva et al., 2005; Yokobayashi et al., 2005).

Cohesin and its role in Drosophila meiosis have been particularly murky. The Drosophila genome includes single SMC1 and SMC3 genes and two members each of the SCC1 (RAD21 and C(2)M), and SCC3/SA (SA and SNM) families, but there is no clear functional REC8 ortholog (Adams et al., 2000). Rad21's functions in mitosis have been examined (Warren et al., 2000a, Warren et al., 2000b; Vass et al., 2003; Valdeolmillos et al., 2004) but a role in meiotic cohesion has not been reported. C(2)M, which exhibits weak similarity to SCC1 and REC8, localizes to the lateral elements of synaptonemal complex during prophase I in oocytes and is required for synapsis, but is absent after mid-prophase I and dispensable for chiasma stability, sister chromatid cohesion and recruitment of centromeric cohesin (Manheim and McKim, 2003; Heidmann et al.,

2004; Khetani and Bickel, 2007). SNM, a meiosis-specific SCC3/SA paralog, is required for stable homolog pairing in achiasmate meiosis of Drosophila males in which homologs pair and segregate without crossing over, SC or chiasmata, but is not required for sister chromatid cohesion in males or for any aspect of female meiosis (Thomas et al., 2005).

Although cohesin mutants are lacking, mutations in three Drosophila genes have been shown to disrupt meiotic cohesion. Mutations in *mei-S332*, which encodes a Shugoshin homolog that localizes to meiotic centromeres from prometaphase I through anaphase II, cause precocious sister chromatid separation (PSCS) beginning at anaphase I and result in high frequencies of meiosis II NDJ (Kerrebrock et al., 1992; Kerrebrock et al., 1995). Mutations in *orientation disruptor (ord)*, which encodes a meiosis-specific protein that localizes to centromeres from late prophase I through anaphase II, cause PSCS and chromatid mis-segregation in both meiosis I and II. ORD has no recognizable domains and no orthologs outside of the genus Drosophila and its molecular function is unclear (Miyazaki and Orr-Weaver, 1992). Mutations in the INCENP protein, a component of the Aurora B kinase complex present on both mitotic and meiotic centromeres, disrupt cohesion prior to metaphase I, leading to chromatid nondisjunction (NDJ) at both meiosis I and meiosis II (Carmena and Earnshaw, 2003; Resnick et al., 2006); however, the cohesion component which INCENP interacts with has not been identified.

Here we describe a novel Drosophila protein, SOLO (Sisters On the LOose), that is required for sister chromatid cohesion in both meiosis I and

meiosis II and for sister centromere co-orientation during meiosis I. Mutations in *solo* cause high nondisjunction of sex chromosomes and autosomes in both meiotic divisions and premature separation of sister centromeres during prophase I. SOLO and the cohesin protein SMC1 co-localize to meiotic centromeres from early prophase I until anaphase II, and both proteins are removed prematurely from centromeres in mei-S332 mutants. Moreover, SOLO is required for centromere localization of SMC1 at all stages of meiosis. Our data indicate that both SOLO and SMC1 play direct roles in sister chromatid cohesion during Drosophila meiosis.

# MATERIALS AND METHODS

## Fly stocks, special chromosomes and Drosophila culture methods.

The *solo* mutations in this paper were from the Zuker-2 (Z2) collection of more than 6000 lines with EMS-mutagenized second chromosomes (Koundakjian et al., 2004). The Z2 lines used in this study were identified in a screen for paternal $4^{th}$ chromosome loss and were kindly provided by B. Wakimoto (Wakimoto et al., 2004). *vas* alleles were obtained from M. Ashburner (Cambridge University), P. Lasko (McGill University), D. Montell (John Hopkins University), and the Bloomington Stock Center at the University of Indiana. *mei-S332* alleles were kindly donated by T. Orr-Weaver (Whitehead Institute, MIT). *ord* alleles and its deficiency were kindly provided by S. Bickel at (Dartmouth College). Compound chromosomes and markers are described in Flybase (2007a). Unless otherwise specified, tested males were crossed singly to two or three females in shell vials. All flies were maintained at 23°C on standard cornmeal molasses medium. Parents were removed from the vial on day 10 and progeny were counted between day 13 and day 22.

## Sex chromosome NDJ assays

To measure X-Y NDJ, $+/B^{s}Yy^{+}$ males were crossed singly to 2-3 females carrying structurally normal X chromosomes marked with $y^{1}$ and $w^{1118}$. Regular progeny are + females and $y^{+}$ w $B^{S}$ males; paternal NDJ generates $y^{+}$ $w^{+}$ $B^{S}$

female and y w $B^+$ male progeny. %NDJ = 100 x ($y^+$ $w^+$ $B^S$ ♀♀ + y w $B^+$ ♂♂)/N. To discriminate between NDJ of homologs and sister chromatids, +/$B^sYy^+$ males were crossed singly to 2-3 *C(1)RM,* $y^2$ *su(*$w^a$*)* $w^a$*/0* females in which both X chromosomes are attached to a single centromere. These females produce eggs that are diplo-X and nullo-X at approximately equal frequencies. When nullo-X eggs are fertilized, the cross yields progeny derived from both XX sperm and XY sperm (+ females and $B^S$ males, respectively), which are diagnostic of sister chromatid and homolog NDJ, respectively, as well as progeny of nullo-XY (O) sperm ($y^2$ *su(*$w^a$*)* $w^a$*)* females), which can result from either type of NDJ. Progeny from XXY, XYY and XXYY sperm were very rare and thus they were neglected in the analysis.

**Measuring 2nd chromosome NDJ**

*solo*$^{Z2\text{-}0198}$*, cn bw/b vas*$^7$ *pr* males were crossed singly with three *C(2)EN, bw sp* females. *vas*$^7$ is null for both *vas* and *solo* function. *C(2)EN* females carry two copies of each arm of chromosome 2 attached to a single centromere and produce only diplo-2 (2^2) and nullo-2 (O) eggs, so the only viable progeny are the products of paternal chromosome 2 NDJ (2/2 and O sperm). Thus the frequency of 2nd chromosome NDJ is proportional to progeny per male. Since sibling conrtol males produce one chromosome if there is no autonomous NDJ, the sperm with one paternal second chromosome combining with the eggs with either 2 second chromosomes or null chromosome would produce no progeny. The control experiment confirmed that in 54 males tested only one male

produced 1 progeny due to autonomous NDJ in male. Since the paternal second chromosomes carry different recessive markers (cn bw and b pr, respectively), the relative frequencies of sister chromatid NDJ and homolog NDJ can be estimated from the proportions of NDJ progeny derived from 2/2 sperm that express the heterozygous markers. cn bw and b pr progeny result from sister chromatid NDJ whereas wild-type (WT, *cn bw/b pr*) progeny result from homolog NDJ.

**Mapping and identification of *solo* mutations**

*solo* alleles were mapped by deficiency complementation against the "deficiency kit" obtained from the National Drosophila Stock Center at Indiana University, Bloomington, using the X-Y NDJ phenotype. *solo* was mapped to the 35B region where *vas* is located. More detailed deficiency mapping using *vas*-region deficiencies (Ashburner et al., 1990 obtained from M. Ashburner (Cambridge University) demonstrated that the *solo* mutations lie within the *vas* locus (data not shown).

All exons and the third intron of *vas* were amplified from genomic DNA of flies homozygous for each of the three *solo* mutations and sequenced using ABI BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems). No mutations were detected in *vas* exons but the two large ORFs in the third intron of *vas* contained single mutations in each of the three *solo* alleles, each of which result in a nonsense mutation (shown in Fig. 2-4). Sequencing also showed that the pre-existing $vas^{RJ36}$ allele (Schupbach and Wieschaus, 1991) has an 8bp

insertion in the first intronic ORF resulting in a frame shift mutation.

**Characterization of *solo* transcripts**

To characterize the *solo* transcription unit, total RNA was prepared from wild type (strain *Zuker-2, cn bw*) adults using TRI Reagent (Sigma). After DNase treatment, the total RNA was used for cDNA synthesis using Superscript™ First-Strand Synthesis System (Invitrogen). Then *solo* cDNAs were amplified by PCR using primers: GTGAGAACTTTGTCACTCGG and TTTATGGGAGGCAGTAAGGC. A following nested PCR reaction was carried out using primers: CAATTCGAGTAGTGGTCAGC and GAATCCGAATACCCTGTTGC. This procedure yielded a specific amplification product of 972 base pairs that contains parts of the two large ORFs from intron 3 of *vas* spliced together to generate a continuous reading frame. To identify the 5' and 3' ends of the solo transcript, 5' RACE and 3' RACE reactions were performed (BD SMART RACE cDNA Amplification Kit) and a cDNA (EST clone AT08465) obtained from Berkeley Drosophila Genome Project (BDGP) was sequenced. These experiments revealed that the second intronic ORF terminates at a stop codon located 92 bases upstream of a poly-A site and 294 bases upstream of the fourth exon of *vas*. At the 5' end, AT08645 includes all sequences in the first three exons of *vas* except for the first 22 base pairs of exon 1. It is not clear if this difference reflects different transcription start sites for the two genes or if AT08645 is incomplete at the 5' end. The primer sequences used in the RACE experiments are available upon request.

The sequence of the SOLO cDNA reported in this paper has been deposited in Genbank as accession # DQ851162.

**Construction of SOLO fusion clones and generation of transgenic flies**

Two SOLO fusion constructs, UASp:Venus-SOLO and UASp:SOLO-Venus, were made. For Venus-SOLO, the *solo* coding sequence and 3'UTR were amplified from the EST clone AT08465 using Pfx polymerase (Invitrogen) and primers CACCATGTCTGACGACTGGGATG and CACCCGACATAGATGCCTCG. For SOLO-Venus, the following primers were used: CACCATGTCTGACGACTGGGATG and GAGCAGCCCGAAAAATCTACC. The PCR products were cloned into the pENTR™/D-TOPO entry vector (Invitrogen) and the resulting products were sequenced.

Both entry constructs were recombined into Gateway P-element vectors pPVW and pPWV (from Drosophila Genomics Resource Center (BDGC)), generating the germ-line transformation vectors *P[w$^{+mC}$, UASp:Venus-SOLO]* and *P[w$^{+mC}$, UASp:SOLO-Venus]*. Both vectors include Venus, UAS sequences for transcriptional activation by GAL4 and *mini-white* to detect germ-line transformants. Both constructs were transformed into *w$^{1118}$* flies (BestGene Inc.). Transformants were mapped by standard procedures. Transformant lines carrying UASp:SOLO-Venus are named 1910-1-# and transformant lines carrying UASp:Venus-SOLO are named 1910-2-#.

**Transgene rescue experiments**

*+/$B^S$Yy+; Df(2L)A267, cn/solo$^{Z2\text{-}0198}$, cn; [UASp:Venus-SOLO]/[nanos:Gal4-VP16]* males and sibling controls without *[UASp:Venus-SOLO]* or *[nanos:Gal4-VP16]* were crossed to *y w* females to measure sex chromosome NDJ. The rescue experiments for UASp:SOLO-Venus transgenic flies were carried out by similar methods.

**Testis Immunostaining**

$\alpha$-tubulin/DAPI staining of testes was carried out as described (Thomas et al., 2005). Immunostaining was performed with modification according to (Bonaccorsi et al., 2000). The primary antibodies used: 1:500 anti-CID (chicken) (provided by Dr. G. Karpen), 1:1000 anti-CID (rabbit) (Abcam), 1:250 anti-SNM C-terminal (rabbit) (Thomas et al., 2005), 1:250 Anti-SMC1 (rabbit) (Thomas et al., 2005), 1:500 anti-GFP (rabbit) (Molecular Probes). The secondary antibodies used: Alexa Fluor 555 goat anti-chicken IgG (H+L) (Molecular Probes), Alexa Fluor 546 goat anti-rabbit IgG (H+L) (Molecular Probes), Alexa Fluor 647 goat anti-rabbit IgG (H + L) (Molecular Probes). Venus-SOLO expression was induced by *nanos:Gal4-VP16* (Doren et al., 1998) and fluorescent signals were detected in the FITC channel or detected using anti-GFP antibody. Acetic orcein staining of male meiotic chromosomes was carried out according to Stapleton et al. (2001).

**Assaying arm cohesion in spermatogonia and spermatocytes**

Arm cohesion was assayed by counting GFP spots in spermatogonia and spermatocytes from males hemizygous for a chromosome 2 transgene carrying a 256mer tandem array of *lac*O repeats and heterozygous for a transgene (also on chromosome 2) expressing a GFP-LacI chimeric protein under control of the *hsp83* promoter (Robinett et al., 1996; Straight et al., 1996; Vaquez et al., 2002; Thomas et al., 2005). The genotype of the tested males was $w^{1118}$*/Y; Df(2L)A267, [GFP-LacI] [lacO]/solo*$^{Z2\text{-}0198}$. Testes were dissected from third instar larvae, pupae or young adults in testes buffer (183 mM KCl, 47 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, 1 mM PMSF) and gently squashed in testes buffer. GFP-LacI foci were imaged by native fluorescence. DNA was stained with DAPI.

**Microscopy and image processing**

All images were collected using an Axioplan (ZEISS) microscope equipped with an HBO 100-W mercury lamp and high-resolution CCD camera (Roper). Image data were collected and merged using Metamorph Software (Universal Imaging Corporation). For CID signals and some SMC1 and Venus-SOLO images, sum or maximum projections of deconvolved Z-series planes were applied using Metamorph Software. Images were processed with Adobe Photoshop CS2.

# RESULTS

## NDJ of homologous and sister chromatids in *solo* males

We identified three alleles of *solo* among a group of EMS-induced mutations that interfere with paternal transmission of the small 4$^{th}$ chromosome (Koundakjian et al., 2004; Wakimoto et al., 2004) and mapped *solo* to the 35B2-35C1 region of chromosome arm 2L by deficiency complementation.

To determine whether *solo* mutations disrupt segregation of the sex chromosomes in male meiosis, and to discriminate between homolog versus sister chromatid NDJ, *solo* males carrying a dominantly marked Y chromosome were crossed to females carrying the attached-X (X^X) chromosome *C(1)RM* (Table 2-1). Males hemizygous for all three *solo* alleles exhibited similar NDJ frequencies, averaging 55.8%. Similar NDJ frequencies were observed in trans-heterozygous (Table 2-1) and homozygous males (data not shown), indicating that all three alleles are genetically null. XY, XX and nullo-XY sperm were generated at frequencies averaging 10.7%, 4.4% and 36%, respectively. Since XY and XX sperm result exclusively from homolog and sister chromatid NDJ, respectively, these data indicate that *solo* mutations perturb segregation of both homologous and sister chromatids. *solo* males also exhibited high sister chromatid and homolog NDJ frequencies for the autosomal 2$^{nd}$ and 4$^{th}$ chromosomes (Table 2-2 and not shown). Taken together, these results suggest the importance of *solo* in both homolog and sister chromatid segregation.

Table 2-1. Sex chromosome nondisjunction in *solo* males.

| | Sperm Genotype[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Male Genotype[b] | X | Y+YY | XY | XX | O | N[c] | %NDJ | P/m[d] |
| *Z2-0198/Df* | 438 | 505 | 172 | 104 | 658 | 1877 | 56.4 | |
| *Z2-3534/Df* | 357 | 309 | 181 | 53 | 551 | 1443 | 58.0 | |
| *Z2-0338/Df* | 472 | 478 | 199 | 76 | 720 | 1945 | 55.1 | |
| *Z2-0198/Z2-3534* | 94 | 105 | 58 | 14 | 132 | 403 | 54.1 | |
| Total *solo* | 1361 | 1397 | 610 | 247 | 2061 | 5676 | 55.8 | 21.6 |
| Gamete frequency | 24.0% | 24.6% | 10.7% | 4.4% | 36.3% | 100% | | |
| *solo; snm*[e] | 137 | 97 | 43 | 9 | 227 | 513 | 56.1 | 2.9 |
| Gamete frequency | 26.7% | 18.9% | 8.4% | 1.8% | 44.2% | 100% | | |

[a] $+/B^{s}Yy^{+}$ males with the indicated 2nd chromosome genotypes were crossed singly to 2-3 *C(1)RM,* $y^{2}$ *su(*$w^{a}$*)* $w^{a}$*/0* females. [b]*Df = Df (2L)A267* (35B1; 35C1), in which the *solo* locus is completely deleted (Alex and Lee, 2005). [c]N: total number of progeny scored. [d]P/m = progeny/male. [e] $+/B^{s}Yy^{+}$; $solo^{Z2\text{-}0198}/solo^{Z2\text{-}3534}$; $snm^{Z3\text{-}0317}/snm^{Z3\text{-}2138}$.

Table 2-2. 2nd chromosome NDJ

| Sperm genotype | NDJ Type | Egg genotype | Progeny Phenotype | # Progeny | Parameters | |
|---|---|---|---|---|---|---|
| *b pr/cn bw* | Homolog | O | WT | 414 | # Males tested | 75 |
| *b pr/b pr* | Sister | O | b pr | 60 | Total progeny | 1182 |
| *cn bw/cn bw* | Sister | O | cn bw | 71 | Progeny/Male | 15.8 |
| O | Both | *2^2, bw sp* | bw sp | 637 | S/H | 0.32 |

WT = wild-type for all markers. S/H = sister/homolog NDJ = (b pr + cn bw)/WT. Since in the 54 control males tested only one male produced one progeny due to its autonomous NDJ, the data is not shown in the table.

**_solo_ males do not exhibit PSCS before anaphase I**

Although elevated homolog and sister chromatid NDJ could result from separate segregation defects at meiosis I and II, respectively, a more parsimonious scenario is that the four chromatids that make up each bivalent separate prematurely, prior to the first meiotic division, and then segregate randomly through both meiosis I and II, as has been suggested for *ord* mutant males which produce a similar mix of NDJ products (Miyazaki and Orr-Weaver, 1992; Bickel et al., 1997). To gain insight into the mechanism of NDJ, we compared spermatocytes from *solo* and wild-type males that had been stained to visualize both DNA and spindles. More than 90% of *solo* spermatocytes in metaphase II exhibited DAPI-stained (DNA) masses that were both smaller than and more numerous than in wild-type males, indicative of PSCS (Fig. 2-1A). Chromosome segregation at anaphase II appeared disorganized. Laggards were observed in approximately 38% of anaphase II nuclei and meiosis II poles exhibited clearly unequal amounts of DNA in 88% of nuclei, indicating high rates of meiosis II nondisjunction. Consistent with these findings, staining of secondary spermatocytes in prophase II or metaphase II with the non-fluorescent dye orcein, which reveals more detail, showed nuclei containing fully separated sister chromatids (Fig. 2-1A).

Surprisingly, however, chromosome morphology and behavior during meiosis I in *solo* primary spermatocytes appeared normal or nearly so (Fig. 2-1B). Single chromatids were virtually never seen prior to anaphase I. Three condensed and separate DAPI-stained masses, corresponding to the three major

Figure 2-1. Chromosome segregation in *solo* and *solo; snm* spermatocytes.

Testes from wild type (WT) and mutants were stained with anti-α-tubulin to visualize spindles and with DAPI to visualize DNA. More than 50 cells were analyzed for each stage. Scale bar: 5 μm.

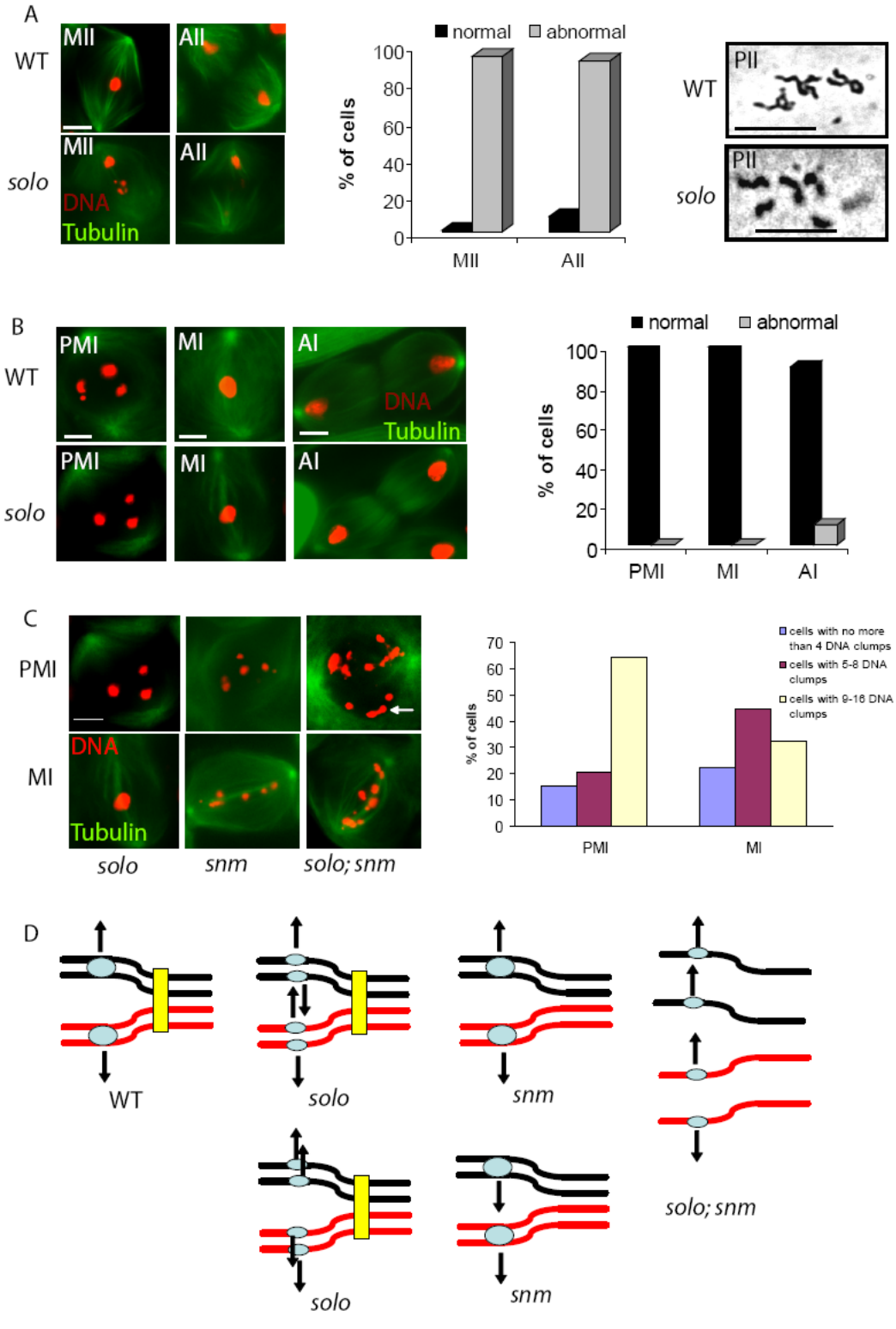
(A) Meiosis II of *solo* mutants and WT. Left panel: sister chromatids separate precociously at metaphase II (MII) in $solo^{Z2\text{-}0198}$/*Df(2L)A267* spermatocytes and segregate unequally to opposite poles at anaphase II (AII). Middle panel: quantification of cytological phenotypes of MII and AII. Abnormal cells were defined as follows. MII (metaphase II): cells with more than one DNA clump; AII (anaphase II): cells with unequal poles or one or more laggards. Right panel: prophase II chromosomes from WT and $solo^{Z2\text{-}0198}$ homozygous males stained with acetic orcein. Centromeric cohesion is clearly present in all of the WT chromosomes but in the *solo* spermatocyte eight fully separated sister chromatids can be seen (the two fourth chromatids and one chromatid of a large chromosome are somewhat out of focus).

(B) Meiosis I of *solo* mutants and WT. Both WT and *solo* exhibit three compact and separate chromatin masses representing the three large bivalents at prometaphase I (PMI). Chromosomes successfully align at metaphase I (MI) in both genotypes. Chromosomes segregate equally to opposite poles at anaphase I in both genotypes (left panel). Quantification of cytological phenotypes. Abnormal cells were defined as follows. PMI (prometaphase I): cells with more than three large DNA clumps; MI (metaphase I): cells with more than one DNA

(Figure 2-1 cont'd) clump; AI (anaphase I): cells with unequal poles or one or more laggards (right panel).

(C) Sister chromatids separate prematurely in *solo*; *snm* double mutants ($solo^{Z2\text{-}0198}/solo^{Z2\text{-}3534}$; $snm^{Z3\text{-}2138}/snm^{Z3\text{-}0317}$). The arrow points to a chromosome territory with four clearly separated, partially condensed DAPI-stained masses (right panel). Quantification of cytological phenotypes in *solo; snm* spermatocytes (right panel).

(D) Schematic drawing of the behavior of homologous chromosomes (red and black) and sister chromatids in meiosis I. Note that the cohesion between sister chromatids in WT and *snm* are omitted for clarity but the cohesion in *solo* and *solo; snm* is actually lost due to *solo* mutation. The grey ovals on the chromosomes indicate centromeres. The yellow rectangles represent possible conjunction complex that hold homologs and sister chromatids simultaneously. In WT, sister centromeres are co-oriented and the conjunction complex creates tension. In *solo* mutants, sister centromeres are oriented randomly but sister chromatids can equationally (mitosis-like) segregate to opposite spindle poles at anaphase I due to the tension created by the conjunction complex. In *snm* mutant, homologs orientate randomly and segregate unequally to opposite poles at anaphase I. In *solo; snm* double mutant, sister chromatids orient randomly and segregate unequally at anaphase I.


A
WT
solo
MII
AII
DNA
Tubulin
normal
abnormal
% of cells
100
80
60
40
20
0
MII
AII
PII
B
PMI
MI
AI
PMI
MI
AI
C
PMI
MI
DNA
Tubulin
solo
snm
solo; snm
cells with no more than 4 DNA clumps
cells with 5-8 DNA clumps
cells with 9-16 DNA clumps
70
60
50
40
30
20
10
0
PMI
MI
D
WT
solo
snm
solo
snm
solo; snm

bivalents, were regularly seen (176/176) in prometaphase I. The bivalents congressed normally, forming compact metaphase I configurations with the bivalents equidistant from the two poles, then segregated synchronously, generating poles of roughly equal DNA content in 62/69 anaphase I spermatocytes, suggesting that approximately equal numbers of chromatids segregate to each pole at the first division. Thus despite the genetic data indicating that homologous chromatids (we use the term “homologous chromatids” to refer to two chromatids that are from either of homologous chromosomes, such as X and Y chromatids) segregate to the same pole at high frequencies in *solo* males, the cytological data revealed visible PSCS only in meiosis II but not before anaphase I and provided little evidence for disturbed segregation at meiosis I.

**Sister centromere cohesion is lost prior to PMI in *solo* spermatocytes**

Although sister chromatids do not dissociate prior to anaphase I in *solo* spermatocytes, sister centromeres might nevertheless separate prematurely due to loss of cohesion. This precocious separation of centromeres could cause failure of co-orientation of sister centromeres and thereby perturb the reductional division, causing homologous chromatids move to the same spindle poles at anaphase I, as revealed by the cross data. Although the mechanism of sister centromere co-orientation is poorly understood, in both *S. pombe* and *Arabidopsis* cohesion between sister centromeres is required for their co-orientation and the failure of co-orientation shift the reductional pattern of

chromosome segregation at meiosis I to equational chromosome segregation (Watanabe and Nurse, 1999; Watanabe et al., 2001; Chelysheva et al., 2005).

To test for effects of *solo* mutations on centromere cohesion, we monitored centromere behavior in wild-type and *solo* males with an antibody against CID (centromere identifier), a centromere-specific histone H3-like protein (Ahmad and Henikoff, 2001; Blower and Karpen, 2001) (Fig. 2-2). In wild-type spermatocytes, the number of anti-CID foci per nucleus never exceeded the number of homologous chromosomes (eight in meiosis I and four in meiosis II). During late prophase I when the four bivalents occupy well-separated territories, two CID spots could often be seen in each chromosome territory.

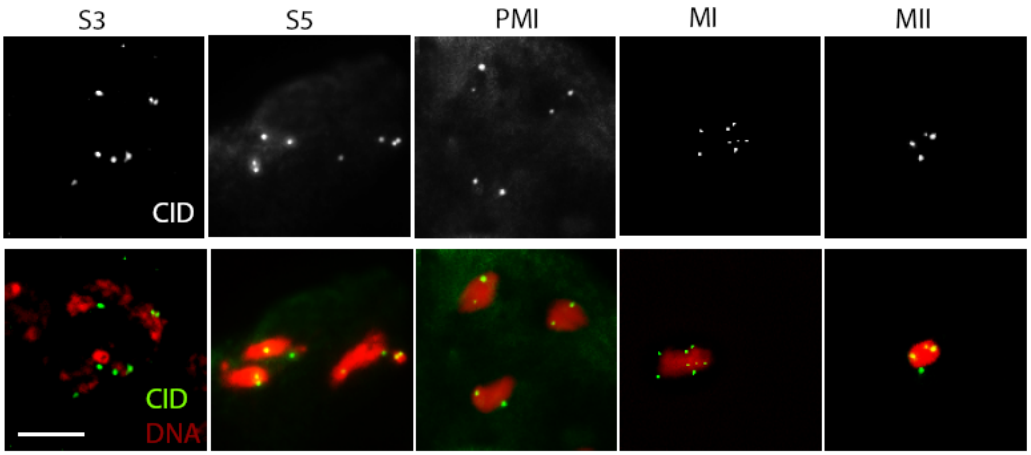
The numbers of anti-CID foci in *solo* mutants were similar to wild-type throughout early and mid-prophase I (stages S1-S4 (Cenci et al., 1994). However, from late prophase I (stages S5 & S6, (Cenci et al., 1994)) through metaphase I, many bivalents in *solo* spermatocytes exhibited 3 or 4 spots instead of the normal 2. Virtually all nuclei exhibited more than 8 spots, with the number ranging up to 16, the number of sister centromeres in a diploid nucleus (Fig. 2-2C). *solo* mutants also exhibited too many CID spots during meiosis II, up to eight instead of four. These observations indicate that sister chromatid cohesion is lost prior to prometaphase I in *solo* mutants.

Thus although bivalents remain intact in *solo* males until anaphase I and align properly on the meiosis I spindle, sister centromeres separate from one another much earlier in meiosis I than anaphase I. These observations strongly suggest that the aberrant meiosis I segregation patterns apparent in the cross

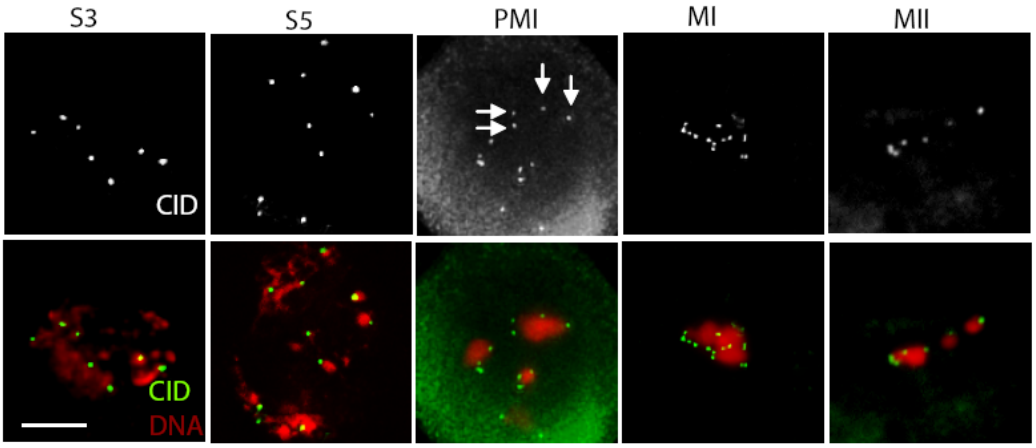
Figure 2-2. Sister centromeres separate prematurely in *solo* mutants.

Testes from wild type (A), *solo/Df(2L)A267* (B) and rescued *solo* (*solo/Df(2L)A267; [UASp:Venus-SOLO](1910-2-1A)/[nanos:Gal4-VP16]*) (D) males stained with anti-CID antibody to identify centromeres and with DAPI to visualize DNA. Sum or maximum projections of 3D deconvolved Z-series stacks were carried out to obtain CID signals. Scale bar: 5 μm. No more than eight CID spots are present in wild-type meiosis I at any stage while *solo* spermatocytes show more than eight CID spots at S5, PMI and MI (11, 13 and 15 spots, respectively, in the nuclei shown); arrows indicate a bivalent in which sister centromeres completely separate. (C) Quantification of CID spots in ($solo^{Z2\text{-}0198}/Df$) and sibling control at different stages. Percentage shows the spermatocytes with more than 8 CID spots. The number of scored nuclei is in parentheses.

A. Wild type



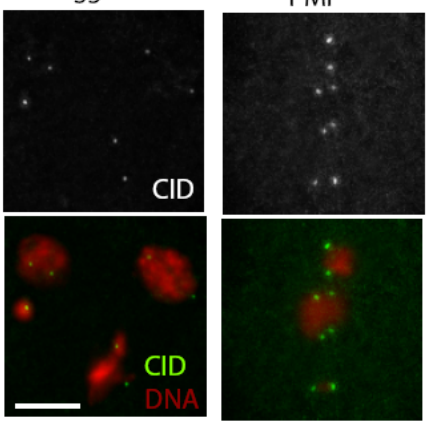

B. *solo*




C

| | *solo* | Control |
|---|---|---|
| Late prophase I | 95% (47) | 0% (74) |
| Prometaphase I | 100% (31) | 0% (58) |
| Metaphase I | 100% (10) | 0% (19) |

D. rescued *solo*



data result from premature loss of centromeric cohesion, allowing sister centromeres to orient randomly with respect to one another on the meiosis I spindle and resulting in abnormal homolog disjunction in meiosis I.

In many nuclei in late prophase I and prometaphase I, CID spots that appeared to represent sister centromeres were located a considerable distance from one another (e.g., the spots indicated by arrows in Fig. 2-2B). As the inter-spot distance in such cases considerably exceeded the diameter of CID spots and no apparent abnormal morphology of heterochromatin was observed, this observation suggests that *solo* mutations may lead to loss of cohesion of large heterochromatic domains that flank the centromeres as well as of the centromeres themselves.

**The homolog conjunction proteins SNM and MNM prevent complete separation of homologous and sister chromatids in *solo* mutants**

If cohesion is lost at centromeres and in pericentric heterochromatin domains by late PI in *solo* males, why do the chromatids not separate completely? Cohesion in the euchromatic arms cannot be responsible as arm cohesion is lost by stage S3 in wild type males (Vazquez et al., 2002). We suspected that conjunction between homologous chromosomes could account for the residual connections between chromatids in bivalents of *solo* males. SNM and MNM proteins are required for stable bivalent formation in male meiosis and co-localize to a prominent dense focus on the X-Y bivalent in both wild-type (Thomas et al., 2005) and *solo* (Fig. 2-3) spermatocytes. Both proteins also

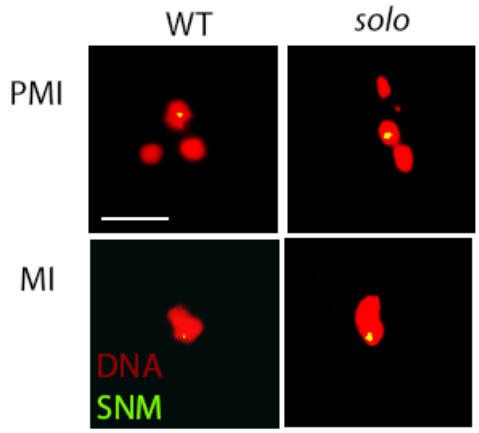



Figure 2-3. SNM localizes normally to the X-Y bivalent during meiosis I in solo mutants.

Testes from *solo*$^{Z2\text{-}0198}$/*Df(2L)A267* males were stained with anti-SNM antibody to visualize the conjunction between homologous chromosomes and with DAPI to visualize DNA. PMI (prometaphase I), MI (metaphase I). Scale bar: 5 μm.

localize to foci on all three autosomal pairs (Thomas et al., 2005; unpublished observations). To test the possibility that SNM can hold sister chromatids together in the absence of SOLO, males doubly mutant for *solo* and *snm* (*solo*$^{Z2\text{-}0198}$/*solo*$^{Z2\text{-}3534}$; *snm*$^{Z3\text{-}2138}$/*snm*$^{Z3\text{-}0317}$) were generated and their chromosomes examined by DAPI staining. As shown in Fig. 2-1C, the double mutants exhibited a much more severe phenotype during meiosis I than *solo* single mutants, which have no significant effect on chromosome morphology prior to anaphase I, or *snm* single mutants, which cause premature separation of homologous chromosomes, leading to the presence of up to eight univalents from late prophase I through metaphase I. Instead of eight univalents, *solo; snm* double mutants often exhibited 9-16 chromatin clumps during prometaphase I and metaphase I (64% and 32.5%, respectively, Fig. 2-1C). We interpret these clumps to be single chromatids, as we never observed more than 16 per nucleus and frequently observed four adjacent, same-sized clumps within one chromosome territory (e.g., arrow in Figure 2-1C). We conclude that sister chromatid cohesion is severely impaired by late prophase I in *solo* males, but that this absence is masked until anaphase I by the homolog conjunction complex which can hold sister chromatids as well as homologs together. The more severe phenotype of chromosome segregation in *solo; snm* double mutants is consistent with the severity of its fertility defect as revealed in the genetic assay. In single *solo* mutants, each male produced average approximately 22 progeny (Table 2-1). In contrast, each male produced only about 3 progeny in the double mutant.

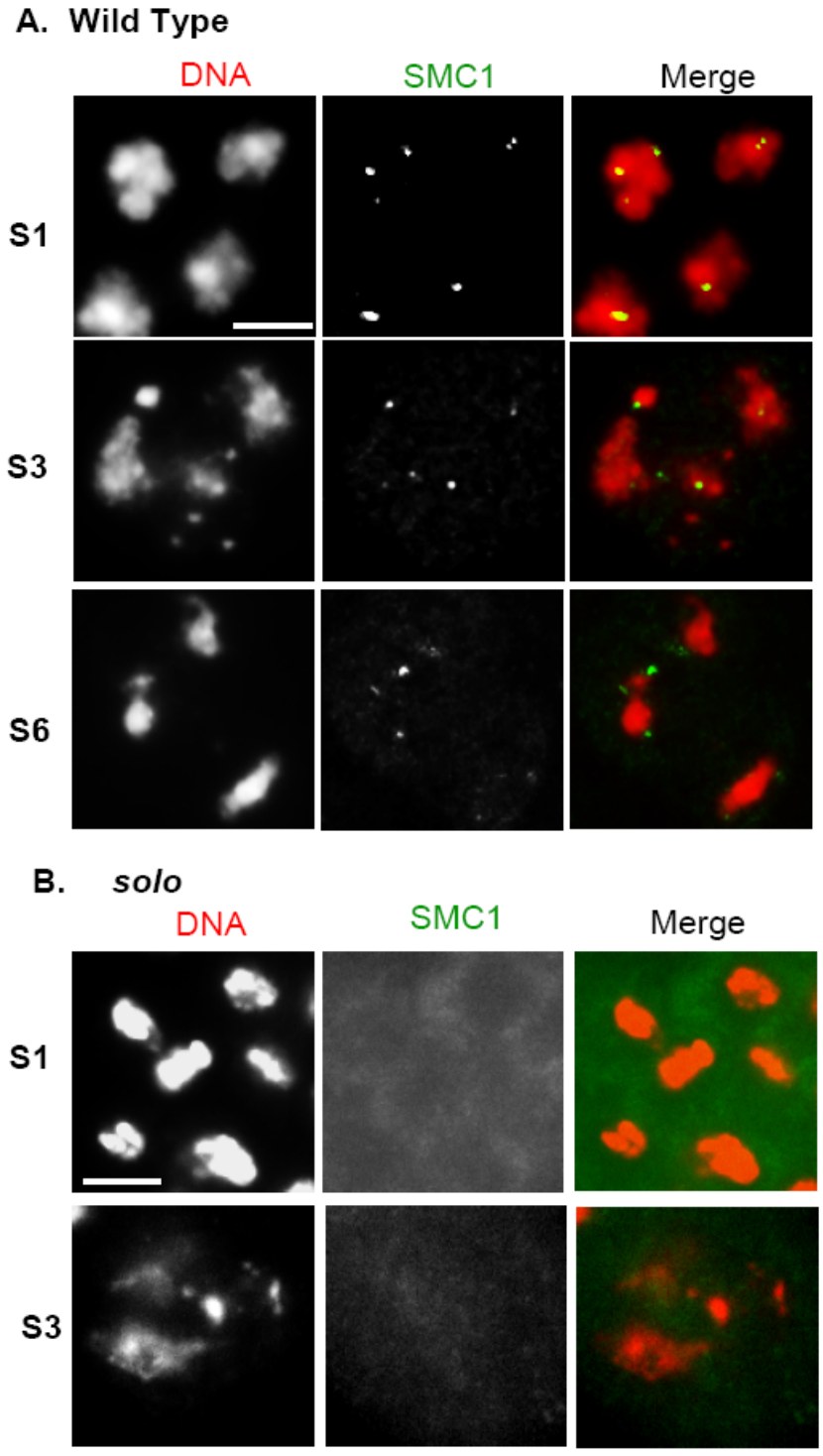
**_solo_ mutations eliminate centromeric SMC1 foci**

In both *S. cerevisiae* and *S. pombe*, meiotic centromere cohesion is mediated by cohesin (Watanabe and Nurse, 1999; Klein et al., 1999). It has not been clear whether this is also the case in Drosophila. Recently, we generated an antibody against Drosophila SMC1 protein, which is specific in western blot (Thomas et al., 2005), and found that SMC1 co-localizes with CID in male meiosis (data not shown). If these SMC1 foci represent complexes that are responsible for maintaining centromeric cohesion and if SOLO is required for stable localization of cohesin, then mutations in *solo* might be expected to perturb the SMC1 localization pattern. To test this prediction, wild-type and *solo* mutant spermatocytes were stained with anti-SMC1 (Fig. 2-4). In wild-type spermatocytes, anti-SMC1 foci were present at at centromeric regions in all stages of meiosis I and metaphase II (Fig. 2-4 and Fig. 2-10). During early prophase I, the anti-SMC1 foci varied in number, usually from 1-3, reflecting the variable number of chromocenters present during this period. In late prophase I, one or two foci could be detected in most chromosome territories. However, in *solo* mutant spermatocytes, no distinct anti-SMC1 foci were detected at any stage of meiosis. The failure to observe centromeric SMC1 foci is not due to failure to form normal centromeric heterochromatin since morphologically normal anti-CID foci are present throughout meiosis I in *solo* spermatocytes (Fig. 2-2).

We conclude that SOLO is required for localization of cohesin to centromeres from the beginning of prophase I in male meiosis, and suggest that SOLO may be required not only for maintenance of cohesion at centromeres but

Figure 2-4. Localization of SMC1 in wild-type and *solo* spermatocytes.

SMC1 foci were detected by anti-SMC1 and DNA was stained with DAPI. Centromeric SMC1 foci are visible throughout prophase I in wild-type (A) but are completely absent in *solo* spermatocytes (B). Mutant spermatocytes are from $solo^{Z2\text{-}0198}/solo^{Z2\text{-}0198}$ adult males. All images are sum projections of 3D deconvolved Z-series planes. Size bar: 5 μm.


A. Wild Type
DNA
SMC1
Merge
S1
S3
S6
B. solo
DNA
SMC1
Merge
S1
S3

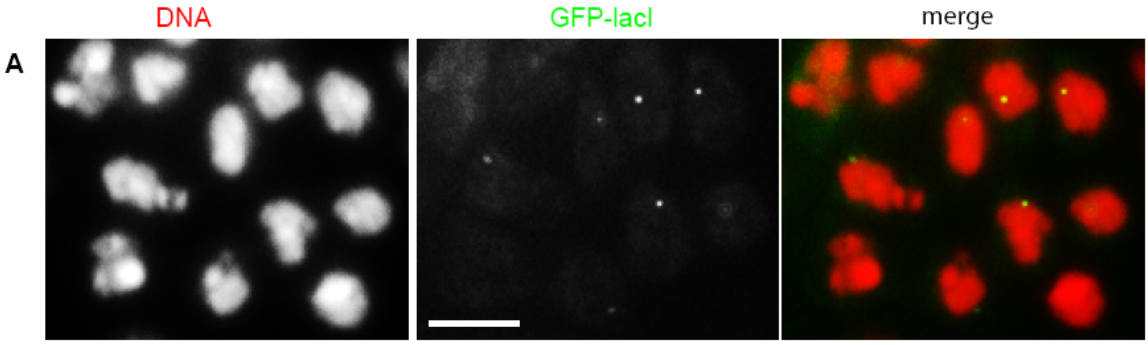
also for its establishment. These data also provide the first concrete evidence that centromere cohesion in Drosophila male meiosis is mediated by a cohesin complex.

**SOLO is not required for arm cohesion or for mitotic chromatid segregation.**

In wild-type males, cohesion between sister chromatid arms is maintained throughout the early stages of prophase I (S1 and S2), as shown by fusion of GFP-LacI foci bound to *lac*O inserted arrays on sister chromatids (Vazquez et al., 2002). *solo* mutants exhibited normal frequencies of arm cohesion during early prophase I, indicating that the role of *solo* in male meiotic cohesion is restricted to centromeric and heterochromatic domains (Fig. 2-5), like that of *ord* (Balicky et al., 2002). These experiments also provided evidence that *solo* is dispensable for cohesion and sister chromatid segregation in pre-meiotic spermatogonia. Mitotic NDJ in *lac*O heterozygotes yields trisomic spermatocytes that exhibit four GFP spots during late prophase I instead of the normal two. No spermatocytes with more than two GFP-LacI spots were observed in *solo* males hemizygous for the *lac*O array (data not shown).

**SOLO is a novel protein encoded by an alternative splice product of *vasa***

*solo* was mapped by deficiency complementation to the *vasa (vas)* locus on chromosome 2 (Fig. 2-6A), which encodes a conserved DEAD-box RNA




B

| | % of one spot | % of two spots | N |
|---|---|---|---|
| gonia | 99 | 1 | 290 |
| S1 | 96.5 | 3.5 | 86 |
| S2 | 92.1 | 7.9 | 102 |

Figure 2-5. Arm cohesion in spermatogonia and early prophase I spermatocytes in solo males hemizygous for an inserted lacO array on chromosome 2.

(A) GFP-LacI foci in early prophase I spermatocytes. Image is of stage S1 nuclei from unfixed testis preparations from *w*$^{1118}$*/Y; Df(2L)A267, [GFP-LacI], [lacO]/solo*$^{Z2\text{-}0198}$ males. Only one spot is evident in each nucleus although there are two copies of the *lac*O array on sister chromatids of one of the 2$^{nd}$ chromosomes, indicative of arm cohesion. Scale bar: 5 μm. (B) Quantification of GFP-LacI foci in spermatogonia and early prophase I spermatocytes. N shows the numbers of the scored nucleus.

helicase involved in germline establishment and axis specification in oocytes and early embryos (Styhler et al., 1998; Tinker et al, 1998). DNA sequence analysis revealed no mutations in the *vas* coding sequences in any of the *solo* alleles. However, sequence alterations were found within the third intron of *vas* which contains two large open reading frames (ORFs). Each of the three *solo* alleles exhibited a single-base substitution that creates a premature stop codon in one of those ORFs; *Z2-0338* and *Z2-0198* disrupt the upstream ORF whereas *Z2-3534* disrupts the downstream ORF.

To characterize the *solo* transcription unit, we sequenced a nearly full-length cDNA as well as several RT-PCR and 5' and 3' RACE fragments that include part or all of the intronic ORFs. Those analyses revealed that in addition to the two intronic ORFs, *solo* transcripts also include the three upstream *vas* exons, which encode several RGG repeats found in RNA-binding proteins (Alex and Lee, 2005), but lack the five downstream *vas* exons which encode the RNA helicase domain. The three upstream *vas* exons and the two intronic ORFs are spliced together to create a continuous open reading frame that extends from the translation start site of VASA in exon 2 to a stop codon in the downstream intronic ORF and that could encode a protein 1031 amino acids in length (Fig. 2-6B).

Complementation analysis between *solo* and *vas* mutations confirmed our proposed exon structure of *solo* (Fig. 2-6A). *solo* alleles complemented all *vas* alleles containing mutations in any of the five C-terminal exons (Liang et al.,

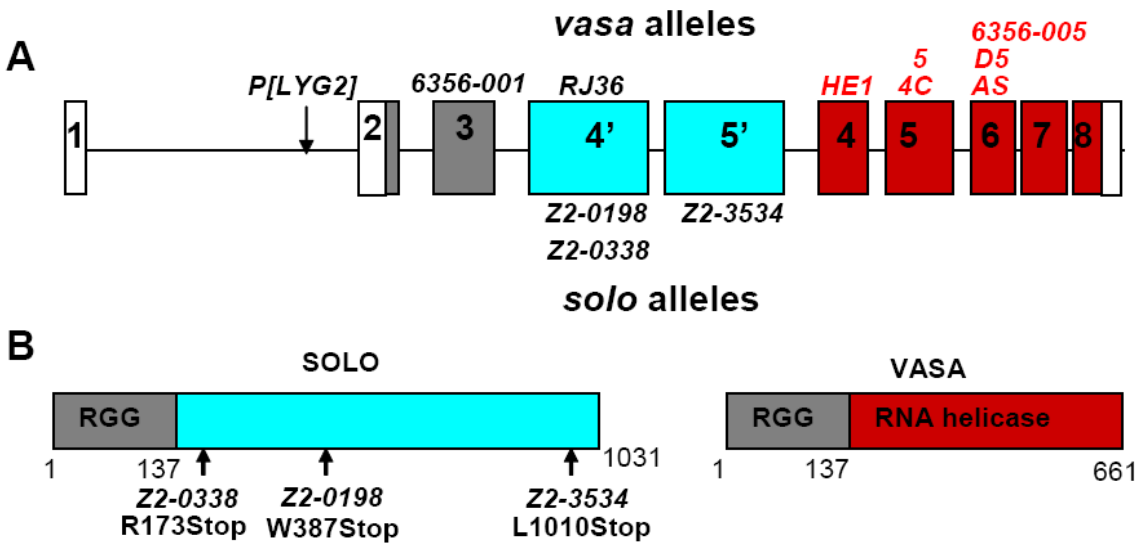



Figure 2-6. Molecular characterization of *solo*.

(A) The genomic structure of *solo* and *vas*. The *solo* and *vas* transcription units share exons 1, 2, and 3. Grey shading represents shared translated sequences; white represents the 5' and 3' UTR. Exons 4' and 5' (blue) are unique to *solo* and exons 4-8 (red) are unique to *vas*. Mutations above the locus are *vas* alleles; those in red fully complement *solo*; those in black fail to complement *solo*. *solo* mutations are shown below the locus. *vas* alleles: $vas^{HE1}$, $vas^{5}$, $vas^{D5}$, $vas^{AS}$, $vas^{4C}$ (Liang et al., 1994); $vas^{6356\text{-}005}$, $vas^{6356\text{-}001}$ (Tinker et al., 1998); $vas^{LYG2}$ (Styhler et al., 1998). (B) Predicted structures of SOLO and VASA proteins, and mutation sites of *solo* alleles.

1994), which encode the VASA helicase domain, indicating that the C-terminus of VASA is not shared by SOLO. However, *vas* mutations that map upstream of the SOLO-specific ORFs, including one nonsense mutation in exon 3, $vas^{6356\text{-}001}$ (Tinker et al., 1998) failed to complement the *solo* alleles, indicating that the 137 amino acids encoded by the upstream exons are present in both proteins. It is unlikely that the SOLO-specific exons are expressed independently of *vas* in addition to being expressed as a fusion product with the N terminus of *vas*, as $vas^{6356\text{-}001}$ behaves as a null allele of *solo*, giving X-Y NDJ frequencies of 41-44% in trans with *solo* alleles. We conclude that *solo* encodes a protein that includes the N-terminal 137 amino acids of VASA fused to 894 amino acids encoded within the 3rd intron of *vas*.

Single homologs of SOLO were identified by BLAST analysis in the genomes of all 10 Drosophila species for which sequenced genomes were available (Flybase, 2007b). Overall conservation is fairly low; *D. melanogaster* SOLO exhibits only around 30% amino acid identity with its homologs in *D. virilis* and *D. pseudoobscura*. However, in all of the Drosophila genomes, the *solo* sequences are nested within the third intron of *vas,* and SOLO appears capable of being expressed by the same alternative splice mechanism used in *D. melanogaster*.

No homologs of SOLO were identified outside of the genus Drosophila, not even in the genome of the mosquito *Anopheles gambiae*, another Dipteran insect. While it is possible that *solo* exists in Anopheles but is unrecognizable due to divergence, it would have to be located elsewhere in the genome as there

are no large ORFs nested within introns of the Anopheles *vas* gene. Other than the RGG motifs in the common N-terminus, SOLO exhibits no significant homologies with other proteins in the sequence database.

**Venus-SOLO co-localizes with CID and SMC1 from early prophase I until anaphase II**

To study the intracellular localization pattern of SOLO, transgenic insertions of two P-element constructs containing the *solo* cDNA tagged at its N or C terminus with Venus (an enhanced yellow-fluorescent protein) cloned downstream of yeast UAS sequences were generated (see Supplementary Experimental Procedures). Expression of the fusion proteins was induced by the GAL4-VP16 transcription activator under control of the Drosophila *nanos* promoter, which is active in most male germ cells (Doren et al., 1998). Two 3$^{rd}$ chromosome insertions of *[UASp:Venus-SOLO]* and one 3$^{rd}$ chromosome insertion of *[UASp:SOLO-Venus]* were tested for ability to complement the meiotic phenotypes of *solo* mutants. One copy of each SOLO transgene sufficed to provide virtually complete rescue of *solo* meiotic phenotypes. Sex chromosome NDJ was reduced to background levels (Table 2-3) and cytological analysis indicated that meiosis II segregation is regular in *solo/Df; [UASp:Venus-SOLO]/[nanos:Gal4-VP16]* males (data not shown). Venus-SOLO also suppressed the centromere cohesion defect of *solo* mutants. Late prophase I and prometaphase I nuclei from rescued males showed a maximum of two CID spots per bivalent (Fig. 2-2D), whereas nuclei from unrescued sibling *solo* males

Table 2-3. Transgene rescue data.

| Transgene[a] | Line | % X-Y NDJ (N)[b] |
|---|---|---|
| *[UASp:Venus-SOLO]* | 1910-2-2A | 0.31% (2217) |
| | Control | 46.4% (649) |
| *[UASp:Venus-SOLO]* | 1910-2-1A | 0.53% (2059) |
| | Control | 44.0% (234) |
| *[UASp:SOLO-Venus]* | 1910-1-1 | 1.47% (612) |
| | Control | 46.0% (211) |

[a]Indicated transgenes were carried on the 3rd chromosome and present in one copy in the crosses. [b]X-Y NDJ was measured by crossing *+/$B^S$Yy$^+$; solo$^{Z2\text{-}0198}$/Df(2L)A267; [UASp:Venus-SOLO]* or *[UASp:SOLO-Venus]/[nanos:Gal4-VP16]* males to *y w* females. Controls were *solo$^{Z2\text{-}0198}$/Df(2L)A267* siblings carrying either the SOLO transgene or the Gal4 driver but not both. N = number of progeny scored.

typically showed three or four CID spots per bivalent (data not shown). These data indicate that the tagged SOLO proteins function similarly to endogenous SOLO in male meiosis.

The localization pattern of Venus-SOLO in spermatocytes was examined using *[nanos:Gal4-VP16]* to induce expression. Bright Venus foci were seen in nuclei of mitotic spermatogonia and meiotic spermatocytes of all stages up to and including metaphase II but were absent at anaphase II and subsequent stages (Fig. 2-7 and Fig. 2-8A). Moreover, at all stages, the Venus foci overlapped CID foci (Figure 5), indicating that Venus-SOLO localizes to centromeres. The number of Venus foci per nucleus varied with stage. Young primary spermatocytes (stages S1 and S2), in which non-homologous centromeres form variable numbers of clusters, typically exhibited one to four foci, whereas spermatocytes in mid-late prophase I (stages S3-S6) and in prometaphase I and metaphase I exhibited up to eight foci per nucleus, typically two foci per bivalent.

Secondary spermatocytes typically exhibited 3-4 foci. SOLO-Venus exhibited a similar localization pattern (data not shown). In addition, nuclei in late prophase I often exhibited diffuse Venus-SOLO foci that localized to chromosomal domains considerably larger than the centromeres (Fig. 2-9). This observation is consistent with the idea that SOLO localizes not only to centromere regions but more generally to heterochromatic domains of spermatocyte chromosomes.

Figure 2-7. Co-localization of Venus-SOLO and CID on meiotic centromeres.

Venus-SOLO were detected by FITC channel or stained with anti-GFP in *[UASp:Venus-SOLO]/[nanos:GAL4-VP16]* males. The transgenic line is (1910-2-2A). CID was stained with anti-CID antibodies and DNA was stained with DAPI. All images are sum projections of 3D deconvolved Z-series planes. Scale bar: 5um.

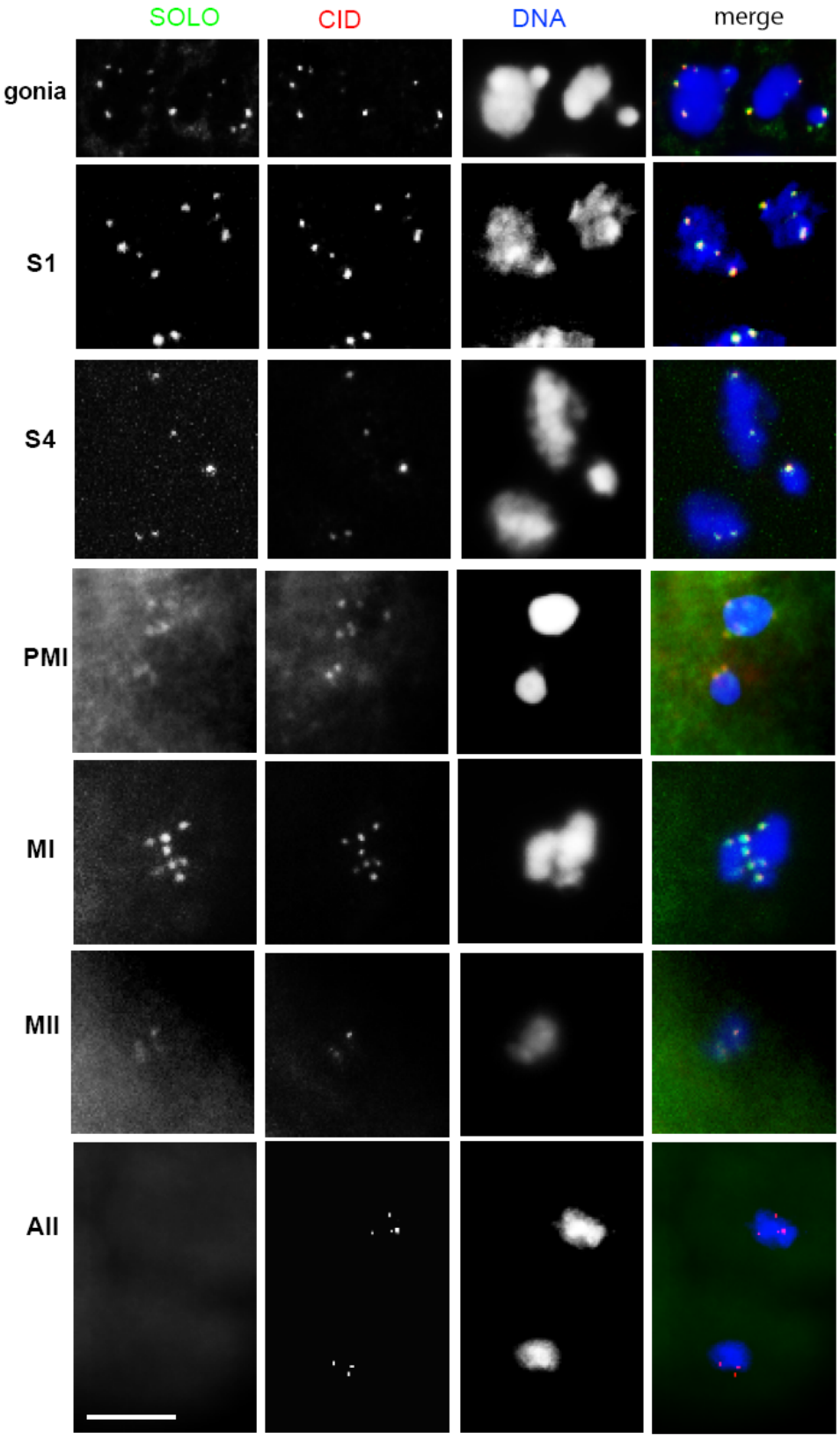
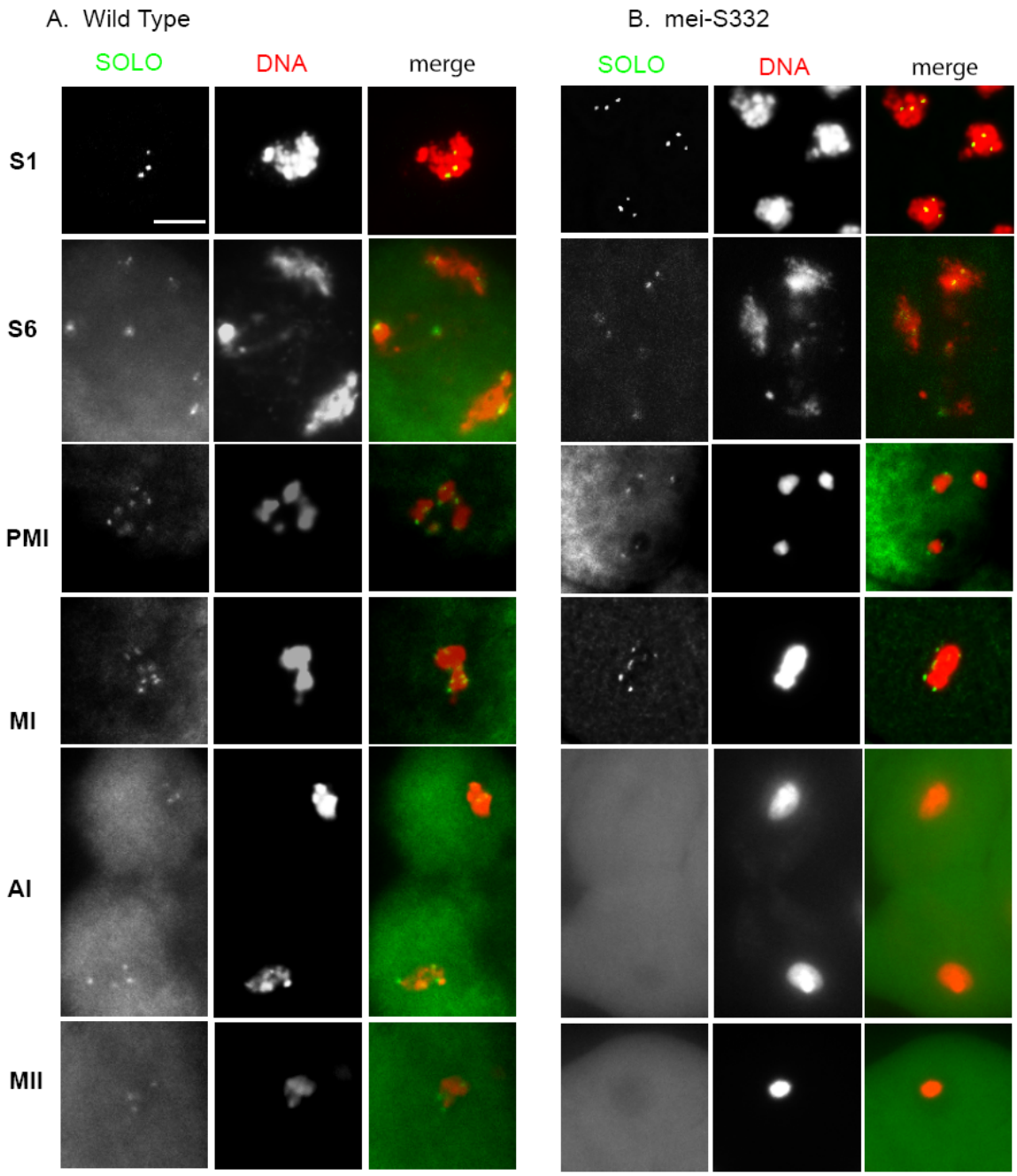

SOLO
CID
DNA
merge
gonia
S1
S4
PMI
MI
MII
AII

Figure 2-8. Venus-SOLO foci in wild-type (A) and mei-S332 (B) spermatocytes.

Venus foci were detected by native fluorescence. Expression of Venus-SOLO was induced by *nanos:Gal4-VP16*. Mutant spermatocytes are from *mei-S332$^{4}$/mei-S332$^{8}$*; *[nanos:GAL4-VP16]/[UASp:Venus-SOLO]* (transgenic line 1910-2-2A) males. All images are sum or maximum projections of 3D deconvolved Z-series planes. Scale bar: 5 μm.


A. Wild Type
B. mei-S332
SOLO
DNA
merge
SOLO
DNA
merge
S1
S6
PMI
MI
AI
MII

To determine whether SOLO functions together with a member of the cohesin complex, we compared the localization patterns of SOLO and the cohesin protein SMC1 in spermatocytes from males expressing Venus-SOLO and stained with anti-SMC1 antibody (Fig. 2-10A). We found that anti-SMC1 and Venus-SOLO foci co-localized throughout meiosis until anaphase II, when both proteins became undetectable. These data strongly suggest that SOLO and SMC1 function together to maintain cohesion between sister centromeres in male meiosis.

**Centromere localization of Venus-SOLO and SMC1 from anaphase I until metaphase II depend on the Shugoshin protein MEI-S332**

In *mei-S332* mutants, centromere cohesion is lost prematurely at anaphase I (Kerrebrock et al., 1992; Kerrebrock et al., 1995). MEI-S332 is a distant homolog of yeast Shugoshin proteins, in which mutations cause premature removal of centromeric cohesin at anaphase I (Kerrebrock et al., 1995; Katis et al., 2004; Kitajima et al., 2004; Rabitsch et al., 2004). To test whether *mei-S332* mutations cause premature loss of SMC1 and/or SOLO, we compared the Venus-SOLO and SMC1 localization patterns in *mei-S332* trans-heterozygous mutant spermatocytes with those in wild type ones (Fig. 2-10B, Fig. 2-8B). Venus-SOLO and SMC1 foci were present throughout meiosis I until metaphase I in *mei-S332* spermatocytes and were morphologically similar to those in wild-type spermatocytes. However, unlike wild-type spermatocytes in

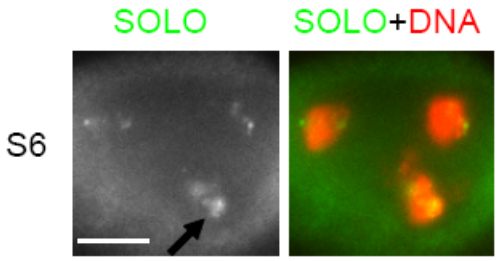
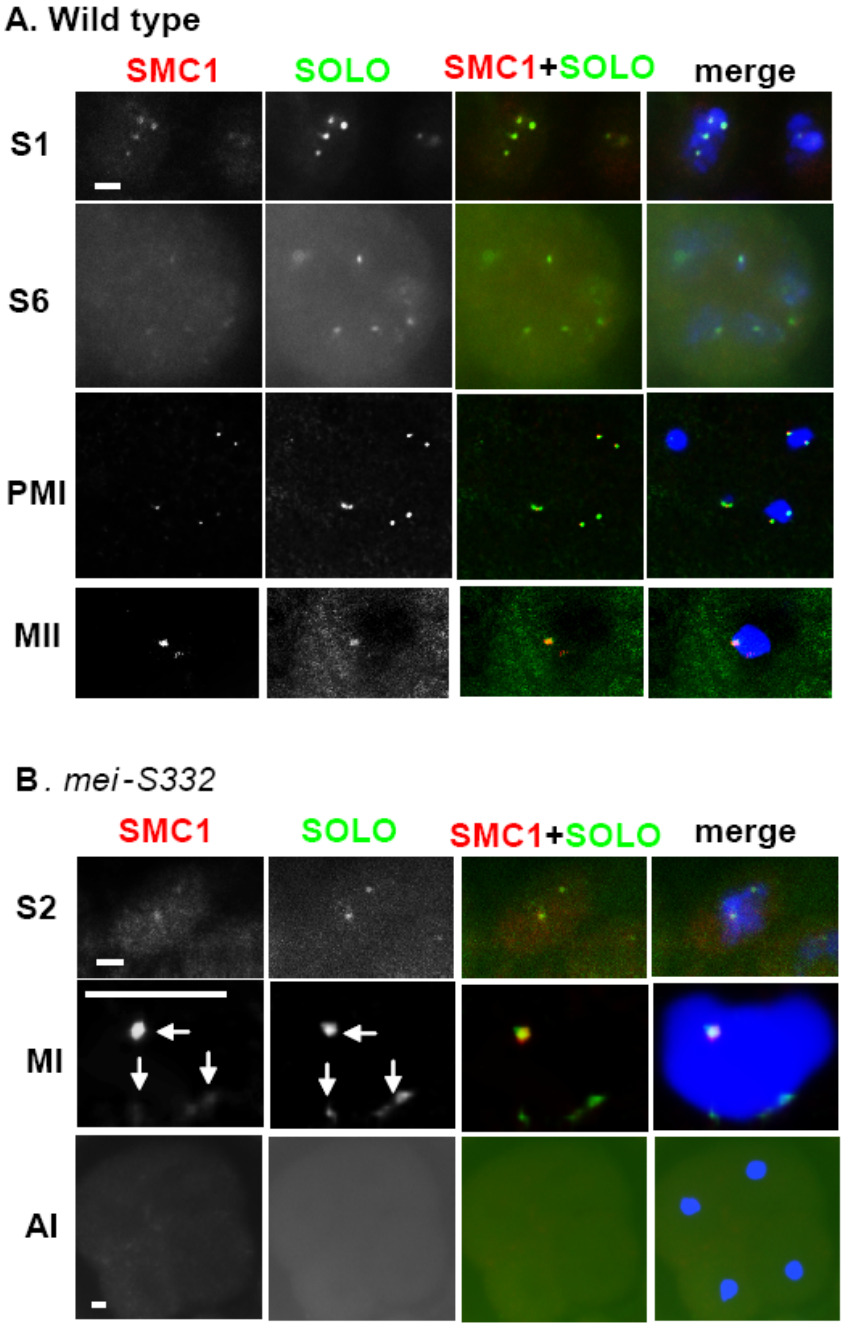



Figure 2-9. Diffuse Venus-SOLO foci during late prophase I.

Testes from wild-type *[nanos:GAL4-VP16]/[UASp:Venus-SOLO]* (transgenic line 1910-2-2A) males were stained with DAPI. One bivalent (arrow) often shows more staining than the others. This is probably the X-Y bivalent since the X-Y bivalent contains more heterochromatin than the 2$^{nd}$ or 3$^{rd}$ chromosome bivalents, but this conjecture has not yet been directly tested. Scale bar: 5 μm.

Figure 2-10. Co-localization of Venus-SOLO and SMC1 foci on centromeres in wild-type and *mei-S332* spermatocytes.

Venus-SOLO and SMC1 foci were detected by anti-GFP and anti-SMC1 antibodies, respectively, and DNA was stained with DAPI. Venus-SOLO and SMC1 foci co-localize until anaphase II in wild-type (A) but are lost by anaphase I in mei-S332 (B). White arrows in MI panel point to co-localizing foci. Mutant spermatocytes are from *mei-s332*$^{4}$*/mei-s332*$^{8}$; *[UASp:Venus-SOLO]/[nanos:GAL4-VP16]* (transgenic line 1910-2-2A) males. Two anaphase I spermatocytes are shown in the bottom panel. All images are sum projections of 3D deconvolved Z-series stacks. Scale bar: 2 μm.


A. Wild type
SMC1
SOLO
SMC1+SOLO
merge
S1
S6
PMI
MII
B. mei-S332
SMC1
SOLO
SMC1+SOLO
merge
S2
MI
AI

which Venus-SOLO and SMC1 foci were present until metaphase II, no foci of either protein were detected at anaphase I or later stages of meiosis in *mei-S332* spermatocytes. Therefore, we conclude that persistence of SOLO and SMC1 on meiotic centromeres after metaphase I is dependent on the Shugoshin protein MEI-S332. This result provides further evidence that SMC1 and SOLO collaborate in maintaining centromeric cohesion in meiosis. It also provides the first direct evidence that MEI-S332 functions to stabilize a cohesin protein on meiotic centromeres between anaphase I and anaphase II, like yeast Shugoshins.

**Centromeric Venus-SOLO localization is not maintained in *ord* mutants.**

ORD has also been shown to localize to centromeres and maintain sister chromatid cohesion (Miyazaki and Orr-Weaver, 1992; Bickel et al., 1996; Bickel et al., 1997). We therefore wondered if ord mutations would disrupt maintenance of SOLO on centromeres. To study the effect of *ord* on SOLO, we examined centromeric localization of Venus-SOLO in *ord* null mutants (*ord*$^{5}$/*Df(2R)WI370*; +/*UASp:Venus-SOLO nanos:Gal4-VP16*). Centromeric Venus-SOLO foci were absent throughout meiosis I whereas centromeric SOLO localization was not affected in spermatogonia (Fig. 2-11). In contrast, centromeric localization of SOLO was not affected during meiosis in *ord* sibling controls. In some 16-cell cysts weak SOLO spots were present in some cells but SOLO signals were completely absent in other cells (data not shown). These results suggest that SOLO might localize to centromeres but it cannot be retained in the absence of

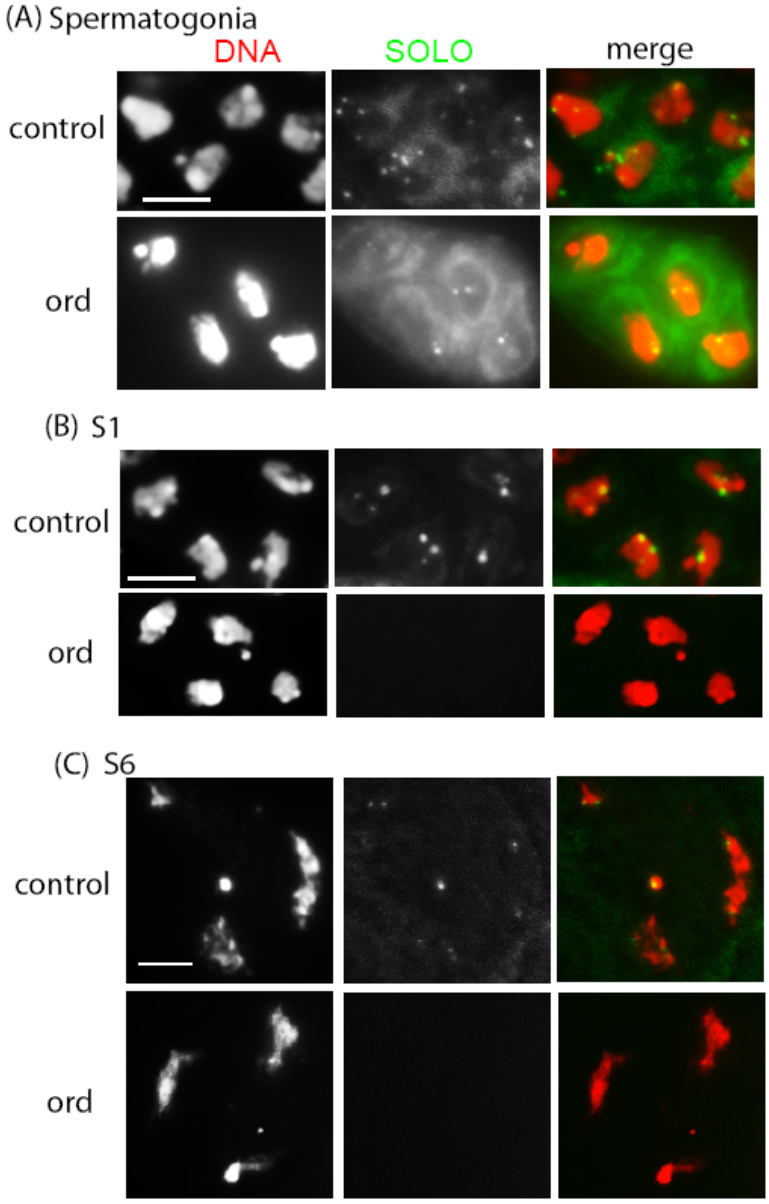



Figure 2- 11. SOLO is absent at centromeres from early prophase I but present in spermatogonia.

Venus-SOLO (green) was detected by FITC channel and DNA was stained with DAPI (red). Venus-SOLO foci is present in spermatogonia (A) but absent in S1(B) and S6 stages of prophase I (C). Scale bar: 5μm.

ORD. Previous genetic studies have shown *ord* functions earlier than *mei-S332* in maintaining cohesion during meiosis. Our results in which SOLO disappears at early prophase I in *ord* mutants while it is lost until anaphase I in *mei-S332* provide further evidence at cytological level.

# DISCUSSION

**SOLO is required for centromere cohesion and for co-orientation of sister centromeres.**

Our results show that SOLO is required in male meiosis for sister centromere cohesion during both meiosis I and meiosis II. Homologous and sister chromatids of both sex chromosome and autosomal bivalents segregate approximately randomly from one another in genetic crosses of *solo* males. Although bivalents remain intact through meiosis I, sister centromeres are visibly separated in most bivalents by stage S5 of prophase I and sister chromatids are fully separated during meiosis II.

Taken together, these observations indicate that both sister centromere cohesion and co-orientation of sister centromeres during meiosis I are disrupted by *solo* mutations. We presume that the failure of sister centromeres to co-orient is a consequence of their premature loss of cohesion, rather than an indication of a second function for SOLO. In both *S. cerevisiae* and *S. pombe*, there are specialized proteins required for sister centromere co-orientation, Monopolin in *S. cerevisiae* and Moa1 in *S. pombe* (Toth et al., 2000; Yokobayashi and Watanabe, 2005). However, in both organisms centromeric cohesion is also essential, as loss of *rec8* function leads either to random chromatid orientation (*S. cerevisiae*) or to predominantly equational orientation (*S. pombe*) at meiosis I (Klein et al., 1999; Watanabe and Nurse, 1999; Watanabe et al., 2001). Similar

observations have been made in *rec8* mutants in both *C. elegans* and *Arabidopsis* (Pasierbek et al., 2001; Xu et al., 2005). It remains to be determined whether other proteins analogous to Monopolin or Moa1 are also required for centromere co-orientation in Drosophila.

Since Venus-SOLO foci were present by stage S1 of prophase I and *solo* mutations abolished centromere localization of SMC1 from stage S1 on, it is surprising that sister centromere CID foci were not visibly separate prior to stage S5 in *solo* mutants. Why the loss of cohesion due to *solo* mutations does not cause separation of sister centromeres at early and mid prophase I is at present unclear. The complete separation of sister centromeres at later meiotic stages argues against residual activity of *solo* being responsible for holding sister centromeres together at early and mid prophase I. The molecular data showing that all three *solo* are null alleles also confirm that there is no residual SOLO activity. Other pathways unrelated to cohesion, like catenation between sister centromeres, might account for the lack of apparent separation of sister centromeres at early and mid prophase I. We note that our finding is consistent with observations in *S. pombe* (Molnar et al., 1995), where *rec8* mutations did not affect centromere clustering at meiotic prophase.

**The roles of SMC1, SOLO and ORD in meiotic cohesion**

In yeast, multiple meiotic cohesion functions are carried out by cohesin complexes that include meiosis-specific subunits such as REC8, which replaces the mitotic kleisin subunit RAD21. REC8 is conserved among most eukaryotes

and has been shown in several model plants and animals to be critical for many of the same meiotic functions identified in yeast (Pasierbek et al., 2001; Cai et al., 2003; chelysheva et al., 2003; Petronczki et al., 2003; Schleiffer et al., 2003; Xu et al., 2005). In Drosophila, however, no true REC8 ortholog has been identified and no mutations in cohesin genes have been available. Thus the role of cohesin in Drosophila meiotic cohesion has been unclear.

The phenotypes of *solo* mutations are similar to those of *ord* mutations in Drosophila and *rec8* mutations in other eukaryotes. Both *solo* and *ord* mutations cause premature loss of centromere cohesion during meiosis I, leading to mis-segregation of both homologous and sister chromatids (Miyazaki and Orr-Weaver, 1992; Bickel et al., 1997). Like SOLO, ORD is a centromere protein, but there are significant differences in the localization patterns of the two proteins in spermatocytes. ORD localizes predominantly to the interchromosomal spaces in early prophase I of male meiosis, then to the chromosome arms in late prophase I (after the loss of arm cohesion in mid-prophase I) and finally concentrates on centromeres at prometaphase I where it remains until anaphase II (Balicky et al., 2002). SOLO localizes to centromeres during pre-meiotic stages and the earliest stages of prophase I and remains on the centromeres until anaphase II, similar to the timing of REC8 localization in budding yeast (Watanabe et al., 2001). The fact that some SOLO-Venus foci were markedly more extended than CID foci suggests that SOLO may also localize to non-centromeric heterochromatin as well as to centromeres. However, we found no evidence that SOLO localizes to euchromatic arms or to inter-chromosomal spaces. Interestingly, centromeric

foci of SOLO are absent throughout meiosis in *ord* mutants although ORD does not localize to centromeres at prophase I. Thus these data suggest that ORD may affect SOLO indirectly. It seems unlikely that they work as cohesin partners.

Our localization data strongly suggest that SOLO and SMC1 function as partners in mediating centromere cohesion in Drosophila meiosis. Anti-SMC1 and Venus-SOLO foci overlap extensively on centromeres throughout meiosis until anaphase II when both proteins disappear. In addition, both Venus-SOLO and anti-SMC1 foci disappear prematurely at anaphase I in *mei-S332* mutants, coincident with the loss of centromere cohesion in that genotype. Finally, centromere localization of SMC1 is abolished at all stages of meiosis in *solo* spermatocytes. These results suggest that SOLO is required both for the establishment of cohesin-mediated centromere cohesion and for its maintenance throughout meiosis.

The exact role of SOLO in meiotic cohesion remains to be determined. One possibility is that it is a regulatory protein required for stable localization of cohesin to centromeres. Several cohesin co-factors have been described that are required for specific aspects of cohesin function, such as chromosomal loading, establishment of cohesion, removal of cohesin during prophase, protection of centromeric cohesin or for undetermined functions (Lee and Orr-Weaver, 2001; Nasmyth, 2001; Petronczki et al., 2003; Hauf and Watanabe, 2004; Uhlmann, 2004; Watanabe, 2005). SOLO appears to play a more general role than most of these co-factors: it is involved both in stable chromosome

association of cohesin and in the establishment and maintenance of cohesion throughout meiosis. Moreover, unlike the known cohesin co-factors which associate with cohesin during certain stages of the cell cycle, SOLO co-localizes with SMC1 throughout meiosis. Thus, except for the lack of homology to any of the four families of cohesin proteins, our data are strongly consistent with the possibility that SOLO is a novel and essential component of a meiosis-specific cohesin complex. It will be of considerable interest to determine the subunit composition of the meiotic cohesin complex(es) in Drosophila and to investigate what proteins SOLO interacts with.

**The role of *mei-S332* in meiotic cohesion**

Mutations in *mei-S332* lead to premature loss of centromere cohesion during anaphase I, resulting in high frequencies of sister chromatid NDJ during meiosis II (Kerrebrock et al., 1992). Although *mei-S332* was recently shown to be a distant homolog of Shugoshin proteins (Watanabe, 2005), its precise molecular function has remained unclear. Mutations in yeast Shugoshins lead to premature, Separase-dependent removal of REC8 cohesin from meiotic centromeres at anaphase I, indicating that Shugoshins function to protect REC8 cohesin from cleavage by Separase (Watanabe, 2005). However, because Drosophila meiotic cohesins have not previously been identified, it has not been clear whether MEI-S332 plays a similar role in Drosophila meiosis. Our data indicate that MEI-S332 functions like other Shugoshins to protect a meiotic cohesin complex from premature removal from centromeres at anaphase I.

Whether it does so by preventing Separase-mediated cleavage of a kleisin subunit of such a complex, or by some other means, remains to be determined.

**A role for SNM and MNM in connecting sister chromatids?**

We have previously shown that SNM and MNM are required for homolog conjunction and segregation during meiosis I but not for sister chromatid cohesion during meiosis II or sister kinetochore orientation during meiosis I (Thomas et al., 2005). Here we have shown that SNM localizes normally to meiosis I chromosomes in *solo* mutants and that *solo* mutations do not greatly perturb bivalent stability during meiosis I. Taken together, these observations suggest that the homolog conjunction and sister centromere cohesion pathways are largely independent in Drosophila male meiosis. However, simultaneous loss of both *solo* and *snm* causes complete separation of sister chromatids prior to prometaphase I, a phenotype never seen in either *solo* or *snm* single mutants. This implies that the homolog conjunction complex is able to maintain connections between sister chromatids in a bivalent as well as between homologs in the absence of SOLO. These SNM/MNM-mediated connections are evidently restricted to non-centromeric sites since both centromeres are often well-separated during late prophase I in *solo* spermatocytes. In addition, they differ from SOLO-mediated sister connections in being unable to fully support sister centromere co-orientation. The location and nature of the sister chromatid connections mediated by SNM and MNM are at present unknown. Perhaps SNM and MNM connect sister chromatids at the same chromosomal sites at which

they connect homologs (e.g. rDNA locus on X and Y chromosomes (Thomas et al., 2005), and might not distinguish between sister and homologous chromatids. However, we cannot rule out more complex scenarios in which the SNM/MNM complex has separate functions to connect homologs and sister chromatids simultaneously.

A surprising feature of meiosis in *solo* mutants is that anaphase I poles usually contain approximately equal amounts of DNA despite the presence of four separate centromeres at prometaphase I and despite the evidence from genetic crosses that sister and homologous chromatids segregate nearly randomly. We propose that the conjunction complex containing SNM and MNM still holds all four sister chromatids in a bivalent at pairing sites when the SOLO-containing complex that holds sister chromatids at centromeres is absent. The conjunction complex creates tension when the four sister centromeres in a bivalent are attached by microtubules from opposite spindle poles. A checkpoint with reduced-efficiency may exist in Drosophila male meiosis (Basu et al., 1999; Rebollo and Gonzalez, 2000; Malmanche et al., 2006; Malmanche et al., 2007) to monitor the presence or absence of bipolar tension at kinetochores. The checkpoint causes tension balanced among four sister chromatids in a bivalent. This would shift reductional division at meiosis I to an equal but random chromosome segregation pattern of four chromatids, i.e. any combinations of two chromatids segregate to one spindle pole while the remaining two go to the opposite pole (Fig. 2-1D). The equal but random segregation of chromatids lead to equal DNA amount to each pole at anaphase I, as shown in Fig. 2-1B. The

model showed in Fig. 2-1D predicts that cytologically detectable PSCS will not occur until anaphase I, which is confirmed by our observations that obvious PSCS can be seen at prophase II but not before anaphase I. This model also predicts PSCS can be seen at telophase I, however, decondensation of chromosomes at telophase I hinders the cytological analyses of PSCS at this stage.

## Conclusions

SOLO is a novel cohesion protein required for meiotic sister chromatid cohesion in Drosophila. *solo* mutations disrupt centromere cohesion during both meiosis I and meiosis II and randomize orientation of sister centromeres on the meiosis I spindle. Since SOLO localizes to centromeres throughout meiosis I and meiosis II but is absent from anaphase II on, and since its persistence on centromeres after anaphase I is dependent on the Shugoshin protein MEI-S332, we propose that SOLO is a component of the machinery that acts to maintain cohesion at centromeres in Drosophila meiosis. Further, since SOLO colocalizes with SMC1 on meiotic centromeres and is required for centromere localization of SMC1, we suggest that SOLO is either a regulatory protein essential for stable localization of cohesin to meiotic centromeres or an essential, albeit non-canonical, member of a meiosis-specific cohesin complex in Drosophila.

## Acknowledgments

We thank B. Wakimoto, P. Lasko, M. Ashburner, D. Montell, T. Orr-

Weaver, S. Bickel and Bloomington Stock Center for providing fly stocks and G. Karpen for providing chicken anti-CID antibody. This work was supported by NIH grant # R01 GM40489 to B.D.M.

# CHAPTER 3 - SOLO IS A COHESION PROTEIN REQUIRED FOR FORMATION OF SYNAPTONEMAL COMPLEX, MAINTENANCE OF CHIASMATA, AND PROMOTING HOMOLOG RECOMBINATION IN DROSOPHILA MEIOSIS

**Abstract**

Sister chromatid cohesion is essential for proper chromosome segregation during meiosis. It is required for normal homologous recombination, homolog synapsis, and chiasmata maintenance. However, the mechanism of sister chromatid cohesion in these processes in Drosophila is not well understood. Mutations of *solo* cause severely reduced fertility and high nondisjunction (NDJ) of sex chromosomes and autosomes in Drosophila female meiosis. Homolog and sister chromatid NDJs of autosomes were observed in *solo* mutants. The frequencies of homologous recombination of X chromosomes and autosomes are reduced and the distribution of crossovers is altered in *solo* females. In contrast, the level of exchanges between sister chromatids increases in the absence of SOLO. Our cytological evidence shows that SOLO appears before the meiotic stages and colocalizes with SMC1 and C(3)G in meiosis. SC assembly is severely disrupted in the earliest meiotic stage in *solo* mutants. These data suggest SOLO is a component of cohesin and synaptonemal complex (SC) and is required for SC formation. Additionally, SOLO is required for stabilizing chiasmata generated from residual recombination events. Our studies about SOLO in Drosophila female meiosis suggest that SOLO acts as a cohesin protein to promote formation of crossovers and following chiasmata. Furthermore, the timing of SOLO expression and disruption of SC in *solo* mutants suggest SOLO may be involved in initiation of SC assembly.

# INTRODUCTION

Sister chromatid cohesion is essential for chromosome segregation during meiosis and mitosis. Meiotic cohesion is not only necessary for the distinct dynamic behavior of sister chromatids in both divisions but also is essential for proper progression of homologous chromosomes (homologs) during meiosis I (Lee and Orr-Weaver, 2001; Nasmyth 2001).

Meiotic recombination is required for correct homolog segregation in Drosophila females. DSBs generated by Mei-W68 (an ortholog of spo11 in Drosophila) are repaired to generate crossovers that are required for connecting and orienting homologs in order to ensure their segregation at meiosis I (McKim and Hayashi-Hagihara, 1998). Studies have shown that DSBs and crossovers are not random events but are finely regulated (McKim et al., 2002). The frequency and distribution of crossovers varies irregularly along chromosomes and with genetic background. Under normal conditions, the recombination frequency is lower near centromeres and telomeres than in medial regions. In addition, crossovers rarely occur close to an existing crossover, a phenomenon called interference (Muller, 1916). The changes of genetic context may result in alterations of the distribution of crossovers besides changing recombination frequency, like precondition mutations that are widely studied (Carpenter and Sandler, 1974; Bhagat et al., 2004). Mutations of any genes involved in meiotic recombination would lead to chaotic chromosome segregation during meiosis I.

In Drosophila females, DSBs and crossovers occur in the context of synaptonemal complex (SC). SC is a conserved proteinaceous structure that is assembled between two homologs during prophase I (Page and Hawley, 2004). At early prophase I, sister chromatid axes undergo shortening and are assembled into lateral elements (LEs). When homologs achieve synapsis at pachytene, transverse filaments connect LEs and central elements that are midway between two LEs (van Heemst and Heyting, 2000). In Drosophila, C(3)G and C(2)M have been identified as components of the transverse filaments and lateral elements, respectively (Page and Hawley, 2001; Manheim and McKim, 2003). C(2)M is necessary for assembly of C(3)G into SC (Manheim and McKim, 2003). Sister chromatid cohesion is essential for the assembly of SC because the mutations that disrupt cohesion reduce SC formation or maintenance and eventually reduce meiotic crossovers, which could affect homolog segregation. Studies from yeast, flies, worms, plants, and mammals have confirmed the essential role of cohesion in SC assembly and revealed that cohesins are components of lateral elements of SC (Klein, et al., 1999; Cai et al., 2003; Chan et al., 2003; Eijpe, et al., 2003; Mercier et al., 2003; Revenkova, et al., 2004; Webber et al., 2004).

SC is disassembled at mid prophase I and the resulting chiasmata (cytological manifestations of crossovers) generated by meiotic repair process serve to link homologs (Petronczki et al., 2003). However, chiasmata alone are not enough to hold homologs together, sister chromatid cohesion distal to chiamata is necessary to stabilize chiasmata (Pentroczki et al., 2003).

In Drosophila, ORD (Orientation disruptor), a cohesion protein, is required for maintaining meiotic sister chromatid cohesion in both sexes (Miyazaki and Orr-Weaver, 1992; Bickel et al., 1997). The lack of ORD causes random chromosome segregation in both meiotic divisions (Miyazaki and Orr-Weaver, 1992). In addition, *ord* is necessary for SC maintenance but not for initiating of SC assembly during prophase I and *ord* mutations lead to reduced recombination between homologs (Bickel et al., 2002; Webber et al., 2004). Furthermore, the loading of SMC1 and SMC3 to centromeres in oocytes requires functional ORD (Khetani et al., 2007). Correspondingly, ORD localizes to centromeres and chromosome arms during meiosis (Webber, et al., 2004; Khetani et al., 2007). SMC1 and SMC3 have recently been shown to localize to SC (Khetani et al., 2007), probably as components of the lateral elements. A recent study showed a spindle checkpoint protein BubR1 is also essential for maintenance of SC (Malmanche et al., 2007), suggesting complexity of regulation of SC. However, these studies do not answer the question whether meiotic cohesion is required for initiating (establishment) of SC.

We have identified a novel cohesion protein SOLO (Sisters On the LOose) that is required for sister chromatid cohesion in both meiotic divisions in Drosophila males, which undergo meiosis without SC, recombination or chiasmata. SOLO localizes to centromeres and colocalizes with centromeric foci of SMC1. The mutations of *solo* cause loss of centromeric cohesion at the first meiotic division and disrupt the co-orientation of sister centromeres, thereby leading to high nondisjunction (NDJ) of chromosome segregation. In *mei-S332*

mutants SOLO and SMC1 centromeric foci disappear at anaphase I while they are protected in normal situations.

Here, we show SOLO's key role in homolog synapsis in Drosophila female meiosis. *solo* mutations cause reduced female fertility and high nondisjunction (NDJ) on X chromosomes and autosomes. Both homolog and sister chromatid nondisjunction occurs in *solo* females. The homologous recombination frequency is reduced and the distribution of crossovers is altered in the absence of SOLO, probably due to the failure to form SC and to inhibit sister chromatid exchange. A fluorescently tagged SOLO protein that completely rescues female meiotic phenotypes localizes to centromeres in oocytes and colocalizes with SMC1 and C(3)G, suggesting that SOLO is a cohesion protein and is a component of SC. Moreover, the few chiasmata that are generated in *solo* females are not effectively maintained. Our studies provide further evidence for cohesion's key role in successful meiosis in Drosophila.

# MATERIALS AND METHODS

## Fly strains and culture methods

The *solo* mutations used in this paper were from the Zuker-2 (Z2) collection of more than 6000 lines with EMS-mutagenized second chromosomes (Koundakjian et al., 2004) and have been described in Chapter 2. *b vas*$^{7}$ *pr* stock was obtained from M. Ashburner (Cambridge University, England). Other flies are from Bloomington Stock Center at the University of Indiana. Unless otherwise specified, the females being tested were crossed singly to two or three males in shell vials. All flies were maintained at 23°C on standard cornmeal molasses medium. Parents were removed from the vial on day 10 and progeny were counted between day 13 and day 22.

## Assaying X chromosome NDJ and recombination in females

*+/y pn cv m f* females were crossed with *YSX.YL, In(1)EN, y B/Y* males. The regular progeny from this cross are: (a) B females and (b) $B^+$ males. Female NDJ yields (c) $B^+$ females and (d) y B males. % X NDJ = 2(c + d)/(N + c + d). Recombination along the marked X was scored in the regular ($B^+$) sons.

## Assaying 2$^{nd}$ chromosome recombination

2$^{nd}$ chromosome recombination was assayed among regular disjunctional progeny by crossing *solo*$^{Z2\text{-}0198}$*, cn bw/b vas*$^{7}$ *pr* females to *b cn bw* males and

scoring the progeny for the frequency of crossovers between *b* and *cn* which are on opposite sides of the centromere in proximal 2L and proximal 2R, respectively, and between *cn* and *bw*, which flank most of 2R. *vas*$^{7}$ is a strong *vas* allele (Liang et al., 1994) that also acts as a null allele of *solo* (data not shown).

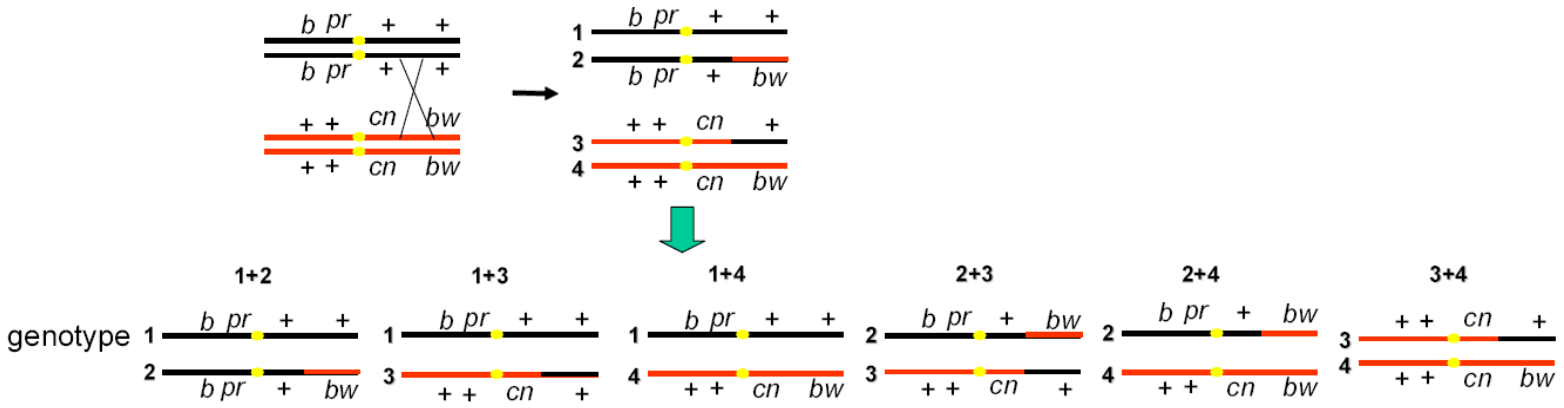
**Assaying NDJ and chiasmata instability on chromosome 2**

*solo*$^{Z2\text{-}0198}$*, cn bw/b vas*$^{7}$ *pr* females were crossed singly to two or three *C(2)EN, bw sp* males. *C(2)EN* flies generate only diplo-2 (22), *bw sp* and nullo-2 (0) eggs, so viable, euploid progeny are produced only from fertilization by reciprocally aneuploid NDJ eggs. In the absence of recombination, four classes of progeny are recovered: a) *cn bw*, b) *b pr*, c) +, and d) *bw sp*. Class a and b progeny result from 22 gametes that are homozygous for either of the two paternal 2$^{nd}$ chromosomes and thus represent sister chromatid NDJ. Class c progeny are heterozygous for the two paternal 2$^{nd}$ chromosomes and thus represent homolog NDJ. Class d progeny result from nullo-2 sperm that can arise from either type of NDJ. Since regular haplo-2 sperm are not recovered, there is no direct measure of total NDJ. However, crosses with wild-type males produce less than one progeny per male and fecundity in this cross is roughly proportional to the chromosome 2 NDJ frequency.

In the presence of recombination, additional classes arise as a result of recombination prior to NDJ (Fig. 3-1). In particular, recombination within the long *cn-bw* interval followed by NDJ of sister centromeres yields $b^{+}$ $pr^{+}$ *cn bw/*$b^{+}$ $pr^{+}$ *cn*

Figure 3-1. The chromosome segregation pattern and chiasmata stability test in *solo* females when recombination occurs.

Residual recombination may occur, yielding recombinant chromatids. The $b^+$ $pr^+$ $cn^+$ $bw^+$ F1 males from the cross of $solo^{Z2\text{-}0198}$, *cn bw/b* $vas^7$ *pr* females to C(2)EN, *bw sp* males were crossed to *b cn bw* females. The F2 progeny were scored for the presence and distribution of the markers b, cn and bw. The presence of two reciprocal exchange chromosomes in the progeny (chromatid 2 and 3 in the figure) indicates that chiasma is not effectively maintained in *solo* females.




phenotype

b pr

$b^+$ $pr^+$ $cn^+$ $bw^+$ ♂ X *b cn bw* ♀

$b\ cn^+\ bw^+$

$b^+\ cn\ bw^+$

$b^+$ $pr^+$ $cn^+$ $bw^+$ ♂ X *b cn bw* ♀

$b\ cn^+\ bw^+$

$b^+\ cn\ bw$

$b^+$ $pr^+$ $cn^+$ $bw^+$ ♂ X

$b\ cn^+\ bw$

$b^+\ cn\ bw^+$

$b^+$ bw

cn

Chiasma stability assay

*bw*$^{+}$ progeny (cinnabar eyes) that are readily distinguished from other classes, and *b pr cn*$^{+}$ *bw/ b pr cn*$^{+}$ *bw*$^{+}$ progeny that show the same phenotype as sister chromatid NDJ of *b pr* in the absence of recombination. Recombination within the *cn-bw* interval followed by homologous NDJ generates b pr cn$^{+}$ bw/b$^{+}$ pr$^{+}$ cn bw along with three classes of b$^{+}$ pr$^{+}$ cn$^{+}$ bw$^{+}$ progeny, of which the former are readily distinguished from other classes. The ratio of sister chromatid NDJ to homolog NDJ under the condition of meiotic recombination was calculated by the ratio of *cn bw*$^{+}$ progeny to *b*$^{+}$ *bw* progeny.

In order to obtain an estimate of *cn-bw* recombination in the NDJ progeny, the total number of *cn bw*$^{+}$ and *b*$^{+}$ *bw* recombinants were multiplied by 3 (to account for the fact that *cn bw*$^{+}$ recombinants are detectable only when they segregate with the *cn bw* chromatid, but not when they segregate with the *cn*$^{+}$ *bw* or + + chromatids, which segregations are presumed to be equally likely) and then divided by the number of recovered chromatids (2XN).

To investigate chiasma instability on chromosome 2, the b$^{+}$ pr$^{+}$ cn$^{+}$ bw$^{+}$ F1 males from the cross of *solo*$^{Z2\text{-}0198}$*, cn bw/b vas*$^{7}$ *pr* females to C(2)EN, *bw sp* males were crossed to *b cn bw* females with structurally normal 2$^{nd}$ chromosomes. The F2 progeny were scored for the presence and distribution of the markers b, cn and bw. b$^{+}$ pr$^{+}$ cn$^{+}$ bw$^{+}$ F1 males that resulted from homolog NDJ may contain 0, 1 and 2 exchange chromosomes. The presence of two reciprocal exchange chromosomes in the progeny indicates that the absence of an effective chiasma in meiosis in *solo* females.

**Assaying sister chromatid exchange**

Sister chromatid exchange in *solo* and wild type females was assayed by monitoring the ratio of a Ring X chromosome to a Rod (normally linear) X chromosome in the progeny. Since an odd number of crossovers occurs between Ring sister chromatids generate a dicentric double ring chromosome which cannot be transmitted to the progeny, reduced recovery of the Ring X chromosome from *solo* female mutants then indicates the increased level of sister chromatid exchange.

*R(1)2, $y^1$ $f^1$/$B^S$Y$y^+$* males (Bloomington, stock # 4330) were crossed to *Df(2L)A267, b cn bw*/*CyO, cn* females. The *R(1)2, $y^1$ $f^1$*/+; *Df(2L)A267, b cn bw*/+ female progeny were crossed to *y w*/Y; *solo, cn bw*/CyO, *cn* males to generate *R(1)2, $y^1$ $f^1$*/y w; *Df(2L)A267, b cn bw*/*solo, cn bw* females and the sibling control *R(1)2, $y^1$ $f^1$*/y w; +/CyO, *cn* females. These females were crossed to $w^{1118}$/Y males to test sister chromatid exchange. The crosses were carried out without an X chromosome balancer to estimate changes of sister chromatid exchange under the condition in which both homolog and sister chromatid exchange occur in mutant and wild type. The progeny classes were distinguished by eye color, body color and bristle phenotype. The recovered Ring X chromosome generated $y^+$ $w^+$ $f^+$ females and *y* $w^+$ *f* males (Ring progeny). In contrast, the recovered Rod X chromosome generated $y^+$ *w* $f^+$ females and *y w* $f^+$ males (Rod progeny). These four categories were used to calculate the ratio of Ring/Rod. Additionally, sister chromatid and homolog NDJ generated *y* $w^+$ *f* or *y w* $f^+$, and *y* $w^+$ $f^+$ females, respectively. $y^+$ *w* $f^+$ XO males are recovered from null oocytes. Rarely, *y w f*

and *y* $w^+$ $f^+$ males were observed due to double exchanges between homologous ring and rod sister chromatids. Since *R(1), y f* is actually bobbed and the *R(1), y f* homozygotes grow slower than normal females, the progeny that were from sister chromatid NDJ were not used for evaluating sister chromatid exchange in this cross experiment.

**Rescue experiments**

*+/w; Df(2L)A267, cn/solo*$^{Z2\text{-}0198}$*, cn; [UAS:Venus-SOLO]/[nanos:Gal4-VP16]* and sibling control females (lacking *[UAS:Venus-SOLO]* were crossed to *X^Y, y B* males to measure X chromosome NDJ. The rescue experiments for UAS:SOLO-Venus transgenic flies were carried out by similar methods.

**Immunostaining in whole-mount ovaries**

Newly eclosed females were fattened 2-3 days in vials with yeast paste and males and then ovaries were dissected in 1 X PBS. Ovary immunostaining was performed according to Page and Hawley (2001). After immunostaining, ovaries were separated into individual ovarioles and transferred to slides and mounted with Prolong Antifade reagent (Invitrogen). Venus-SOLO expression was induced by *nanos:Gal4-VP16* (Doren et al., 1998) and fluorescent signals were detected in the FITC channel or detected using anti-GFP antibody.

**Chromosome spread**

Chromosome spreads were performed according to Webber et al. (2004). This method takes advantages of drying-down techniques to get higher resolution of meiotic cells, and thus is beneficial to better understand the localization of SOLO in females.

**Antibodies used**

Primary antibodies used : 1:500 anti-C(3)G mouse monoclonal antibody (provided by R.S. Hawley), 1:500 rabbit anti-GFP polyclonal antibody (Invitrogen), 1:1000 rabbit anti-CID polyclonla antibody (Abcam), 1:250 anti-SMC1 rabbit polyclonal antibody. Secondary antibodies used: Alexa Fluor 555 donkey anti-mouse IgG (H+L) (Molecular Probes), Alexa Fluor 647 goat anti-rabbit IgG (H+L) (Molecular Probes), Alexa Fluor 546 goat anti-rabbit IgG (H+L) (Molecular Probes).

**Microscopy and image processing**

All images were collected using an Axioplan (ZEISS) microscope equipped with an HBO 100-W mercury lamp and high-resolution CCD camera (Roper). Image data were collected and merged using Metamorph Software (Universal Imaging Corporation). For signals and some Venus-SOLO images, maximum or sum projections of deconvolved Z-series were applied using Metamorph Software. Adobe photoshop CS2 and Illustrator CS2 were used to process images.

# RESULTS

## *solo* mutations cause reduced fertility and elevated NDJ in female meiosis

*solo* mutations have been shown to cause high nondisjunction of sex chromosomes and autosomes in Drosophila male meiosis. To assess the effects of *solo* mutations on sex chromosome segregation in female meiosis, females trans-heterozygous for two different pairs of *solo* alleles were tested for X chromosome NDJ. The results (Table 3-1) show that *solo* females are semi-sterile, producing only about 3.5 progeny per female, compared to 50-80 progeny per female in parallel crosses involving *solo*/+ sibling control females. X NDJ frequencies were also highly elevated in *solo* mutant females: 36.0% in *Z2-3534/Z2-0198* females and 53.0% in *Z2-0338/Z2-0198* compared to less than 1% in the wild-type control crosses. Elevated 2$^{nd}$ chromosome NDJ was also observed in *solo* females. Diplo-2 eggs that carry sister chromatids and homologous chromatids (we use the term “homologous chromatids” to refer to two chromatids that are from either of homologous chromosomes, such as X and Y chromatids) were detected in the test, indicating that *solo* mutations cause both homolog and sister chromatid NDJ in females (Table 3-2).

## *solo* mutations reduce recombination

*solo* females also exhibited reduced recombination frequencies on both the X and 2$^{nd}$ chromosomes. Recombination between the *pn* and *f* loci, which are near the distal and proximal ends of the X euchromatin, totaled only 18.0cM

Table 3-1. X chromosome recombination and nondisjunction in *solo* females.

| ♀ Genotype[a] | #T[b] | N[c] | F[d] | B$^+$♀♀[e] | B♂♂[e] | %NDJ[f] | MD (*y-f*)[g] |
|---|---|---|---|---|---|---|---|
| *Z2-0338/+* (C) | 5 | 415 | 83.0 | 1 | 0 | 0.96 | 44.0cM |
| *Z2-0338/Z2-0198* | 48 | 163 | 3.4 | 43 | 43 | 53.0 | 8.6cM |
| *Z2-3534/+* (C) | 4 | 203 | 50.8 | 0 | 0 | 0 | 43.2cM |
| *Z2-3534/Z2-0198* | 39 | 138 | 3.5 | 5 | 18 | 36.0 | 18.0cM |

[a] *y pn cv m f/+* females of the indicated chromosome 2 genotypes were crossed with *Y$^S$X.Y$^L$, In(1)EN, y B/Y* males *(X^Y/Y)*. [b]Number of females tested. [c]N = total # progeny. [d]Fertility = N/#T. [e]B$^+$♀♀ (X/X/Y) and B♂♂ (X^Y/O) are the viable products of maternal X chromosome NDJ. [f]%NDJ = 2(B$^+$♀♀ + B♂♂)/(N + (B$^+$♀♀ + B♂♂)). [g]MD *(y-f)* = map distance between the *yellow (y)* and *forked (f)* genes.

Table 3-2. Second chromosome NDJ.

| Egg genotype | NDJ Type | Sperm genotype | Progeny Phenotype | # Progeny |
|---|---|---|---|---|
| *b pr/cn bw* | Homolog | O | WT | 1012 |
| *b pr/b pr* | Sister | O | b pr | 144 |
| *cn bw/cn bw* | Sister | O | cn bw | 106 |
| *cn bw$^{+}$/cn bw* | Sister | O | cn | 37 |
| *cn$^{+}$ bw/cn bw* | Homolog | O | bw | 36 |
| O | Both | *2^2, bw sp* | bw sp | 360 |

*solo$^{Z2\text{-}0198}$ cn bw/b vas$^{7}$ pr* females were crossed with *C(2)EN, bw sp* males. *vas$^{7}$* is null for both *vas* and *solo* function. S/H (sister chromatid NDJ/homolog NDJ = (144 + 106 + 37)/(1012 +36) = 0.27. The estimated map distance between *cn* and *bw* is 100 x (37+36) x3 /((1012+144+106+37+3) x 2) = 8.2cM (see Materials and Methods).

and 8.6cM in solo$^{Z2\text{-}3534}$/*solo*$^{Z2\text{-}0198}$ and solo$^{Z2\text{-}0338}$/*solo*$^{Z2\text{-}0198}$ females, respectively, compared to 43.2cM and 44.0cM in the corresponding wild-type control crosses (Table 3-1). On chromosome 2, recombination in the large euchromatic intervals flanked by the *cinnabar (cn)* and *brown (bw)* loci was strongly reduced in *solo*$^{Z2\text{-}0198}$ females (6.8cM (Table 3-3) and 8.2cM (Table 3-2) compared to wild-type control females (41.9cM) (Table 3-3). However, recombination was actually slightly higher in *solo* than in wild-type females (5.1 cM versus 3.9 cM) in the *b-cn* interval that flanks the centromere, indicating that the requirement for *solo* function in recombination is much greater in distal euchromatic regions than in centromere-proximal regions. This pattern is typical of precondition mutations that reduce recombination and alter crossover distribution (Carpenter and Sandler, 1974; Bhagat et al., 2004).

**Sister chromatid exchange is elevated in *solo* mutants**

The reduced meiotic recombination frequency could be due to a decrease of all recombination events including both sister chromatid and homologous recombination. Alternatively, if SOLO is required for the meiotic “homolog bias”, sister chromatid exchange could be increased at the expense of homologous recombination in *solo* mutants. To explore this possibility, we tested the transmission of a Ring X chromosome during meiosis in *solo* mutants. A ring chromosome cannot be transmitted efficiently when an odd number of crossovers occurs between two Ring sister chromatids because it creates a

Table 3-3. 2nd chromosome recombination in *solo* and control females.

| | Map Distances | | | | |
|---|---|---|---|---|---|
| Female Genotypes | *b-cn* | *cn-bw* | N[a] | #T[b] | F[c] |
| *solo*$^{Z2\text{-}0198}$ *cn bw/ b vas*$^{7}$ *pr* (E) | 5.1 cM | 6.8 cM | 118 | 47 | 2.51 |
| *b cn bw*/+ + + (C) | 3.9 cM | 41.9 cM | 1167 | 15 | 77.8 |
| E/C[d](%) | 131 | 16.2 | | | 3.2 |

The indicated females were crossed with *b cn bw* males. E – experimental cross; C - control cross. [a]Number of progeny. [b]Number of females tested. [c]Fertility = N/#T. [d]Ratio of the experimental to the control value.

dicentric Ring chromosome, whereas Ring chromosomes can be transmitted efficiently when an even number of crossovers occurs between two Ring sister chromatids. In contrast, Rod X chromosomes (linear chromosomes) can be transmitted efficiently following sister chromatid crossovers. Although the exchanges between Ring and Rod also produce dicentric products, they equally decrease the transmission of Ring and Rod chromosomes. Therefore, if the inhibition of sister chromatid exchange is lost in *solo* mutants, the progeny derived from Ring X chromosome-bearing eggs will be greatly decreased related to those derived from normal Rod chromosome-bearing eggs.

We monitored the meiotic transmission of the *R(1), y f* chromosome in wild type and hemizygous *solo* females. As shown in Table 3-4, the recovered ratio of Ring/Rod is approximately 0.35 among the progeny of both $solo^{Z2\text{-}0198}$ and $solo^{Z2\text{-}3534}$ females, which is significantly reduced compared to that in wild type (0.83), strongly suggesting that SOLO is required for inhibiting sister chromatid recombination to promote homologous recombination in Drosophila female meiosis.

**Chiasma stability is defective in *solo* mutants**

Chiasmata must be effectively maintained before anaphase I in order to separate homologs properly. Otherwise, homologs cannot be correctly disjoined to opposite spindle poles. Our previous study has shown $vas^{7}$ is not only a vas mutant but also a null allele of *solo*. *solo* females with one $vas^{7}$ chromosome marked with *b* and *pr* and the other $solo^{z2\text{-}0198}$ marked with *cn bw* were crossed

Table 3-4. Sister chromatid exchange is reduced in *solo* mutants.

| Genotype | Ring progeny | Rod progeny | Ring/Rod[b] |
|---|---|---|---|
| [a]*R(1)2, y f /yw; +/+* | 958 | 1156 | 0.83 |
| *R(1)2, y f/y w; Df(2L)A267/solo*$^{Z2\text{-}0198}$ | 209 | 605 | 0.35 |
| *R(1)2, y f/y w; Df(2L)A267/solo*$^{Z2\text{-}3534}$ | 216 | 604 | 0.36 |

[a]The number of progeny from *R(1)2, y f/y w; Df(2L)A267/solo* or wild type control females crossed to $w^{1118}/Y$ males was scored. [b]Only numbers of progeny showing regular Ring and Rod chromosome are used in the table. The numbers of NDJ flies are shown in appendix.

to *C(2)EN, bw sp* males, which produce nullo-2 sperm and diplo-2 sperm. Eggs that carry two chromatids that are either sister chromatids and homologous chromatids are viable when fertilized by null sperm. We propose that if residual meiotic recombination occurs in *solo* mutants and the chiasmata cannot be maintained in the absence of *solo*, some of the $b^+$ $pr^+$ $cn^+$ $bw^+$ progeny contain chromosomes with reciprocal exchange between *b* and *bw*. The genetic analysis confirmed our hypothesis. Among the 450 tested $b^+$ $pr^+$ $cn^+$ $bw^+$ male progeny, 12 carried a pair of reciprocal exchange second chromosomes derived from one crossover (Table 3-5), 9 were from the exchanges between *cn* and *bw* and the other three were from the exchanges between *b* and *cn*. The map distance between *cn* and *bw* in this chiasma stability test is 6.7 cM (9+1) x 3/(184+9+3+1+1+252) (The frequencies of the combinations among [(2+3) + (1+4)], [(1+3) + (2+4)], and [(1+2) + (3+4)] are presumed to be equal, thus is multiplied by the factor 3), which is not significantly different from the map distance in the recombination test (6.8 cM) (Table 3-3) or in the $2^{nd}$ chromosome NDJ test (8.2 cM) (Table 3-2). Therefore our genetic test demonstrated that crossovers were not effective in orienting the crossover chromatids to opposite meiosis I poles even though they were formed in the absence of sister chromatid cohesion due to *solo* mutations.

If all four sister chromatids segregate randomly when solo is mutated, the frequency of (3+4), (2+4), and (2+3) segregation should be equal (Fig. 3-1). The frequency of (2+3) segregation in total 1012 progeny is adjusted to 1012 X

Table 3-5. Chiasmata stability is defective in *solo* mutant.

| | |
|---|---|
| [ab]# of father producing progeny b $cn^+$ $bw^+$ and $b^+$ cn bw | 184 |
| [c]# of father producing progeny $b^+$ cn and b bw | 9 |
| [c]# of father producing progeny $b^+$ $cn^+$ $bw^+$ and b cn bw | 3 |
| [d]# of father producing progeny $b^+$ cn and b $cn^+$ $bw^+$ | 1 |
| [e]# of father producing progeny b cn and $b^+$ bw | 1 |
| [f]# of father producing no progeny | 252 |

[a]The homolog NDJ male progeny ($b^+$ $cn^+$ $bw^+$) that were taken from the same cross showed in Table 3-2 in which homolog and sister chromatid NDJ were tested were crossed to *b cn bw* females to test chiasma instability. Totally 450 males were tested.

[b]represents the homolog NDJ males without crossovers between interval *b* and *bw*.

[c]represents the males with the chromosomes due to failure of chiasmata maintenance. The crossovers between *cn bw* or between *b cn* failed to be maintained and thus moved to the same spindle pole in *solo* mutants.

[d]represents the (1+3) segregation in Fig. 3-1.

[e]represents a double exchange between b and bw loci.

[f]the majority were sterile, probably XO males, due to the nullo-X eggs from *solo* females fertilized with one X chromosome from *C(2)EN, bw sp* males (252/450).

9/(184+9+3) = 46.5. If the residual chiasmata can direct the exchange chromosome to the exchange chromosome to opposite poles, the frequency of (2+3) segregation should be much lower than that of (3+4) and (2+4) segregation, which is 37 and 36, respectively. The frequency of (2+3) segregation is actually higher. We noticed that the frequency of (2+3) segregation is much higher than that of (1+3). They should be theorectically equal. The reason why this occurs is not known now.

**Synaptonemal complex formation is defective in *solo* females**

The alterations of recombination pattern may be due to defects in the SC, a machinery that is involved in meiotic recombination. To assess the effects of *solo* mutations on SC formation, we stained dissected ovarioles with an antibody against C(3)G, a structural component of the SC that localizes to the central region of SC and functions as part of the transverse filaments to link homologous chromosome axes (Page and Hawley 2001; Anderson et al. 2005).

In *solo* mutants, much less anti-C(3)G staining was present at all stages than in wild-type (Fig. 3-2). In wild-type females, anti-C(3)G staining is particularly prominent in germaria, where the early stages of meiotic prophase take place (Page and Hawley 2001). Linear C(3)G structures were evident in region 2a of germaria, corresponding to the zygotene stage when SC formation is initiated, and in regions 2b and 3, corresponding to pachytene, when full-length SCs are present. In *solo* germaria, some staining was usually present but in many fewer nuclei than in wild-type. Moreover, C(3)G staining in *solo* germaria

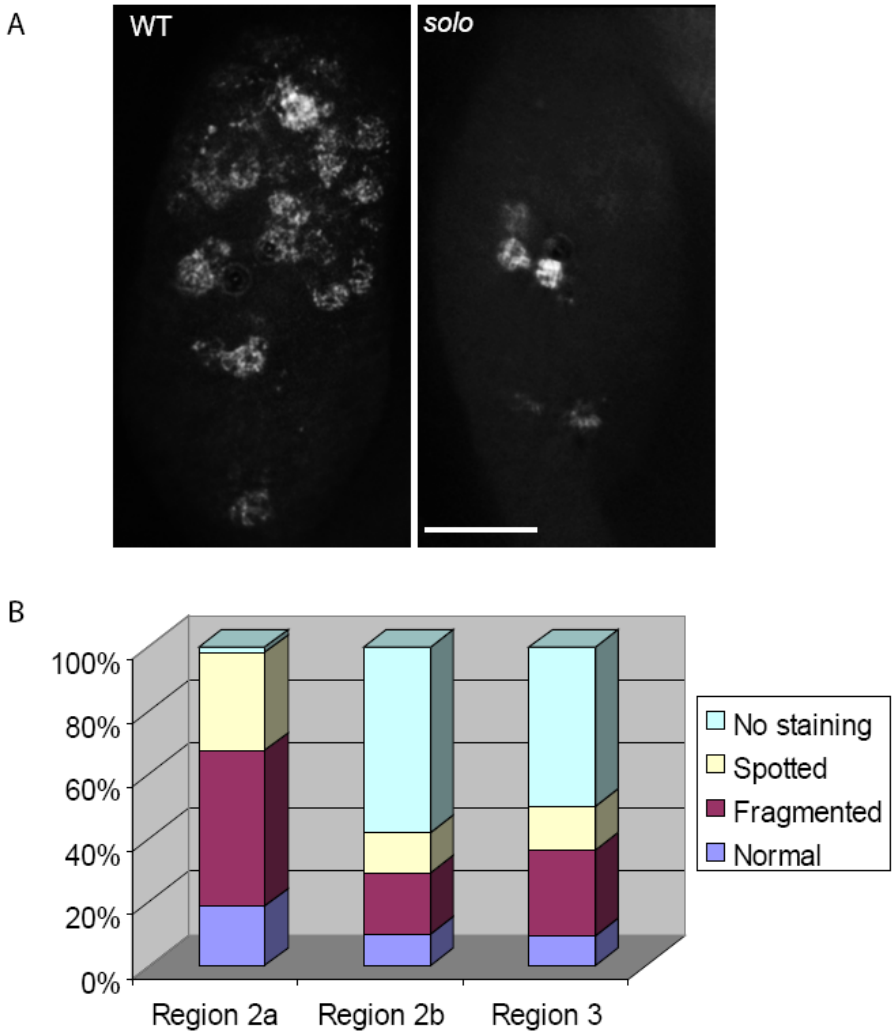



Figure 3-2. *solo* mutations disrupt the assembly and maintenance of the synaptonemal complex.

Each image comes from a maximum projection of a 3D deconvolved z-series. Scale bar: 10 μm. *(A)* C(3)G forms linear structures in wild type germaria. In *solo* mutants (*solo*$^{Z2\text{-}0198}$/*Df*), a few linear C(3)G-stained structures appear in region 2a, but most C(3)G staining fails to exhibit linear structures by region 3. *(B)* Quantification of types of C(3)G staining in germaria of *solo* mutants.

was often punctate rather than linear. Occasionally, normal-looking short linear structures were seen in regions 2a and 2b but full-length SCs were very rarely seen at any stage. Overall, normal C(3)G staining was observed in less than 20% of nuclei in region 2A and less than 10% of nuclei in regions 2B and 3 of the germarium (Fig. 3-2B). These data indicate that SOLO is required for synapsis. In addition, germ cells in ovarioles of *solo* mutants were significantly reduced in number compared to wild type, suggesting a defect in germ cell proliferation or development, consistent with the poor fertility of *solo* females.

**Venus-SOLO form bright foci at centromeres in oocytes**

We generated transgene constructs expressing *UAS:Venus-SOLO* and *UAS:SOLO-Venus* and transformed them into flies. One copy of each construct was previously shown to completely rescue male phenotype when their expression were induced by *nanos (nos):Gal4-VP16*. Similar rescue experiments showed that *UAS:Venus-SOLO* and *UAS:SOLO-Venus* also rescued female NDJ to background level induced by *nos:Gal4-VP*16 (Table 3-6), suggesting that Venus-SOLO is fully functional in both male and female meiosis. SOLO localizes to centromeres in Drosophila males, corresponding to its role in centromeric cohesion. In order to explore the localization of SOLO in female, Venus-SOLO was induced by *nos:Gal4-VP16*. We found that Venus-SOLO formed one to three very bright foci in nuclei of female germ cells of various stages. The and Venus-SOLO foci co-localized with foci of anti-CID, which detects a centromere-specific variant of histone H3. In germaria, Venus-SOLO

Table 3-6. Venus-SOLO transgenes completely rescue *solo* phenotypes in females.

| Transgene | Line | %NDJ Female[a] |
|---|---|---|
| *[UAS:Venus-SOLO]* | 1910-2-2A | 0 (190) |
| | Control | 60.7 (63) |
| *[UAS:Venus-SOLO]* | 1910-2-1A | 0 (523) |
| | Control | ND |
| *[UAS:SOLO-Venus]* | 1910-1-1 | 0 (212) |
| | Control | ND |

[a]To rescue *solo* female phenotypes, X-X NDJ was measured by crossing +/+; $solo^{Z2\text{-}0198}$*/Df(2L)A267; [UAS:Venus-SOLO]* or *[UAS:SOLO- Venus]/[nanos:Gal4-VP16]* females to YSX.YL, In(1)EN, y B males. Controls were $solo^{Z2\text{-}0198}$*/Df(2L)A267* siblings carrying either the SOLO transgene or the Gal4 driver but not both. ND = not done.

and CID colocalized in oogonia undergoing mitosis in region 1, and in pro-oocytes, oocytes and nurse cells in regions 2 and 3 (Fig. 3-3A). In later meiotic stages, Venus-SOLO continued to colocalize with CID in oocytes and nurse cells (Fig. 3-3B and 3C). No Venus-SOLO foci were detected in the somatic follicle cells.

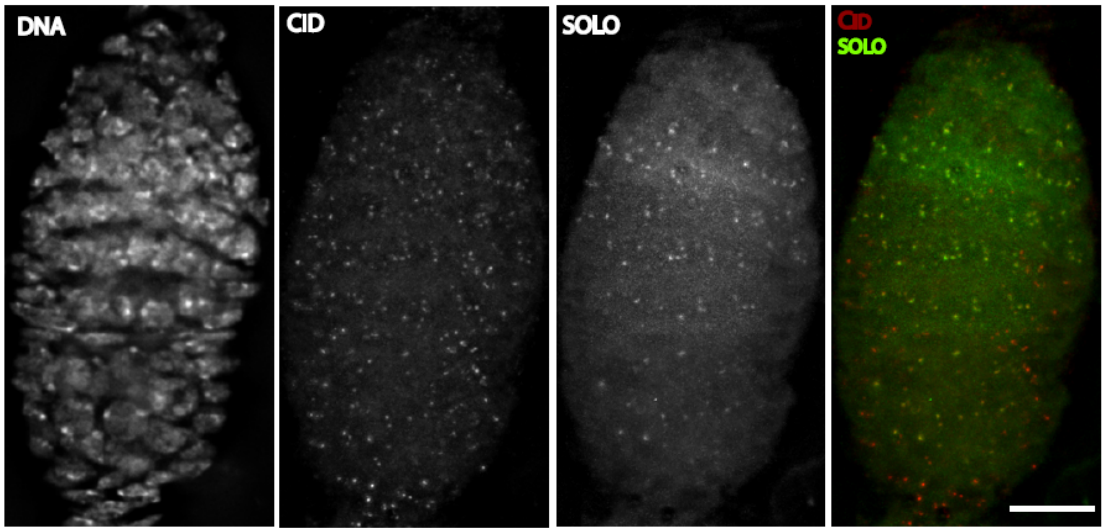
**SOLO localizes to synaptonemal complexes**

Meiotic recombination in Drosophila females occurs in the context of SC (Page and Hawley 2003). The reduced recombination frequency, altered distribution of crossovers, and the defects of SC in *solo* mutants suggest that SOLO is probably a component of SC. To test this possibility, we simultaneously stained germaria for SOLO and C(3)G, a component of the transverse filaments of SC (Fig. 3-4). In addition to forming bright foci in nuclei of female germ cells at centromeres as shown in Fig. 3-3, Venus-SOLO also localized more generally within germ cell nuclei and appeared to be especially abundant in region 2 of the germarium, where germ cells undergo early meiotic prophase. Besides very bright staining at centromeres, weak thread-like Venus-SOLO staining could sometimes be seen in regions 2a, region 2b, and region 3. The thread-like SOLO staining appeared to overlap with C(3)G. In region 2a, thread-like SOLO staining colocalizes with C(3)G in several nuclei. The colocalization of SOLO and C(3)G occurred in pro-oocytes, oocytes, and nurse cells. This is not surprising since previous studies have shown that temporary SC formation

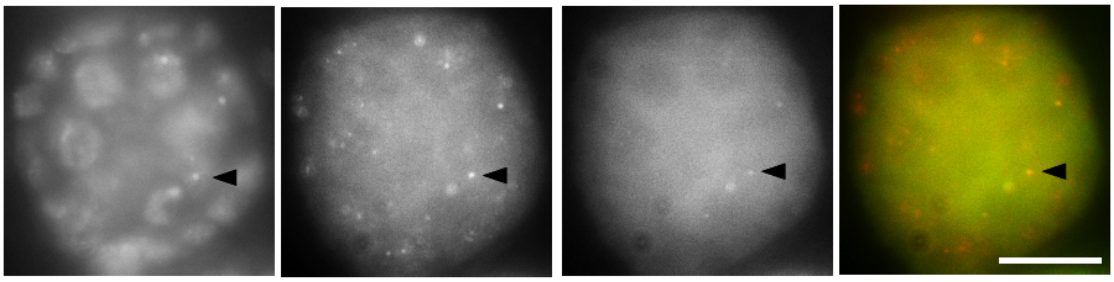
Figure 3-3. Venus-SOLO foci are abundant at centromeres.

Expression of Venus-SOLO was induced by *nanos:Gal4-VP16* in *[UAS:Venus-SOLO]/[nanos:Gal4-VP16]* females and detected at FITC channal. Centromeres were visualized with CID staining and DNA was stained with DAPI. The transgenic line is 1910-2-2A. Venus-SOLO colocalizes with CID in oogonia, pro-oocytes, oocytes, and nurse cells of germaria. The images represent sum projections of 3D-deconvolved Z-series (A). Venus-SOLO colocalizes with CID in oocytes and nurse cells at stage 2 (B) and stage 4(C). Arrow heads indicate the oocytes, showing colocalization of SOLO and CID at centromeres. No Venus-SOLO foci but CID foci appear on follicle cells. Scale bars: 10 μm.

A. Germarium




B. Stage 2



C. Stage 4

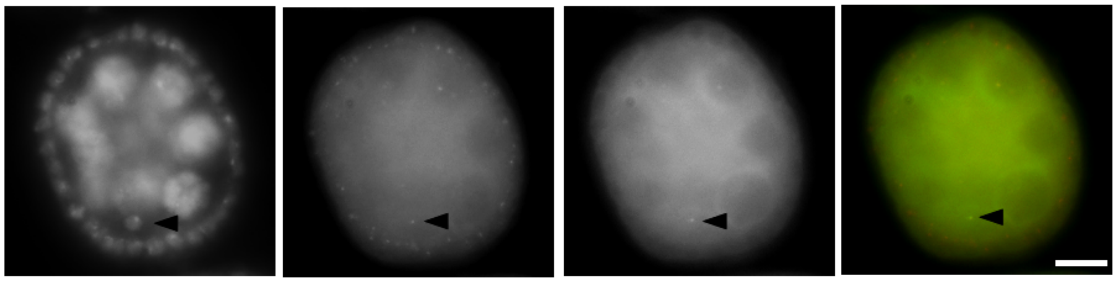


Figure 3-4. Thread-like SOLO structures localizes to SC.

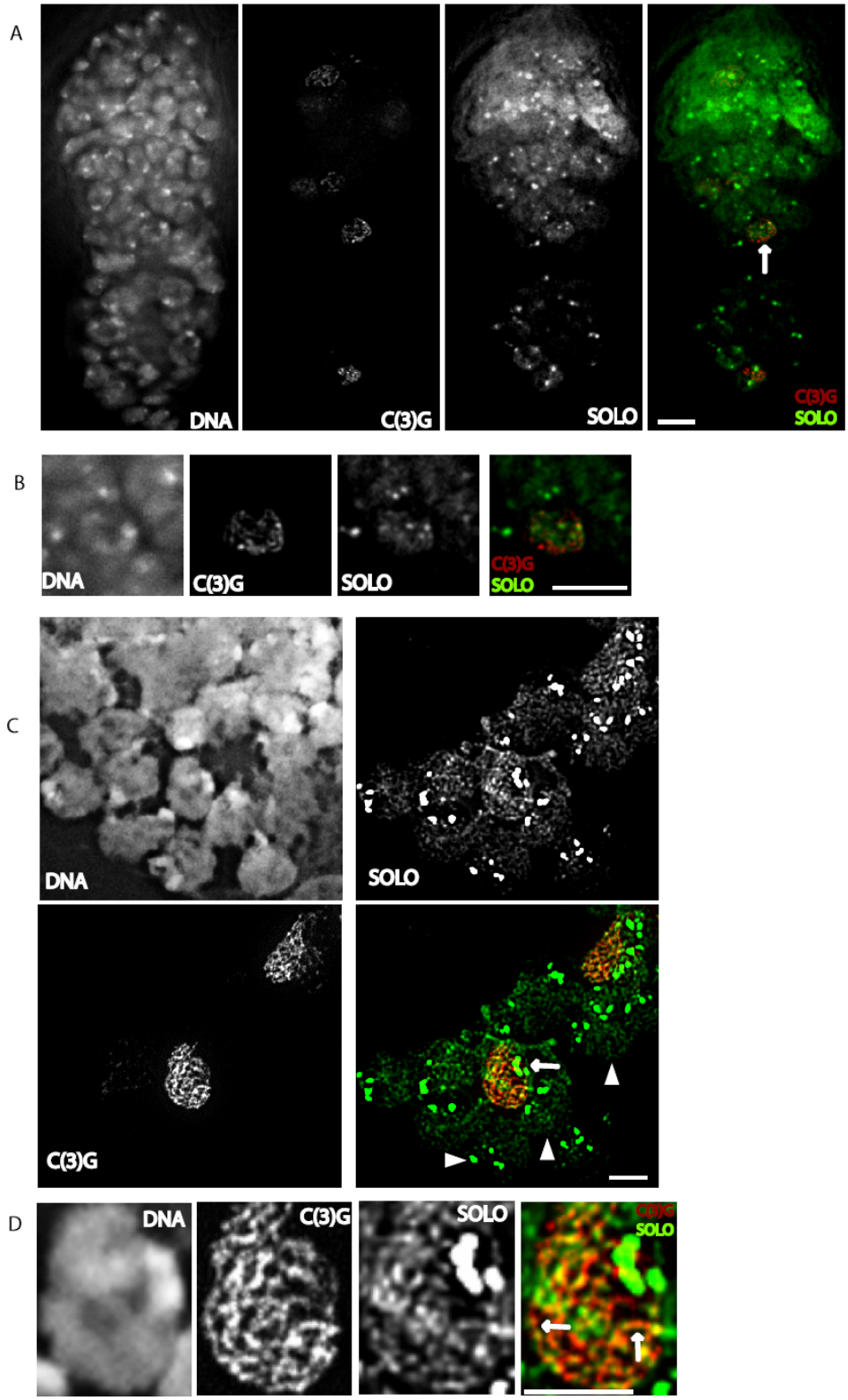
Expression of Venus-SOLO was induced by *nanos:Gal4-VP16* in *[UAS:Venus-SOLO]/[nanos:Gal4-VP16]* females and stained with anti-GFP antibody. The transgenic line is 1910-2-2A. SC was visualized by C(3)G staining and DNA was stained with DAPI staining. All images represent sum projections of 3D-deconvolved Z-series stacks. Scale bars: 5 μm.

(A) Some thread-like Venus-SOLO staining colocalizes with C(3)G staining in a whole-mount germarium (arrow).

(B) Magnification of a pro-oocyte marked by arrow in (A).

(C) SOLO and C(3)G thread-like staining is seen in germ cell nuclei preparation by chromosome spread. Thread-like SOLO staining apparently colocalizes with C(3)G staining (arrow). Weak thread-like SOLO staining also can be seen in the cells without C(3)G staining (arrowheads).

(D) Magnification of a pro-oocyte marked by arrow in (C). Most of thread-like SOLO staining colocalizes with C(3)G, as pointed by arrows.


A
DNA
C(3)G
SOLO
C(3)G
SOLO
B
DNA
C(3)G
SOLO
C(3)G
SOLO
C
DNA
SOLO
C(3)G
D
DNA
C(3)G
SOLO
C(3)G
SOLO

occurs in nurse cells besides pro-oocytes and oocytes (Carpenter 1979). With the cells move to posterior end of germarium, the cells with diffuse SOLO staining become fewer and finally thread-like SOLO staining is restricted in oocytes at region 3. In addition, the linear C(3)G elements were generally longer and more prominent than the linear Venus-SOLO elements and linear Venus-SOLO staining did not always coreside with C(3)G staining.

To further analyze thread-like SOLO staining SC in ooctyes, we took advantage of the chromosome spread method, which improves greatly the resolution of SC (Webber et al., 2004). Using this method, we found that SOLO apparently associated with the chromatin of most or all germ cells that were from one cyst (Fig. 3-4C and 4D). Thread-like SOLO structures formed in the cells with thread-like C(3)G staining (arrow). In the cells without C(3)G staining (arrowheads) SOLO staining is weaker and discontinuous, showing fragmented and spotty SOLO staining, although very bright SOLO foci at centromeres were still seen. In the cells with C(3)G staining, most or all linear structures of SOLO colocalized with linear C(3)G staining. Linear C(3)G staining was often brighter than that of SOLO staining, confirming the observation in whole-mount ovaries (Fig. 3-4A and B). In contrast, less C(3)G staining was observed in the region of bright foci of SOLO that includes centromeres and pericentromeric heterochromatin (Fig. 3-4D).

**SOLO colocalizes with SMC1 at centromeric region and chromosome arm**

The chaos of sister chromatid segregation in *solo* males and females suggests that SOLO is probably a cohesion protein. To explore this possibility, we simultaneously stained germaria for SOLO and SMC1, a component of cohesin (Fig. 3-5). SMC1 formed bright foci that represent centromeres and ribbon-like structures that represent SC structures. SOLO began to colocalize with SMC1 to form bright foci at centromeres within region 1. The thread-like structures of SOLO and SMC1 first appeared at region 2a where SC assembly begins and continue to region 2b and region 3. The colocalization of SMC1 and SOLO occured in pro-oocytes, oocytes, and nurse cells. These data strongly suggest SOLO is a component of cohesin. Interestingly, thread-like SMC1 staining seemed to be more prominent than that of SOLO. We again applied the detail. As shown in Fig. 3-5B and 5C, SOLO showed very bright foci at centromeric region but weak thread-like staining on chromosome arms compared to SMC1 staining although they almost completely colocalized. The reason for distinct staining pattern is at present not known. Other cohesion proteins, like ORD (Webber et al., 2004), might be contributive to the distinct pattern through forming complexes at distinct chromosome domain. However, we cannot rule out the possibility of other complex scenario.

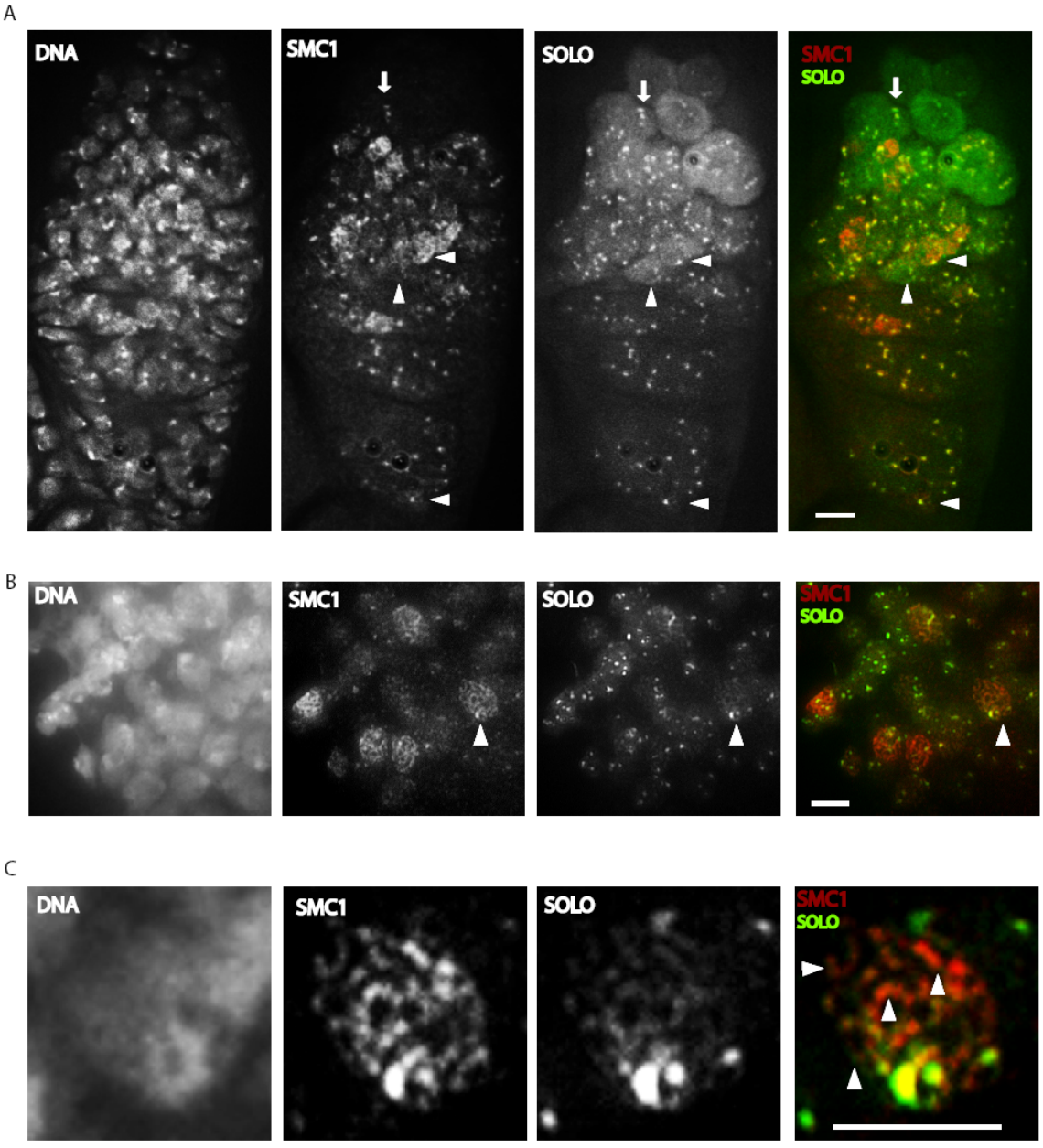
Figure 3-5. SOLO and SMC1 colocalize together.

Expression of Venus-SOLO was induced by *nanos:Gal4-VP16* in *[UAS:Venus-SOLO]/[nanos:Gal4-VP16]* females and stained with anti-GFP antibody. The transgenic line is 1910-2-2A. SMC1 was stained with anti-SMC1 antibody and DNA was visualized with DAPI staining. All images represent sum projections of 3D-deconvolved Z-series stacks. Scale bars: 5 μm.

(A) Both SOLO and SMC1 form bright foci at centromeres and thread-like staining at chromosome arms in a whole-mount germarium and colocalize. Both of them localize to centromeres but do not form thread-like structures at region 1 (arrows). SOLO and SMC1 form thread-like structures at chromosome arm while form bright foci at centromeric region at region 2a, 2b and 3 (arrowheads).

(B) Centromeric foci and thread-like staining of SOLO and SMC1 appear in germ cell nuclei preparation by chromosome spread. SOLO forms relatively brighter foci compared to SMC1 at centromeres whereas the thread-like staining of SOLO is relatively weaker compared to SMC1 although both of them colocalize at centromeres and chromosome arms.

(C) Magnification of a pro-oocyte marked by arrowheads in (B). SOLO and SMC1 apparently colocalize (arrowheads).


A
DNA
SMC1
SOLO
SMC1
SOLO
B
DNA
SMC1
SOLO
SMC1
SOLO
C
DNA
SMC1
SOLO
SMC1
SOLO

# DISCUSSION

Although sister chromatid cohesion is required for the generation of crossovers during meiosis, which is essential for homolog segregation, the mechanism of how sister chromatid cohesion functions in meiotic recombination is not well understood in higher eukaryotes. Here we show that meiotic cohesion is required for formation of SC, promoting homologous recombination by inhibiting recombination between sister chromatids, and maintaining chiasmata stability for homolog segregation, thus providing evidence for cohesion's key role in homolog segregation during meiosis I.

**SOLO is a cohesion protein that is required for normal homolog and sister chromatid segregation.**

We have showed that SOLO is essential for homolog and sister chromatid segregation in Drosophila male meiosis. Our results show that in females both homolog and sister chromatid NDJ occur when *solo* is not functional, suggesting SOLO's role in Drosophila meiosis is not sex-specific but it is universally required for both homolog and sister chromatid segregation in meiosis of both sexes although they undergo meiosis through different pathways. The colocalization of SMC1 and SOLO from pre-meiotic stages to late prophase I in females and their colocalization from pre-meiotic stages to anaphase II in males strongly suggest that SOLO is probably a component of cohesin that requires SMC1. Interestingly, the strength of SOLO and SMC1 staining is distinct at distinct

chromosome domains. SOLO usually shows weaker thread-like staining at chromosome arms compared to SMC1. Although it could be a reflection of three-dimensional distortion of cohesin due to the twisted SC as some studies suggested (Carpenter 1979; Zickler and Kleckner 1999), it is unlikely since, if cohesin is twisted, SOLO would be expected to be brighter than SMC1 at some chromosome arm regions, but actually SOLO is rarely brighter than SMC1 on chromosome arms. Alternatively, SMC1 may form different cohesion complexes with different cohesion proteins at distinct regions depending on their different functions. Besides SOLO, SMC1 may assemble cohesion complex on chromosome arms with C(2)M, an α-Kleisin protein, which is not required for sister chromatid cohesion and does not form bright foci at centromeres but is a component of the lateral elements of SC (Manheim and McKim 2003; Heidmann et al., 2004). This idea that SOLO and C(2)M functions redundantly on chromosome arms is supported by the recent study in which SMC1/SMC3 showed weak staining on chromosome arms in *c(2)M* mutant whereas the staining is robust in wild type (Khetani and Bickel 2007). Moreover, this model also gives an explanation to why about 20% residual recombination still exists in *solo* or *c(2)M* single mutants. If our model is correct, the frequency of recombination in *solo* and *c(2)M* double mutantswould be very low, much less than 20%. Indeed, distinct cohesion complexes form at distinct chromosome domain (kitajima et al., 2003). However, more complex scenario might be still possible. It will be very intriguing to explore the mechanism underlying it through identifying the distinct cohesion complex in Drosophila meiosis.

**SOLO is required for promoting homologous recombination and inhibiting sister chromatid exchange during Drosophila female meiosis.**

Our results showed that SOLO is required for normal homologous recombination occurred on X chromosomes and autosomes since the lack of SOLO causes reduced recombination along chromosome arm. However, the recombination frequency of intervals spanning centromeres actually increases, showing that both of the distribution and frequency of recombination are altered. Thus *solo* belongs to the precondition type of mutation. The phenotype of *solo* is similar to *ord*, which alter distribution and frequency of recombination. Moreover, the homolog recombination is completely abolished in the absence of C(3)G (Page and Hawley, 2003), suggesting that SC is required for homolog recombination. Thus the defects of SC due to the loss of SOLO disrupt the tendency of SC to promote homolog recombination, i.e. make crossovers between homologous chromatids from DSBs.

Cohesion is required for mitotic and meiotic recombination besides chromosome segregation (Hirano 2000; Jessberger 2002). The cohesion-dependent recombination in meiosis occurs preferentially between homologs (Schwacha and Kleckner 1997; Webber et al. 2004) whereas the recombination in mitosis is more apt to occur between sister chromatids (Johnson and Jasin 2000). Our Ring chromosome exchange assay argues that *solo* mutations disrupt homolog recombination bias. Our study showed that Ring/Rod ratio in wild type is less than 1, which is consistent with other studys (Manheim and McKim, 2003; Webber et al., 2004; McKee personal communication) and

probably reflects the normal sister chromatid exchange level of Drosophila female meiosis. However, the recovery of Ring/Rod ratios in *solo* mutants is significantly lower than that in wild type. The increased sister chromatid exchange is not due to the disruption of SC since previous studies have shown that sister chromatid exchange level is not elevated in C(3)G mutants (Sandler et al. 1974). The significantly elevated sister chromatid exchange is probably due to the loss of sister chromatid cohesion when SOLO is absent.

Thus our data suggest the reduced homolog recombination in *solo* mutants might result both from a failure to promote inter-homolog recombination, since SC promotes homolog recombination and *solo* mutations disrupt SC formation, and a loss of the inhibition of sister chromatid exchange, since sister chromatid cohesion is required for inhibition of recombination between sister chromatids and sister chromatid cohesion is lost in *solo* mutants.

**SOLO is a novel component of SC required for meiotic recombination.**

SOLO localizes to the entire chromosome arm besides forming bright foci at centromeric regions, which is consistent with the genetic and cytological observation that SOLO is required for arm and centromeric cohesion during female meiosis. Although thread-like SOLO staining appears in pro-oocytes and pro-nurse cells, the linear structure of SOLO colocalizes with C(3)G along chromosome arms in the cells showing C(3)G thread-like staining, suggesting SOLO is a novel SC component. The severe defects of C(3)G in *solo* mutants provide further support for this idea. SC assembly in the absence of SOLO is

defective in region 2a, the stage when meiotic recombination begins. The SC phenotype of *solo* is earlier and more severe than that of *ord*, which only causes severe defects of SC in region 3, where meiotic recombination is actually completed (McKim et al., 2003). Our data suggest that SOLO, unlike ORD, which is required for SC maintenance, is essential for SC formation. However, the null alleles of *solo* and *ord* show similar reduced recombination frequency (this study; Miyazaki and Orr-Weaver, 1992). The reason why the distinct SC phenotype of *solo* and *ord* resulted in the similar reduced recombination frequency is not clear yet. The defective ultrastructure of SC in region 2a in ord mutants uncovered by EM observation may provide one explanation (Webber et al., 2004).

**Chiasmata stability requires SOLO**

Chiasmata are essential for proper separation of homologs during meiosis I. It has been demonstrated that at least one chiasma per bivalent exists in Drosophila female meiosis (Carpenter and Sandler 1974; Hawley, 1988). In *mei-218* mutants chiasmata remain stable until the onset of anaphase I (Bickel et al. 2002) if they are formed from residual recombination events (McKim et al. 1996, Bhagat et al. 2004). Our genetic analyses have shown that approximately 16-20% of the frequency of meiotic recombination in wild type occurs in *solo* mutants (6.7cM-8.2cM/42cM), which is almost double of that of *mei-218* (8%) (McKim et al. 1996). Thus we would expect that more chiasmata may form in *solo* mutants if they are stable and they separate successfully to opposite poles

in meiosis I. However, if chiasmata that form in oocytes are not stable in the absence of sister chromatid cohesion due to *solo* mutations, the homologous chromatids that undergo one crossover would separate to the same spindle pole after following meiosis II NDJ. Our data demonstrate that crossovers do form in *solo* mutants but all of them are not stable in meiosis I. Stable chiasmata were also not observed in *ord* null mutants (Bickel et al. 2002). Rec8 that is essential for sister chromatid cohesion is required for maintenance, and the failure of resolution of Rec8 blocks homolog segregation in S. cerevisae and C. elegans (Buonomo et al. 2000; Siomos et al. 2002). With these studies our data suggest that the requirement of sister chromatid cohesion for stabilizing chiasmata until anaphase I might be a conserved mechanism among eukaryotes. Without sister chromatid cohesion, they are not effectively maintained to ensure proper chromosome segregation during meiosis I although they can form.

**Conclusions**

SOLO is a novel cohesion protein required for homolog recombination, synapsis, chiamata maintenance, and proper segregation of homologs and sister chromatids in Drosophila female meiosis. *solo* mutations cause reduced meiotic recombination due to the disruption of promoting inter-homolog recombination and inhibiting sister chromatid exchange. The SC and residual chiasmata are not stable in the absence of SOLO, probably due to the loss of sister chromatid cohesion in *solo* mutants. Furthermore, SOLO localizes to centromeres and SC and colocalizes with SMC1. Combining the data in female meiosis with the

observation of SOLO's function and localization pattern in male meiosis, we propose that SOLO is a novel component of a meiosis-specific cohesin complex in Drosophila meiosis.

**Acknowledgments**

We thank B. Wakimoto, M. Ashburner, and Bloomington Stock Center for providing fly stocks, S. Page and R.S. Hawley for providing C(3)G monoclonal antibody. This work was supported by NIH grant # R01 GM40489 to B.D.M.

# APPENDICES

Table A-1. Ring/Rod and NDJ test for *solo* mutants

| | Ring | Rod | Ring/ Ring | Rod/ Rod | Ring/ Rod | null | [b]DCO - 1 | DCO -2 |
|---|---|---|---|---|---|---|---|---|
| genotype | [a]R/$w^{1118}$ R/Y | y w/w y w/Y | | | | | | |
| phenotype | $y^+$ $w^+$ $f^+$ ♀♀ y $w^+$ f ♂♂ | $y^+$ w $f^+$♀♀ y w $f^+$♂♂ | y $w^+$ f ♀♀ | y w $f^+$ ♀♀ | y $w^+$ $f^+$ ♀♀ | $y^+$ w $f^+$ ♂♂ | y $w^+$$f^+$ ♂♂ | y w f ♂♂ |
| control | 958 | 1156 | 0 | 0 | 0 | 50 | 8 | 21 |
| Z2-0198 | 209 | 605 | 6 | 58 | 68 | 306 | 6 | 3 |
| Z2-3534 | 216 | 604 | 12 | 51 | 65 | 318 | 7 | 2 |

The number of progeny from *R(1)2, y f/y w; Df(2L)A267/solo* or wild type control females crossed to $w^{1118}$*/Y* males was scored.

[a]R represents *R(1)2, y f*.

[b]DCO represents double crossovers.

# CHAPTER 4 - NOVEL ROLES OF VASA IN DROSOPHILA MALE MEIOSIS: HOMOLOGOUS RECOMBINATION AND CHROMOSOME SEGREGATION

**Abstract**

Drosophila males undergo meiosis without recombination under normal conditions whereas recombination is required for female meiosis. How males prevent exchanges between homologous chromosomes is not known. *vasa* (*vas*) is a translation initiation factor and is involved in oogenesis and embryogenesis. Here we report novel functions of *vas* in male meiosis. Mutations of *vas* caused X-Y exchanges at elevated frequency in meiosis, producing sterile progeny that exhibited position effect variegation (PEV). The PEV and sterility of the progeny resulted from the partial loss of Y chromosome due to X-Y exchange. Cytological analysis revealed formation of chromatin bridges at anaphase I and II. Chromatin masses were also found in the midzone at anaphase I and II. *vas* and *solo* double mutants showed higher nondisjunction than either *solo* or *vas* single mutants. Additionally, *vas* and *solo* double mutant showed precocious segregation of homologs at metaphase I in addition to chromatin bridges at anaphase I and II. Our data thus for the first time demonstrate that inhibition of meiotic recombination during male meiosis requires *vas* function. Furthermore, interaction between *vas* and *solo* regulates chromosome dynamics in male meiosis.

# INTRODUCTION

Accurate chromosome segregation during meiosis is essential for proper transmission of genetic material in sexual reproduction. Errors in meiosis are the primary cause of miscarriages and genetic diseases in human beings (Hassold and Hunt, 2001).

Homolog pairing and segregation at the first meiotic division (meiosis I) is required for successful meiosis (McKee, 2004). In most eukaryotes, including *Drosophila* females, tight homolog pairing requires homologous recombination and formation of synaptonemal complexes (SCs), proteinaceous structures that connect aligned homologs from end to end (Page and Hawley 2004). SC is disassembled and the tight homolog pairing is lost during mid prophase I and homologs thereafter are only connected at discrete sites by chiasmata, which are the cytological manifestations of crossovers, the reciprocal products of meiotic recombination (Page and Hawley, 2003; Petronczki et al., 2003). Chiasmata hold homologs together until the onset of anaphase I when homologs segregate and are essential for bi-orientation of homologs during late prophase I and metaphase I (Hawley 1988).

In contrast to females, Drosophila males do not form SCs (Meyer 1960, Rasmussen, 1973) and do not recombine and form chiasmata during meiosis (McKee, 2004), i.e. achiasmate meiosis. However, homologs do pair and segregate efficiently in males. Recent studies revealed that homolog pairing and segregation during Drosophila male meiosis requires two proteins: SNM

(Stromalin in Meiosis), a homolog of SCC3/SA cohesion protein, and MNM (Mod(mdg4) in meiosis), a BTB domain protein that is involved in many protein-protein interactions (Thomas et al., 2005, Thomas and McKee, 2007). Moreover, the genetic and cytological studies carried out by McKee and his collaborators have revealed that X-Y chromosomes pair at specific sites: 240bp repeated sequence within the intergenic spacers of the ribosomal RNA gene arrays (rDNA) on both X and Y chromosomes (McKee et al., 1992; Merrill et al., 1992; McKee, 1996). The rDNA arrays are located in the middle of the heterochromatin of X chromosome and on the short arm of Y chromosome proximal to the centromere and consist of total 200-250 tandem copies of the genes for the 18S, 5.8S, and 28S ribosomal RNAs (rRNAs) (Ritossa 1976). Deletions of the entire rDNA arrays disrupt X-Y chromosome pairing and cause random X-Y segregation (McKee and Lindsley 1987). The transgenes containing either rDNA repeats or 240bp repeats can partially restore the ability of X-Y pairing (McKee and Karpen 1990; McKee et al., 1992; Merrill et al., 1992). Moreover, the 240bp repeats are required for the pairing between mini-X chromosome and normal X-Y bivalents (Thomas and McKee, 2007).

The frequency of spontaneous exchanges between X and Y is very low (0.01%). When the males are treated by ionizing agent, like irradiation, the frequency may increase up to 2% (Ashburner et al., 2004). Recently Maggert and Golic (2005) took advantage of the presence of a sequence very similar to the recognition site for the I-CreI restriction endonuclease in the 28S rDNA to generate DSBs on X and Y chromosome. Heat-shock induced I-CreI expression

caused very high exchange (about 20%) between X and Y chromosomes. .

Mei-W68 is the Drosophila ortholog of Spo11, which generates double strand breaks (DSBs) that are required for recombination. *mei-W68* is transcribed in testis although at low level, suggesting DSBs are probably generated in spermatogonia and spermatocytes. The extremely low frequency of spontaneous exchange suggests that there is a mechanism to prevent chromosome exchanges in males. How the males prevent exchange between homologous chromosomes and between sister chromatids is elusive. No mutants that affect this process have been identified.

The Drosophila gene *vas* encodes a DEAD-box RNA helicase that is required for pole cell development and dorsal-ventral axis specification during oogenesis and embryogenesis (Hay et al., 1988; Lasko and Ashburner, 1988; Liang et al., 1994; Styhler et al., 1998; Tinker et al., 1998; Tomancak et al., 1998). VASA shares sequence similarity with the translation initiation factor eIF4A and controls the translation of some key germline-specific mRNAs that are critical for oocyte patterning, e.g. Oskar and Gurken (Hay et al., 1988; Lasko and Ashburner, 1988; Liang et al., 1994; Dahanukar and Wharton, 1996; Styhler et al., 1998; Tinker et al., 1998;). Although *vas* is also expressed in male germline cells, particularly strong in spermatogonia and young spermatocytes (Hay et al., 1988), *vas* males are fully fertile and no phenotypes in males have been reported.

Here we report the novel functions of *vas* in male meiosis. *vas* mutations cause high frequency of X-Y chromosome exchange at rDNA loci and low

nondisjunction (NDJ) of sex chromosomes. The majority of recombinants between X and Y chromosomes are sterile and show position effect variegation due to the loss of Y-chromosome heterochromatin including fertility factors. The recovery of chromatin bridges at anaphase I and anaphase II together with the evidence that majority of recombinants are not clusters indicate that most, if not all, of the recombination events occur during meiosis. In addition, *vas*, *solo* double mutants cause premature loss of linkage between homologous chromosomes in addition to chromatin bridges at anaphase I, suggesting that *solo* and *vas* may interact with each other in male meiosis.

# MATERIALS AND METHODS

## Drosophila stocks and strains

All flies were maintained at 23°C on standard cornmeal molasses medium. *vas* alleles were obtained from M. Ashburner (Cambridge University), P. Lasko (McGill University), D. Montell (John Hopkins University), and the Bloomington Stock Center at the University of Indiana. All chromosomes and markers are described in Flybase (2007). Unless otherwise specified, tested males were crossed singly to two or three females in shell vials. Parents were removed from the vial on day 10 and progeny were counted between day 13 and day 22.

## Sex chromosome NDJ and position effect variation (PEV) assays

To measure X-Y NDJ, $+/B^{S}Yy^{+}$ ; *vas/Df(2L)A267* males were crossed singly to 2-3 females carrying structurally normal X chromosomes marked with $y^{1}$ and $w^{1118}$. Regular progeny are + females and $y^{+}$ w $B^{S}$ males; paternal NDJ generates $y^{+}$ $w^{+}$ $B^{S}$ female and y w $B^{+}$ male progeny. %NDJ = 100 x ($y^{+}$ $w^{+}$ $B^{S}$ ♀♀ + y w $B^{+}$ ♂♂)/N. Besides normal and NDJ progeny, other kinds of exceptional males, $y^{+}$ w $B^{+}$ and y w $B^{S}$, were also recovered. For simplicity of discussion, the Y chromosomes in both males were denoted hereafter as $Y_{ab}^{B+\ y+}$ and $Y_{ab}^{B\ y}$, respectively. All of the $y^{+}$ w $B^{+}$ males showed strong variegation of pigment ($y^{var}$) with patches of yellow and $yellow^{+}$ pigment intermingled in the abdomen.

Fertility test

$y^+$ w $B^+$, y w $B^S$, and XO (from NDJ) male progeny from the above NDJ and PEV assay were crossed to *y w* females. Whether there were progeny was observed.

PEV and sterility inhibition assays

*+/$B^S$Y$y^+$; Df(2L)A267, b cn bw/vas* males were crossed to *YSX.YL, In(1)EN, y B/y w* females. The bar eyes are larger in females with B marker than those in males with $B^S$ marker. When B and $B^S$ are combined, the bar eyes are even smaller. The males with B $w^+$ eyes, representing the males with *YSX.YL, In(1)EN, y B*/$Y_{ab}^{B+\ y+}$ were selected and crossed to *y w* females. The $Y_{ab}^{B+\ y+}$ chromosome combined with *y w* X chromosome produced PEV males again. The PEV males were crossed to *y w* females again to test their fertility. Three *vas* alleles $vas^3$, $vas^5$, and $vas^{RG53}$ were analyzed.

X-Y chromosome exchange test

*+/$B^S$Y$y^+$; Df(2L)A267, b cn bw/vas* males were crossed to *C(1)RM/YS* females, respectively. *C(1)RM/YS* females produced diplo-X and YS eggs at approximately equal frequency. Among the sperm produced by the males, two kinds of them are +/BSYy+, from NDJ, and $B^S$YL.$Xy^+$ $w^+$, from X-Y recombination. When these two kinds of sperm were fertilized by YS eggs, both of them yielded the males with $B^S$ $y^+$ $w^+$ eyes that were not distinguishable. The males with $B^S$ $y^+$ $w^+$ eyes were selected and crossed to y w females. If the male

is from NDJ, it produces 4 types of progeny: $B^S$ $y^+$ w males, $B^+$ $y^+$ $w^+$ females, $B^+$ y w males, $B^S$ $y^+$ $w^+$ females. If the male is from X-Y recombination, it only produces two types of progeny: $B^+$ y w males, $B^S$ $y^+$ $w^+$ females, i.e., all $B^S$ flies are females. Thus the X-Y exchange can be tested.

**Testis Immunostaining**

$\alpha$-tubulin/DAPI staining of testes was carried out as described (Thomas et al. 2005). MNM-GFP live imaging was performed according to Thomas et al. (2005).

**Microscopy and image processing**

All images were collected using an Axioplan (ZEISS) microscope equipped with an HBO 100-W mercury lamp and high-resolution CCD camera (Roper). Image data were collected and merged using Metamorph Software (Universal Imaging Corporation). Images were processed with Adobe Photoshop CS2.

# RESULTS

## *vas* mutations cause low NDJ but high PEV

To study whether *vas* mutations affect sex chromosome segregation in male meiosis, *vas* males carrying a dominantly marked Y chromosome ($B^{S}Yy^{+}$) were crossed to *y w* females (Table 4-1). Males hemizygous for all *vas* single mutants showed similar low NDJ, averaging 2.76%, suggesting that *vas* only has a small effect on chromosome segregation. Surprisingly, in all *vas* alleles additional types of males that did not result from normal or NDJ sperm were recovered. One class is the males with $B^{+}$ w eyes and with the abdomen showing patches of yellow and yellow+ pigment intermingled (Fig. 4-1). These flies showing strong variegation of $yellow^{+}$ pigment ($y/y^{+}$) were referred hereafter as $B^{+}$ $y^{var}$ males and the corresponding Y chromosome in the flies was $Y_{ab}{}^{B+\ y+}$. The other one is the males with $B^{S}$ w eyes and yellow body; they are referred as B y males hereafter. The frequency of $B^{+}$ $y^{var}$ and B y males is approximately 3.7% on average, which is very high (approximately 400 times) compared to the frequency in wild type (0.01%) and *solo* single mutant flies since we never found $B^{+}$ $y^{var}$ males in *solo* cross experiments. The finding of these additional males suggests that *vas* has novel functions in meiosis, or at least in spermatogenesis. In the three tested *vas* and *solo* double mutants, NDJ was 65%, on average, higher than the approximately 50% NDJ in *solo* single mutants and far higher than the 3% NDJ in *vas* single mutants. Moreover, the extremely high ratio of

Table 4-1. X-Y NDJ and exchange in crosses of *+/B$^S$Yy$^+$; vas/Df* males X y w females.

| sperm class [a] | | Reg. X or Ex | Reg. Y | NDJ | NDJ or Ex | Ex | Ex | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| sperm genotype | | Xy$^+$ w$^+$ or Ex | B$^S$Yy$^+$ | O | +/ B$^S$Yy$^+$ or Ex | | | | | |
| | | Progeny | | | | | | | | |
| Allele [b] | mutation type | +♀♀ | y$^+$ w B ♂♂ | yw B$^+$ ♂♂ | B$^S$ ♀♀ | y$^{var}$ w B$^+$♂ ♂ | B y w ♂♂ | total | %X-Y NDJ | %X-Y Ex |
| 3 | Unknown | 949 | 659 | 33 | 19 | 19 | 4 | 1683 | 3.09% | 3.37% |
| 5 | Missense | 2315 | 1784 | 34 | 30 | 37 | 6 | 4205 | 1.52% | 2.35% |
| 6356-005 | Missense | 2896 | 2059 | 89 | 14 | 38 | 4 | 5100 | 1.82% | 2.00% |
| RG53 | Unknown | 2105 | 1222 | 150 | 13 | 38 | 3 | 3531 | 4.61% | 3.25% |
| 7 | Unknown | 155 | 93 | 400 | 12 | 1 | 1 | 662 | 77.30% | 2.11% |
| 6356-001 | Nonsense | 220 | 149 | 698 | 24 | 3 | 0 | 1094 | 66.00% | 1.97% |
| PH165 | Deletion | 392 | 215 | 578 | 77 | 25 | 1 | 1288 | 51.60% | 10.80% |
| total | | 9032 | 6181 | 1982 | 189 | 161 | 19 | 17563 | | 3.69% |

[a]Reg. X = regular X chromosome; NDJ = nondisjunction; Ex. = exchange; see Fig. 4-2 for exchange classes. [b]Green = *vas* alleles; red = *vas solo* alleles. Note: PH165 is also a *vig* allele.

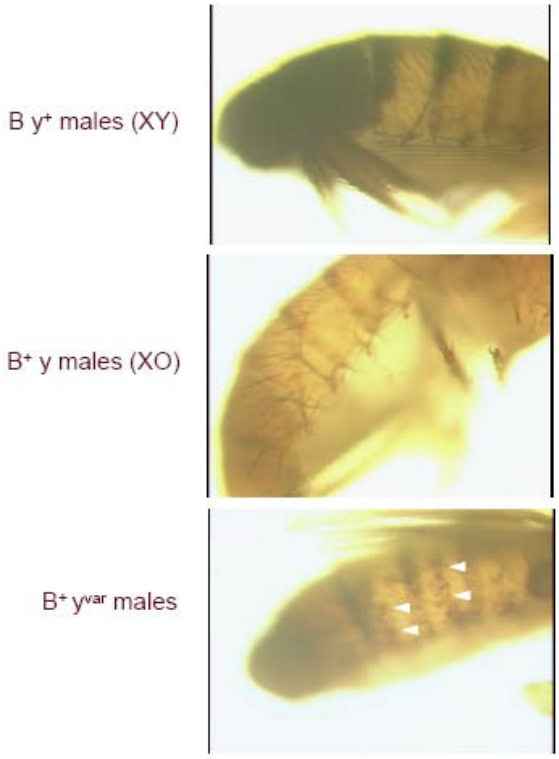



Figure 4-1. Abdomen pigment in different males.

The male progeny are from crosses of *+/B$^{S}$Yy$^{+}$; Df/vas* males to *y w* females. The arrowheads show yellow$^{+}$ pigment patches on a yellow abdomen. The eye shapes of different males are not shown.

XO progeny indicates that the majority of NDJ sperm in *solo* and *vas* double mutants were null sperm, which is very unlike in *vas* and *solo* single mutants. These results suggest that SOLO and VAS might co-cooperate in male meiosis in addition to having their own distinct effects. Furthermore, in all *vas* mutants, progeny from nullo-XY sperm were more frequent than the progeny from XY sperm. Particularly, in $vas^{RG53}$, $vas^{7}$, $vas^{6356-001}$, and $vas^{PH165}$ mutants, the ratio of progeny from nullo-XY sperm to that from XY sperm is about 10 or even higher. However, we need to be very cautious to make this conclusion due to the limitation of our cross. The $B^{S}$ $y^{+}$ females could be either NDJ (XXY) or the recombinant ($B^{S}$YL.X,$y^{+}$/yw) and could not be distinguished from their phenotypes. It was possible therefore that there was no true NDJ in *vas* mutants, i.e. there were no or almost no XXY progeny instead of recombinant. XO progeny may come from chromosome loss (e.g. from anaphase I and anaphase II chromatin bridges) rathter than NDJ. For similar reason, the ratios of the XY:O bias in *vas* and *solo* double mutants were not accurate either. Nevertheless, our data showed X/Y recovery bias among regular progeny, 1.46 on average (9032/6181). Similar observation was made in the progeny of homeless, another DEAD/DEAH box RNA helicase (Stapleton et al., 2001). This bias is not observed in *solo* and *snm* mutants, which are required for sister chromatid cohesion and homolog pairing, respectively, suggesting that *vas* has a distinct functions from *solo* and *snm* in meiosis, and sister chromatid cohesion and homolog pairing are not involved in the bias.

**The PEV males are sterile due to loss of fertility factors on Y chromosomes**

The $y^+$ variegation of $B^+$ $y^{var}$ male progeny seemed likely to be due to heterochromatic position-effect since the $y^+$ gene on the $B^S Y y^+$ chromosome is adjacent to heterochromatin. Considering the fact that the reduced heterochromatic content of the genome would enhance PEV (Gowen and Gay 1934), we speculated the Y chromosome in the exceptional $B^+$ $y^{var}$ and B y males may not be intact due to chromosome loss or recombination. In order to test this idea, we first tested the fertility of these males. They were crossed to y w females. Corresponding to our speculation, more than 50 tested $B^+$ $y^{var}$ males (primarily from the crosses of three different *vas* alleles, $vas^3$, $vas^5$, $vas^{RG53}$) were all sterile, suggesting one or more fertility factors were lost along with $B^S$ marker. This also strengthened our explanation for PEV of these sterile males because the heterochromatin content of their Y chromosomes was reduced. In the fertility test for B y males, which were also crossed to y w females, 16 out of a total of 18 flies were sterile while the other two were fertile. Careful examination revealed that fertile B y males had extremely small Bar eyes ($B^{ext}$ y).

To further test the idea that PEV and sterility of $B^+$ $y^{var}$ males resulted from the partial loss of the Y chromosome, *+/$B^S Y y^+$; vas/Df* were crossed to *YSX.YL, In(1)EN, y B/y w* females. The Bar eyes are larger in females with B marker than those in males with $B^S$ marker. When B and $B^S$ are combined, the bar eyes are even smaller. In total, 23 males with B $w^+$ eyes (from three crosses: $vas^3$, $vas^5$, $vas^{RG53}$), representing the males with *YSX.YL, In(1)EN, y B*/$Y_{ab}^{B+\ y+}$ were selected and crossed to *y w* females. All of these males exhibited normal,

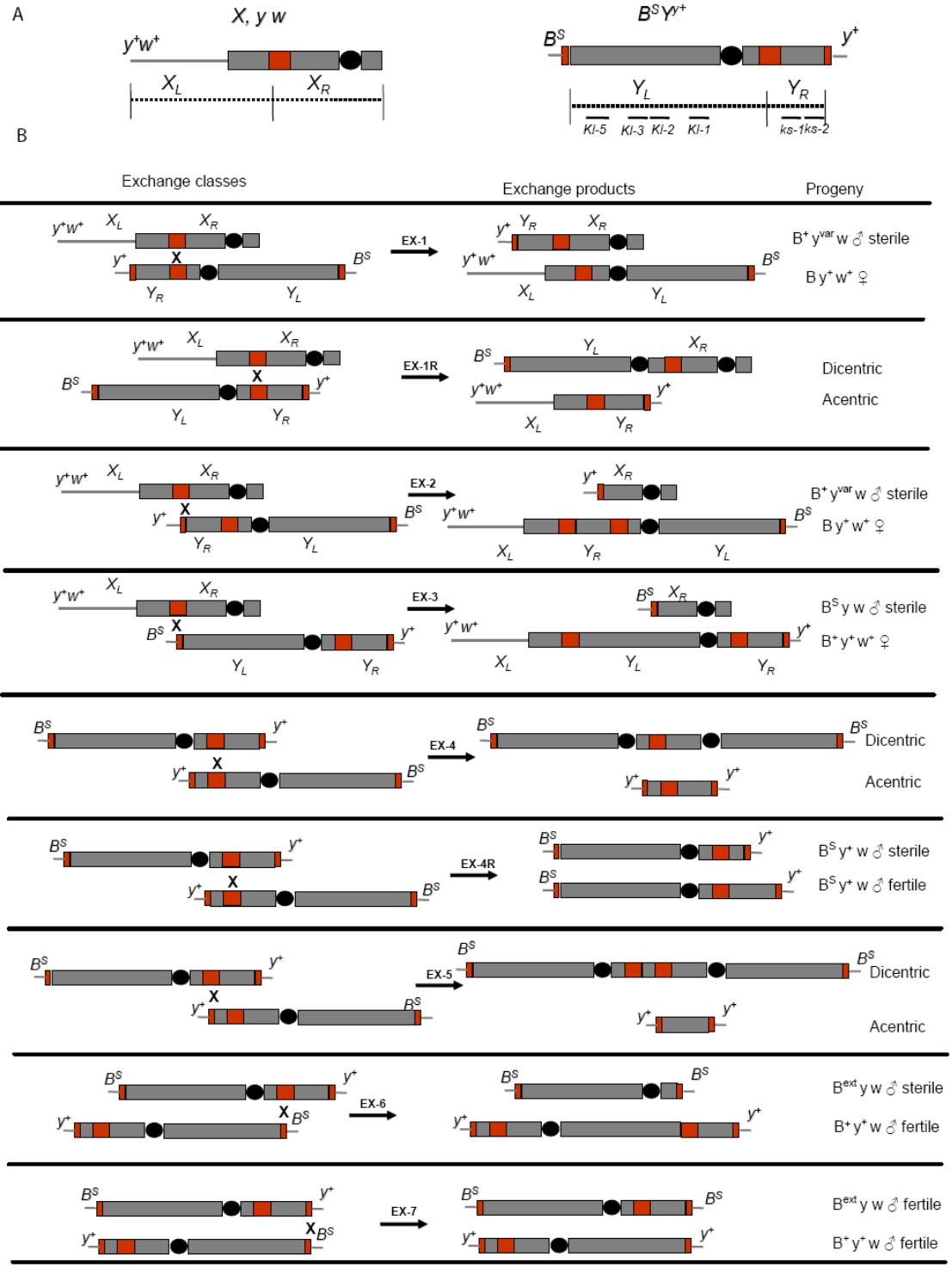
unvariegated expression of the y+ marker on the Y chromosome. The crosses yielded $B^+$ $y^{var}$ males, suggesting that their sex chromosomes were $Y_{ab}^{B+\ y+}$ chromosome and normal *y w* X chromosome. This suggested the PEV and sterility were suppressed by an additional Y chromosome. All the $B^+$ $y^{var}$ males were crossed to *y w* females again and proved to be sterile, further confirming that PEV and sterility resulted from incomplete Y chromosome.

**The possible exchange patterns between X and Y chromosomes**

The uniform loss of fertility and dominant marker of the $B^+$ $y^{var}$ male progeny indicates that $B^+$ $y^{var}$ and B y male progeny may be primarily arisen from X-Y chromosome exchange, rather than random Y chromosome breakage. Four rDNA blocks exist within the X and Y chromosomes used in the crosses (Fig. 4-2A) and several kinds of exchanges may occur among them. The X chromosome used in the crosses is normal in structure carrying y and w recessive mutations. For simplicity of discussion, we refer to the region between the very short arm of X chromosome and the rDNA region as $X_R$, and the region distal to rDNA locus as $X_L$. The $B^S Yy^+$ chromosome is a normal Y chromosome except that it is appended with $B^S$ and $y^+$ markers that were derived from X chromosome on both the long and short arm, respectively. Besides the two markers, two additional sex chromosome heterochromatin blocks that contain rDNA arrays were on the long arm just proximal to $B^S$, and on the short arm just proximal to $y^+$ (Gatti and Pimpinelli 1983). For simplicity of discussion, we define the region of long arm containing fertility factors *kl1, kl2, kl3*, and *kl5*, centromere

Figure 4-2. The structure of X and Y chromosomes used in crosses and the predicted exchange patterns at rDNA loci.

(A) The structures of X and Y chromosomes in *+/B$^{S}$Yy$^{+}$; Df/vas* males. (B) Predicted exchange patterns for *+/B$^{S}$Yy$^{+}$; Df/vas* males. EX-1, 1R, 2, 3 are possible X-Y exchanges. EX-1R is an exchange between rDNA arrays in opposite orientation relative to their centromeres. The exchanges at rDNA loci in EX-2, 3 patterns may occur in opposite orientation relative to their centromeres, and result in dicentric and acentric exchange products, too, although they are omitted in the figure. EX4, 4R, 5, 6, 7 are the patterns of exchange between sister Y chromatids. EX-4R is an exchange between rDNA arrays in opposite orientation relative to their centromeres. The exchanges at rDNA loci in EX-5, 6, 7 patterns may occur in opposite orientation relative to their centromeres, and result in dicentric and acentric exchange products. These possible exchanges are omitted in the figure.


A
X, y w
y+w+
XL
XR
BS Y y+
BS
y+
YL
YR
Kl-5
Kl-3
Kl-2
Kl-1
ks-1
ks-2
B
Exchange classes
Exchange products
Progeny
EX-1
B+ yvar w ♂ sterile
B y+ w+ ♀
EX-1R
Dicentric
Acentric
EX-2
B+ yvar w ♂ sterile
B y+ w+ ♀
EX-3
BS y w ♂ sterile
B+ y+ w+ ♀
EX-4
Dicentric
Acentric
EX-4R
BS y+ w ♂ sterile
BS y+ w ♂ fertile
EX-5
Dicentric
Acentric
EX-6
Bext y w ♂ sterile
B+ y+ w ♂ fertile
EX-7
Bext y w ♂ fertile
B+ y+ w ♂ fertile

and part of the short arm heterochromatin proximal to the centromere as $Y_L$, and the region located distal to the rDNA arrays as $Y_R$.

We considered nine types of exchanges that could generate the exceptional progeny in the cross: three types of inter-chromosomal exchanges between X and Y chromosomes, one type of inter-chromosomal exchange in opposite orientation relative to their centromeres, four types of intra-chromosomal exchanges between two Y sister chromatids, and one type of intra-chromosomal exchange in opposite orientation relative to their centromeres (Fig. 4-2B). The three inter-chromosomal exchanges would generate $B^+$ $y^{var}$ and B y males that are sterile, and $B^+$ $y^+$ $w^+$ and B $y^+$ $w^+$ females that are fertile and cannot be distinguished from regular and NDJ female progeny, respectively, when they are fertilized with y w females. Sterile $B^+$ $y^{var}$ and B y males were recovered in the cross, demonstrating that exchanges between X and Y chromosomes at rDNA loci occurred as we predicted. The crosses yielded more $B^+$ $y^{var}$ males than B y males, probably due to more rDNA repeats were involved in type of EX-1 exchange than that of EX-3 type of exchange. The five intra-chromosomal exchanges would generate fertile $B^+$ $y^+$ w and $B^{ext}$ y w males. One fertile $B^+$ $y^+$ w and two $B^{ext}$ y w fertile males were recovered in the crosses of *+/$B^S$Y$y^+$; Df/vas* to *y w* females, suggesting that exchanges occur between sister chromatids.

To test for the generation of XL.YL $B^S$ chromosome in the crosses of *vas*, which would generate B $y^+$ $w^+$ female progeny and cannot be distinguished from XXY NDJ females, we crossed *+/$B^S$Y$y^+$; vas/Df* males to *C(1)RM, y/YS* females.

The C(1)RM females (an attached-X) produce the eggs with attached X and YS eggs at approximately equal frequency. As expected, the cross generated B $y^+$ sons, carrying the recombinant $y^+$ $w^+$ XL.YL $B^S$ chromosome. The B $y^+$ sons from the C(1)RM/YS cross were all fertile. The B $y^+$ sons from C(1)RM/$Y^S$ cross were expected to result from two sources: XY recombinant ($y^+$ $w^+$ XL.YL $B^S$/YS) or XY NDJ males (+/$B^S$Y$y^+$/YS). A recombinant male mated to y w females produces only two kinds of progeny: $B^+$ y w males and B $y^+$ $w^+$ females, i.e. all B flies are females. An XY NDJ male if mated to y w females produces four types of progeny: B $y^+$ w males, $B^+$ $y^+$ $w^+$ females, $B^+$ y w males, B $y^+$ $w^+$ females. In the tested 24 B $y^+$ males (from three crosses: *vas*$^3$, *vas*$^5$, *vas*$^{RG53}$), four were proved to from XY NDJ while 20 were proved to carry the XY chromosome recombinant ($y^+$ $w^+$ XL.YL $B^S$), and all Bar flies in the progeny were females. These data confirmed our prediction about the structure of X-Y chromosome recombinant and that the sterility of the recombinant males could be rescued by an additional $Y^S$ chromosome.

We also analyzed the distribution of exchanges among singly crossed males. In the 127 *vas/Df* males producing $B^+$ $y^{var}$ males, 1 male generated 5 $B^+$ $y^{var}$ males, which is almost certainly a result of a single mitotic exchange. 12 out of 127 *vas/Df* males produced three $B^+$ $y^{var}$ males in the progeny. 24 out of 127 *vas/Df* males produced 2 $B^+$ $y^{var}$ males in the progeny, which were probably from meiotic recombination. 90 out of 127 (71%) males produced only one $B^+$ $y^{var}$ progeny, which were almost certainly from meiotic recombination. These data suggest most of the recombinants were generated in meiotic stages.

**Cytological analysis of anaphase I and II of vas mutants**

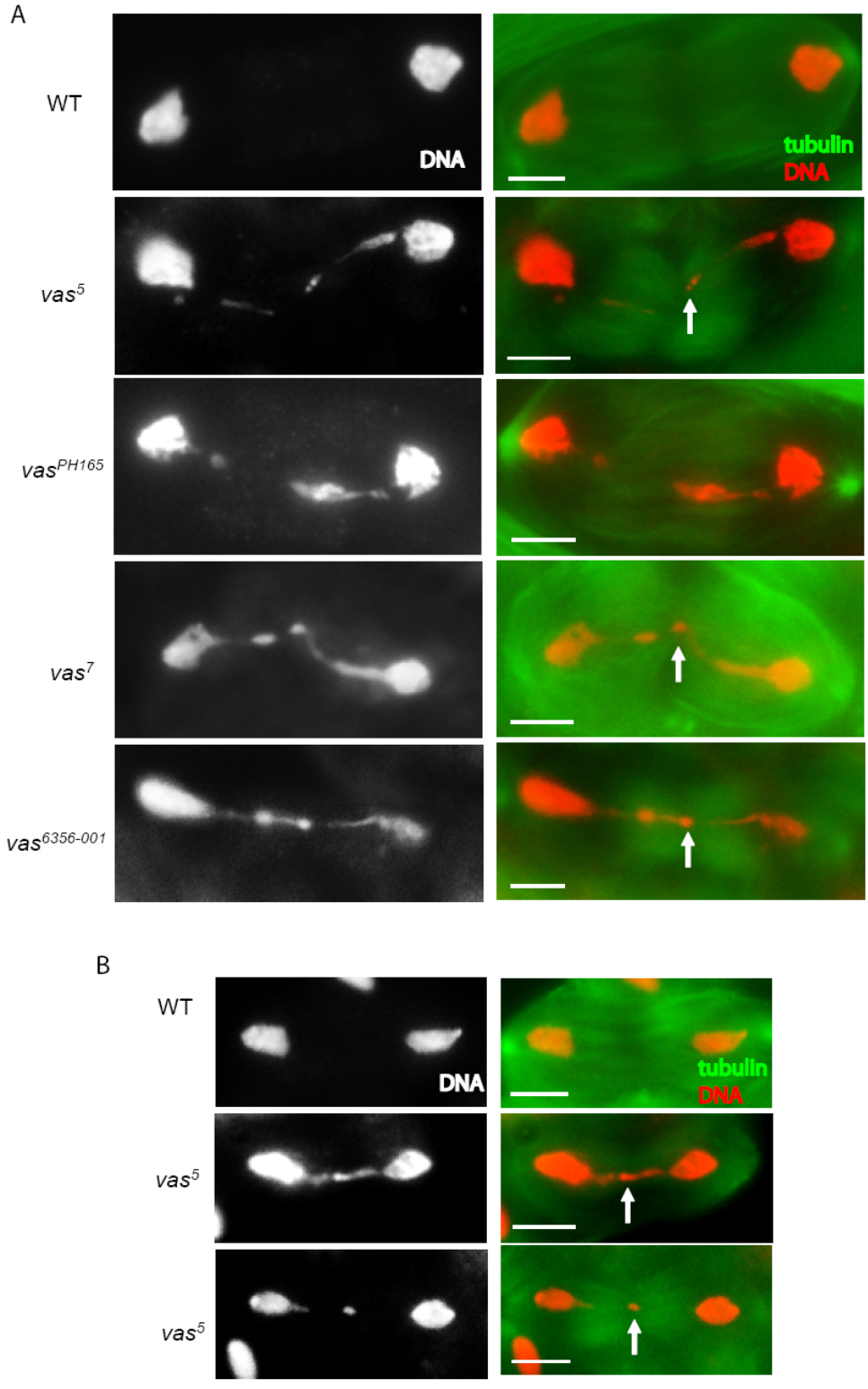
The multiple phenotypes of *vas* mutations could be manifested at cytological level. We didn't try to identify the chromosome recombinant, like XL.YL $B^S$, since they were technically not easy to be detected at cytological level. Instead we explored chromatin bridges that could be generated when dicentric recombination occur between homologs and between sister chromatids (Fig. 4-3). Spermatocytes from *vas/Df* were stained to visualize DNA and spindle. As we predicted, long, thin chromatin bridges were seen at anaphase I in all tested *vas* alleles (Fig. 4-3A), suggesting dicentric recombination occur between homologs during meiosis. Some discontinuous-like chromatin bridges were also observed. They might be connected but were not detectable at some spots. Short chromatin bridges were observed at anaphase II in tested *vas* alleles (Fig. 4-3B), suggesting that exchanges between sister chromatids occurred. Although at this point we do not know what the bridges represent and how they related to other phenotypes of *vas*, we prefer the idea they are dicentric chromosomes that result from X-Y recombination. Chromatin clumps were also observed in the midbody of spindle of anaphase I and II (Fig. 4-3), suggesting that acentric recombination products also occurred. Thus our cytological data confirmed that meiotic recombination occur when *vas* is mutated.

***vas* and *solo* double mutations cause precocious segregation of homologs.**

Interestingly, $vas^7$, a *vas* and *solo* double mutant, showed precocious segregation of homologs. Eight DNA clumps were apparently seen in $vas^7$

Figure 4-3. *vas* spermatocytes exhibit chromatin bridges and chromatin mass at anaphase I and II.

Testes from wild type (WT) and mutants stained with anti-α-tubulin to visualize spindles and with DAPI to visualize DNA. Arrows represent chromatin mass in midbody of anaphase spindles. The chromatin mass in midbody in vasPH165 anaphase I spindle is not visible probably because the chromatin mass is too small to be detected. Scale bar: 5 μm.


A
WT
DNA
tubulin
DNA
vas5
vasPH165
vas7
vas6356-001
B
WT
DNA
tubulin
DNA
vas5
vas5

metaphase I spermatocytes while in wild type metaphase I spermatocytes sex chromosomes and three autosomes align at metaphase plate showing one big DNA clump (Fig. 4-4). The univalents must be due to either *vas* alone or interactions between *vas* and *solo* since *solo* mutations only cause precocious loss of cohesion at centromeres but do not affect the conjunction of homologs.

We wondered that the conjunction between homologous chromosomes could be defective. SNM and MNM proteins are required for stable bivalent formation in male meiosis and co-localize to a prominent dense focus on the X-Y bivalent in both wild-type (Thomas et al., 2005) and *solo* spermatocytes. We tested localization patterns of a fully functional MNM-GFP fusion protein in $vas^{6356\text{-}001}$ mutants, which was molecularly identified as a nonsense mutant for both *vas* and *solo* (Tinker et al., 1998). MNM-GFP showed the same localization pattern in both $vas^{6356\text{-}001}$ and wild type spermatocytes: MNM-GFP foci cluster at nucleolus during late prophase I and form bright foci at prometaphase I and metaphase I (Fig. 4-5). This result suggested that conjunction function is normal in $vas^{6356\text{-}001}$. We do not know at present whether normal localization can be applied to $vas^{7}$ since allele-specificity may exist. We have sequenced all the exons of *vas* and *solo*, and the first big intron (about 3.3 kb) of *vas* and found no mutation in $vas^{7}$ alleles. We also do not know whether normal localization of MNM-GFP may only occur in the normal-looking spermatocytes since the normal localization of MNM-GFP happened in normal-looking spermatocytes. It will be intriguing to know the localization of MNM-GFP in the exceptional spermatocytes.

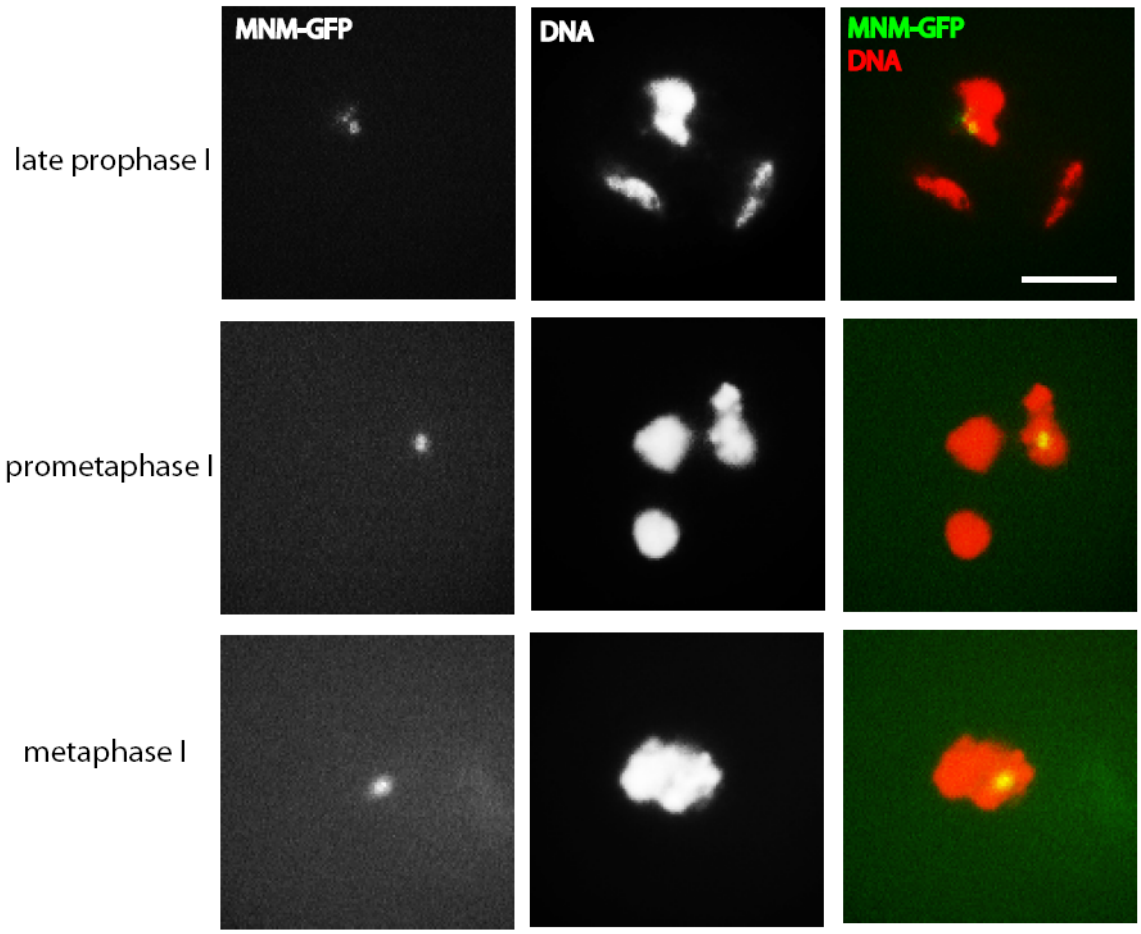



Figure 4-4. Localization of MNM in *vas*$^{6356-001}$ spermatocytes.

MNM-GFP signals were detected at FITC channel and DNA was stained with DAPI. MNM-GFP formed foci cluster during late prophase I and bright foci at prometaphase I and metaphase I. Size bar: 5 μm.

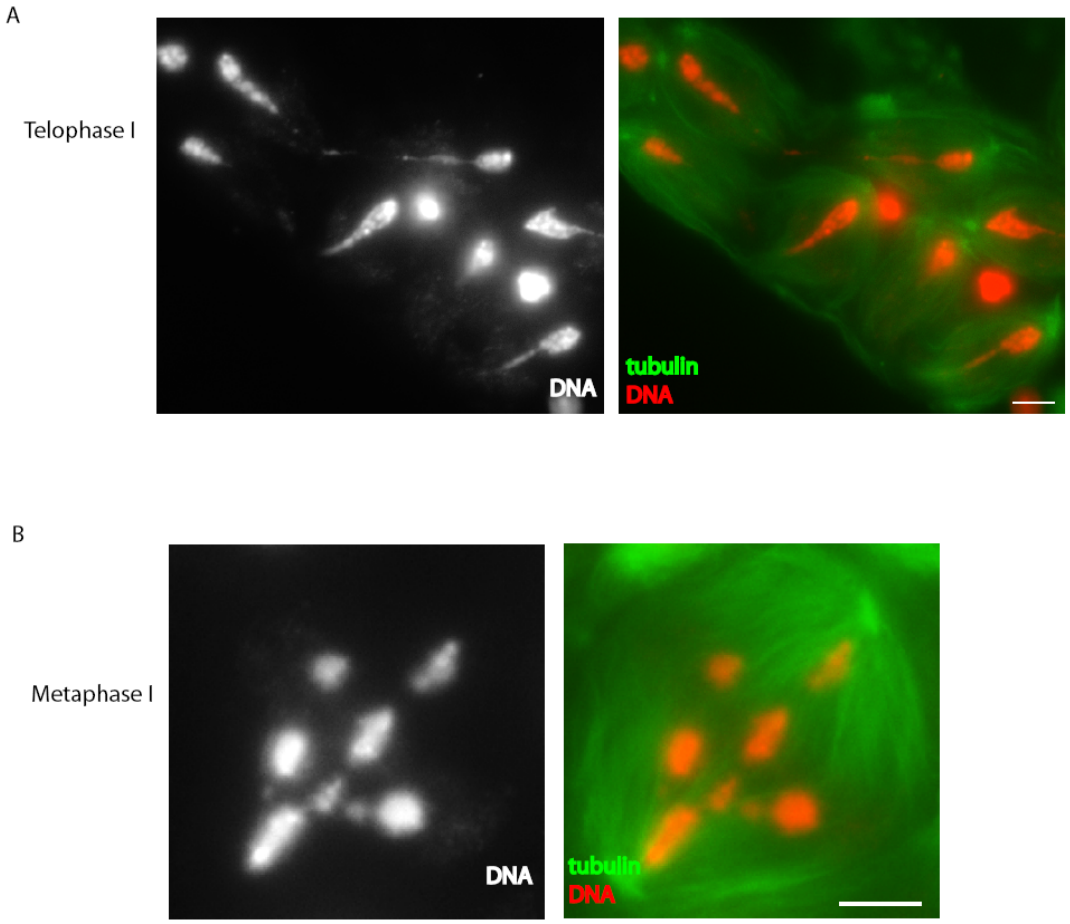



Figure 4-5. Cytological phenotypes of *vas*$^{7}$ spermatocytes.

Testes from *vas*$^{7}$ mutant were stained with anti-α-tubulin to visualize spindles and with DAPI to visualize DNA. *vas*$^{7}$ spermatocytes show chromatin bridges at Telophase I (A) and conjunction failure at metaphase I (B). Scale bar: 5 μm.

# DISCUSSION

## Meiotic recombination in Drosophila males

Recombination is normally absent during male meiosis although meiotic recombination in Drosophila females is a prerequisite for the successful progression of oogenesis (McKee, 2004). However, very little is known how Drosophila spermatocytes respond to endogenous and exogenously-induced DSBs and prevent recombination between homologous sequences during meiosis. We demonstrated here that in a specific case Drosophila males undergo meiosis with meiotic recombination. Our data indicate that *vas* mutations cause high frequencies of recombination between sex chromosomes compared to the very low frequency of spontaneous recombination in normal condition (Ashburner et al., 2004). Meiotic recombination may occur only between sex chromosomes since there is no recombination between *rucuca* (*ru h st th cu sr e ca*) and a wild type 3$^{rd}$ chromosome in *vas*$^{5}$*/Df* or *vas*$^{D5}$*/Df* (B.D. McKee, personal communication). Our cytological analysis further confirmed the existence of dicentric and acentric recombinant products by revealing chromatin bridges at anaphase I and II and the presence of chromatin clumps at midbody of anaphase I and II. The fact that the majority of recombinants are recovered singly rather than on clusters suggests that recombination occurs mainly during meiosis. Mei-W68 appears in young spermatocytes (McKim and Hayashi-Hagihara, 1998), suggesting meiotic recombination may occur in these stages. However, the mechanism of meiotic recombination is unclear at present.

Although it is not known whether recombination occurs at other repetitive sequence besides rDNA arrays (it doesn't occur in 3rd chromosome heterochromatin (McKee, personal communication)) and how *vas*-induced recombination is related to homolog pairing and segregation during meiosis I, our studies strongly suggest that meiotic recombination does occur when *vas* is mutated. Thus our data suggest that *vas* may suppress the occurrence of meiotic recombination during male meiosis. If this is true, it would provide a clue to why Drosophila males undergo meiosis without recombination.

**_vas_ shows pleiotropic functions in Drosophila male meiosis**

*vas* has been shown to be required for pole cell development and embryonic axis specification in oogenesis and embryogenesis (Hay et al., 1988; Lasko and Ashburner, 1988). No phenotypes were reported in spermatogenesis although vas is expressed in spermatogonia and young spermatocytes. Our studies definitely show *vas* functions in suppressing recombination and chromosome segregation in Drosophila male meiosis. In all tested *vas* alleles *vas* mutations cause high frequency of meiotic exchange between X and Y chromosomes. *vas* mutations also cause NDJ of sex chromosome although the frequency is low but it is significantly higher than that of wild type. Since in Drosophila males sex chromosomes pair at rDNA loci and meiotic recombination between X and Y chromosome may lead to increase or decrease of rDNA copy number, the fidelity of pairing of X and Y chromosome at rDNA may be damaged. This would lead to NDJ of sex chromosomes in *vas* mutants. Furthermore, $vas^{7}$,

an allele of *vas* and *solo* double mutant, causes precocious segregation of homologous chromosomes, a phenomenon that does not happen in single *solo* mutants. Although *vas* mutations show pleiotropic phenotypes, *vas* may play indirect roles in these phenotypes since *vas* is a translation initiation factor and localized to nucleoplasm but not to chromatin.

**Conjunction failure in *vas* and *solo* double mutant?**

Homologous chromosomes are linked by a mechanism involving SNM and MNM during male meiosis (Thomas et al., 2005). $vas^{7}$, which is a double mutant for *vas* and *solo* through genetic test but the mutation site are not identified, revealed conjunction failure at metaphase I. Eight chromatin clumps that apparently represent univalents were seen. We have not found any evidence that *solo* single mutants cause conjunction failure, so the conjunction failure in $vas^{7}$ must result from *vas* alone or the interaction between *vas* and *solo*. Interesting, *solo* and *snm* double mutants cause precocious segregation of homolog and sister chromatids, leading to the appearance of up to 16 sister chromatids in prometaphase I. How the sister chromatids were kept together while homologs were separated in $vas^{7}$ mutant is not known. Another *vas* allele $vas^{6356\text{-}001}$ that has been molecularly identified as a null mutant of *solo* and *vas* showed chromatin bridges at anaphase I. The failure of homolog conjunction was not observed and MNM location appeared to be normal in this mutant. Thus it is possible that SNM or MNM still hold sister chromatids (univalents) but the linkage between two univalents is disrupted. Further experiments need to be

done to learn whether SNM or MNM still exist in the separated univalents, which would provide strong evidence of whether SNM and MNM hold two sister chromatids in a univalent at pairing sites (e. g. rDNA in sex chromosomes) other than centromeres that is held together by classical cohesion complex. However, we cannot rule out the failure of homolog conjunction due to mutation complexity of *vas*$^{7}$ allele. Nevertheless, our finding suggests that besides SNM and MNM, other mechanisms exist to control homolog segregation during male meiosis.

# CHAPTER 5 – GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

# GENERAL CONCLUSIONS

The main goal of this study is to explore the mechanism of meiosis in Drosophila melanogaster. Plenty of studies have been carried out in yeast to study cohesion's function in meiosis and revealed many important principles of meiosis (Nasmyth, 2001; Petronczki et al., 2003; Marston and Amon, 2004). However, the mechanism in higher eukaryotes still remains elusive to large extent and some observations that are controversial to yeast emerged (Prieto et al., 2002; Eijpe et al, 2003; Valdeolmillos et al., 2007). Our studies have identified a novel cohesin protein, SOLO, which is essential for all aspects of sister chromatid cohesion in Drosophila meiosis. It is required for homologous recombination, homolog synapsis, maintenance of chiasmata in female meiosis. It is essential for maintaining centromeric cohesion in meiosis I and establishing orientation of sister centromeres in meiosis I and II spindles.

**SOLO is essential for homologous recombination, homolog synapsis, and maintenance of chiasmata.**

Sister chromatid cohesin is required for homologous chromosome recombination and maintaining the resulting chiasmata, which are required for proper homolog segregation during meiosis I (Petronczki et al., 2003). In Drosophila females, ORD, a protein required for maintaining cohesion, is involved in homolog recombination and maintaining chiasmata (Bickel et al.,

2002; Webber et al., 2004). BubR1, a checkpoint protein in mitosis, is required for synapsis (Malmanche et al., 2007). SOLO is a novel sister chromatid cohesion protein. *solo* mutations cause reduced frequency of homologous recombination and altered distribution of crossovers. Synapsis is severely disrupted at the early prophase I when *solo* is mutated. In *solo* females, residual chiasmata cannot be maintained. Thus SOLO is essential for proper homolog dynamics during meiosis I.

**SOLO is essential for orientation of sister centromeres.**

In *solo* males, sister centromeres segregate precociously before prometaphase I. Correspondingly, the cohesin containing SMC1 is lost at centromeres at the earliest meiotic stage. Genetic analyses show that homologs disjoin together in male and female meiosis. However, complete separation of sister chromatids is not observed. These data suggest that precocious separation of sister chromatids does not occur before meiosis I although sister centromere co-orientation is disrupted and sister centromeres orient randomly in meiosis I when *solo* is mutated.

**SOLO and SMC1 function together in Drosophila meiosis.**

Since the Drosophila genome contains only one copy of the SMC1 and SMC3 genes, one can imagine that SMC1 must be one of the components if a meiotic cohesin exists. Our data show centromeric foci of SMC1 appear at the

earliest meiotic stage and throughout meiosis I and until anaphase II when centromeric cohesion is degraded to allow sister chromatids segregate, suggesting that a cohesin containing SMC1 does exist in Drosophila meiosis. SOLO appears from the earliest meiotic stage to metaphase II and then disappears at anaphase II, and colocalizes with SMC1 throughout. *ord*, a protein required for sister chromatid cohesion, appears to bind chromatin at mid prophase I and localize to centromeres from prometaphase I to anaphase II (Balicky et al., 2002). Thus, SOLO becomes the first cohesion protein that shows the exactly same localization pattern with SMC1, strongly indicating that SOLO and SMC1 work together as partners. Moreover, the evidence that *solo* mutations cause disappearance of SMC1 at centromeres from the earliest meiotic stage further support the idea that SOLO and SMC1 are the components of a cohesin in Drosophila, albeit it may be non-canonical.

**Meiotic recombination is not suppressed in *vas* mutants.**

Drosophila males undergo meiosis without recombination. This phenomenon was observed long time ago (Morgan, 1912). By contrast, Drosophila females undergo meiosis with high frequency of recombination. Why there is no recombination in male meiosis is not known. Our findings demonstrate that meiotic recombination occur in the case of *vas* mutations. *vas* mutations cause about 300 fold X-Y exchanges compared to the frequency of spontaneous X-Y exchange (Ashburner et al., 2004). Cytological analyses

reveal chromatin bridges that are the products of dicentric exchange at anaphase I and anaphase II. Although we don't know the mechanism at present, our observations reveal that meiotic recombination in Drosophila males is inhibited by VASA.

# FUTURE DIRECTIONS

Our studies have been focusing on the mechanism of Drosophila meiosis. Our finding of the novel cohesion protein SOLO and the novel functions of VASA in meiosis would enrich knowledge of meiosis.

The studies about mutant phenotypes and localization pattern of SOLO provide substantial support to the idea that SOLO is a component of cohesin containing SMC1 in Drosophila meiosis. However, SOLO is not conserved outside of Drosophila species and does not show high sequence similarity with known cohesion proteins by current bioinformatics methods. To prove that SOLO is a cohesin component, the most straightforward way to determine whether SOLO interact with SMC1 or other cohesin component *in vitro* and *in vivo* at molecular level. The current Venus-SOLO transgenic flies can be used to perform co-immunoprecipitation experiments using antibodies against GFP, which is successful in yeast (Cristea et al., 2005). If GFP pull-down method is not applicable in Drosophila, classical pull-down using anti-HA or Flag can be used, which were successfully applied in Drosophila (Heidmann et al., 2004). Our Venus-SOLO is prepared by Gateway technology and is very easy to change the Venus tag to HA or Flag. After pull-down experiments, western blot and MALDI mass spectrometry can be applied to identify the potential proteins that interact with SOLO.

Another very interesting issue is to learn whether expression of SOLO

during pre-meiotic S phase is required for establishment of proper cohesion in meiosis. In yeast cohesin must be present at chromatin region during pre-meiotic S phase to establish functional cohesion in meiosis (Watanabe et al., 2001). SOLO is expressed in the spermatogonia in males and oogonia in females to form foci at centromeres and thus is present during pre-meiotic stage. *solo* and *vas* share the 5' UTR and the first three exons, suggesting they may share the same promoter. Both Nanos and VASA are expressed in embryogenesis, suggesting the SOLO expression pattern induced by nanos:Gal4 is probably true, which is supported by complete rescue of UAS:Venus-SOLO when induced by nanos:Gal4. The upstream of *vas* will be fused to Venus-SOLO to express SOLO by its native promoter. If the construct can rescue the phenotypes of *solo*, it indicates the cloned upstream contains the true promoter of *solo*. Then the construct will be used for study whether SOLO is expressed before or during pre-meiotic S phase. Using specific G2 (after meiotic S phase) promoter to forcibly express SOLO in *solo* background, whether SOLO expression before or during pre-meiotic S phase is required for establish proper cohesin can be determined.

It will be very intriguing to determine the interactions between SOLO and other cohesion proteins. As revealed by studies in other model organisms, meiotic cohesion is probably not mediated only by classical cohesin complex, other meiotic cohesion proteins, even some cohesion proteins that are first thought mitotic-specific were found to be involved in meiosis (Prieto et al., 2002; Eijpe et al, 2003). Although classical meiotic cohesin is not identified in

Drosophila until now, proteins required for sister chromatid cohesion have been identified, including ORD, SOLO, SUN (McKee, personal communication). Exploring interplays between SOLO and these meiotic cohesion proteins and some mitotic cohesion protein, like dRAD21, etc. will be helpful to understand the mechanism of meiotic cohesion in Drosophila.

Drosophila males undergo meiosis without recombination. *vas*, the alternative splicing products of *solo*, is surprisingly involved in the suppression of meiotic recombination. *vas* mutations cause high X-Y exchange during meiosis. Since the homology sequences in X and Y chromosomes are primarily rDNA repeats, it is important to further know whether recombinations occur at the rDNA repeated sequences although we have already narrowed the recombination region containing rDNA loci. This can be done by using bobbed X chromosome flies, or X heterochromatin deficiency flies with rDNA transgenes. They will yield less X-Y exchange by compared to that of the normal X chromosome. It is also important to know the origin of chromatin bridges in anaphase I. rDNA or even 240bp repeat probes will be the first candidate probes and then other tandem repeats will be tried if rDNA or 240bp repeats are not.

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# VITA

Rihui Yan was bron in Loudi City, Hunan Province, China in 1971. He attended Wuhan University in 1989 and graduated with a Bachelor of Science in 1993. He graduated with a Master of Sciences from Shanghai Institute of Plant Physiology, Chinese Academy of Sciences in 1997. He then worked in FosunPharma, a biotechnology company. He began his graduate study in 2002 at the University of Tennessee, Knoxville. He defended his dissertation in 2007.