

Spindle mechanics and dynamics during mitosis in *Drosophila*

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***Drosophila melanogaster* is an excellent model for studying mitosis. Syncytial embryos are amenable to time-lapse imaging of hundreds of synchronously dividing spindles, allowing the quantitation of spindle and chromosome dynamics with unprecedented fidelity. Other *Drosophila* cell types, including neuroblasts, cultured cells, spermatocytes and oocytes, contain spindles that differ in their design, providing cells amenable to different types of experiments and allowing identification of common core mechanisms. The function of mitotic proteins can be studied using mutants, inhibitor microinjection and RNA interference (RNAi) to identify the full inventory of mitotic proteins encoded by the genome. Here, we review recent advances in understanding how ensembles of mitotic proteins coordinate spindle assembly and chromosome motion in this system.**

Mitosis, the process by which identical copies of the replicated genome are distributed to the daughter cells at each cell division, depends upon the action of a bipolar protein machine – the mitotic spindle. The related process, meiosis, uses a similar molecular machine, the meiotic spindle, to reduce the size of the genome by half during the generation of haploid gametes. Consequently, the actions of mitotic and meiotic spindles are crucial for the formation, maintenance and reproduction of healthy organisms.

All mitotic and meiotic spindles use common molecules and mechanisms based on microtubules (MTs) and MT-associated proteins (MAPs) to assemble and coordinate chromosome segregation; in *Drosophila*, however, different types of spindles exist in different cell types (Figure 1). For example, spindles in embryos, neuroblasts and male meiotic spermatocytes [1,2] are generally ‘amphistastral’; this means that they are formed by a centrosome-directed assembly pathway involving the interaction between two separating asters [CENTROSOMES (see Glossary) and associated MTs]. By contrast, female meiosis-I spindles [3] are ‘anastral’ meaning that they lack centrosomes and ASTRAL MTs and use a chromosome-directed assembly pathway to become organized into bipolar structures with narrow and tapered poles (Box 1) [3–5]. Cultured S2 cells of *Drosophila* normally contain amphistastral spindles;

however, when the centrosome-directed pathway fails, they are capable of using the noncentrosomal mechanism of pole formation as a backup to produce functional monastral bipolar spindles [6]. Mitosis in the *Drosophila* neuroblasts is also of interest, because it serves as a model for studying an asymmetric mitosis that leads to an asymmetric cleavage, producing cells of different size and thus different fates [7,8].

This review focuses on recent work that exploits the peculiar advantages of *Drosophila* embryos for studying the molecular mechanisms underlying the dynamics of mitotic spindles *in vivo*. In addition, to illustrate those fundamental principles of spindle mechanics and dynamics that are probably common to all types of spindles, the review compares embryo spindles with the

Glossary

Central Spindle: The midregion of bipolar spindles where microtubules of two half spindles overlap and interact with each other.

Centrosome: An organelle composed of a pair of centrioles and surrounding amorphous material called pericentriolar material (PCM); it includes γ -tubulin and CP190 and is responsible for MT nucleation. In most animal cells, centrosomes function as microtubule-organizing centers (MTOCs) to nucleate MTs with uniform polarity; consequently, the minus ends of MTs, attached to the γ -tubulin-ring complex, are embedded in centrosomes and their plus ends radiate outward.

Kinetochore: A protein complex associated with centromeric regions of chromosomes involved in capturing chromosomes and pulling chromatids poleward.

Astral Mts: MTs that radiate from centrosomes out into the cytoplasm and are capable of interacting with chromosome arms or cell cortices

Interpolar Mts: Bundles of parallel MTs emanating from opposite poles that interdigitate with one another to form antiparallel arrays in the overlap region at the central spindle; here, plus- and minus-end-directed MT-sliding motors can generate outward and inward forces, respectively.

Kinetochore Mts: Bundles of MTs that link spindle poles to the kinetochore, which is a protein complex associated with centromeric regions of chromosomes involved in capturing chromosomes and pulling chromatids poleward.

MT motors: ATP-dependent force-generating enzymes including two families – the dyneins and the kinesins. Kinesins are subcategorized to Kin N, Kin I (MT depolymerases) and Kin C (minus-end-directed kinesin), depending on the location of the motor domain at the N terminus, internal region and C-terminus of the polypeptides, respectively.

MT Polymer-Ratchets: MTs that polymerize or depolymerize to generate pushing and pulling forces, respectively.

Salt-Stripped Centrosomes: Centrosome scaffolds that do not have MT nucleation activity because of loss of soluble factors during salt extraction.

Spindle-Assembly Checkpoint: A mechanism by which the spindle monitors whether all kinetochores are attached, bi-oriented and under tension; it occurs at a point at which cell-cycle progression can be halted to ensure proper chromosome segregation. Its silencing allows progression from metaphase to anaphase.

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Box 1. Spindle morphogenesis: (a) centrosome-directed amphistral and (b) chromosome-directed anastral spindle assembly pathways

Amphistral (from the Greek meaning stars from both sides) bipolar spindles form by the centrosome-directed or 'outside-in' mechanism; this involves the overlap of two radial arrays of astral microtubules (MTs), emanating from centrosomes at the poles and forming antiparallel bundles in the central-spindle region (Figure 1a). By contrast, anastral spindles lack centrosomes and establish a bipolar structure by using a chromosome-directed or 'inside-out' mechanism; this involves sequential steps of random nucleation of MTs around chromosomes in response to a RAN-GTP gradient, followed by MT sorting into a bipolar array and, finally, the 'zipping' together of bundled MTs to form focused spindle poles (Figure 1b).

Although these two assembly pathways are thought to be deployed in different cell types, it is plausible that amphistral spindles might use them together to enhance the fidelity of spindle assembly [6,25]. Where they coexist, the centrosome-directed pathway is dominant; this conclusion is based on *in vitro* studies showing that the addition of single centrosomes to the spindles assembling around chromosomes introduces a bias to the formation of monopolar spindles [65]. It is also interesting to speculate why amphistral and anastral spindles exist in different cells, and what advantages each might have. The presence of centrosomes might enhance the efficiency of mitosis by providing

amphistral spindles with two efficient MT-organizing centers that nucleate the formation of two overlapping uniform polarity arrays, thereby circumventing the need for motor-dependent sorting of MT arrays assembled around chromatin. Centrosomes also appear to enhance mitotic fidelity by playing roles in regulating cell-cycle progression. For example, *Drosophila* embryos contain a checkpoint kinase that responds to DNA damage by inactivating centrosomes and removing their associated damaged nuclei, thereby preventing them from contributing aneuploid nuclei to the developing embryo [66]. Finally, centrosomes and their associated asters play important roles in positioning spindles in response to positional cues during asymmetric cell divisions [7,22,46]. The advantages of anastral spindles are less obvious, although cells, such as *Drosophila* oocytes and all cells of flowering plants, have evolved efficient meiosis and mitosis in the absence of centrosomes and centrioles. In oocytes, one advantage might be prevention of having supernumerary centrosomes after fertilization when the sperm supplies a centriolar precursor (basal body). Different organisms appear to have adapted to centriole loss either during oogenesis (e.g. *Drosophila*, human and worm) or later during polar-body formation (e.g. in most other organisms).

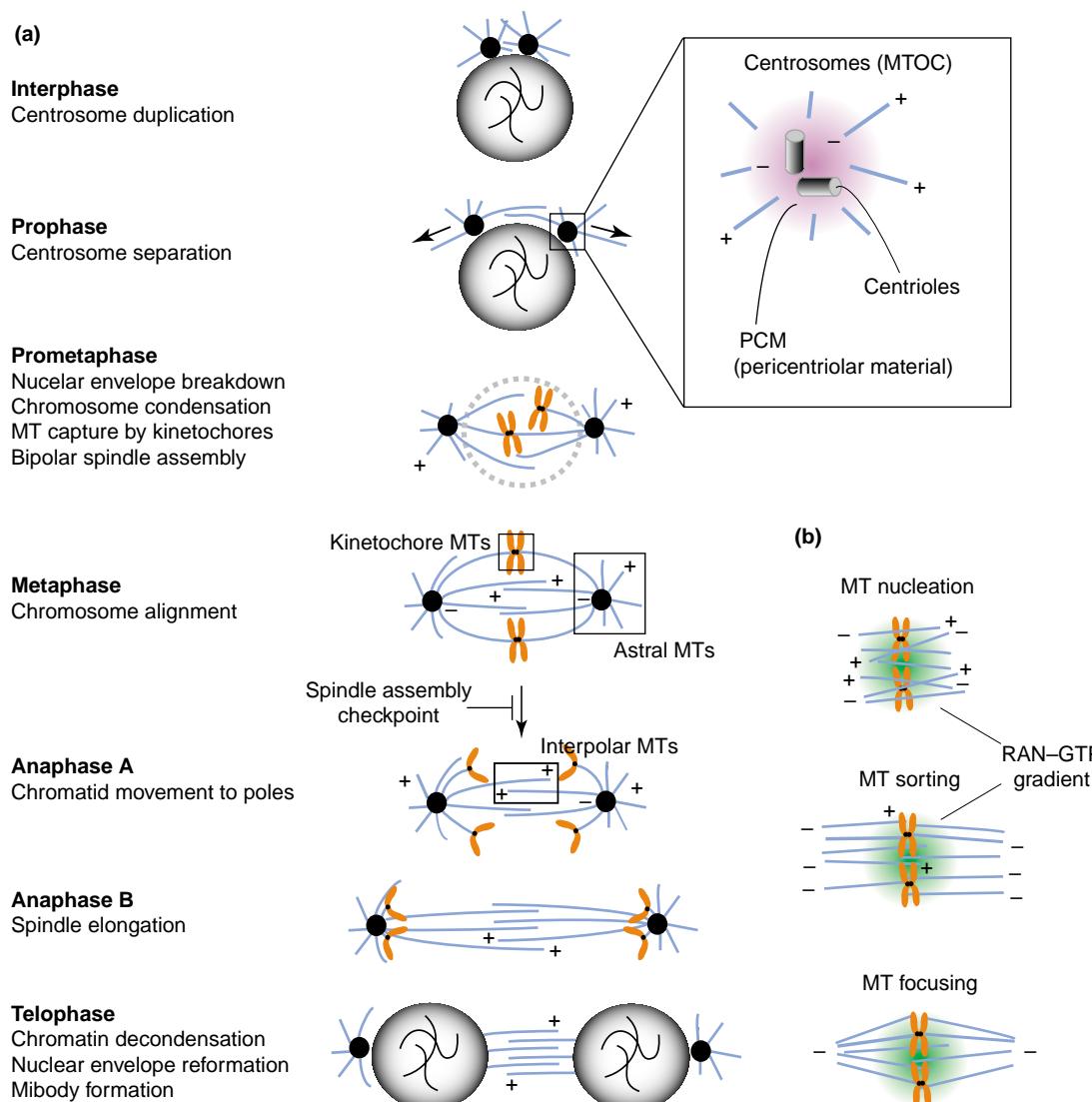


Figure 1.

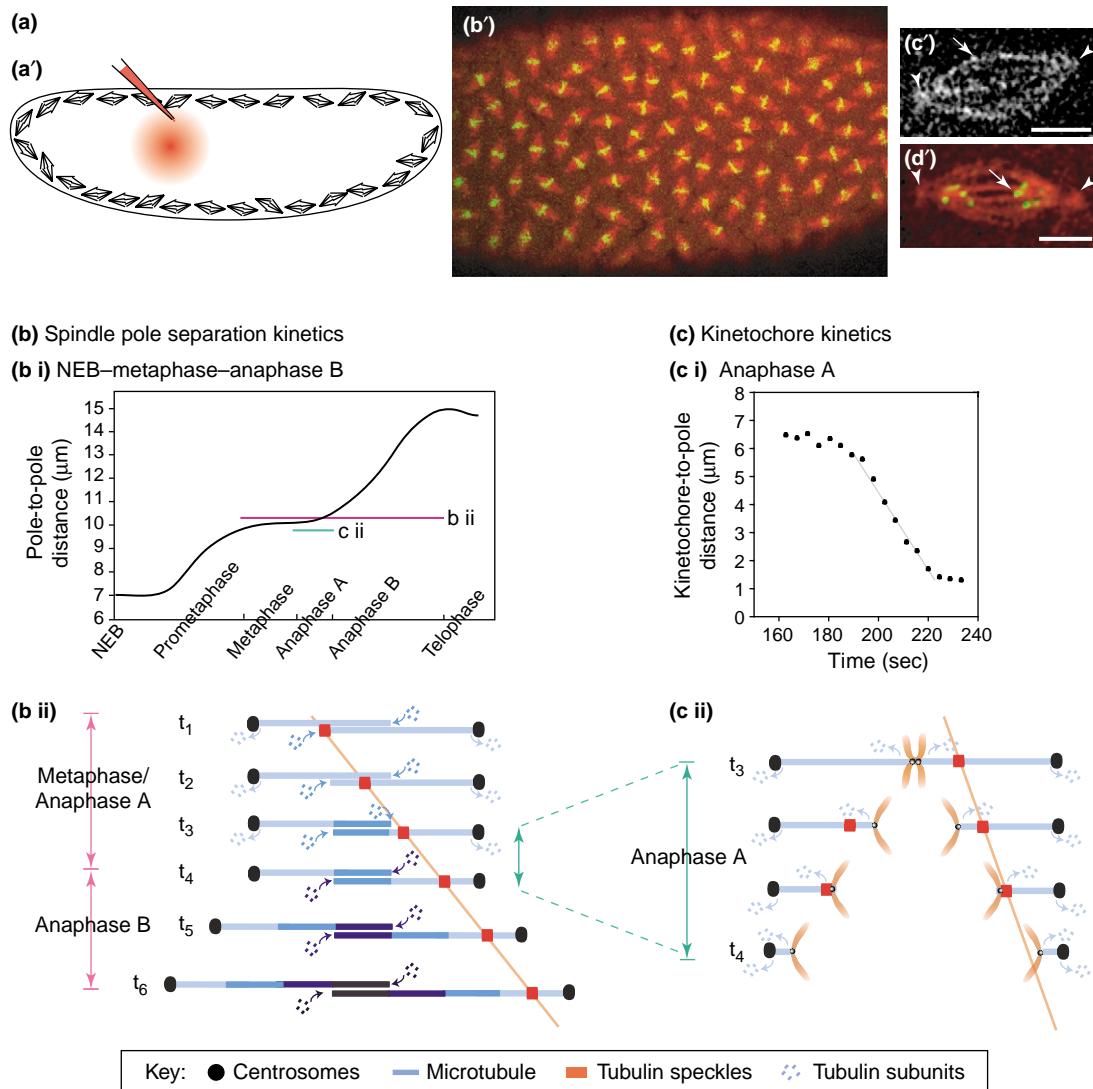
Box 2. Dynamics of MTs, chromosomes and spindle poles in Drosophila embryos

(a) Cartoon and real images of *Drosophila* syncytial embryos [Figure 1(a')]. The embryo can be microinjected with fluorescent probes or soluble inhibitors, such as antibodies, and cortical mitoses can be visualized with high precision in large numbers of spindles using time-lapse confocal microscopy. (b') Rhodamine–tubulin-injected GFP–histone-expressing embryo. High-magnification images obtained using fluorescence speckle microscopy (FSM) (c') and confocal microscopy (d') show spindle poles (arrowheads) and tubulin speckles and kinetochores (arrows), respectively. Bars, 5 μ m.

(b) Spindle-pole dynamics. Plots of spindle-pole separation versus time, from nuclear-envelope breakdown (NEB) –to anaphase B [b(i)], reveal a ‘punctuated-equilibrium’ pathway in which bursts of rapid pole–pole separation are interspersed with quiescent pauses, which is proposed to depend upon antagonistic inward and outward forces acting on the poles. When the difference between the outward and inward forces is great, the poles separate rapidly; as the difference falls, the rate of pole–pole separation decreases; and when the outward and inward forces equilibrate, pole–pole separation distance is maintained constant and the spindle is now a transient steady-state structure. Steady states form at prophase, prometaphase, metaphase and telophase and might represent stages when the spindle pauses to allow other events occur (e.g. NEB, chromosome capture, chromosome alignment and maintenance of spacing between daughter nuclei).

Studies using fluorescence speckle microscopy (FSM) and inhibitors elucidate the contributions of MT polymer-ratchets and mitotic motors to spindle-pole dynamics, providing insights into the mechanisms of anaphase B [b(ii)]. Simultaneous tracking of tubulin speckles on MTs (orange) relative to spindle poles (black) reveals that (i) speckles flux towards the poles at a constant rate ($\sim 0.03\text{--}0.06 \mu\text{m sec}^{-1}$) throughout metaphase–anaphase-A (t1–t4); and (ii) flux stops at the onset of anaphase B as tubulin speckles and poles move at the same rate (t4–t6). Thus, it is thought that net motor forces are counterbalanced by MT depolymerization during metaphase–anaphase-A keeping the poles stationary (t1–t4). The switch from poleward flux to MT–MT sliding occurs at the onset of anaphase B when the cessation of MT depolymerization engages a motor-dependent sliding-filament mechanism that drives spindle elongation.

(c) Chromatid dynamics. Plot of kinetochore-to-pole distance versus time during anaphase A [c(i)] and model showing the relative contributions of pacman (kinetochore-based mechanism) and poleward flux to chromatid movement during anaphase [c(ii)]. In embryos, anaphase A is essentially complete before anaphase B begins so that spindle length is constant as kinetochores move to poles at $\sim 0.11 \mu\text{m sec}^{-1}$; however, poleward flux of tubulin subunits occurs at $0.03\text{--}0.06 \mu\text{m sec}^{-1}$. Thus, flux contributes only 30–60% to anaphase-A rates, with the remainder contributed by pacman-driven kinetochore movement.

**Figure I.**

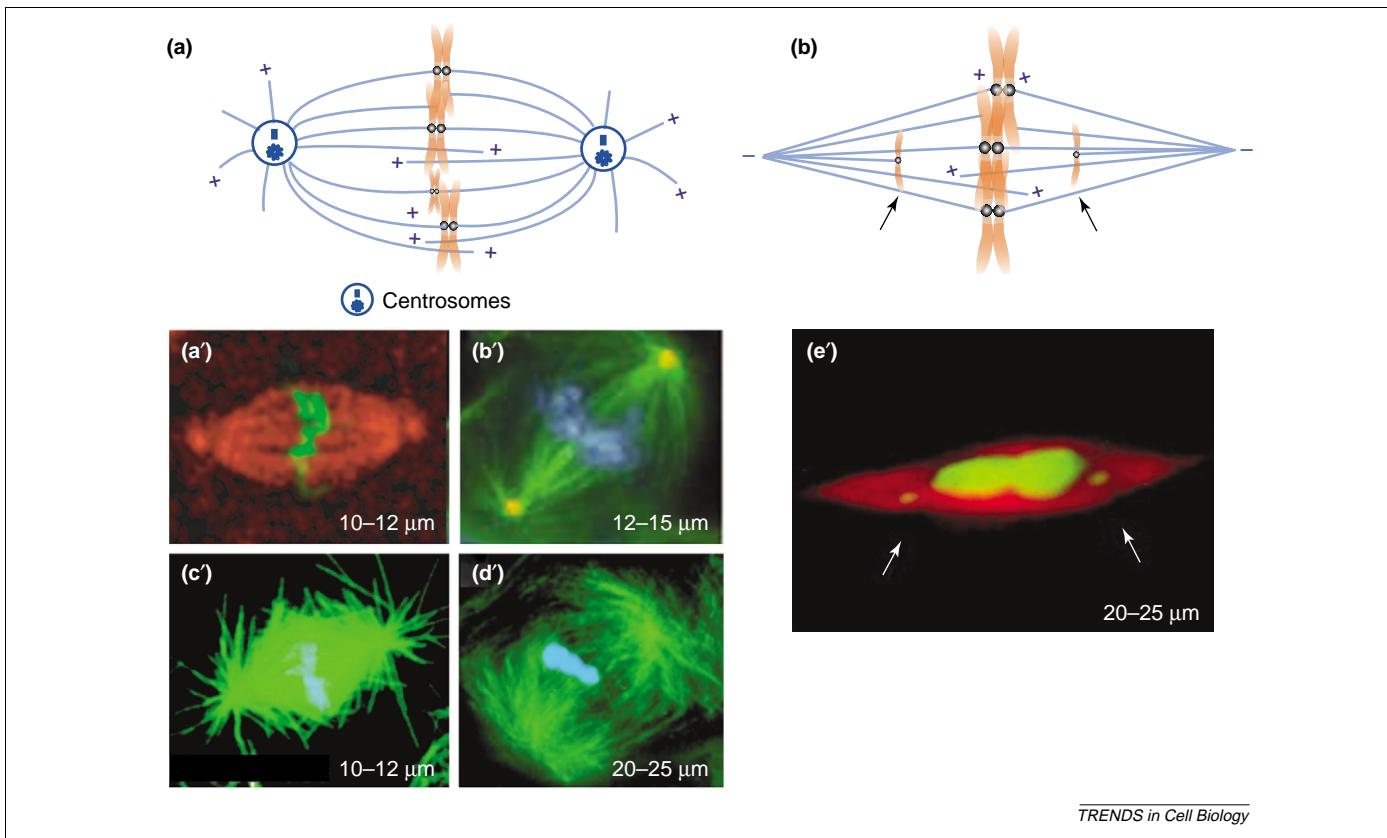


Figure 1. Amphiastral and anastral spindles in different *Drosophila* cell types. Cartoons and micrographs show *Drosophila* centrosome-containing 'amphiastral' spindles [(a) (a'-d')] and noncentrosome-containing 'anastral' spindles [(b) (e')]. Lower panels show confocal fluorescence images of typical metaphase spindles in *Drosophila* syncytial embryos (a'), larval neuroblasts (b'), cultured S2 cells (c'), spermatocytes (d') and meiosis-I oocytes (e'). The meiotic spindle of *Drosophila* oocyte has narrow focused poles, and although most bivalents lie on the metaphase equator, the small nonexchange fourth chromosomes lie midway between the equator and the pole, arrows in (b). (a',e') Tubulin is shown in red and chromosomes in green; (b'-d') tubulin is in green and chromosomes in blue. Average length of metaphase spindle is given below each spindle. (b'-e') Images are adapted from [23,46,63,64], respectively.

various types of spindles found in other *Drosophila* cell types. To the limited extent possible, the review speculates on the poorly addressed question of how different spindles are adapted to their specific niche and what advantages a particular design of spindle might confer. The interesting topics of asymmetric cell divisions [7], how the mitotic machinery is regulated or how the cell division cycle is modulated during development [9] are not discussed here.

Dynamics of the mitotic machinery in *Drosophila*

Chromosome and spindle dynamics in *Drosophila* embryos

The value of the powerful genetics and genomics of *Drosophila* is well known; however, the advantages of using the living syncytial embryo of *Drosophila* for studies of the mechanisms of mitosis are generally less well appreciated, despite decades of productive use of this system [1,10]. The syncytial embryo is specialized for rapid mitoses, functioning as a living factory to repetitively assemble multiple spindles; these divide the single zygotic nucleus into thousands of nuclei that populate the embryonic cortex within approximately two hours (Box 2a). The nuclei, which form a monolayer just under the cell membrane, divide rapidly and synchronously within a common cytoplasm without any intervening cytokinesis. This makes the *Drosophila* embryo ideal for

observing live mitosis and allows the acquisition of high-fidelity kinetic information on spindle and chromosome dynamics based on the observation of a large number of spindles (Box 2). For example, (i) KINETOCHORE dynamics can be monitored using confocal fluorescence microscopy of living transgenic embryos expressing GFP-CID or GFP-MEI S332 kinetochore markers; (ii) spindle-pole dynamics can be monitored in embryos labeled with fluorescent tubulin; and (iii) poleward flux, which describes the persistent movement of tubulin subunits along the MT polymer lattice towards poles, can be distinguished by tracking both polymer ends and tubulin subunits within the polymer using fluorescence speckle microscopy (FSM) [11–13]. This approach has facilitated the recognition that certain subtle aspects of mitotic movements, such as pauses in spindle pole movements, occur in a highly reproducible and stereotypical fashion [14,15]; in addition, when used in conjunction with microinjection of antibodies or dominant-negative inhibitors of specific mitotic proteins, it begins to answer fundamental questions about the sites of force generation within the spindle and the roles of dynamic MT POLYMER-RATCHETS and MT-sliding motors in driving mitotic movements.

Movements of chromosomes to the poles are thought to depend upon two force-generating mechanisms acting on KINETOCHORE MTS (kMTs). These are (i) the 'pacman' mechanism, in which kinetochores catalyze

the depolymerization of kMTs at their plus ends (facing the equator), thereby moving to the poles by ‘chewing up’ MT tracks; and (ii) the ‘poleward flux’ mechanism, in which kMTs are pulled towards the poles through the depolymerization of kMTs at their minus ends (facing the poles) [13]. Recently, using FSM, poleward flux was directly measured in *Drosophila* embryos, as a way of testing its contribution to movements of anaphase-A kinetochores [11–13]. A comparison of the rates of anaphase A and poleward flux reveals that poleward flux is not sufficient to drive anaphase A, suggesting that, in *Drosophila* embryos, a combined flux–pacman mechanism is needed for efficient chromatid movement towards the poles (Box 2c) [11–13]. As discussed below, very recently this has allowed progress in identifying the underlying molecular machinery.

Kinetic analysis of spindle-pole dynamics in live *Drosophila* embryos reveals that, during its assembly and elongation, the spindle passes through multiple transient steady-state structures, which reflect the action of multiple antagonistic and/or complementary forces generated by motors and MT polymer-ratchets (Box 2b) [14–15]. These force generators exert outward and inward forces on the poles; consequently, when the outward forces exceed the inward forces, the spindle poles move apart and when these forces balance, the spindle poles are maintained at a constant spacing, characteristic of the transient steady states. For example, measurements of MT flux reveal that the spindle maintains a constant length during the metaphase–anaphase-A steady state, with net polymerization of INTERPOLAR (IP) MTS at the cell equator and depolymerization at the poles producing constant poleward flux [11]. However, at the onset of anaphase B, MT flux within ipMTs ceases, and consequently, tubulin speckles and spindle poles are observed to move away from the equator at the same rate, suggesting that poleward flux is converted to MT–MT sliding [11]. This indicates that preanaphase-B motors work in a ‘futile cycle’, hydrolyzing ATP to slide apart the slippery tracks of fluxing MTs but exerting no force on the poles; however, at the onset of anaphase B, the sliding apart of nonfluxing MTs starts to push apart the poles, thereby driving spindle elongation. Thus, MT flux might act as a regulatory switch to engage the ipMT-sliding motors that drive spindle elongation by a sliding filament mechanism [11].

Cell division in other Drosophila cell types

Other *Drosophila* cell types have distinct advantages for studying aspects of cell division. For example, analysis of spindle morphology in larval neuroblasts from mutants displaying late larval or pupal lethality has long been used to identify essential mitotic genes and their functions [16,17]. The spindles of meiosis-I oocytes are anastral but contain highly tapered poles, making them very suitable for studies of the mechanism of anastral spindle-pole formation [3–5,18]. Spermatocytes have large spindles and thus are well suited for cytological and micromanipulation studies; because they appear to lack stringent spindle checkpoints, they are also amenable to studies of later mitotic events such as CENTRAL-SPINDLE assembly and cytokinesis [2,19,20]. Recently, with the completion of

the *Drosophila* genome sequence and the application of RNAi techniques, cultured S2 cells have proven to be suitable for the rapid preliminary screening of proteins essential for cell division on a large scale [6,21]; however, detailed analysis is inevitably required for anything beyond a superficial understanding of spindle-protein function.

The long cell-cycle times, relative asynchrony and stringent checkpoints in neuroblasts and S2 cells make it more difficult to perform live cell imaging and kinetic analysis on these other cell types; however, comparative studies on spindle and chromosome dynamics have been valuable in identifying the significance of common and specialized mitotic mechanisms [5,6,8,19]. For example, as noted earlier, a major difference in the pathway of amphialstral and anastral spindle morphogenesis is the use of centrosome-directed and chromosome-directed mechanisms, respectively (Box 1) [3–5,18]. Despite the differences between the amphialstral and the anastral pathways, a good deal of evidence suggests that both mechanisms can coexist, and the chromosome-directed pathway might be conserved even in cell types that contain centrosomes. For example, in somatic cells of *cnn* and *asterless* mutants, which have dramatically reduced levels of centrosomes and asters, the chromosome-directed pathway appears to assemble functional bipolar spindles with fairly focused poles capable of separating chromosomes and completing mitosis [22,23]. More recently, real-time imaging of cultured neuroblasts and S2 cells has revealed that a fraction of wild-type bipolar spindles assemble by the fusion of multiple dispersed polar regions or MT-organizing centers (MTOCs) [6,8]; this suggests that, despite their different spindle architecture, amphialstral spindles can use the assembly mechanism of anastral spindles as an alternative or backup mechanism to establish spindle bipolarity through *de novo* pole formation when the normal mechanism fails [24,25].

Spindle protein-machinery

The completion of the *Drosophila* genome sequence led to the identification of the full inventory of potential mitotic proteins that probably act in this system; these include: five α -, five β - and two γ -tubulins (no ε - or δ -tubulin homologs were found), several nonmotor MAPs, centrosome proteins and mitotic kinases, as well as the entire complement of mitotic motors, which includes 24 kinesins and 12 dynein heavy-chains [26]. An impressive array of tools is available for perturbing the function of these mitotic proteins, including (i) forward genetic screens and mitotic mutant analysis, (ii) microinjection of inhibitors such as function-blocking antibodies or dominant-negative constructs (especially in embryos) (Box 2) and (iii) RNAi for genome-wide screening of protein function in cultured S2 cells. Such studies are uncovering the contribution that each mitotic protein makes to spindle mechanics and dynamics in *Drosophila* (Table 1).

MT-associated motor proteins

On the basis of both the effects of inhibiting a variety of motors by mutation or inhibitor microinjection in *Drosophila* embryos and knockdown of every kinesin and

Table 1. Proteins involved in mitosis in *Drosophila melanogaster*

Protein ^a	Family	Localization	Function	Phenotypes ^b	Refs
MT-associated motor proteins					
KLP61F	Eg5, BimC (Bipolar)	Central spindle	Bipolar spindle maintenance and elongation	Spindle collapse, monopolar spindle ^{a,c,d}	[6,14,27,28]
Ncd	Kin C	Central spindle	Regulation of pole separation rate, MTOC and/or *pole formation	Premature pole separation during interphase ^b , disorganized poles ^{d,f}	[5,6,14,18,28]
KLP10A	Kin I	Spindle poles Kinetochores	Poleward flux	Monopolar or long bipolar spindles ^{c,d} , congression defects and slow anaphase A ^c	[6,13]
KLP59C	Kin I	Kinetochores	Congression and anaphase A	Congression defects and slow anaphase A ^c	[6,13]
KLP67A	Kip3	Astral MT ends	MT destabilization	Unseparated Ctr ^e , monopolar or long bipolar spindles ^d	[6,31]
CENP-meta	CENP-E	Kinetochores	^{+Cs assembly, cytokinesis} Chr congression	Chr misalignment ^{a,b,d}	[6,35]
KLP3A	Chromo K	Chr central spindle	Spindle elongation, Chr alignment, ^{+Cs assembly/cytokinesis}	Short bipolar spindles ^c , disorganized ipMTs and/or Cs ^c , Chr misalignment ^d	[2,6,20,30]
Nod	Chromo K (KID)	Chr	Chr alignment, *nonexchange Chr positioning	Chr misalignment ^{d,f}	[3,6]
KLP38B (<i>tiovivo</i>)	Unc104	Chr	Spindle bipolarity, cytokinesis	Monopolar spindles ^{b,c} , cytokinesis defect ^a	[32,33,39,67]
PAV-KLP (<i>pavarotti</i>)	MKLP, CHO1, ZEN4	Ctr, Cs, cortical actomyosin ring	Cs assembly and/or maintenance, cytokinesis	Disorganized Cs and cleavage furrow, cytokinesis defect ^{b,d}	[6,21,37,38]
Dhc64C	Cytoplasmic dhc	Cell cortex, Kinetochores	Spindle pole separation, Chr congression, anaphase A, mitotic exit	Unseparated Ctr ^{b,c} , short spindles ^c , slow anaphase A ^{c,e} , delay in mitotic exit ^{a,d}	[6,14,19,29,34,36]
Nonmotor MAPs and centrosomal proteins					
Asp	No homolog	Minus end region of MTs at Ctr and Cs	Organization of MTOC and Cs, cytokinesis	Bipolar spindles with dispersed γ -tubulin foci (in wild-type or <i>asl</i> mutant background) ^a	[47–49,60]
Msps	Dis1, TOG, XMAP215	Ctr, MTs, *acentrosomal poles	Spindle integrity, MTOC and *pole formation (by D-TACC)	Spindle disorganization ^a Tripolar female meiotic-spindles ^f	[18,24,51]
Mast or orbit	CLASPs	Ctr, kinetochores, MTs and Cs	Spindle bipolarity, kinetochore attachment and congression,	Spindle collapse, monopolar spindle ^{a,b,d} , misaligned Chr ^{b,d}	[41,42,44]
EB1	Bim1	Plus ends of MTs	Spindle positioning and elongation, Chr segregation	Defects in spindle orientation ^d , elongation ^c , Chr segregation ^c , decrease in aster size ^d	[46]
CNN	No homolog	Mitotic Ctr	γ -Tubulin recruitment to Ctr for MTOC and/or aster formation	Bipolar spindles with decreased γ -tubulin and aster size ^{a,d} , spindle misorientation ^a	[22,54]
D-TACC	TACC	Ctr, *acentrosomal poles	Recruitment of Msps, aster or *pole formation	Bipolar spindles with fewer astral MTs ^{a,b} , tripolar female meiotic spindles ^f	[18,24,52,53]

^aThe words in parentheses indicate the mutant name when it is different from the protein name.

^bThe type of gene disruption and cell is indicated with superscripts as follows: (a) mutant neuroblasts, (b) mutant embryos, (c) inhibition with antibodies in embryos, (d) RNAi in S2 cells, (e) mutant spermatocytes and (f) mutant oocytes.

^cAbbreviations: Asl, asterless; Asp, abnormal spindle; Chr, chromosome; CNN, centrosomin; Cs, central spindle; Ctr, centrosomes; D-TACC, transforming acidic coiled coil; ipMT, interpolar microtubule; Msps, mini spindles; MT, microtubule; MTOC, MT-organizing center.

^dMeiotic roles are shown for male meiosis.

^eMeiotic roles are shown for female meiosis.

cytoplasmic dynein using RNAi in cultured S2 cells, it is clear that at least 11 MT MOTORS act as mitotic motors in *Drosophila* spindles. These motors exert force to transport mitotic cargos or regulate MT dynamics, thus contributing to spindle assembly, control of spindle length and chromatid motility (Table 1).

In various *Drosophila* cell types (as in other organisms), a key player in the morphogenesis and elongation of bipolar amphiastral spindles is the bipolar kinesin KLP61F; the bipolar structure of this protein consists of motor domains on opposite ends of a rod, allowing it to crosslink ipMTs and exert outward forces on spindle poles

using a sliding filament mechanism [6,14,15,27,28]. For example, the function of KLP61F has been examined in the spindles of syncytial blastoderm. In this experiment, spindle-pole dynamics were monitored following the microinjection of motor inhibitors into (i) wild-type embryos, which induces the collapse of prometaphase spindles [28] and (ii) Ncd-null mutant embryos, which circumvents this collapse but inhibits spindle elongation during anaphase B (Box 2) [14,28]. This approach also allowed a dissection of the pathway by which KLP61F cooperates with the C-terminal kinesin Ncd, the chromokinesin KLP3A and cortical dynein to assemble, maintain

and elongate the embryo spindles in a controlled manner [14,29,30]. The best-understood aspect of this pathway is transition from interphase to prophase. During this stage, most mitotic motors are sequestered in the nucleus, and the centrosomes migrate around the nucleus under the influence of a balance of forces. Outward-pulling forces are generated by cortical dynein, which is thought to slide astral MTs relative to cortical actin, and antagonistic inward forces are generated by the minus-end-directed MT-sliding motor Ncd, acting on ipMT bundles. The inward Ncd-generated force persists from the onset of centrosome separation until the onset of anaphase B [14]. Following nuclear envelope breakdown (NEB), motors, in addition to Ncd and dynein, interact with spindle MTs to coordinate metaphase spindle assembly by a poorly understood pathway. Based on the data from the aforementioned microinjection experiments, a KLP61F-dependent sliding-filament mechanism is thought to maintain bipolar spindle assembly during prometaphase and drive the elongation of anaphase-B spindles, with cortical dynein augmenting its action at late stages of anaphase B [14]. Another crucial player is the chromokinesin KLP3A, which associates with mitotic chromosomes and the central spindle, where it organizes bundles of ipMTs and contributes to spindle elongation during prometaphase and anaphase B [30]. Interestingly, KLP3A plays an important role in a switch from flux to sliding that engages the KLP61F-driven elongation of anaphase spindles: KLP3A inhibition leads to persistent poleward flux (that normally stops) during anaphase B and a corresponding decrease in spindle elongation (I. Brust-Mascher *et al.*, pers. commun.).

Two other kinesins that participate in spindle morphogenesis, the Kin-I motor KLP10A and the KIP3 motor KLP67A, modulate the dynamic properties of spindle MTs and contribute to spindle assembly by controlling spindle length; the inhibition of these kinesins leads to strikingly similar phenotypes characterized by extremely long bipolar spindles or monopolar spindles with long MTs [6,13,31]. KLP10A localizes to the poles of embryonic spindles and, based on biochemical data, directly depolymerizes MTs [13]. FSM reveals that, in embryos, this motor plays a crucial role in poleward flux by depolymerizing MTs at the poles and possibly drawing them poleward by a polymer-ratchet mechanism. This MT depolymerization could constrain spindle length during metaphase, and its suppression at the onset of anaphase B might allow KLP61F to drive spindle elongation [11,13]. In contrast to KLP10A, the mechanism of KLP67A is less well understood; it might depolymerize spindle MTs directly, or given its plus-end-directed MT-translocating activity, it might transport true MT depolymerizers, such as Kin-I motors, to their site of action [31].

In addition to KLP3A, *Drosophila* contains two other chromokinesins – Nod and KLP38B. As in other systems, these chromokinesins are thought to associate with chromosome arms and appear to play diverse roles associated with spindles functions in *Drosophila*. For example, the simultaneous inhibition of chromokinesins by RNAi in S2 cells leads to defects in chromosome alignment, suggesting roles in chromosome positioning

[6]. KLP38B is proposed to contribute to the assembly and maintenance of bipolar spindles through chromosome–MT interactions in neuroblasts and embryos [32,33]. By contrast, a primary function of KLP3A is to organize ipMTs into bundles in embryonic spindles, thereby contributing to spindle elongation during prometaphase and anaphase B [30]. Interestingly, KLP3A also appears to be required for normal rates of chromatid movement during anaphase A, because after KLP3A inhibition, anaphase A is slower, raising the intriguing possibility that ipMTs provide structural support for efficient chromatid movement [30]. Some chromokinesins, such as KLP3A and KLP38B, show a dynamic distribution, relocating from chromosomes to central spindles at specific stages of mitosis; thus, the multiple roles of these motors might not be surprising.

The mechanisms that drive chromatid segregation during anaphase have been investigated for over a century, and new work in *Drosophila* embryos has identified two Kin-I mitotic motors, KLP10A and KLP59C, that appear to drive a concerted pacman and poleward flux mechanism [13]. In contrast to other organisms, in *Drosophila*, these motors are spatially segregated, with KLP59C being localized to kinetochores and KLP10A to the poles. This allows specific inhibition of both KLP59C, which slows down anaphase A to a rate identical to that of poleward flux, and KLP10A, which slows anaphase A to a rate corresponding to kinetochore-based pacman mechanism [13]. These results suggest that anaphase A utilizes a Kin-I-dependent flux–pacman mechanism in which KLP10A at the poles and KLP59C at the kinetochores depolymerize both ends of kMTs. Kinetochore-associated dynein is thought to contribute to this mechanism by ‘feeding’ MTs into the kinetochore for efficient KLP59C-dependent depolymerization; consequently, the functional perturbation of dynein also slows down anaphase A [19,34].

The kinetochore is also a site of association for many checkpoint proteins that detect MT attachment and/or tension; the kinetochore motors dynein and CENP-meta might participate in this control system. In embryos that do not have a robust SPINDLE ASSEMBLY CHECKPOINT, the disruption of CENP-meta and dynein results in chromosome congression defects, suggesting that, the main function of these motors is accurate positioning of kinetochores [34,35]. However, in cells such as neuroblasts and S2 cells, which have more stringent checkpoints, dynein probably has roles in mitotic exit by transporting regulatory checkpoint proteins away from the kinetochore [6,36].

The deployment of motors to carry out different tasks in different cell types are apparent in the case of Ncd, underscoring the fact that findings from one type of spindle might not be applicable to all spindles. In spindles of S2 cells and oocytes, the main role of Ncd is focused pole formation [5,6,18], whereas, in embryos, Ncd serves as a brake that restrains spindle elongation by antagonizing KLP61F [14,28]. The formation of spindles with dispersed polar regions in Ncd-disrupted cells is thought to reflect a role for Ncd in either crosslinking MTs at poles or transporting MT-stabilizing proteins, such as Msps

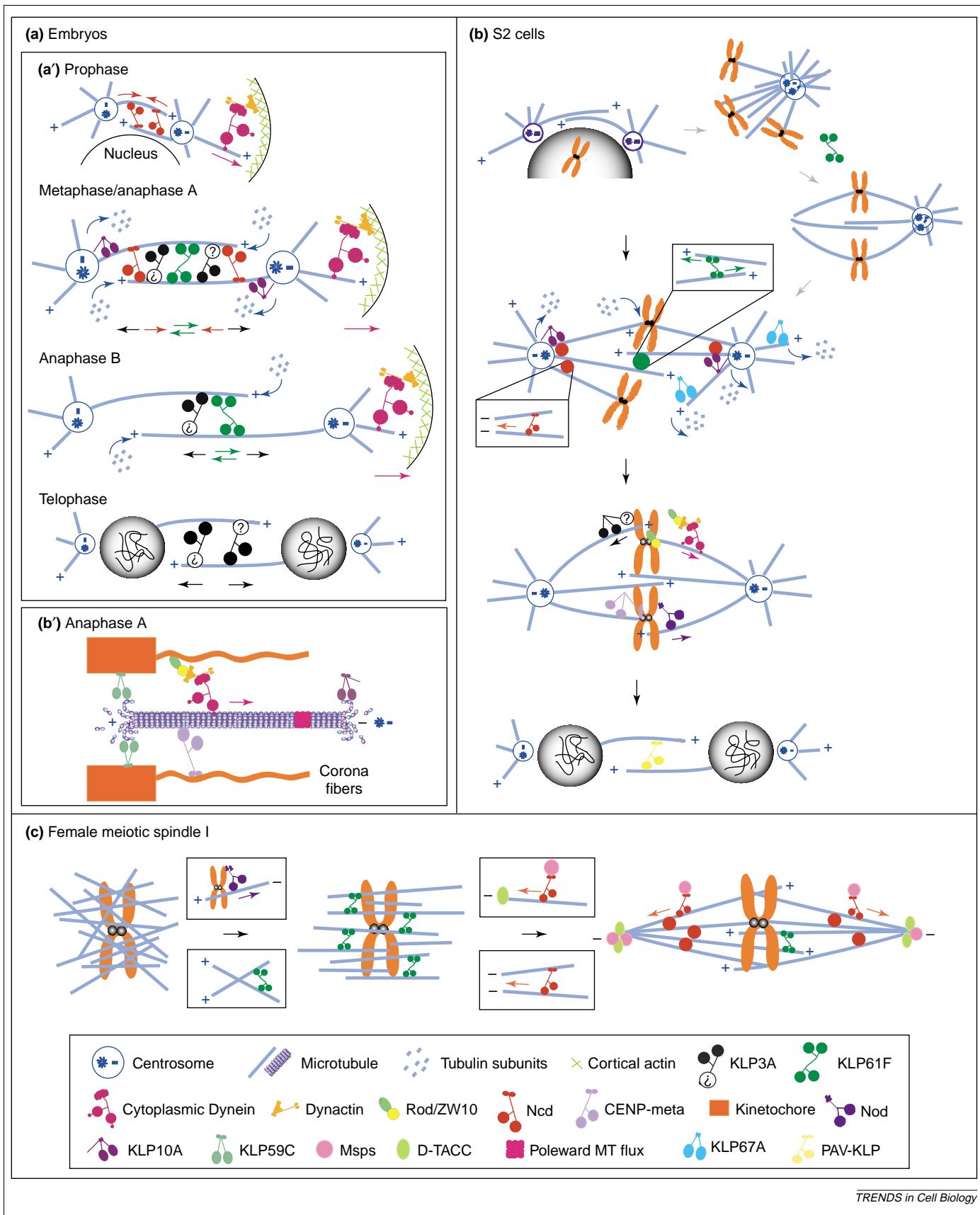


Figure 2. Models for spindle formation and function in different *Drosophila* cell types. **(a)** Spindle morphogenesis and chromatid segregation in embryos: **(a')** Spindle assembly and elongation by a force–balance transient steady-state mechanism [11,30,62] (model modified from [30]). During prophase, centrosome separation depends upon antagonistic outward and inward forces generated by cortical dynein and Ncd on interpolar (ip) microtubules (MTs), which pull astral MTs at the cortex and draw centrosomes together, respectively. After nuclear envelope breakdown, KLP61F and KLP3A can act on MTs in the central spindle and around chromosomes to augment the outward force, whereas Ncd continues to generate an antagonistic inward force; together these motors drive the prometaphase-to-metaphase transition. During

(the *Drosophila* homolog of *Xenopus* XMAP215), to the poles [5,6,18].

Finally, cytokinesis in different cell types seems to require different kinesins. Several motors, including KLP3A, PAV-KLP, KLP38B and KLP67A, play roles in organizing central spindles for efficient cell division in various *Drosophila* cell types [2,6,20,21,30,31,37–39].

MAPs and centrosomal proteins

Several *Drosophila* nonmotor MAPs (Table 1) are thought to act primarily by modulating MT dynamics, contributing to force generation in the spindle by influencing the polymer-ratchet action of dynamic MTs or linking spindle MTs to other structures.

Two MAPs, Mast (also called orbit – the protein was identified and named independently in two mutant screens) and EB1, appear to mediate interactions between the plus ends of MTs and either kinetochores or the cell cortex. Mast is a CLASP (CLIP-associating protein) ortholog that interacts with plus-end-tracking proteins [40]; it localizes to kinetochores, where it might control kMT dynamics, thereby contributing to kinetochore positioning and the stability of bipolar spindle [41–43]. This explains why S2 cells treated with Mast–RNAi, as well as *mast*-mutant embryos, display defects in kinetochore attachment and/or congression and also display spindle collapse [44]. Studies on mast homologs in yeast suggest that Mast could also function cooperatively with KLP61F to stabilize spindle MTs and to support spindle bipolarity [45]. *Drosophila* EB1 also localizes to the growing plus ends of MTs and its role has been examined by RNAi in S2 cells and by time-lapse microscopy of live embryos following microinjection of EB1 antibody [46]. Loss of EB1 function produced short mispositioned anastral spindles with defects in anaphase elongation, suggesting that EB1 is required for the positioning and dynamics of spindles. EB1 might promote the growth of astral and ipMTs, thereby allowing cortical dynein and KLP61F to act on these MT arrays and mediate efficient spindle elongation. Also, the observed defects in chromosome segregation suggest that, during mitosis, EB1 might regulate kMT dynamics and kinetochore–MT attachment.

The coiled-coil protein Asp (abnormal spindle) localizes to spindle poles and to central spindles; it is proposed to bind to the minus ends of spindle MTs [47–49]. The finding that Asp can confer MT nucleation capability to SALT-STRIPPED CENTROSOMES suggested that Asp might play an important role as a MT nucleator [48,50]. However, *asp* mutants have splayed spindle poles, particularly in acentrosomal spindles; this indicates that Asp functions mainly to focus MTs previously nucleated by either polar MTOCs or chromosomes to produce tapered poles [48,49]. The MT-dependent centrosome-independent localization of Asp to the spindle poles has led to proposals that it might be a functional ortholog of NuMA (nuclear mitotic-apparatus protein) in *Drosophila*, serving to organize spindle poles by stabilizing or bundling MTs at their minus ends [49].

Msp (mini spindles) localizes to mitotic centrosomes, anastral spindle poles and spindle MTs, where it performs a general role in maintaining spindle integrity and controlling MT length in spindles [18,51]. During mitosis, Msp is thought to cooperate with the centrosomal protein D-TACC (the *Drosophila* homolog of TACC protein) to stabilize astral MTs in amphiastral mitotic spindles; its inhibition leads to the formation of small asters, whereas overexpression of the conserved TACC domain induces Msp-dependent ectopic asters that do not contain γ -Tubulin [24,52,53]. Further studies are needed to test whether Msp promotes the formation of long MTs by acting directly on MT dynamics.

Meiotic spindles of anastral females appear to deploy the same mitotic proteins in different ways. For example, the organization of tapered acentrosomal poles requires the concerted activity of a MAP–motor complex consisting of D-TACC, Msp and Ncd, because meiotic spindles of *msps*, *d-tacc* or *ncd* mutants are either tripolar or display unfocused poles [5,18]. The observation of decreased Msp accumulation at the polar regions of *d-tacc* and *ncd* mutants lead to the intriguing proposal that Ncd transports Msp to polar regions, whereas the Msp–D-TACC interaction is required to anchor MTs for the formation of focused poles.

The mitotic centrosome-associated protein, centrosomin (CNN), seems to be crucial for both the centrosome-directed pathway of spindle assembly and the stability of

metaphase–anaphase-A transition, the outward force generated by central-spindle motors and dynein is balanced by poleward flux driven by KLP10A so the poles maintain a constant spacing. At the onset of anaphase B, the downregulation of Ncd and the cessation of poleward flux trigger the switch from poleward flux to MT–MT sliding, so that forces generated by ipMT-sliding motors act on the poles to drive spindle elongation. During telophase, KLP3A functions in the assembly of central spindle and the maintenance of spacing between daughter nuclei. (b) Kinesin-dependent ‘flux–pacman’ model for anaphase A (adapted from [13]). The concerted activity of KLP59C (Kin I) and dynein produces a ‘pacman’ mechanism in which the kinetochore depolymerizes the plus ends of kMTs and ‘chews’ its way to the poles. At kinetochores, CENP-meta probably contributes to this mechanism but its precise role is unclear [35]. At the poles, KLP10A (Kin I) actively depolymerizes the minus ends of kMTs to drive poleward flux, which contributes to ~30–60% of anaphase A in this system. (b) Spindle assembly in cultured S2 cells. In S2 cells, spindle bipolarity requires the cooperation of multiple mitotic motors including KLP61F, which serves to crosslink ipMTs; Ncd, which is required to focus spindle poles; and the plus-end motor KLP67A and the Kin-I motor KLP10A, which cooperate to regulate spindle length by destabilizing spindle MTs at their plus ends and the poles, respectively. In S2 cells, collapsed monopolar spindles resulting from KLP10A or KLP67A inhibition can be rescued to form bipolar spindles; this is achieved through a backup mechanism that depends upon KLP61F and utilizes *de novo* pole formation by focusing MTs to produce bipolar monastral spindles (pathway shown with gray arrows). Chromosome alignment to the metaphase plate in S2 cells requires the coordinated activity of chromokinesins, such as Nod and KLP3A, and also the kinetochore motor CENP-meta. In contrast to the obvious requirement for dynein in embryonic mitosis, in S2 cells, dynein does not appear to have any function associated with the mitotic spindle; however, it might serve to control the timing of anaphase-A onset, presumably by transporting checkpoint proteins, such as Rod/ZW10, outward from the kinetochore towards poles along the kMTs. Currently, it is difficult to study anaphase A and B in S2 cells, and thus no information is available. During telophase, however, PAV–KLP organizes and maintains central spindle for successful cytokinesis. (c) Anastral spindle assembly during female meiosis. Meiosis-I spindles in female *Drosophila* lack centrosomes but can form through an ‘inside-out’ chromosome-dependent assembly pathway. Initially, the MTs that are randomly nucleated around chromosomes become aligned and sorted into bipolar arrays by the MT-cross-linking motor KLP61F, and probably by plus-end-directed chromokinesins that can push minus ends of MTs away from the chromosomes. The Ncd–D-TACC–Msp pathway serves to focus polar MTs into highly tapered poles by a mechanism in which Ncd transports Msp to centrosomes, where they anchor MTs to polar regions through their interaction with D-TACC [18]. In addition, Ncd is capable of crosslinking MTs and zipping them together as it translocates towards the minus ends of MTs, thus focusing the poles [5].

astral MTs; *cnn* mutants form bipolar spindles containing no detectable γ -Tubulin or astral MTs, presumably by a chromosome-directed pathway [22,54]. In addition, over-expression of CNN induces ectopic asters that contain γ -Tubulin, supporting the idea that CNN primarily functions by recruiting MT-nucleating γ -Tubulin complexes to centrosomes [54].

Mitotic kinases

How are the activities of spindle components regulated to coordinate the dynamics of mitosis? Unfortunately, the important but poorly understood question of the regulation of force generation by mitotic MTs, motors and MAPs is considered to be beyond the scope of the current review. However, several recent studies have elucidated the regulatory function of *Drosophila* mitotic kinases, particularly the aurora and polo kinases, in spindle and chromosome dynamics, as well as in cytokinesis [2,20,24,27,37,53–61]. As progress in this area continues, its impact on studies of spindle mechanics and dynamics will become increasingly more significant.

Models for mitosis in *Drosophila*

The information described above on the mechanics and dynamics of the spindle machinery in *Drosophila*, combined with the properties of individual mitotic proteins, has allowed investigators to propose qualitative molecular models for how multiple mitotic proteins cooperate as ensembles to drive the events of mitosis and meiosis with high precision (Figure 2). These models are only a start, but they begin to explain events such as the molecular mechanisms of poleward flux, spindle pole and chromatid movements in embryos, the function of multiple mitotic motors in S2 cells, and the chromosome-directed spindle-assembly pathway in oocytes (Figure 2). Among the most notable advances made using *Drosophila* are:

- the proposal of a force–balance-dependent transient steady-state model for spindle assembly and elongation in embryos (Figure 2a, a');
- the discovery of the chromosome-directed anastral spindle-assembly pathway in oocytes (Figure 2c) and its deployment as a backup mechanism in cultured S2 cells, which normally use the centrosome-directed assembly pathway (Figure 2b);
- the striking discovery of a concerted Kin-I-dependent flux–pacman mechanism for chromatid-to-pole movement during anaphase A (Figure 2a, b').

Beyond these qualitative models, the extensive data obtained in this system allow workers to propose quantitative models in which the dynamics of mitosis observed in living *Drosophila* cells is explained in terms of the properties of individual underlying force-generating molecules. For example, a mathematical force–balance model for spindle pole movement during early spindle morphogenesis has been proposed and is now being extended to other aspects of mitosis [62].

Concluding remarks

A reasonable qualitative understanding of the mechanism of mitosis, at least in outline, has been gained; however,

much remains to be learned, and *Drosophila* will very likely remain an important system for future advances. One area that probably will progress rapidly is the identification of the full inventory of key mitotic proteins, including nonmotor MAPs, mitotic kinases and centrosome components, through the use of RNAi in S2 cells. By complementing these studies with high-fidelity analysis of spindle and chromosome dynamics, in the hundreds of synchronously dividing syncytial blastoderm nuclei, it should be possible to determine the precise contributions of mitotic proteins, working individually and as ensembles, to spindle mechanics and dynamics. These studies will probably improve understanding of issues such as the role of MAPs in the regulation of spindle-MT dynamics, including poleward flux, dynamic instability and MT–MT sliding *in vivo*, and how MAPs and motors cooperate in pathways such as the Ncd–Msps–D-TACC pathway or the Mast–KLP61F pathway. An important and emerging topic is understanding how mitotic force generators are regulated in relation to the cell cycle by modulation of their level of expression, functional activity or subcellular distribution. Further progress is anticipated by the complementation of these *in vivo* approaches with biochemical and biophysical analysis of mitotic proteins, many of which can be purified in large amounts and in an active state from insect (Sf9) cells. This will facilitate direct measurements of force and movement generated by *Drosophila* mitotic motors *in vitro*, assays of the effects of purified MAPs on MT dynamics, and examination of the role of mitotic kinases on these *in vitro* activities. Finally, the augmentation of these experimental approaches, with further mathematical modeling, has the potential for providing a deep understanding of the basic molecular and physical mechanisms underlying mitosis, and might begin to elucidate how the different designs of spindle that are found in different cell types within the same organism are adapted to their specific cellular or developmental niche.

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