4

Dynamics of Cellular Components in Meiotic and Premeiotic Divisions in *Drosophila* Males

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1. Introduction

Meiosis in higher organisms is programmed as a part of gametogenesis. It should be discussed separately from a yeast meiosis that initiates in response to extracellular nutrient conditions. In this chapter, we would like to introduce current knowledge including our new findings about dynamics of meiosis in *Drosophila* males. At first, we will introduce general views of cell divisions and cell growth during *Drosophila* spermatogenesis as we illustrated in Fig. 1A. At the tip of the testis in adult *Drosophila* males, several germline stem cells (GSCs) are surrounding next to a cluster of smaller hub cells. The GSCs receive a signal to maintain their multi-potential stem cell characteristics secreted from the adjoining hub cells. A secreted protein encoded by the *unpaired* gene acts as a ligand of the maintenance signal (Kiger, *et al*., 2001, Fuller & Spradling, 2007). The insulin-like peptides in hemolymph first induce the cell cycle progression of the male GSCs from G2 phase to M phase (Ueishi et al., 2009). Between two daughter cells derived from asymmetric division of the GSC, a proximal daughter cell exclusively receives the Unpaird signal and becomes a self-renewed GSC. The other distal daughter cell leaves the niche and begins to differentiate as a spermatogonium. The spermatogonium then undergoes four cell cycles and generates a 16 cell unit known as a cyst (as a review Fuller, 1993). In ever mitosis, all spermatogonia within a cyst undergo cell divisions synchronously. Cytokinesis in spermatogonia mitoses as well as in following meiotic divisions terminates incompletely. The cleavage furrow ingression is arrested at the middle of cytokinesis and then the contractile rings transform into cytoplasmic bridges called ring canals (Hime et al., 1996). These 16 spermatocytes synchronously enter a growth phase during which the cells remarkably increase in volume by up to 25 times. Following completion of the enormous cell growth, primary spermatocytes carry out two consecutive meiotic divisions. A cyst of 16 spermatocytes gives rise to 64 spermatids simultaneously as a consequence of meiotic divisions. Every spermatid in a cyst at onion stage contains equally sized nucleus and Nebenkern that is a single large aggregate derived from mitochondria. This should be achieved as a consequence of proper chromosome segregation and cytokinesis in germ line cells as well as equal partition of mitochondria (Castrillon et al., 1993, Ichihara et al., 2007).

Next, we describe that the meiotic cells perform dynamic alterations of their cellular components as meiosis progress. If one would like to examine alterations of intracellular structures such as cytoskeletons and organelles during cell division, *Drosophila* male meiotic

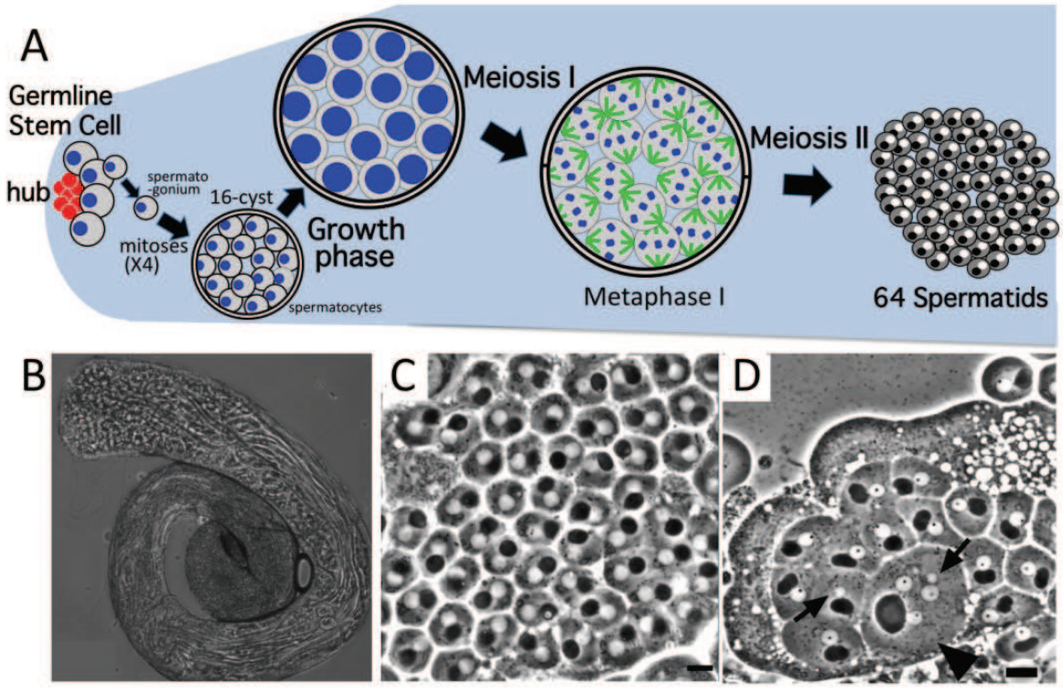

Fig. 1. A. Illustration of premeiotic cell division, cell growth of spermatocytes and meiotic divisions in testis. B. A micrograph of *Drosophila* testis. C, D. A phase contrast micrograph of spermatids at onion stage from wild-type (C) and *orbit*^{7} mutant males. Each arrow indicates a smaller nucleus derived from unequal chromosome segregation. Arrowhead shows a larger spermatid containing a large Nebenkern and four nuclei, suggesting a failure of cytokinesis in both meiotic divisions. Bars, 10 μm. Panel C and D were reprinted from Inoue et al., (2004)

cells have several advantages. In *Drosophila*, good cultured cell lines that proliferate well in a standard culture condition are also available (Rogers & Rogers, 2008). However, their cell size, particularly cytoplasmic volume, is much smaller than that of mammalian cells. This is a disadvantage in examination of cellular components during cell division. Spermatocytes, on the other hand, achieve distinct cell growth before initiation of first meiotic division. The primary spermatocyte is the largest diploid cells among proliferative cells in *Drosophila* whole body. Thus, one can easily perform detailed observation of cellular structures in dividing cells using optical microscopes. In *Drosophila melanogaster*, well-advanced and sophisticated genetic techniques are available. Meiotic defects in chromosome segregation and in cytokinesis appear in cellular organization of spermatids just after completion of 2nd meiotic division. By observation of such early spermatids, one can easily find out even subtle meiotic abnormalities. Many of genes essential for cytokinesis have been identified by this method, as we discuss later. Furthermore, if a loss of microtubule integrity or its dynamics would have occurred in normal cultured cells, their cell cycle progression should be arrested before metaphase. Therefore, it is hard to examine how microtubules would influence later processes of cell divisions in the somatic cells. As spermatocytes, on the other hand, are less sensitive to microtubule abnormalities at microtubule assembly checkpoint before metaphase. A colchicine treatment of spermatocytes causes a delay of meiotic cell cycle but it does not make a cell cycle arrest (Rebollo & Gonzalez, 2000). One can, therefore, examine a role of microtubule-related genes in cytokinesis without arresting cell cycle. This is another great advantage of male meiotic cells in cell division studies.

We and other groups have established systems to facilitate dynamics of chromosomes or microtubules by expression of proteins with GFP fluorescence tag (Clarkson & Saint, 1999, Inoue et al., 2004). We describe behavior of chromosomes or chromatids in meiotic divisions and summarize about distribution of homologous chromosomes after premeiotic DNA replication to prophase I. And further, we can also observe other cellular components such as microtubules, actin filaments, endoplasmic reticulum, Golgi apparatuses or mitochondria during male meiosis by a simultaneous expression of proteins with different fluorescence tags. Not only chromosomes but these cellular components also perform dynamic distribution and are equally partitioned in *Drosophila* meiosis. In addition to meiotic divisions, we will also describe here on premeiotic mitoses to generate meiotic spermatocytes. As those cell division, growth and differentiation seen in *Drosophila* spermatogenesis are well conserved among higher eukaryotes, we believe that readers studying on other organisms should also be interested in dynamics of meiotic and premeiotic divisions in *Drosophila* males.

2. Chromosome dynamics in *Drosophila* male meiosis

2.1 Characteristics of chromosome behavior in male meiosis I

Unlike female meiosis, male meiosis in *Drosophila melanogaster* is unique in the following aspects. No chiasmata formation indicating crossing over is observed in male meiosis. Another consistent observation that synaptonemal complex is not formed at prophase I were also reported (Rasmussen, 1973). Thus, meiotic recombination does not occur in *D. melanogaster* males. Even so, the synapsis formation between homologous chromosomes, its maintenance until metaphase I and bivalent disjunction at anaphase I takes a place properly. In past studies, primary spermatocytes dissected from testis were incubated in a culture condition and living

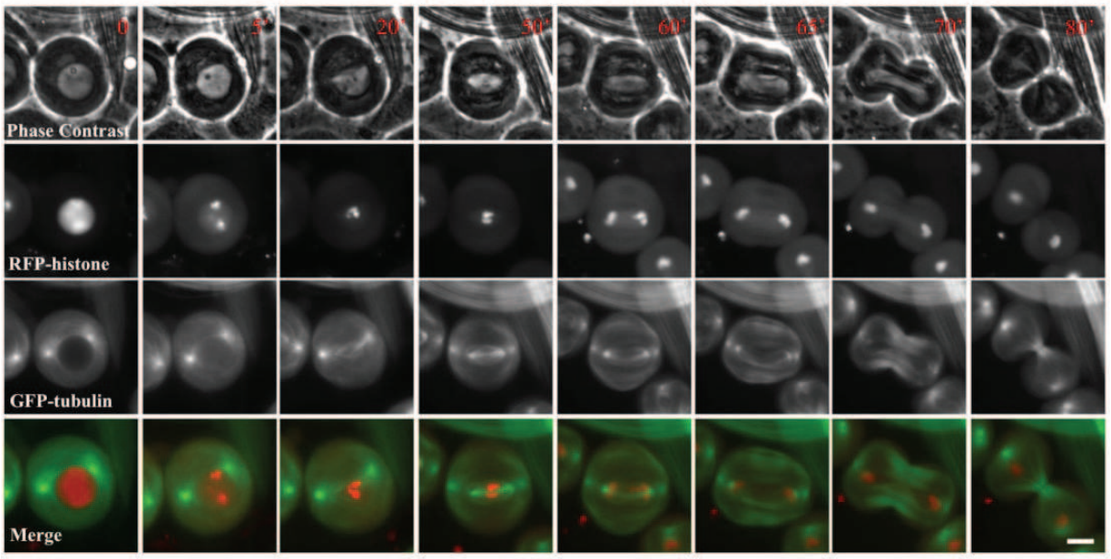

Fig. 2. Time-lapse observation of a living primary spermatocyte expressing GFP-βtubulin and mRFP-Histone2Av simultaneously. Selected fluorescence images of the primary spermatocyte showing chromosomes (red in bottom row, white in second row), microtubules (green in bottom, white in third) and the corresponding phase contrast images to observe cleavage furrow and ER-based cell organelles inside (top). Bar, 10 μm.

spermatocytes were observed under Phase contrast or Differential Interference Contrast (DIC) microscopy equipped video camera (Church & Lin, 1985). In order to trace chromosome behavior together with cell division apparatuses, we have induced simultaneous expression of histone 2Av and βtubulin fused with GFP and mRFP tag, respectively. Time-lapse observation of two kinds of fluorescence makes it possible to examine chromosome behavior such as condensation, congression and segregation as alteration of spindle microtubules (Fig.2).

2.1.1 Chromosome dynamics at meiotic prophase I

Vazquez and colleagues described the distribution of homologous chromosomes at premeiotic stage in primary spermatocytes (Vazquez, et al., 2002). They had established fly stocks in which *LacO* sequences from *E. coli* have been inserted into specific sites of *Drosophila* chromosomes. They then induced expression of GFP-LacI repressors to visualize a distribution of two homologous chromosomes within a nucleus. In spermatocytes at G1 phase just after a completion of 16-cell cyst, homologous chromosomes originally distribute closely each other. This is a characteristic known as a somatic pairing commonly observed in insect cells. This distribution of homologous chromosomes is maintained until meiotic prophase through premeiotic DNA replication. What is a molecular mechanism to fulfill the stable bivalent formation in male meiosis? It has been shown that the homologous chromosomes form a synapsis using specific chromosome regions so-called pairing sites. Church and Lin (1985) proposed that the pairing and separation of sex chromosome X-Y bivalents is controlled by a different mechanism from that of autosome bivalents. It was reported that X-Y pairing depends on a specific pairing site on heterochromatin region of sex chromosomes (Cooper, 1964, McKee and Karpen, 1990), while regions required for autosome pairing distribute across the euchromatin region (Ashburner et al., 2004). The X-Y pairing site seems to correspond to the rDNA gene localized in heterochromatin on X and Y chromosomes. The intergenic spacer regions of rDNA gene clusters play a role in X-Y pairing independent on nucleolus formation (McKee et al., 1992). On the other hand, there seems to be no pairing sites in heterochromatin regions of autosomes (Yamamoto, 1979, Ashburner et al., 2004). The autosome bivalents can be paired along entire chromosome regions. Trans-acting factors required for proper execution of meiotic chromosome pairing has also been identified. It was recently reported that a loss of a chromosomal passenger protein, Australin results in defects in chromosome segregation in *Drosophila* male meiosis (Gao et al., 2008). As the Australin is exclusively required for male meiosis, these genetic data is consistent with evidences that chromosome segregation in male meiotic divisions is regulated by different mechanisms from that in females. And further, mutations of *tef* gene encoding a chromatin binding protein with Zn finger disrupted autosomal pairing without affecting X-Y pairing and segregation in male meiosis I (Tomkiel et al., 2001). Common factors essential for the pairing of all chromosomes have been also identified. The *mod(mdg4)* encoding a chromosome binding protein required for segregation of all chromosomes in male meiosis (Soltani-Bejnood et al., 2007). These genetic data suggest that some of molecular mechanisms in meiotic chromosome segregation are different from each other in females and males but others are common in both sexes.

2.1.2 Chromosome movement in prometaphase I to telophase I

Church and Lin (1985) examined chromosome movement in meiosis I by time-lapse video microscopy. They observed unpredictable movement and frequent reorientation at

prometaphase-like stage before the bivalents achieve a stable bipolar orientation. They described that *Drosophila* primary spermatocyte does not form a well-defined metaphase plate. We have observed four foci of GFP-Histone 2Av corresponding to bivalents between two major autosomes, X-Y chromosomes and tiny 4th chromosomes in living primary spermatocytes at prometaphase I (Fig. 2). *Drosophila melanogaster* cells contain 3 major chromosomes and one small chromosome 4. Bivalents of smaller chromosomes are not usually observed because of overlapping with major chromosomes. Those three foci appear to congress into a single chromosome mass at the middle of the primary spermatocytes. Savoian and colleagues found that each chromosome carried out one or more rapid poleward movement with an average velocity of 11.2 ± 1.2 μm/min until bipolar kinetochore attachment (Savoian et. al., 2000, Savoian et al., 2004). However, it is not necessary for anaphase initiation to make all bivalents align at the cell equator. It was also revealed that state of microtubule assembly is surveyed at the M-phase checkpoint, although the checkpoint at male meiosis is less strict than that in somatic cells (Rebollo & Gonzalez, 2000). Past studies reported that chromosome movement at Anaphase I was highly irregular in velocity (Church and Lin, 1985). Savoian and colleagues described that Anaphase I takes 8 ± 1 min and that the chromosomes moved polewards at 1.9 ± 0.1 μm/min after dyad disjoining.

3. Dynamics of cytoskeletons and cell organelles as a progression of male meiosis

3.1 Dynamics of cytoskeletons in spermatocytes

It is well known that cytoskeletons perform dramatic changes in their structures during cell divisions. In *Drosophila* germline cysts, the most characteristic future concerning cytoskeletons in spermatocytes is a presence of fusome that is a germline specific cell organelle (Hime et al., 1996). This is the F-actin based branching structure containing alpha-Spectrin, ß-Spectrin, adducin-like protein (Hu-li tai shao) and Ankyrin. The fusome traverses cytoplasmic bridges to connect clonally related spermatogonia and premeiotic spermatocytes within a cyst. Male germline stem cells and early spermatogonia contain a spherical type of the fusome, called a spectrosome (Yamashita et al., 2003, Wilson, 2005). During the four rounds mitoses of spermatogonia, the fusome forms one large branched structure that extends though the ring canals into all the cells within a cyst. Before meiotic division I, the characteristic branched structure of fusome has disappeared and its fragmented remnants appear during meiotic divisions. And then similar branched structures devoid of F-actin appear to penetrate post-meiotic ring canals (Hime et al., 1996). It is possible to speculate that these fusome structures play a role in connecting individual spermatogonia with each other and in determination of their division axis as we discuss later.

3.1.1 Actin cytoskeletons

At late anaphase, a contractile ring consisting of F-actin and myosin II is constructed on the middle of cell cortex in spermatocytes. Myosin II moves along the F-actin filaments by using the free energy of ATP hydrolysis. Shrinking of the ring constricts the cell membrane to form a cleavage furrow (Egger et al., 2006). Cytokinesis in spermatogonia mitoses as well as in meiotic divisions terminates incompletely. The cleavage furrow ingression is arrested at the middle of cytokinesis and then the contractile rings transform into cytoplasmic bridges called ring canals.

F-actin or Myosin II is no longer found in the ring canals of spermatocytes but instead large amount of phospho-tyrosin, anillin and septins are contained in the bridge architecture (Hime et al., 19996). It has been revealed that the position of contractile ring formation is determined by central spindles (as a review, Goldberg et al., 1998). The bundle of interdigitated microtubules emanating from both spindle poles formed between the separating homologous chromosomes (Inoue et al., 2004). Interestingly, the actin-based contractile ring shows a cooperative interaction with the central spindle microtubules. Both mutations of *chic* gene encoding a profillin homolog essential for actin polymerization and treatment of testis cells with cytochalasin B showed a similar cytokinesis phenotype. In both cases, not only a formation of the actin-based contractile ring but the central spindle microtubules also failed to be constructed (Giansanti et al., 1998). On the contrary, for example, mutations for *orbit* gene encoding a microtubule-associated protein caused a sever disruption of central spindle microtubules and they also resulted in a failure of cytokinesis devoid of the F-actin rings (Inoue et al., 2004). These results strongly suggest that the central spindle microtubules and the contractile ring consisting actomyosin are interdependent structures, at least during cytokinesis in male meiosis. A molecular mechanism to link these intracellular structures remains to be uncovered. It should be necessary to identify a key molecule(s) to interact directly with both the central spindle microtubules and components of the contractile ring.

3.1.2 Microtubule structures

Drosophila primary spermatocytes possess characteristic microtubule structures for a preparation of meiotic divisions. Particularly, well-developed astral microtubules become to be prominent as initiation of meiosis I in primary spermatocytes. The astral microtubules are easy to recognize from spindle microtubules that are formed in the nuclear space surrounded by multiple membrane layers known as parafusorial membranes (Tates, 1971, Fuller, 2004, Bonaccorsi et al., 2000). To visualize astral and spindle microtubules and to examine the dynamics of microtubule behaviour in living primary spermatocytes, Inoue and colleagues used a transgenic line ubiquitously expressing GFP-tagged β-tubulin (Inoue et al., 2004). They performed simultaneous observation of multiple cellular structures by DIC microscopy as well as fluorescence microscopy. We presented here several selected figures of a primary spermatocyte undergoing meiosis I simultaneously expressing mRFP-histone 2Av and GFP-βtubulin (Fig. 2). As chromatin inside of nucleus has unevenly distributed at late prophase (t=0 minute), microtubules around both spindle poles become to be prominent. At prometaphase I, four condensed bivalent chromosomes would be observed within a nucleus in which nuclear membrane seems to be intact (t=5, under phase contrast microscope). Only two dyads seem to be in focus in Fig. 2. Developing asters have moved around nuclear membrane as to reach at opposite poles. Then, at 20 minutes later, all chromosome complements congress at the centre of the bipolar spindle structure. At this stage, nuclear morphology has already disintegrated and spindle microtubules free to elongate into inside of nuclear space. The kinetochore microtubules seem to capture the chromosomes and put them in the centre. At onset of anaphase I (t=50), A multilayer of nuclear membrane see as phase-dark structure surrounding around nuclear space separate spindle microtubules including thick kinetochore microtubules from well developed astral microtubules nucleated around each spindle pole. Two populations of central spindle microtubules appear after disjunction of bivalents (t=60 to 70, Fig. 1). A peripheral set of the microtubules elongating from spindle pole regions become more dynamic as if they look for

the cytoplasm towards the cell equator (Inoue et al., 2004). Another set of the microtubule bundles corresponding spindle microtubules is localised interiorly at the middle of the cell. The peripheral microtubules from opposite poles met together at equator and form bubble-like structures protruding outwards (t=60). The interior and most of the peripheral central spindles then are released from each pole and they formed independent bundles at the equator (Inoue et al., 2004, Savoian et al., 2004). Furrow ingression was observed soon after the peripheral microtubules from both poles contacted the cell cortex. Thus, we speculate that interaction between the peripheral microtubules and the cell cortex plays a role in determination where and when cleavage furrow ingression initiate in male meiosis.

3.2 Mitochondria

It had been believed that inheritance of cytoplasmic organelles such as mitochondria is achieved passively as a consequence of equal cytokinesis (Shima and Warren, 1998). However, it has been described that mitochondria are transmitted toward a daughter cell by the active transport system in fission yeast mitosis (Yaffe et al., 2003). In *Drosophila* male meiosis, the mitochondria line up along nuclear membranes and are equally divided to two daughter cells at each division of *Drosophila* male meiosis (Fuller, 1993). Ichihara and colleagues examined carefully distribution of mitochondria during meiotic divisions in *Drosophila* males (Fig. 3 from Ichihara et al., 2007). At a beginning of prophase (Fig. 3A),

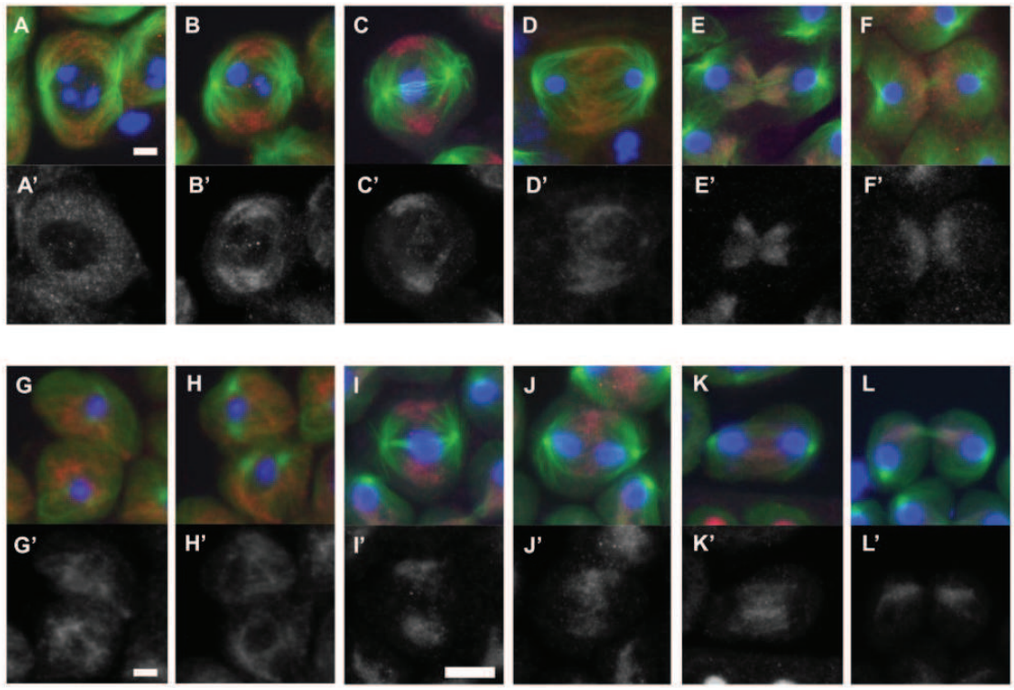

Fig. 3. Distribution of mitochondria with aster and spindle microtubules at meiotic stages in male meiosis I and II. Normal spermatocytes undergoing meiosis I (A-F) or II (G-L) were stained to visualize microtubules (green), mitochondria (red) and chromosomes (blue). Prophase (A, H), prometaphase (B), metaphase (C, I), anaphase A (D, J), anaphase B (E, K), telophase (F, L) and interphase (G), respectively. Bars, 10 μm. These all panels were reprinted from Ichihara et al., (2007).

mitochondria are homogenously distributed throughout cytoplasm. As aster microtubules are developing at prometaphase (Fig. 3B), mitochondria are expelled from inside of the asters and then assembled toward plus ends of aster microtubules. At metaphase, the mitochondria are clustered at the equator of a peripheral cytoplasmic region between facing two asters (Fig. 3C). As central spindle microtubules are formed between separating sister chromatids at anaphase (Fig. 3D), these mitochondria distribute along central spindles as if to decorate the microtubule structures. The mitochondria dispersed into cytoplasm (Fig. 3F), as the central spindles disintegrated. At the end of meiotic division II, mitochondria release from midbody microtubules and assemble to form single large aggregate called Nebenkern (Fig. 1). This transition of mitochondria is regulated by an ordered mode rather than a stochastic partitioning strategy. As spermatozoa require a consumption of higher amount of ATP for the motility, existence of regulation to facilitate equal inheritance of mitochondria in male meiosis may be of advantage that can produce a large number of spermatozoa with homogeneous quality at the same time.

3.3 Golgi stacks

During cell division, it was shown that some cell organelles lose their function and are fragmented by modification of their key components (Rabouille and Jokitalo, 2003; Rabouille and Klumperman, 2005). Golgi apparatus in mammalian cells is consisting of flattened membrane-bound compartments. The stacks interconnect each other and form a single large organelle so-called Golgi ribbon beside nucleus. The mammalian Golgi apparatus undergoes disassembly at onset of mitosis and reassembles into the ribbon structure at telophase (Misteli, 1997). A major difference in the Golgi organization between the mammalian and *Drosophila* cells is that a single Golgi ribbon as seen in mammals is not observed in *Drosophila*. Instead, multiple sets of smaller tER-Golgi unit that is a complex consisting of tER site and a piece of Golgi stacks dispersed throughout the cytoplasm (Kondylis & Rabouille, 2009). As membranous intracellular structures such as parafusorial membranes are well developed in spermatocytes (Tates 1971, Fuller, 2003), it is interesting to take up a dynamic behavior of membrane-based organelles in premeiotic and meiotic cells. It is considered that the Golgi stacks in *Drosophila* cultured cells also display a cycle of disassembly and reassembly during mitosis (Kondylis et al., 2007). We also observed carefully distribution of Golgi stack components visualized by immunostaining with antibody against a cis-Golgi protein, GM130 at several meiotic stages (Fig. 4). After spermatocytes initiate meiosis I, the Golgi-derived vesicles seem to increase in the number (compare Fig. 4A with B). The Golgi-derived vesicles were first uniformly distributed throughout the cytoplasm (Fig. 4A). They were then assembled into two groups containing similar amounts of Golgi vesicles prior to chromosome segregation (Fig. 4B). The Golgi vesicles were accumulated around each spindle pole until mid-telophase. At the end of cytokinesis after stage E in Fig. 4, the Golgi vesicles become to be redistributed throughout cytoplasm. These observations remind us equal partition of mitochondria in male meiosis described above. It might be possible to interpret that the Golgi-derived vesicles would be also partitioned equally between two daughter cells in male meiotic divisions. However, it was reported that vesicles containing another Golgi component, Rab11 was concentrated on the Golgi stacks and at the nuclear envelope from prophase to metaphase and that the Golgi-derived vesicles were subsequently accumulated at the cell poles (Giansanti, et al., 2007). At mid-telophase, the vesicles became to concentrate at cell equator possibly to

contribute new membrane insertion in cytokinesis. Accumulation of Rab11-containing Golgi vesicles at each cell pole may be no more than storage of vesicles required at cleavage furrow site. Further examinations using other Golgi-related vesicles should be necessary to conclude significance of Golgi-derived vesicle distribution during male meiotic divisions.

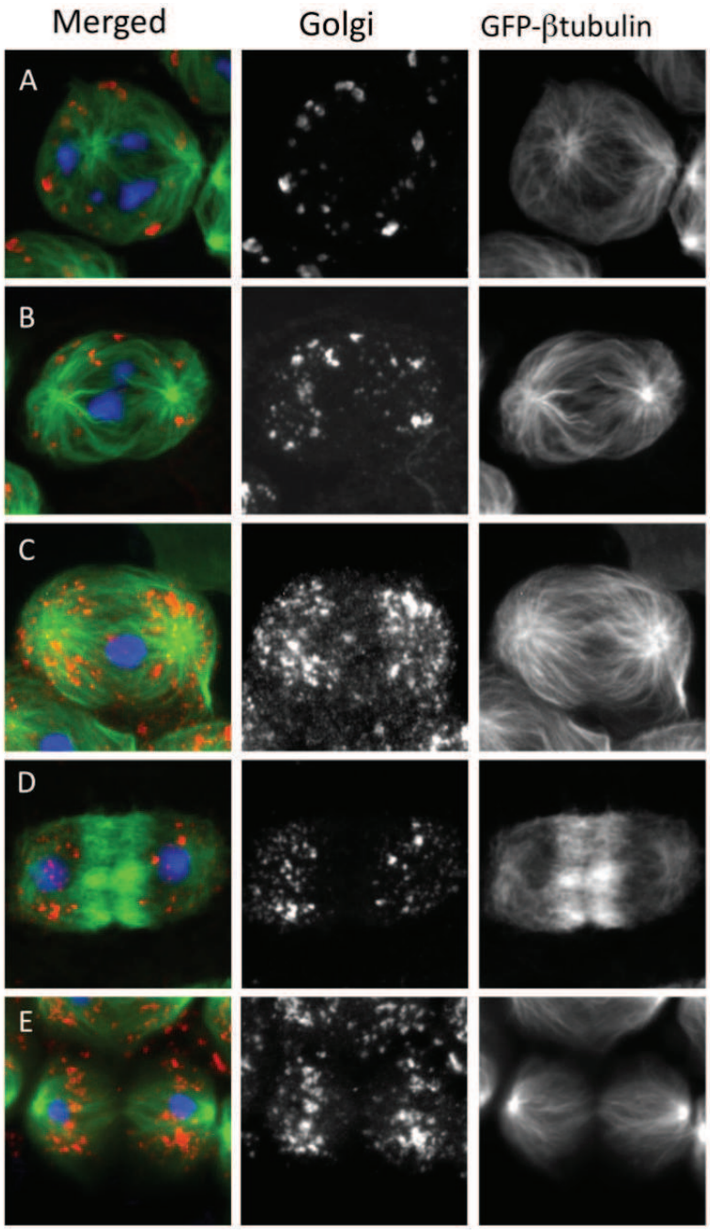

Fig. 4. Immunostainig of primary spermatocytes undergoing meiosis I with antibody against the cis-Golgi protein. Interphase or prophase I (A), prometaphase I (B), metaphase I (C), late anaphase I (D), telophase I (E). (Red) immunostaining with anti-GM130 antibody that recognizes a cis-Golgi protein. (Green) microtubules visualized by GFP-βtubulin, (blue) DNA staining. Prophase (A, H), prometaphase (B), metaphase (C, I), anaphase A (D, J), anaphase B (E, K), telophase (F, L) and interphase (G), respectively. Bars, 10μm

3.4 Endoplasmic Reticulum- based structures

Expression of Rtnl1 proteins residing predominantly in endoplasmic reticulum (ER) fused with GFP tag allowed us to observe its dynamics in primary spermatocytes. The ER-residing proteins are distributed on phase-dense structures observed around the nuclear space under phase contrast microscope (Miyauchi and Inoue, unpublished). This observation showed that the ER constructs distinct intracellular structures in spermatocytes during male meiotic divisions. Dorogova and colleagues have reported that ER network has distinctive reticular morphology immediately before initiation of male meiosis (Dorogova et al., 2009). The ER forms concentric circles on the outside of nuclear membrane at meiotic prophase. This ER network then dramatically changes as follows. As a progression of meiotic divisions, the ER networks form multi-layers of branchless membranous sheets appeared as phase-dense structure around nuclear space (Fig. 2 upper row). After prometaphase to telophase, the membranous structure develop very well around astral microtubules. This structure possibly corresponds to cellular organelles known as astral membrane in *Drosophila* embryos. This membranous organelles in embryo seem to be mainly consisted of ER sheets (Bobinnec et al., 2003). The reticular ER structure elongates along a direction of division axis and it transforms into multiple layers of membranes known as spindle envelopes, which surround the nuclear space. These two ER-based structures, astral membranes and spindle envelopes are especially characteristic in male meiotic cells. And they seem to be closely associated with astral microtubules and central spindle microtubules, respectively. These meiotic configurations restore to the reticular distribution around nucleus in next interphase. It is reasonable to speculate that these ER-based structures would interact with other cellular components and may have a role to facilitate dynamic changes of cellular components.

4. Identification of genes essential for proper execution of chromosome segregation and cytokinesis in male meiosis

Drosophila male meiosis provides us an advantage in examination of chromosome segregation and cytokinesis (Fuller, 1993; Maines and Wasserman, 1998). After a completion of meiotic divisions, every spermatid in a cyst consisting 64 cells contains equally sized single nucleus and a Nebenkern. This should be achieved as a consequence of proper chromosome segregation and cytokinesis in germ line cells. This is a quite convenient and sensitive method to find out those cell division defects in either or both meiotic divisions in living spermatids (Castrillon *et al*., 1993). Mutants for either chromosome segregation or cytokinesis or even both in male meiotic divisions could easily be distinguished by the spermatid morphology at onion stage. The evidences that the volume of spermatid nuclei is proportional to numbers of chromosome complements allow us to find out defects in chromosome segregation on the base of a presence of different sized spermatids (Gonzalez et al., 1989). A failure of cytokinesis results in generation of spermatids carrying abnormally large Nebenkern and multiple nuclei. Another advantage of *Drosophila* male meiosis is its less strict checkpoint to monitor spindle assembly before metaphase. Even if meiotic cells carry spindle defects, they only delays anaphase onset rather than arresting the cell cycle before metaphase as observed in somatic cells. Therefore, one can examine influence of microtubules on later stages after

microtubule assemble checkpoint such as chromosome segregation at anaphase or cytokinesis. After picking up mutant candidates by spermatid morphology, one can perform further careful examination in primary spermatocyte that is the largest proliferative cell. Using this convenient examination system, many of genes required for meiotic divisions have so far been identified. However, disadvantage of the cell type is that mutants for essential genes cannot usually be examined due to their earlier lethality. Hypomorphic mutations that can overcome the lethality at earlier developmental stages are useful for this purpose. And furthermore, we and other group have recently succeeded to perform knockdown experiments by induction of dsRNA for genes in premeiotic spermatocytes using the Gal4/UAS system (Goldbach et al., 2010 , Kitazawa et al., submitted). We put here a partial list of the genes required for meiotic cytokinesis in *Drosophila* male with especial focus on genes involved in a formation of central spindle microtubules and contractile ring (Table 1). The responsible genes for cytokinesis mutants includes genes encoding factors involved in dynamics of actin filaments, microtubules, motor proteins along cytoskeletons and so on. It is well known that spindle microtubules and contractile rings consisting actomyosin display mutual dependency in construction and function (Giansanti et al., 1998). To understand molecular mechanism of crosstalk between these two cytoskeletons, these *Drosophila* mutants and dsRNA stocks should be valuable genetic tools. These cytokinesis phenotypes appeared in *Drosophila* male meiosis, however, have not been completely confirmed by knockdown experiments in S2 cultured cells (Somma et al., 2002). For example, knockdown of *chic*, *fwd* and *klp3A* that have previously identified as essential genes for male meiosis did not impair cytokinesis of S2 cells treated with dsRNA. It is possible to speculate that a requirement of these gene products might differ from each cell type. It is important to continue comparative studies between somatic cells and meiotic cells for understanding general mechanisms to control cell divisions.

Protein name	**Symbol**	**Biochemical activity**	**Cell function**	**orthologue**
abnormal spindle	asp	microtubule-associated protein (MAP)	microtubulesorganization & cytokinesis	ASPM(mammal)
Adenomatous polyposis coli homolog 2	Apc2	adenomatous polyposis coli homolog	centrosome localization in Germline Stem cells (GSCs)	Apc
Apc-like	Apc	adenomatous polyposis coli homolog	centrosome localization in GSCs	Apc
Anillin	scra	component of the contractile ring	cytokinesis	Anillin
arrest	aret	RNA-binding protein	regulation of mRNAs involved in gametogenesis	
asterless	asl	centriolar or PCM protein	centrosome organization	
aurora B	ial	Serine/Threonine kinase	chromosome condensation and cytokinesis	Aurora B
auxilin	aux	Serine/Threonine kinase	cytokinesis	cyclin G associated kinase

Table 1. Proteins involved in organization of microtubules and actin filaments in *Drosophila* male meiosis.

Protein name	Symbol	Biochemical activity	Cell function	orthologue
bag-of-marbles	bam	Serine/Threonine kinase	spermatogonia deivision	
james bond	bond	Serine/Threonine kinase	contractile ring assembly	GNS1/SUR4 (yeast)
Btk family kinase at 29A	Btk29A	protein-tyrosine kinase	maintaining the equilibrium between G- and F-actin	Btk
Bub1	bub1	Serine/Threonine kinase	microtubule assembly checkpoint	Bub1
Centrosomin	cnn	pericentrosomal matrix protein	centrosome organization	CDK5 RAP2(mammal)
Chickadee	chic	profilin homolog	regulates polymerization of the actin cytoskeletone	Profilin
CIN85 and CD2AP orthologue	cindr	adaptor protein	links cell surface junctions and adhesion proteins	CIN85, CD2AP
Cortactin	cortactin	cytoskeletal component	stimulate actin polymerizationx	cortactin
courtless	crl	ubiquitin conjugating enzyme	male meiosis	Ubiquitin-conjugating enzyme, E2
diaphanous	dia	Formin homology domain protein	cytokinesis	mDia(mammal)
Dynamin related protein 1	drp1	Dynamin family	mitochondrial fusion	Dynamin
effete	eff	UbcD1	germline stem cell maintenance	
exo84	exo84	Cullin repeat	contractile ring constriction	exo8
Fmr1	Fmr1	RNA binding		FMR1
four wheel drive	fwd	Phosholipid kinase	contractile ring constriction	1-phosphatidylinositol 4-kinase
four way stop	fws		contractile ring constriction	Cog5
Fps oncogene analog	Fps85D	protein tyrosin kinase	protein tyrosin kinase cytoskeletal reorganization	Fps
sec8	sec8	exocyst complex component	contractile ring constriction	sec8
gilgamesh	gish	Ser/Thr kinase sperm indivisualization	Ser/Thr kinase sperm indivisualization	casein kinase
Kinesin-like protein at 61F	klp61F	kinesin-5 family protein	microtubule crosslinking and sliding activities	Eg5
Kinesin-like protein at 3A	klp3A	kinesin-4 family	cytokinesis	KIF4A
loquacious	loqs	RNA binding miRNA processing	germline stem cell maintenance	TARBP2
mei-s332	mei-s332	Shugoshin related	sister chromatid cohesion	Shugoshin related

Table 1. Proteins involved in organization of microtubules and actin filament in *Drosophila* male meiosis. (Continuation)

Protein name	Symbol	Biochemical activity	Cell function	orthologue
Myt1	Myt1	protei kinase	meiosis and spermatid differentiation	Myt1
off-schedule	ofs	eIF4G2 translation initiation	meiosis	elF
orientation disrupter	ord	unknown	meiotic sister chromatid cohesion	
parkin	park	E3 Ubiquichin ligase	mitochondria organization	parkin
pavarotti	pav	kinesin-6 family	spindle organization and cytokinesis	MKLP1 related
peanut	pnut	septin family	contractile ring formatioin	septin
pebble	pbl	RacGAP50C	cytokinesis	ECT2
pelota	pelo	eRF1 domain protein	male meiosis progression	pelota homolog
pericentrin-like protein	cp309	centriolar proetin	spindle pole organization	pericentrin
plk4	plk4	Ser/Thr kinase	centriole duplication	plk4
polo	polo	Ser/Thr kinase	cytokinesis central spindle & contractile ring	plk
Rab-protein 11	Rab11	small GTPase	cytokinesis membrane traffic	Rab11
Rac1	Rac1	small G protein	actin filament organization	Rac1
rhomboid-7	rho-7	rhomboid family proteinase	mitochondrial fusion in spermatogenesis	PARL protease
shutdown	shu	FK506-binding protein domain	germline stem cell regulation	
Spageghetti squash	sqh	Myosin regulatory light chain	formation of central spindle & contractile ring	Myosin regulatory light chain
Spectrin a	aSpec	cytoskeletal protein	fusome organization	Spectrin a
spindle assembly abnormal 6	sas-6	centriolar proetin	centrosome duplication	Sas6
sticky	sti	Rho effector kinase	cytokinesis	citron
subito	sub	kinesin-like protein	meiosis spindle organization	klp9
Syntaxin 5	Syx5	t-SNARE homology	golgi traffic assembly	Syntaxin 5
Transforming growth factor beta at 60A	gbb	TGF ligand	regulate germ line stem cells and other process	TGFb
twinstar	tsr	cofilin	meiosis and spermatid differentiation other process	cofillin
vibrator	vib	Phosphatidylinositol transfer protein	contractile ring constriction	
zipper	zip	myosin heavy chain II	cytokinesis formation of central spindle & contractile ring	Myosin II

Table 1. Proteins involved in organization of microtubules and actin filament in *Drosophila* male meiosis. (Continuation)

Information about each *Drosophila* gene can be obtained from the flybase by linking to the following URL: http://flybase.org/.

5. Signaling pathways to control remarkable cell growth of spermatocytes before meiotic initiation

5.1 Cell growth of premeiotic spermatocytes

The *Drosophila* spermatocytes increase in size up to 25 times during 90 hours after premeiotic DNA replication. The cell growth of premeiotic spermatocytes is the largest cell growth among that seen in *Drosophila* proliferative cells. The growth phase of primary spermatocytes is clarified as S1 to S6 stages (Cenci et al., 1994, Bonaccorsi et al., 2000). After four rounds of spermatogonia mitosis to form a 16-cell cyst, premeiotic DNA replication takes a place at first S1 stage. S2 to S6 stage corresponds to extended G2 phase of cell cycle. The later S6 stage is possibly overlapping to meiotic prophase. In later S2 phase known as polar spermatocyte, mitochondria increase in number and form a cluster at opposite side of the nucleus, while nucleus is dislocated from central position. As the cell volume of spermatocytes has gradually increased after the S2 stage, a plenty of genes including testis-specific gene, βtub85D are highly transcribed at the growth stages. Male fertility factors such as KS-1 are highly expressed so that its transcripts become to be visible under phase contrast microscope (Bonaccorsi et al., 2000). Because chromosomes are highly condensed during meiosis and after later spermatogenesis, most of gene products required for meiotic divisions and later spermatogenesis should be expressed in primary spermatocytes before meiotic initiation. The extreme cell growth may be achieved in coordination with enhanced expression and accumulation of proteins required for later cell divisions and development.

5.2 Growth factors and signaling cascades to induce spermatocyte growth

As described above, the *Drosophila* spermatocytes have achieved most distinctive cell growth up to 25 times after premeiotic DNA replication. What is a molecular mechanism to induce such an enormous spermatocyte growth? Insulin-like peptides (ILPs) play an important role in induction of somatic cell growth (Brogiolo, et al., 2001, Ikeya et al., 2002). Ueishi and colleagues reported that a loss of ILPs by specific apoptosis induction to insulin-producing cells results in reduced growth of spermatocytes, suggesting that the spermatocyte cell growth is required for ILPs (Rulifson et al., 2000, Ueishi et al., 2009). They further showed that an accumulation of active Akt form phosphorylated by its upstream factor, PDK1 in the growing spermatocytes. A diameter of spermatocytes from mutant males for Insulin Receptor (InR) or IRS orthologue encoded by *chico* gene decreased to 70 % of normal size. These genetic data suggest that the insulin signaling plays an essential role in the remarkable cell growth of spermatocytes (Ueishi et al., 2009). We further examined whether PI_3 kinase acting upstream Akt is also involved in cell growth induction. The expression of constitutive active form of PI_3 kinase catalytic subunit was induced in spermatogonia to premeiotic spermatocyte stage (Ogata and Inoue unpublished). Such an induced expression results in 14% increase of spermatocytes in diameter. These genetic data strongly suggest that the ILPs and its signaling cascade through PI_3 kinase to Akt plays a role in induction of spermatocyte cell growth in *Drosophila*. As mammalian insulin can also activate the Ras-MAP kinase cascade after the Insulin receptor (as a review, Avruch, 1998), we further examine whether Ras signaling cascade acting downstream of Drosophila Insulin Receptor homologue (InR) is also involved in the cell growth of spermatocytes before male meiosis. Constitutively activated mutation for Ras85D, $Ras85D^{v12}$ (Kim et al., 2006) also induced approximately 10 % increase of cell diameter in length (Ogata and Inoue, unpublished). Therefore, these genetic data suggest that both PI_3K-

Akt cascade and Ras-MAP kinase cascades acting downstream of InR are essential for induction of the premeiotic spermatocyte growth.

6. Asymmetric division of germline stem cells and directional divisions of spermatogonia before meiosis

At the tip of the testis in adult *Drosophila* males, several germline stem cells (GSCs) can be observed. The GSCs receive a signal to maintain their stem cell characteristics secreted from the adjoining hub cells. A ligand protein encoded by the *unpaired* gene is used as the maintenance signal and the signal is transmitted through the JAK-STAT signaling cascade (Kiger, *et al*., 2001, Tulina & Matunis, 2001). A proximal cell of the two daughter cells derived from an asymmetric division of the GSC exclusively receives the signal and becomes a self-renewed GSC. For self-renewal and differentiation of GSC daughters, it is crucial to set up cell division axis perpendicular to a cluster of the hub cells (Yamashita et al., 2003). What is a molecular mechanism to set up the spindle axis perpendicular? It was shown that mother centrosome in GSC remains to be positioned at the cell cortex contiguous to the hub cells and that daughter centrosome derived from duplication of the mother in GSCs is released from the cortex and migrates toward an opposite pole (Yamashita et al., 2007). The distal daughter cell derived from a GSC division leaves the niche and differentiates as a spermatogonium.

The spermatogonium then initiates four times of cell cycles to generate a cyst consisting of 16 cells. Every spermatogonium in a cyst undergoes these four rounds of cell division synchronously. The orientation of these spermatogonia divisions rotates at 90 degree in every mitosis. Like GSCs, it is possible to speculate that a daughter centrosome derived from the mother anchored to the fusome is free from a connection with the fusome extended over spamatogonia within a cyst and thus it could migrate toward an opposite pole until prometaphase. In this way, spermatogonia could alternate division orientation at 90 degree in every cell division. Such unusual mitoses may be advantageous to store a cyst containing constant numbers of spermatogonia within a limited space of testis.

7. Future researches

The primary spermatocyte is considered as one of the cells most thoroughly examined about cell division together with a S2 cultured cell. In addition to genetic analyses using hypomorphic mutants viable up to developmental stages in which male meiosis can be observed, targeted knockdown of all most of the *Drosophila* genes currently became to be possible in spermatocytes. We can expect that saturation genetic studies to examine variety of phenotypes should be frequently carried out near future. It should be fruitful for us to collect whole information about cell phenotypes appeared in primary spermatocytes from such large scale knock down experiments. These efforts would certainly increase value of male meiotic cell as a model cell for researches on cell proliferation and growth. As the primary spermatocytes have some specific futures in terms of intracellular structures or cell cycle regulation, it is necessary to perform comparative studies using common cultured cells for confirmation of genetic results obtained in male meiotic cells. For more detailed real-time observation to examine dynamics of multiple cellular components simultaneously during male meiosis, it is important to develop cultured system to make it possible to do longer

observation from spermatogonial stage to onion stage of spermatid. We currently succeeded to carry out continuous observation from onset of meiosis to onion stage spermatids. It is also necessary to establish *Drosophila* stocks to induce simultaneous expression of several cellular components fused with different fluorescence tag, GFP, mRFP or CFP. They allow us to perform multi-color time lapse observation that would make it possible to trace alterations of chromosomes, microtubules or actin filaments and other cellular organelles in a single cell. Such a new observation system should stimulate understanding of dynamic feature of male meiosis in *Drosophila*. As a series of cellular events such as cell division, growth, elongation and differentiation in spermatogenesis is considerably conserved between *Drosophila* and mammals (Zhou and Griswold, 2008), we expect that *Drosophila* data would also bring us valuable information to help better understanding of mammalian spermatogenesis.

8. Conclusion

In this chapter, we conclude that *Drosophila* primary spermatocytes undergoing meiosis I is an excellent model cell to examine dynamics of chromosomes and other cellular components such as cell organelles and cytoskeletons. Although chiasmata formation and homologous recombination does not occur in *Drosophila* male meiosis, we can study on more simple chromosome pairing and segregation of homologous chromosomes. We showed a simultaneous observation of chromosomes, microtubules and cell organelles in living primary spermatocytes expressing proteins fused with different fluorescence tags. Microtubules and actin filaments display dynamic alterations as a progression of male meiotic divisions. Furthermore, our observation indicates cell organelles such as mitochondria or golgi foci are also transmitted equally toward two daughter cells dependent on microtubule structures. By examination of hypomorphic mutants or knockdown spermatocytes, it have been shown that a plenty of cell cycle related genes including many novel genes play a important role in male meiotic divisions. Before initiation of meiotic division I, the cells achieve largest extent of cell growth in *Drosophila*. We also discussed molecular mechanisms to induce the distinct cell growth of premeiotic spermatocytes by insulin-like peptides, their signaling pathways and other related pathways showing crosstalk with the insulin cascade. In addition to male meiotic divisions, we briefly referred four round premeiotic divisions of spermatogonia to generate a 16-cell cyst and discussed about its regulatory mechanism. These mitoses are synchronous cell divisions in which spindle axis rotate by 90 degree in every division. Animal meiosis is a part of development programs in gametogenesis. It is basically different from yeast meiosis that can be induced by environment cues. *Drosophila* male provides us a good model to understand common molecular mechanisms to control animal meiosis.

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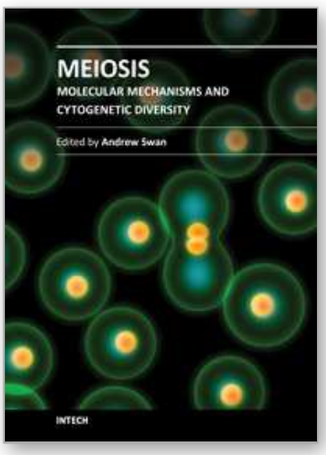

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