PATHWAYS OF SPINDLE FORMATION IN *DROSOPHILA* MITOTIC AND MEIOTIC CELLS

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Spindle assembly relies on three main classes of microtubules (MTs): MTs nucleated by the centrosomes, MTs nucleated near the chromosomes/kinetochores and MTs nucleated from preexisting MTs through the augmin-based pathway. Here, we review the roles of these microtubule generation pathways in *Drosophila* spindle assembly. The extant results indicate that female meiotic cells, male meiotic cells, larval brain cells and S2 tissue culture cells exploit specific pathway combinations for generating the MTs necessary for spindle formation. Thus, different *Drosophila* cell types have specific modes of spindle assembly, which might be related to specific functional and developmental requirements.

Key words: mitosis, meiosis, spindle formation, microtubules, centrosomes, kinetochores, chromosomes.

Abbreviations: MT(s) — microtubule(s), PCM — pericentriolar material.

Mitotic and meiotic spindle assembly requires spatially and temporally controlled microtubule (MT) polymerization. Dividing cells assemble their spindles exploiting three main MT populations: MTs nucleated by the centrosomes, MTs generated near the chromosomes and/or at kinetochores, MTs nucleated within the spindle from preexisting MTs via the augmin-based pathway (reviewed by Duncan, Wakefield, 2011; Gatti et al., 2012). All MTs are nucleated by the γ-tubulin ring complexes (γ-TuRCs), which are embedded into the centrosomes, free in the cytoplasm near the chromosomes, or bound to the sides of spindle MTs (Lüders, Stearns, 2007; Goshima, Kimura, 2010). Spindle formation in different *Drosophila* cells types requires specific contributions from the MT generation pathways. Here, we briefly review the process of spindle formation in female meiosis, larval brain cells and S2 tissue culture cells, and male meiosis. These cell types exhibit different spindle morphologies (Figure) and exploit different combinations of MT generation pathways for spindle assembly.

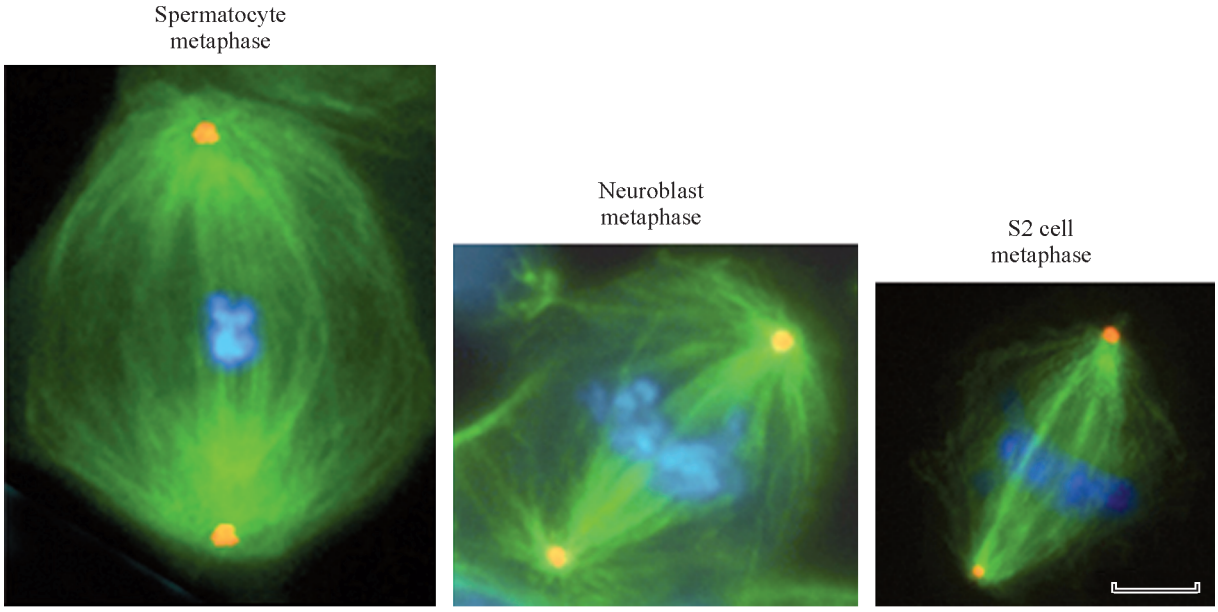
Spindle formation in female meiotic cells

Drosophila oocytes do not contain centrosomes and organize the meiosis I spindle from MTs that grow from the chromosomes (McKim, Hawley, 1995). These MTs are initially randomly oriented and become organized into bipolar spindles by motor proteins such as Ncd and Subito, and MT stabilizing factors such as DTACC and Msps (McKim, Hawley, 1995; Matthies et al., 1996; Cullen, Ohkura, 2001; Giunta et al., 2002). Female meiosis arrests at metaphase I, and resumes after passage of the oocyte through the oviduct. The second meiotic division occurs perpendicularly with respect to the egg surface leading to the formation of pair of tandemly arranged twin spindles. Interestingly, these tandem spindles develop an astral MT array between their innermost poles. Even if this MT array is not nucleated by canonical centriole-containing centrosomes, it is enriched in centrosomal proteins at its center. The biological role of the aster-like MT structure assembled between the two meiosis II spindles is unclear; it might be involved in the migration of the female pronucleus towards the male pronucleus (Riparbelli, Callaini, 1996).

Spindle formation in somatic cells undergoing mitosis

The mechanisms of spindle assembly have been studied in three main systems, the neuroblasts and ganglion mother cells of larval brains and the Schneider 2 (S2) tissue culture cells; S2 cells were established from a primary culture of late stage (20—24 h) embryos (Schneider, 1972). All these cells types possess centrosomes and form their spindles from both centrosome- and chromosome-driven MTs. To address the relative contributions of these pathways to spindle assembly, centrosome or chromosome MT nucleation activities have been disrupted by mutations or RNAi and the ensuing mitotic phenotype analyzed.

The centrosomes are comprised of a pair of centrioles surrounded by pericentriolar material (PCM); they contain hundreds of proteins including those necessary for centroso-



Examples of metaphase spindles observed in spermatocytes undergoing the first meiotic division, larval neuroblasts and S2 cells. Cells are stained for DNA (*blue*), tubulin (*green*) and the centrosome marker Cnn (*red*). *Scale bar* — 5 μm.

me duplication and MT nucleation. Mutations in genes that mediate centriole duplication or affect PCM formation result in cells devoid of centrosomes or disrupt the centrosome nucleating ability. Larval brain cells carrying mutations in conserved genes such as *asterless* (*asl*/*CEP152*; the slash separates the *Drosophila* gene from its human orthologue), *centrosomin* (*cnn*/*CDKRAP2*), *DSas-4*/*CENPJ* or *DSpd2*/*CEP192* fail to form asters but are nonetheless able to assemble functional anastral spindles. Consistent with these results, homozygous *asl*, *cnn*, *DSas-4* or *DSpd2* mutant larvae develop to adulthood (reviewed by Duncan, Wakefield, 2011; Gatti et al., 2012). RNAi-based studies have shown that centrosomal MTs are also dispensable for spindle assembly in S2 cells (see, for example, Somma et al., 2008). Thus, centrosome-driven MT nucleation appears to be dispensable for the assembly of a functional spindle in *Drosophila* somatic cells.

The role of chromosome-driven MTs in spindle formation has been addressed in cells with incompletely separated centrosomes where the chromosomes are distant from the asters. In these cells, kinetochores emanate robust MT bundles with the plus ends that polymerize at kinetochores and the minus ends pointing away from the chromosomes (Maiato et al., 2004). Another approach to study chromosome-driven MT assembly is the analysis of their regrowth following cold- or colcemid- induced spindle depolymerization. While in brain cells MT regrow from both the kinetochores and chromosome arms, in S2 cells MT regrowth seems to be restricted to the kinetochore region (Bucciarelli et al., 2009; Mottier-Pavie et al., 2011). Several genes have been identified that are necessary for proper MT regrowth in S2 and brain cells. In S2 cells, MT regrowth from kinetochores requires each of the 8 the augmin subunits (Bucciarelli et al., 2009). Chromosome-driven MT growth in larval brain cells requires the *misato* (*mst*) gene, whose absence leads to reduced MT density, aster collapse and monopolar spindles, ultimately resulting in larval death (Mottier-Pavie et al., 2011). Recent work has shown that Mst interacts with TCP-1/CCT tubulin chaperone complex; RNAi-mediated *in vivo* depletion of any of the TCP-1 subunits phenocopies the *mst* mutant phenotype, preventing chromosome-driven MT regrowth and leading to larval lethality (Palumbo et al., 2015).

Collectively, these data indicate that centrosomal MTs are dispensable for mitotic spindle assembly in both S2 and larval brain cells. In contrast, kinetochore-driven MTs appear to be essential for spindle assembly in both cell types.

Spindle formation in male meiotic cells

Meiotic spindles of *D. melanogaster* males are much larger than those of somatic cells and contain centrosomes that nucleate prominent asters. It has been recently shown that the large size of these spindles can be linked to a precise developmental requirement. Cytological analysis of male meiosis in six different *Drosophila* species that exhibit dramatic differences in sperm tail length (ranging from 0.3 mm in *D. persimilis* to 58.3 mm in *D. bifurca*) showed that these species also exhibit striking variations in the spindle size, which positively correlates with the sperm length. These results suggest that *Drosophila* primary spermatocytes manufacture and store most of the tubulin needed for sperm tail formation and use it for meiotic spindle assembly (Lattao et al., 2012).

In *asl* and *Dspd2* mutants, dividing spermatocytes fail to form asters but are able to generate MTs around the chromosomes. However, these chromosome-driven MTs are not sufficient for proper spindle formation; *asl* and *Dspd2* mutant spermatocytes assemble highly defective spindles that are unable to mediate chromosome segregation (Bonaccorsi et al., 1998; Giansanti et al., 2008). Thus, the MTs nucleated by the centrosomes have a preponderant role in meiotic spindle assembly in *Drosophila* males. Consistent with this conclusion, spermatocytes have the peculiar ability to assemble a morphologically normal spindle in the absence of chromosomes. *fusolo* (*fsl*) and *solofuso* (*suo*) are male sterile mutants that exhibit severe defects in chromosome segregation and frequently produce secondary spermatocytes that are devoid of chromosomes; *suo* is a weak mutant allele at the *Topoisomerase II* (*Top2*) gene (Bucciarelli et al., 2003; Mengoli

et al., 2014). Surprisingly, the centrosomes of the achromosomal secondary spermatocytes nucleate astral MTs that give rise to bipolar spindles; these peculiar spindles elongate during anaphase and form regular central spindles, which support the formation of an actin-based contractile ring and undergo cytokinesis (Bucciarelli et al., 2003). Thus, *Drosophila* secondary spermatocytes can organize a spindle in the complete absence of chromosome-induced MT-nucleation.

The role of augmin in spindle assembly

Augmin is a conserved multiprotein complex that anchors the γ-tubulin rings to the sides of preexisting MTs, thus mediating MT amplification within the spindle (Goshima, Kimura, 2010; Petry et al., 2013). In S2 cells, augmin is required for kinetochore-driven MT growth and k-fiber formation (Bucciarelli et al., 2009). In contrast, augmin is dispensable for spindle assembly in larval brain cells; mutants in the *wac* and *msd1* genes, both of which encode augmin subunits, are viable and male fertile and do not exhibit detectable defects in larval neuroblast or male meiotic spindles. However, both mutants are female sterile and display defects in female meiotic spindles (Meireles et al., 2009; Wainman et al., 2009). Interestingly, doubly mutant flies homozygous for both *cnn* and *msd1* viable alleles are lethal and exhibit highly aberrant mitotic spindles (Wainman et al., 2009). Taken together, these results suggest that the defects observed in *wac* and *msd1* mutants are primarily due to a reduced augmin-mediated chromosome-driven MT generation, which would be sufficient for mitotic spindle formation in the presence of centrosomes, but insufficient for female meiotic spindle assembly. The residual amount of chromosome-induced MTs in *wac* and *msd1* mutants would be also insufficient for mitotic spindle assembly in the absence of *cnn*-mediated centrosome-driven MT nucleation. The observation that augmin is essential for S2 spindle assembly but dispensable for brain cell mitosis is difficult to explain. We can only postulate that brain cells, besides the augmin pathway, possess an additional pathway for chromosome-driven MT formation and that this second pathway is absent in S2 cells.

Conclusions

Different *Drosophila* cell types use specific combinations of MT generation pathways for spindle assembly. The chromosome-based pathway is essential for brain cell mitosis and female meiosis but dispensable for male meiosis; the centrosome-driven pathway is crucial for male meiosis, non-essential for mitosis and dispensable for female meiosis. The augmin-based pathway appears to be non-essential for both mitosis and male meiosis but is required for female meiosis. Despite the use of different pathways for MT generation the spindles of these three cell types appear to function equally well in mediating chromosome segregation. This suggests that there is no single «best» pathway combination for spindle assembly, and that the flexibility in the modes of spindle formation reflects precise developmental requirements. The chromosome-based spindle formation in female meiosis is simply a consequence of centriole degeneration during oogenesis to ensure a uniparental contribution of the centriole to the zygote. The requirement of centrosome-driven MTs for male meiotic spindle assembly could reflect the large amount of tubulin stored in spermatocytes for sperm tail assembly. Spermatocytes evolved very big centrioles/centrosomes to potentiate their centrosome-driven MT nucleation pathway. Thus, it is quite possible that in the absence of centrosomes spermatocytes are unable to deal with their large tubulin pool and to form a regular spindle (Gatti et al., 2012; Lattao et al., 2012). Spindle formation in brain cells mainly relies on chromosome-driven MTs as in mammalian somatic cells (O'Connell, Khodjakov, 2007), suggesting that this is a basic and efficient mode to build a spindle. However, the great flexibility in the spindle assembly pathways observed in flies strongly suggests that a similar flexibility might be present in different cells of many organisms.

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References

Bonaccorsi S., *Giansanti M. G.*, *Gatti M. 1998.* Spindle self-organization and cytokinesis during male meiosis in *asterless* mutants of *Drosophila melanogaster*. J. Cell Biol. 142 : 751—761.

Bucciarelli E., *Giansanti M. G.*, *Bonaccorsi S.*, *Gatti M. 2003.* Spindle assembly and cytokinesis in the absence of chromosomes during *Drosophila* male meiosis. J. Cell Biol. 160 : 993—999.

Bucciarelli E., *Pellacani C.*, *Naim V.*, *Palena A.*, *Gatti M.*, *Somma M. P. 2009. Drosophila* Dgt6 interacts with Ndc80, Msps/XMAP215, and gamma-tubulin to promote kinetochore-driven MT formation. Curr. Biol. 19 : 1839—1845.

Cullen C. F., *Ohkura H. 2001.* Msps protein is localized to acentrosomal poles to ensure bipolarity of *Drosophila* meiotic spindles. Nat. Cell Biol. 3 : 637—642.

Duncan T., *Wakefield J. G. 2011.* 50 ways to build a spindle: the complexity of microtubule generation during mitosis. Chromosome Res. 19 : 321—333.

Gatti M., *Bucciarelli E.*, *Lattao R.*, *Pellacani C.*, *Mottier-Pavie V.*, *Giansanti M. G.*, *Somma M. P.*, *Bonaccorsi S. 2012.* The relative roles of centrosomal and kinetochore-driven microtubules in *Drosophila* spindle formation. Exp. Cell Res. 318 : 1375—1380.

Giansanti M. G., *Bucciarelli E.*, *Bonaccorsi S.*, *Gatti M. 2008. Drosophila* SPD-2 is an essential centriole component required for PCM recruitment and astral-microtubule nucleation. Curr. Biol. 18 : 303—309.

Giunta K. L., *Jang J. K.*, *Manheim E. A.*, *Subramanian G.*, *McKim K. S. 2002. subito* encodes a kinesin-like protein required for meiotic spindle pole formation in *Drosophila melanogaster*. Genetics. 160 : 1489—1501.

Goshima G., *Kimura A. 2010.* New look inside the spindle: microtubule-dependent microtubule generation within the spindle. Curr. Opin. Cell Biol. 22 : 44—49.

Lattao R., *Bonaccorsi S.*, *Gatti M. 2012.* Giant meiotic spindles in males from *Drosophila* species with giant sperm tails. J. Cell Sci. 125 : 584—588.

Lüders J., *Stearns T. 2007.* Microtubule-organizing centres: a re-evaluation. Nat. Rev. Mol. Cell Biol. 8 : 161—167.

Maiato H., *Rieder C. L.*, *Khodjakov A. 2004.* Kinetochore-driven formation of kinetochore fibers contributes to spindle assembly during animal mitosis. J. Cell Biol. 167 : 831—840.

Matthies H. J., *McDonald H. B.*, *Goldstein L. S.*, *Theurkauf W. E. 1996.* Anastral meiotic spindle morphogenesis: role of the non-claret disjunctional kinesin-like protein. J. Cell Biol. 134 : 455—464.

McKim K. S., *Hawley R. S. 1995.* Chromosomal control of meiotic cell division. Science. 270 : 1595—1601.

Meireles A. M., *Fisher K. H.*, *Colombie N.*, *Wakefield J. G.*, *Ohkura H. 2009.* Wac: a new Augmin subunit required for chromosome alignment but not for acentrosomal microtubule assembly in female meiosis. J. Cell Biol. 184 : 777—784.

Mengoli V., *Bucciarelli E.*, *Lattao R.*, *Piergentili R.*, *Gatti M.*, *Bonaccorsi S. 2014.* The analysis of mutant alleles of different strength reveals multiple functions of topoisomerase 2 in regulation of *Drosophila* chromosome structure. PLoS Genet. 10 : e1004739.

Mottier-Pavie V., *Cenci G.*, *Verni F.*, *Gatti M.*, *Bonaccorsi S. 2011.* Phenotypic analysis of *misato* function reveals roles of non-centrosomal microtubules in *Drosophila* spindle formation. J. Cell Sci. 124 : 706—717.

O'Connell C. B., *Khodjakov A. L. 2007.* Cooperative mechanisms of mitotic spindle formation. J. Cell Sci. 120 : 1717—1722.

Palumbo V., *Pellacani C.*, *Heesom K. J.*, *Rogala K. B.*, *Deane C. M.*, *Mottier-Pavie V.*, *Gatti M.*, *Bonaccorsi S.*, *Wakefield J. G. 2015.* Misato controls mitotic microtubule generation by stabilizing the TCP-1 tubulin chaperone complex. Curr. Biol. 25 : 1777—1783.

Petry S., *Groen A. C.*, *Ishihara K.*, *Mitchison T. J.*, *Vale R. D. 2013.* Branching microtubule nucleation in *Xenopus* egg extracts mediated by augmin and TPX2. Cell. 152 : 768—777.

Riparbelli M. G., *Callaini G. 1996.* Meiotic spindle organization in fertilized *Drosophila* oocyte: presence of centrosomal components in the meiotic apparatus. J. Cell Sci. 109 : 911—918.

Schneider I. 1972. Cell lines derived from late embryonic stages of *Drosophila melanogaster*. J. Embryol. Exp. Morphol. 27 : 353—365.

Somma M. P., *Ceprani F.*, *Bucciarelli E.*, *Naim V.*, *De Arcangelis V.*, *Piergentili R.*, *Palena A.*, *Ciapponi L.*, *Giansanti M. G.*, *Pellacani C.*, *Petrucci R.*, *Cenci G.*, *Verni F.*, *Fasulo B.*, *Goldberg M. L.*, *Di Cunto F.*, *Gatti M. 2008.* Identification of *Drosophila* mitotic genes by combining co-expression analysis and RNA interference. PLoS Genet. 4 : e1000126.

Wainman A., *Buster D. W.*, *Duncan T.*, *Metz J.*, *Ma A.*, *Sharp D.*, *Wakefield J. G. 2009.* A new Augmin subunit, Msd1, demonstrates the importance of mitotic spindle-templated microtubule nucleation in the absence of functioning centrosomes. Genes Dev. 23 : 1876—1881.

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МЕХАНИЗМЫ ФОРМИРОВАНИЯ ВЕРЕТЕНА ДЕЛЕНИЯ В МИТОТИЧЕСКИХ И МЕЙОТИЧЕСКИХ КЛЕТКАХ *DROSOPHILA*

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При сборке веретена деления формирование микротрубочек (МТ) инициируется тремя основными способами: от центросом, от хромосом/кинетохоров и на уже существующих МТ посредством augmin-зависимого механизма. В настоящей работе рассматриваются роли этих способов образования МТ при сборке веретена деления у дрозофилы. Накопленные результаты указывают на то, что для сборки веретена деления в процессах оогенеза, сперматогенеза, а также в клетках нервных ганглиев личинок и в культивируемых клетках S2 задействованы специфические комбинации способов формирования МТ. Таким образом, разные типы клеток дрозофилы используют разные механизмы сборки веретена, которые могут быть обусловлены специфичностью функции и развития этих клеток.

Ключевые слова: митоз, мейоз, сборка веретена, микротрубочки, центросомы, кинетохоры, хромосомы.