Chapter 12
Coordination of Embryogenesis by the Centrosome in *Drosophila melanogaster*

Caitlyn Blake-Hedges and Timothy L. Megraw

Abstract The first 3 h of *Drosophila melanogaster* embryo development are exemplified by rapid nuclear divisions within a large syncytium, transforming the zygote to the cellular blastoderm after 13 successive cleavage divisions. As the syncytial embryo develops, it relies on centrosomes and cytoskeletal dynamics to transport nuclei, maintain uniform nuclear distribution throughout cleavage cycles, ensure generation of germ cells, and coordinate cellularization. For the sake of this review, we classify six early embryo stages that rely on processes coordinated by the centrosome and its regulation of the cytoskeleton. The first stage features migration of one of the female pronuclei toward the male pronucleus following maturation of the first embryonic centrosomes. Two subsequent stages distribute the nuclei first axially and then radially in the embryo. The remaining three stages involve centrosome-actin dynamics that control cortical plasma membrane morphogenesis. In this review, we highlight the dynamics of the centrosome and its role in controlling the six stages that culminate in the cellularization of the blastoderm embryo.

12.1 The Development of the Syncytial Embryo: Six Key Steps

Drosophila early embryo development occurs in a large syncytium in 13 rapid and synchronous nuclear cleavage cycles with 10–13 min separating each mitosis. These divisions occur over approximately 2 h, culminating in roughly 6000 nuclei that cellularize in interphase of cycle 14 to form the cellular blastoderm (Foe et al. 1993; Foe and Alberts 1983). During these early cleavage divisions, the centrosome coordinates cytoskeletal dynamics that are essential for proper development.

The centrosome is the major microtubule organizing center (MTOC) in most animal cells and is composed of two centrioles surrounded by the pericentriolar material (PCM) where microtubule assembly occurs. This coordination of

C. Blake-Hedges (✉) · T. L. Megraw
Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL, USA
e-mail: cb16j@my.fsu.edu; timothy.megraw@med.fsu.edu

microtubule production results in a polar microtubule array with the minus ends of the microtubules anchored at the centrosomes and plus ends that can rapidly grow and shrink. In the syncytial embryo, the centrosome is the only known MTOC. The centrosome has only recently been identified as an actin filament organizing center (Farina et al. 2016) but whether this is the case in the early embryo remains to be determined.

Here we describe the six key cell biological and developmental stages that rely on the centrosome and cytoskeletal dynamics during early embryo development. The first stage involves the maturation of the two centrioles contributed by the sperm, migration of one female pronucleus toward the male pronucleus, and the first zygotic division (Fig. 12.1a). The second stage consists of nuclear migrations that distribute the nuclei axially (Fig. 12.1b). The third stage is a perpendicular nuclear migration toward the cortex that generates the syncytial blastoderm (Fig 12.1c). The fourth, fifth, and sixth stages involve cortical membrane reorganization around each nucleus to generate cells (Fig. 12.1d–e). Each stage utilizes the centrosome in very different modes to organize the nuclei, assist in mitotic divisions, and/or form the first embryonic cells.

The first stage occurs during the initiation of embryogenesis, triggered by sperm entry through the anterior micropyle during fertilization in the uterus. Two paternally supplied centrioles mature and replicate utilizing maternally supplied PCM and centriolar proteins to form the first two embryonic centrosomes (Blachon et al. 2014). These centrosomes nucleate microtubules, termed the sperm aster, that assist in the migration of one female pronucleus toward the male pronucleus (Fig. 12.1a) (Callaini and Riparbelli 1996; Riparbelli et al. 2000). The first zygotic division is orchestrated by the newly formed centrosome pair, and four subsequent cleavage cycles precede the remaining centrosome-dependent stages.

During the second stage, axial nuclear migration, the early nuclei distribute evenly along the anterior-posterior (A-P) axis during cleavage cycles 4–7 (Fig. 12.1b). Localized actomyosin cortical contractions produce cytoplasmic streaming that assists in this nuclear migration (Royou et al. 2002; von Dassow and Schubiger 1994; Wheatley et al. 1995).

The third stage, cortical nuclear migration, positions the majority of the nuclei evenly along the cortex during cleavage cycles 7–9 (Fig 12.1c). Asymmetric microtubules nucleate preferentially toward the interior of the embryo to facilitate in this nuclear migration (Baker et al. 1993). A subset of nuclei, known as the yolk nuclei, remain in the interior of the embryo, complete error-prone replications that result in polyploid nuclei, and eventually lose their centrosomes (Fig. 12.1d) (Foe et al. 1993; Foe and Alberts 1983). Little is known of the molecular regulators of these nuclear migrations, but their function in positioning the nuclei is necessary for subsequent developmental stages (Niki and Okada 1981; Niki 1984; Okada 1982; Hatanaka and Okada 1991).

In the fourth stage, the nuclei that arrive in the posterior pole plasm during cortical migration are the first to cellularize, doing so during cleavage cycles 9–10. These nuclei cellularize before the remainder of the embryo to form the pole cells (primordial germ cells), the future gametic cells of the adult fly (Fig. 12.1d) (Foe and Alberts
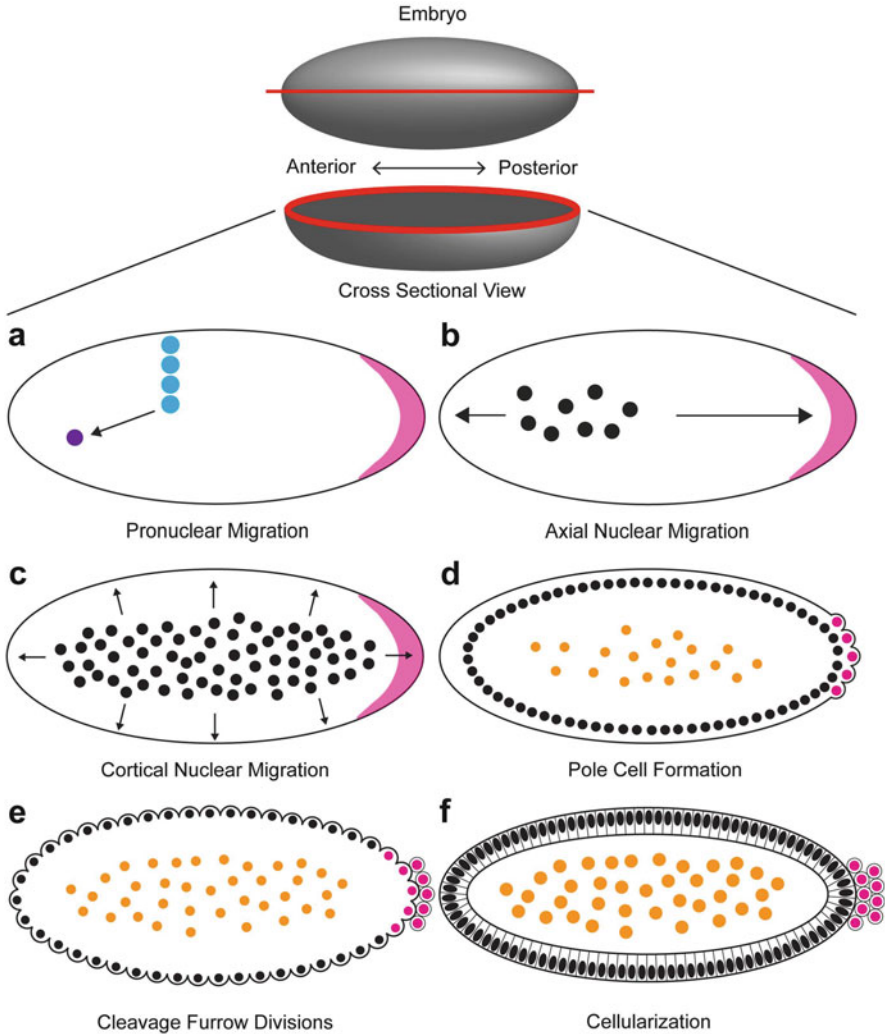
Fig. 12.1 The six stages of the *Drosophila* syncytial embryo that rely on centrosome-cytoskeletal dynamics. Each stage is viewed as a cross section through the anterior-posterior axis (red ring). (**a**) One female pronucleus (blue) migrates toward the male pronucleus (purple) to form the first zygotic nucleus. The pole plasm (pink), which is localized to the posterior of the oocyte during oogenesis, is present at the posterior pole of the embryo. (**b**) During cleavage cycles 4–7, the nuclei migrate along the anterior-posterior axis. (**c**) During cleavage cycles 7–9, a majority of the nuclei migrate to the cortex. (**d**) During cleavage cycles 9–10, a subset of nuclei at the posterior pole cellularize to form the pole cells (pink circles). The yolk nuclei (orange) remain in the interior of the embryo. (**e**) The final four cleavage divisions (10–13) occur at the cortex where membrane invaginations surround each dividing nucleus. (**f**) After the 13th cleavage cycle, the cortical nuclei form distinct cellular membranes during interphase of cycle 14

1983). The pole plasm contains germ cell-specific proteins and mRNAs that are localized to the posterior of the oocyte in a microtubule-dependent manner during oogenesis (Lantz et al. 1999; Mahowald 2001). The pole plasm, which is necessary and sufficient to drive pole cell cellularization, is contained in polar granules that transport to the nuclei dependent on centrosomes and microtubules (Illmensee and Mahowald 1974; Lerit and Gavis 2011; Shamanski and Orr-Weaver 1991). The centrosomes coordinate reorganization of the plasma membrane to surround each nucleus as it divides, until the membrane is pinched off to form separate cells (Fig. 12.1d) (Raff and Glover 1989).

During the fifth stage, the remaining cortical nuclei complete four final divisions that utilize centrosome-dependent actin-microtubule dynamics to reorganize the cortical plasma membrane (Fig. 12.1e). The membrane dynamics resemble the organization of the posterior membrane during the fourth stage, but the membrane does not seal or close to form new cells. These membrane arrangements are termed pseudo-cleavage furrows, or Rappaport furrows (Raff and Glover 1988; Ede and Counce 1956; Turner and Mahowald 1976; Foe and Alberts 1983). These final divisions are important for increasing nuclear numbers and priming the embryo for cellularization.

The final stage succeeds the 13th division and occurs in the 70-min-long interphase of cleavage cycle 14 (Foe and Alberts 1983). The cortical nuclei are surrounded by long membrane invaginations rich in actin and cytokinetic components that cleave at the base to form cells (Fullilove and Jacobson 1971; Warn and Robert-Nicoud 1990; Young et al. 1991) (Fig. 12.1f). The centrosomes and microtubules assist in the membrane invaginations and eventual cell formation. This last step transitions the syncytial embryo to the cellular blastoderm (Zalokar and Erk 1976; Foe and Alberts 1983).

12.2   The Structure of the Embryonic Centrosome and Regulation of Microtubule Assembly

The embryonic centrosome is organized into a pair of centrioles surrounded by the PCM from which microtubules are nucleated and regulated. The embryonic centrioles have a canonical structure similar to differentiated tissue and mammalian centrioles with slight variation in length and the number of radial microtubules. *Drosophila* centrioles do not contain distal and subdistal appendages, structural features found on vertebrate mother centrioles (Callaini et al. 1997). The structure of the syncytial embryo centrioles remains constant throughout all of embryogenesis and into the larval stages, indicating that the structure is not unique to the specificities of the syncytial embryo (Callaini et al. 1997; González et al. 1998).

The embryonic centrioles are ~0.2 μm wide and long, composed of nine doublet microtubules that are all equal in length (Fig. 12.2) (Debec et al. 1999; Moritz et al. 1995; Lattao et al. 2017). The centrioles contain a "cartwheel" structure with a
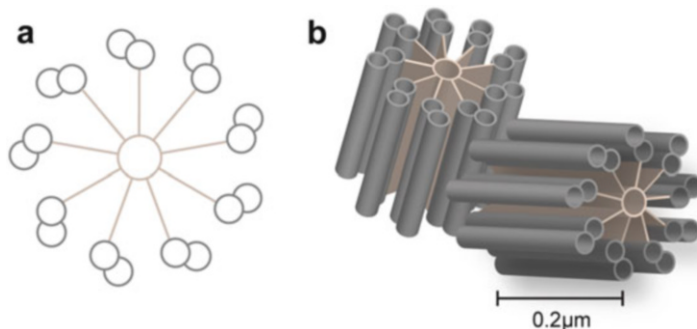
Fig. 12.2 The centrioles of the syncytial *Drosophila* embryo. (**a**) Top view of a centriole containing nine peripheral microtubule doublets (gray) connected through radial spokes to the central hub (brown). (**b**) The centrosome contains two pairs of centrioles that are each as long as they are wide, about 0.2 μm. The centrioles are orthogonal to one another, and the daughter centriole is located at the proximal end of the mother

central hub linked to each peripheral doublet through radial spokes along the entire length (Fig. 12.2) (Debec et al. 1999; Callaini et al. 1997). Differentiated tissues, such as wing epidermal cells (Tucker et al. 1986) and ommatidia sensory bristles (Mogensen et al. 1993), contain microtubule triplets absent of the cartwheel structure, while midgut epithelial cells and rhabdomeric cells contain microtubule doublets with the cartwheel structure (Gottardo et al. 2015). Therefore, over development, some specified cells have different centriolar microtubule compositions. The reasons for these differences and any differential functions they might impart are not known.

The embryo is primed to construct centrioles thanks to the maternal supply of centriolar components, but centriole formation is blocked without the sperm centrioles. This block in activation of centriole biogenesis can be bypassed in unfertilized embryos through overexpression of centriole assembly/replication proteins such as Spindle assembly abnormal 6 (Sas-6) (Peel et al. 2007; Rodrigues-Martins et al. 2007a), Anastral spindle 2 (Ana2), Asterless (Asl) (Stevens et al. 2010), or Polo like kinase 4 (Plk4) (Peel et al. 2007; Rodrigues-Martins et al. 2007b), which drives de novo formation of centrioles.

While embryos are permissive for de novo centriole assembly, ovaries are not. Overexpression of either Spindle assembly abnormal 4 (Sas-4), Sas-6, or Plk4 during oogenesis still allows for the destruction of centrioles in the oocyte, resulting in unfertilized embryos initially absent of centrioles (Peel et al. 2007). De novo centriole formation is never seen in wildtype unfertilized embryos, indicating inhibitory mechanisms that limit centriole formation until fertilization. Dynein plays a negative regulatory role in centriole formation, as a dominant negative form of Dynein Heavy Chain 64C (Dhc64C) in unfertilized embryos causes de novo centriole formation (Belecz et al. 2001).

Most de novo centrioles maintain the typical embryonic architecture, such as during Plk4 overexpression (Rodrigues-Martins et al. 2008), but in some cases the

centrioles have abnormal structures, such as during Sas-6 overexpression or in dominant negative Dynein heavy chain *Dhc64C* embryos. Tube-like structures rather than bona fide centrioles are produced, suggesting a precursory role in centriole biogenesis for Sas-6 and Dynein (Rodrigues-Martins et al. 2007a; Belecz et al. 2001). Although most of these embryos contain a large number of de novo centrioles, it is unclear whether the centrioles can replicate on their own. Rodrigues-Martins et al. showed that de novo centrioles from Plk4 overexpression can form procentrioles (Rodrigues-Martins et al. 2007b), but Peel et al. concluded no replication of de novo centrioles through live imaging of fluorescently tagged and overexpressed Plk4, Sas-4, or Sas-6 embryos (Peel et al. 2007). However, de novo centrioles can recruit PCM and nucleate microtubules (Peel et al. 2007; Rodrigues-Martins et al. 2007a, b, 2008; Stevens et al. 2010; Belecz et al. 2001).

One of the principle components of the PCM, γ-Tubulin, is the main microtubule nucleator at the centrosome. γ-Tubulin is expressed as two isoforms. γTUB37C, a maternal isoform, is expressed only in the ovaries and embryos. γTUB23C is the ubiquitous isoform, but the two isoforms are functionally redundant (Wilson et al. 1997). γ-Tubulin assembles into at least two different complexes that are important for microtubule nucleation and anchoring of the microtubule minus ends to MTOCs. The γ-Tubulin small complex (γ-TuSC) and the γ-Tubulin ring complex (γ-TuRC) are composed of γ-Tubulin complex proteins (GCPs) that contain grip domains which associate with γ-Tubulin and with other GCPs (Gunawardane et al. 2000; Oakley 2000; Farache et al. 2018; Lin et al. 2015; Kollman et al. 2015; Oakley et al. 2015).

γ-Tubulin is essential for syncytial embryo development (Tavosanis et al. 1997). *γTUB37C* mutants cannot nucleate astral microtubules and PCM recruitment is disrupted although spindles can still form (Wilson and Borisy 1998; Llamazares et al. 1999). γ-Tubulin complexes are recruited to the centrosome by the PCM component Centrosomin (Cnn) by the Centrosomin Motif 1 (CM1) domain of Cnn (Zhang and Megraw 2007; Chen et al. 2017).

In somatic tissues, Cnn is required for γ-Tubulin accumulation at the centrosome as well as astral microtubule production and PCM recruitment (Megraw et al. 1999; 2001; Mahoney et al. 2006). In the embryonic cleavage divisions of *cnn* maternal-effect mutants, PCM components such as γ-Tubulin are severely depleted at the centrosomes, but the centrioles can support a reduced amount of microtubule assembly, evident by small astral microtubules (Zhang and Megraw 2007). However, once the centrioles are lost, particularly in the later cleavage cycles, the centrioles cannot be properly maintained at the spindle poles, resulting in no detectable microtubule asters (Lucas and Raff 2007). Other PCM components are absent from the centrosome or are transiently recruited in *cnn* mutants, consistent with its primary role in recruiting PCM components (Zhang and Megraw 2007; Lucas and Raff 2007; Megraw et al. 1999; Vaizel-Ohayon and Schejter 1999).

The microtubules nucleated from γ-Tubulin are stabilized by Transforming acidic coiled-coil protein (Tacc) and Minispindles (Msps), which form a complex that is required for microtubule assembly and regulates astral microtubule length. Tacc-Msps localize at the centrosomes, and Tacc appears to recruit Msps. *tacc* mutants

display a reduction in Msps localization at the centrosomes and a reduction in astral microtubules. Overexpression of Tacc causes a greater density of astral microtubules and more Msps recruitment to the centrosome than wildtype (Gergely et al. 2000a; Lee et al. 2001).

Tacc-Msps localization at the centrosomes is dependent on Aurora A (AurA) and the CM1 domain of Cnn (Gergely et al. 2000b; Barros et al. 2005; Cullen and Ohkura 2001; Lee et al. 2001; Giet et al. 2002; Zhang and Megraw 2007). *aurA* mutants display less localization of Tacc-Msps and shorter astral microtubules (Giet et al. 2002), while null *cnn* or CM1 domain mutants ($cnn^{\Delta 1}$) still partially recruit Tacc-Msps (Zhang and Megraw 2007). The CM1 of Cnn domain also recruits γ-Tubulin, and recent work has revealed that Msps orthologs (Stu2 and Alp14) directly binds to γ-Tubulin complex proteins to assist in microtubule nucleation through their tumor overexpressed gene (TOG) domains (Gunzelmann et al. 2018; Flor-Parra et al. 2018; Nithianantham et al. 2018). Therefore, reduced Tacc-Msps in *cnn* mutants may be due to reduced γ-Tubulin localization at the centrosomes.

For broader coverage of the centrosome and MTOCs, see Centrosomal and Non-centrosomal Microtubule-Organizing Centers (MTOCs) in *Drosophila melanogaster* (Tillery et al. 2018).

12.3   Fertilization and the First Zygotic Division

Embryogenesis of the zygote begins with syngamy of the haploid female and male pronuclei at fertilization. Sperm entry, pronuclear migration, and the first zygotic division all occur within 15 min and rely on the complementary contributions of the paternal centrioles and maternally supplied PCM components (Foe et al. 1993). The sperm enters the egg in the uterus and female meiosis, arrested in metaphase I, is activated by passage through the oviduct (Von Stetina and Orr-Weaver 2011). Meiosis produces four pronuclei that are arranged in a row perpendicular to the cortex (Fig. 12.3a–c). The sperm supplies two centrioles that immediately recruit maternal PCM components and assemble astral microtubules that stretch toward the cortex, termed the sperm aster (Fig. 12.3b–c) (Callaini and Riparbelli 1996; Riparbelli et al. 2000). The female pronucleus farthest from the cortex (and closest to the sperm aster) migrates toward the male pronucleus along the sperm aster until the two pronuclei are in apposition (Fig. 12.3c–d). The first zygotic division proceeds utilizing the newly matured centrioles and their templated daughter centrioles that form the first embryonic centrosomes (Loppin et al. 2015).

Two centrioles are supplied by the sperm: a larger "giant" centriole (GC), derived from the basal body, and the smaller unconventional centriole, referred to as the PCL (proximal centriole-like) (Fig. 12.3a) (Blachon et al. 2009; Blachon et al. 2014). The PCL is unconventional in that it lacks centriolar microtubules (Khire et al. 2016). During centriole maturation, maternal PCM components including Cnn, Asl, γ-Tubulin, Spindle defective 2 (Spd2), Pericentrin-like protein (Plp), and Centrosomal protein 190kD (CP190) localize at the GC and PCL upon sperm
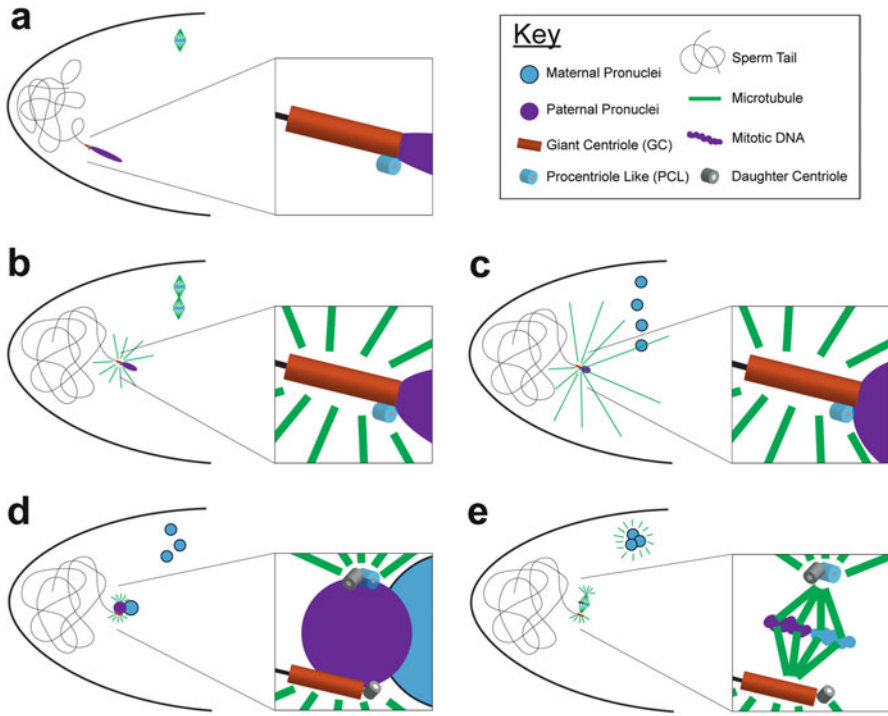
Fig. 12.3 Fertilization in the syncytial embryo utilizes paternally supplied centrioles for pronuclear migration and the first zygotic division. (**a**) The sperm enters the egg from the anterior micropyle, while the female chromosomes reactivate meiosis I. The sperm supplies two centrioles, the larger GC and smaller PCL. (**b**) The GC and PCL nucleate microtubules, termed the sperm aster, while the female chromosomes complete meiosis II. (**c**) The microtubules of the sperm aster reach the female pronucleus furthest from the cortex to facilitate in its migration. (**d**) The female pronucleus migrates toward the male pronucleus and the sperm aster diminishes. The GC and PCL separate to opposite poles of the male pronucleus, divide, and nucleate astral microtubules to prepare for the division. (**e**) The centrioles replicate to form two functioning centrosomes that aid in the gonomeric division of the female and male pronuclei. The remaining female pronuclei condense into polar bodies and utilize cytoskeletal elements to keep them separate and inactive. Figure based on Loppin et al. (2015)

entry (Blachon et al. 2014; Callaini et al. 1999; Khire et al. 2016). PCM recruitment to sperm centrioles is reliant on Spd, as *spd2* mutants recruit Asl but fail to recruit Cnn and have impaired sperm aster formation and pronuclear migration (Dix and Raff 2007). This requirement of Spd2 for sperm centriole maturation differs from somatic cells where loss of Spd2 only partially impedes PCM recruitment, allowing for significant centrosome activity to remain (Dix and Raff 2007; Giansanti et al. 2008).

Following PCM recruitment/maturation of the sperm centrioles and the completion of female meiosis, the sperm aster facilitates in the migration of one female pronucleus to the male pronucleus (Fig. 12.3c–d) (Loppin et al. 2015). The three

remaining female pronuclei become polar bodies, which do not divide and remain throughout the syncytial embryo stages (Fig. 12.3c–d) (Dävring and Sunner 1973).

MTOC function and sperm aster assembly are essential for pronucleus migration. Loss of Asl or Spd2 disrupts sperm aster formation and pronuclear migration fails (Blachon et al. 2014; Varmark et al. 2007; Dix and Raff 2007). Loss of the PCM component, Asp, or the spindle microtubule regulators, Polo or Wispy (Wisp), produces a weaker phenotype where the sperm aster forms but does not fully extend toward the cortex, also preventing pronuclear migration (Riparbelli et al. 2002; Riparbelli et al. 2000; Brent et al. 2000). Loss of Tacc, which regulates microtubule stability, results in pronuclear migration failure. *tacc* mutants that survive to later stages of embryogenesis display diminished aster and spindle microtubules, suggesting failure of pronuclear migration may be due to a diminished sperm aster (Gergely et al. 2000b).

The Linker of Nucleoskeleton and Cytoskeleton (LINC) complex helps maintain centrosome-nucleus connections (Hieda 2017) and facilitates female pronuclear migration in zebrafish and *Caenorhabditis elegans* embryos (Lindeman and Pelegri 2012; Malone et al. 2003). However, in *Drosophila*, Klarischt (Klar) and Muscle-Specific Protein 300 kDa (Msp300), both LINC complex components, are not necessary for pronuclear migration (Technau and Roth 2008). In *C. elegans,* the LINC complex cooperates with microtubule motor proteins to assist in pronuclear migration (Meyerzon et al. 2009), and while the LINC complex is not necessary in *Drosophila*, motors are needed. What tethers nuclei to the cytoskeleton during pronuclear migration and other nuclear movements in early *Drosophila* embryos remains unknown.

The kinesins Non-claret disjunctional (Ncd), Subito (Sub), and Kinesin-like protein at 3A (Klp3A) all play roles in pronuclear migration. The female pronucleus migrates in a minus-end-directed manner, and Ncd, a microtubule minus-end-directed motor, assists in this migration in conjunction with an isoform of α-Tubulin, αTub67C (Komma and Endow 1997). αTub67C is a maternal-specific isoform of α-Tubulin with the special property of conveying faster microtubule assembly (Venkei et al. 2006). Sub is a kinesin involved in antiparallel microtubule bundling and *sub* mutants display similar phenotypes to *polo*, *wispy*, and *α-tub67C* mutants, where the mitotic spindles do not form properly and the embryos arrest early without any zygotic divisions (Giunta et al. 2002; Cesario et al. 2006). Therefore, Sub may play a role in attaching the female pronucleus to the sperm aster through microtubule interactions. Loss of Klp3A prevents pronuclear migration, but Klp3A is a plus-end-directed motor, implicating an indirect role in pronuclear migration. Klp3A recruits Polo, suggesting that it may regulate the formation of the sperm aster, which is necessary for pronuclear migration (Glover 2005; Williams et al. 1997).

During migration the pronuclei swell until the nuclei are in apposition to one another, resulting in a slightly larger female pronucleus (Fig. 12.3d) (Callaini and Riparbelli 1996). The paternal centrioles separate to opposite poles once the pronuclei are apposed and template daughter centrioles to form two functioning centrosomes that aid in the first zygotic division (Fig. 12.3d–e) (Blachon et al. 2014).

This division is gonomeric because the female and male chromosomes remain separated on the metaphase plate until telophase when they join to form two diploid zygotic nuclei (Fig. 12.3e) (Callaini and Riparbelli 1996).

In order to properly replicate and recruit PCM proteins, the PCL requires unique components such as Proteome of centrioles 1 (Poc1). In *poc1* mutant testis, the PCL does not assemble and sperm contain only a GC. In *poc1* paternal effect mutant embryos, PCM proteins are recruited to the GC only (because no PCL is delivered with the sperm), resulting in monopolar spindles that contain only the GC and its replicated daughter centriole (Khire et al. 2016).

The ultrastructure of each centriole goes through multiple changes, and centriolar components such as Anastral spindle 1 (Ana1), Ana2, Asl, Sas-4, and Sas-6 are stripped away during spermatogenesis (Khire et al. 2016; Blachon et al. 2014). These components are maternally supplied in the embryo, and at least Asl is recruited to the sperm centrioles, while Sas-4 and Sas-6 remain absent from the GC and PCL. However, when the centrioles replicate, Sas-4 and Sas-6 are present at the newly formed daughter centrioles (Blachon et al. 2014).

For more on *Drosophila* fertilization, see a recent review (Loppin et al. 2015).

12.4   The Syncytial Embryo Employs an Adapted Cell Cycle

Due to the accelerated pace of nuclear divisions, the syncytial embryo involves a modified cell cycle that does not utilize gap phases but only S and M phases until cellularization (Glover et al. 1989). Because of the fast transitions from mitosis to interphase, there is also a severe reduction in transcription until the maternal to zygotic transition (MZT) during the tenth cleavage division (Lamb and Laird 1976; McKnight et al. 1977; Zalokar 1976; Edgar and Schubiger 1986). Instead, the embryo relies on the activities of maternally supplied proteins and mRNAs to execute the syncytial nuclear divisions (O'Farrell 2015; Lasko 2012).

The major cell cycle regulators, Cyclin-dependent kinases (Cdks) and Cyclins, are important in managing the timing of mitosis in the syncytial blastoderm. Cdks and Cyclins cooperate to regulate the timing of protein activation during various stages of the cell cycle. Typically, Cdk and Cyclin levels are regulated through temporal expression, but the lack of transcription in the early embryo results in a modified mechanism to support cleavage cycle regulation. In the embryo, Cdks diffuse through the embryo in waves, causing subsequent waves of mitosis (Deneke et al. 2016), whereas cyclins are locally degraded at the centrosomes to prevent global destruction that would halt further mitotic cycles (Huang and Raff 1999; Raff et al. 2002).

The syncytial nuclei divide in a synchronous wave regulated by Cdk1 that propagates along the A-P axis. Cdk1 forms complexes with Cyclin A and B to regulate entry into mitosis. Because diffusion would take too long for mitotic activation in the embryo, Cdk1 propagates throughout the embryo as a wave that signals the nuclei to enter mitosis. Particularly in the later cleavage cycle stages,

Cdk1 waves spread throughout the embryo during S phase to trigger mitosis, resulting in a subsequent wave of mitosis of the nuclei (Deneke et al. 2016).

Both Cyclin A and B are involved in regulating the syncytial embryo cell cycle. Cyclin A is localized at the nucleus and regulates the duration of the entire cell cycle, as decreased Cyclin A results in a longer cell cycle, while the mitotic index remains the same (Edgar et al. 1994; Stiffler et al. 1999). Cyclin B plays a more complex role, regulating not only specific mitotic stages but also microtubule length and nuclear velocity in the migration stages. Cyclin B localizes to the spindle microtubules during metaphase and astral microtubules in later mitotic stages (Huang and Raff 1999; Stiffler et al. 1999). Decreasing Cyclin B levels results in longer astral and spindle microtubules, as well as centrosome detachment from their respective nuclei. Increasing Cyclin B levels causes shorter astral and spindle microtubules, resulting in nuclear spacing defects (Stiffler et al. 1999).

Cyclin B is destroyed during metaphase and this destruction is localized to the spindle microtubules, starting at the centrosomes (Huang and Raff 1999) and catalyzed by Cdc20 (Fizzy (Fzy))-dependent Anaphase-promoting complex (APC) activation (Raff et al. 2002; Sigrist et al. 1995). Fzy is localized to the centrosomes, spindle microtubules, and kinetochores during the start of mitosis and begins to disappear during metaphase. The localization of Fzy to the centrosome is microtubule-dependent as colcemid treatment (a microtubule depolymerizer) causes Fzy to localize strictly to the kinetochores. It is hypothesized that the Fzy-APC complexes are activated at the centrosomes and spread to the kinetochores due to the localization of Fzy (Raff et al. 2002) and that centrosome and spindle attachment is necessary for Cyclin B destruction (Wakefield et al. 2000).

When centrosomes detach from the spindle and Cyclin B is not destroyed, spindles arrest in mitosis, which is also seen in *sas-4* mutants (spindle arrest, absent of centrioles), supporting a role for the centrosomes and Cyclin B destruction (Stevens et al. 2007; Wakefield et al. 2000). Pan gu (png) forms a complex with Giant nuclei (Gnu) and Plutonium (Plu) to regulate Cyclin B levels, specifically in the early embryo. It is hypothesized that this complex works to stabilize Cyclin B, as Cyclin B levels are decreased in either *png*, *glu*, or *plu* mutants (Fenger et al. 2000). These mutants also display DNA replication without division resulting in polyploid nuclei, as well as centrosome detachment from the spindles. (Freeman et al. 1986; Freeman and Glover 1987; Elfring et al. 1997; Shamanski and Orr-Weaver 1991). This phenotype is also seen in embryos lacking Cyclin B, supporting the idea of localized Cyclin B destruction at the centrosome (Stiffler et al. 1999). The detached centrosomes continue to replicate, uncoupled from the DNA replication cycle, indicating a mechanism by which the centrosome and DNA replication cycles can be uncoupled (Freeman et al. 1986; Freeman and Glover 1987; Elfring et al. 1997; Shamanski and Orr-Weaver 1991).

In aphidicolin-injected embryos (DNA replication inhibitor) that have a prolonged S phase, centrosomes separate from the nuclei, and over-replication of centrosomes occurs (Raff and Glover 1988; Debec et al. 1996). Also in *dhc64C* mutants, centrosomes separate from the nuclei and continue to replicate, leading to excessive centrosome replication (Belecz et al. 2001). Similarly, in *mcph1* mutants

where S phase is prolonged causing an increase in the DNA replication cycle length, the length of the centrosome replication cycle stays the same, resulting in excessive centriole replication (Brunk et al. 2007).

Centriole replication throughout the syncytial embryo mitoses is regulated by the same components involved in initial centriole formation during fertilization. The centriole assembly proteins, Sas-4, Sas-6, and Plk4, are required for the formation of the daughter centriole during centrosome replication. In *sas-4*, *sas-6*, or *plk4* maternal mutants, centrioles are not formed, and embryos arrest early with very few divisions that have abnormally shaped anastral spindles. Additionally, PCM components such as Cnn are not recruited to the spindle poles (Rodrigues-Martins et al. 2008; Stevens et al. 2007). Overexpression of either Sas-6 or Plk4 causes excessive centrosome replication, due to their role in centriole biogenesis (Peel et al. 2007; Rodrigues-Martins et al. 2007b). Excessive centrosome replication due to Sas-6 overexpression is exacerbated by the loss of Centriole Coiled Coil Protein 110 kDa (CP110). *cp110* mutants cause excessive centrosome replication when either Asl or Ana2 was overexpressed, which do not display abnormal centrosome replication on their own (Franz et al. 2013). Therefore, CP110 negatively regulates centrosome duplication through Sas-6, Asl, and Ana2.

12.5 Centrosome-Nucleus Association

As in most cell types and organisms, centrosomes are closely linked to the nuclei of the syncytial embryo through microtubule interactions, motor proteins, and microtubule-associated proteins (MAPs). Close centrosome-nuclear localization allows for rapid assembly of the mitotic spindle during the quick transitions of the cleavage cycle, as well as aiding in force mechanisms for nuclear migration and positioning. Due to the syncytial nature of the embryo, this association is important to prevent centrosomes from drifting away from their respective nuclei, which is less of a concern in the containment of a cell. Free centrosomes can disrupt nuclear divisions as well as prevent proper nuclear positioning; therefore, the syncytial embryo requires unique mechanisms to keep the centrosome-nuclear association intact.

Nuclear envelope breakdown in the embryo deviates from the canonical cell cycle, as the centrosomes remain extremely close to the nuclei. During prophase, the nuclei become indented near the centrosomes; during prometaphase, portions of the nuclear envelope breakdown at these indents, theoretically due to the astral microtubules piercing the nucleus. Remnants of the nuclear envelope continue to surround the spindle until telophase, when it fully breaks down and reforms at the two newly separated nuclei (Stafstrom and Staehelin 1984; Paddy et al. 1996; Rothwell and Sullivan 2000).

The LINC complex is an obvious candidate for nuclear attachment to the centrosome; however, loss of the LINC complex components Klar, Msp300, or Klaroid (Koi) does not display any obvious centrosome-nuclear attachment defects

(Archambault and Pinson 2010; Technau and Roth 2008). Rather, the microtubule motor protein dynein and PCM proteins are necessary for this attachment. *dhc64c* mutants display detached centrosomes from interphase/prophase nuclei as well as spindle poles, and centrosomes often fail to separate properly during prophase (Robinson et al. 1999). Loss of Polo also causes interphase/prophase centrosome detachment, particularly during the cortical migration stage. This may be due to the recapturing of detached centrosomes during spindle formation when they are still close by, but during the migration stage, the nuclei move too far for the centrosomes to be recaptured, resulting in monopolar spindles. Centrosome detachment is not highly penetrant in *polo* mutants, but this phenotype is exacerbated by the overexpression of Microtubule-associated protein 205 (Map 205) or Greatwall (Gwl) (Archambault et al. 2007; Archambault et al. 2008). Map 205 sequesters Polo to microtubules during interphase, and Gwl antagonizes Polo via inhibition of the regulatory subunit of Protein phosphatase 2A, Twins (Wang et al. 2011).

Mutations in the gene for another MAP, Mars (HURP homolog), shows centrosome detachment from prophase nuclei when depleted, but more often centrosomes detach from the mitotic spindle (Zhang et al. 2009). Centrosome detachment from the spindle is also seen in *asp* mutants, evident by monopolar spindles (González et al. 1990). A syncytial embryo specific MAP, Toucan (Toc), localizes to the nuclear envelope and centrosomes during interphase and the spindle microtubules during mitosis. Mutant *toc* embryos display detached centrosomes from spindles and defective spindle formation. Astral microtubules remain intact and these embryos typically arrest early on in a metaphase state, indicating a specialized role for Toc in regulating syncytial mitotic spindles (Debec et al. 2001; Mirouse et al. 2005).

Microcephalin (MCPH1), which localizes to the centrosomes and spindle, is necessary for centrosome-spindle pole attachment as mutant *mcph1* embryos display detached centrosomes and monopolar spindles. These mutants arrest early on in a metaphase state but also have a delayed S phase, which results in uncoupling of the centrosome and cell cycles. Desynchronization of these cycles can result in over-replication of centrosomes, which may be the cause of the detached centrosomes (Brunk et al. 2007).

For more on centrosome attachment in the syncytial embryo, see Free Centrosomes: Where Do They Come From? (Archambault and Pinson 2010).

12.6 Axial Nuclear Migration Distributes Nuclei along the A-P Axis

The first 3–4 cleavage cycles are skewed toward the anterior end of the embryo where syngamy occurs (Karr 1991). Before the nuclei migrate toward the cortex and form the blastoderm, they organize into a uniform distribution along the A-P axis through axial nuclear migration (also known as axial expansion) (Fig. 12.1b) (Hatanaka and Okada 1991). Axial nuclear migration occurs during cleavage cycles
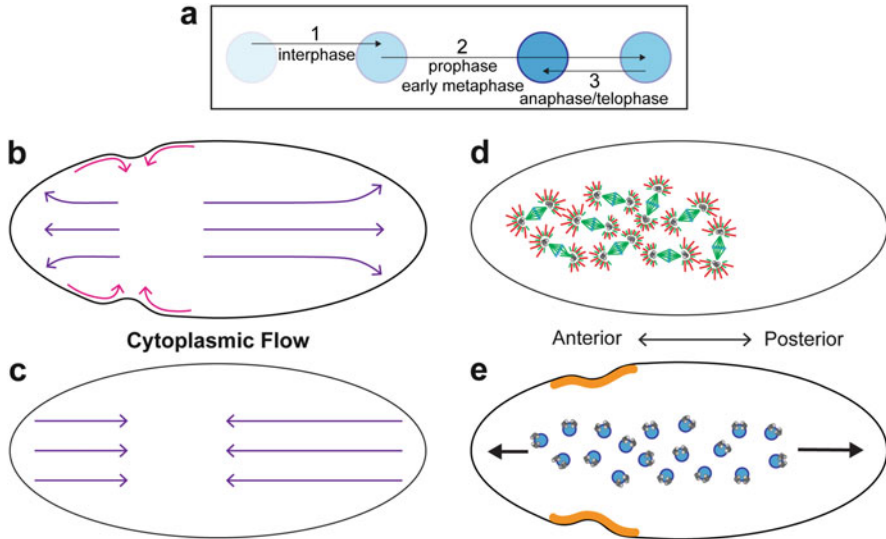
Fig. 12.4 During axial nuclear migration the nuclei migrate along the A-P axis due to localized contractions at the anterior cortex. (**a**) The nuclei (blue) slowly begin to migrate along the A-P axis during interphase. During prophase/early metaphase, the nuclei migrate faster along the A-P axis. The nuclei slightly retract along the A-P axis during anaphase/telophase. (**b**) Cross-sectional view of an embryo along the A-P axis during interphase of axial nuclear migration. The deep cytoplasm (purple arrows) moves toward the poles, while the peripheral cytoplasm (pink arrows) converges at the constriction point, which is slightly anterior at the cortex. (**c**) Cross-sectional view of an embryo along the A-P axis during anaphase/telophase of axial nuclear migration. The deep cytoplasm retracts and moves inwards away from the poles. (**d**) Cross-sectional view of an embryo along the A-P axis during metaphase of cleavage cycle 4. The centrosomes nucleate actin asters (red) that are more intense at the nuclei toward the poles and less intense at the inward nuclei. (**e**) Cross-sectional view of an embryo along the A-P axis during interphase of cleavage cycle 5. Actin and Myosin II (orange) localize to the constriction point, while the nuclei make an overall migration toward the anterior and posterior poles

4–7 and relies on cytoskeletal dynamics that are regulated through the cell cycle and the centrosome.

Actin and non-muscle myosin II (MyoII) localize at the anterior cortex in a cell cycle-dependent manner, where they control physical contractions of the embryo that assist in the axial nuclear migration (Fig. 12.4d) (Royou et al. 2002; von Dassow and Schubiger 1994; Wheatley et al. 1995). It is hypothesized that this contraction causes cytoplasmic streaming that forces the nuclei to migrate along the A-P axis, which is further supported by the cytoplasmic movements during these stages (Fig. 12.4b) (von Dassow and Schubiger 1994). A second hypothesis is that the actin network causes the cytoplasm to become stiffer in the middle of the embryo and looser toward the poles. Interacting microtubules from neighboring centrosomes repel the nuclei away from one another, forcing the nuclei to expand laterally along the A-P axis during these contractions. (Foe et al. 1993; von Dassow and Schubiger

1994). There is support for both of these hypotheses, and both may contribute to the nuclear movements during axial nuclear migration.

Three distinct phases of nuclear movements transpire during each cleavage cycle 4–7 at different stages of the cleavage cycle (Fig. 12.4a). First, the nuclei slowly begin to migrate toward the poles along the A-P axis at the end of interphase. In the second phase, this movement rapidly increases during prophase and early metaphase. Finally, nuclear movement slows down and they regress slightly along the A-P axis, away from the poles, during anaphase and telophase (Baker et al. 1993; von Dassow and Schubiger 1994).

The specificity of cell cycle stages during these nuclear movements suggests a role for cell cycle regulation in axial nuclear migration. In support of this, increasing overall Cyclin B levels in the embryo decreases the velocity of nuclear movements, and decreasing overall Cyclin B levels in the embryo increases the velocity of nuclear movements (Stiffler et al. 1999). Therefore, Cyclin B regulates the nuclear velocity during these three phases of nuclear migration.

The cytoplasm contained inside the entire embryo displays movements that mimic nuclear migration. As the nuclei move toward the pole, the cytoplasm deep in the middle of the embryo moves outward toward the poles as well (Fig. 12.4b). The cytoplasm at the periphery of the cortex flows toward the middle of the embryo, and the two distinct waves, from the posterior and anterior, converge slightly anterior at the cortex, at what is termed the constriction point. Because of the opposing flows of the deep cytoplasm and peripheral cytoplasm, this movement is referred to as fountain streaming. During the final phase of each nuclear movement when the nuclei slightly retract, the cytoplasm also retracts toward the middle of the embryo (Fig. 12.4c) (von Dassow and Schubiger 1994).

Axial nuclear migration relies on actin filaments and not microtubules as colchicine treatment (a microtubule depolymerizer) does not affect A-P nuclear distribution, but cytochalasin (prevents actin polymerization) does inhibit it (Zalokar and Erk 1976; Hatanaka and Okada 1991). Filamentous actin (F-actin) appears to nucleate from the centrosomes starting at metaphase, growing through telophase, and dispersing in interphase. These actin asters are greatest at the outward nuclei, closest to the poles, and weakest at the inward nuclei, furthest from the poles (Fig. 12.4d). Because the outward nuclei move more than the inward nuclei, it is suggested that these actin asters facilitate in axial nuclear migration (von Dassow and Schubiger 1994).

Loss of Grandchildless N26 (Gs(1)N26), Grandchildless N41 (Gs(1)N41), or Paralog (Par), all of which have not been mapped to a physical locus, cause actin to appear as a uniform layer over the cortex with rough aggregates and defective axial nuclear migration (Hatanaka and Okada 1991). This indicates they play a role in regulating actin distribution during axial nuclear migration.

Actin, together with MyoII which is also necessary for axial nuclear migration, shows a distinct localization progressing through cleavage cycles 4–7 (Kiehart et al. 1990; Wheatley et al. 1995; Royou et al. 2002). MyoII localizes at the cortex, slightly anterior, during interphase of the axial nuclear cleavage divisions (Fig. 12.4e). MyoII localizes to the anterior constriction site starting at interphase

4 and increases in intensity during the following interphase cycles until interphase 7, where it starts to disperse along the cortex. By interphase 8, when the nuclei are evenly distributed along the A-P axis, MyoII appears almost entirely disperse at the cortex. Actin also cycles to the cortex in a similar manner (Royou et al. 2002).

The cell cycle-dependent localization of MyoII is regulated by Cdk1 and Cyclin B. Localized degradation of Cyclin B during late anaphase inactivates Cdk1 (Su et al. 1998), and increasing Cyclin B levels prevents MyoII cortical localization, while inhibiting Cdk1 results in abnormal localization of cortical MyoII (Royou et al. 2002). However, cortical MyoII localization is not reliant on actin, as cytochalasin or latrunculin (prevents actin polymerization) injection does not disrupt MyoII localization (Chodagam et al. 2005). Additionally, in mutants for the regulatory light chain of MyoII, Spaghetti squash (Sqh), cortical actin localization is not disrupted, indicating it is independent of MyoII (Royou et al. 2002).

Antimyosin antibody injection or *sqh* mutants display defective axial nuclear migration (Kiehart et al. 1990; Wheatley et al. 1995), specifically, the primary activating phosphorylation site of Sqh, Serine 21 (Karess et al. 1991), is necessary for this migration. Phosphorylation site mutants that either mimic phosphorylation or prevent phosphorylation of Serine 21 both hinder axial nuclear expansion, indicating proper regulation of Sqh phosphorylation is necessary for axial nuclear migration (Jordan and Karess 1997). This site is phosphorylated by Rho kinase (Rok), and inhibition of Rok with Y-27632 also hinders axial nuclear expansion as well as MyoII distribution (Royou et al. 2002).

The centrosome, apparently acting through the centrosomal protein CP190, also plays a role in regulating axial nuclear migration. *cp190* mutants display defective axial nuclear migration and MyoII localization, but actin organization remains intact. The constitutively active Sqh phosphomimetic mutant can partially rescue this phenotype, implicating the centrosome in MyoII regulation. Although originally identified as a centrosomal protein, CP190 is best known as a chromatin insulator (Kellogg et al. 1989; Pai et al. 2004). CP190 localization is regulated during the cell cycle as it localizes to the centrosomes during mitosis and is nuclear during interphase (Chodagam et al. 2005). These localization dynamics may regulate the localization of MyoII during axial nuclear migration. Two unmapped genes regulate axial nuclear migration and affect centrosome localization: *shackleton* (*shkl*) and *out of sync* (*oosy*) mutants display defective axial nuclear migration, and centrosome loss from the spindles. *oosy* mutants also display asynchronous cleavage divisions (Yohn et al. 2003). Therefore, the timing of axial nuclear migration relies on cell cycle regulators that may involve the centrosome in an unknown way.

Overall, axial nuclear migration is a poorly understood process that is regulated by Rho1-dependent actin-MyoII dynamics and has an unclear connection to centrosomes. It is critical for the timely delivery of nuclei to the germ plasm during the cycle 10 window of pole cell formation. It also spreads the nuclei along the A-P axis to establish an even migration to the cortex.

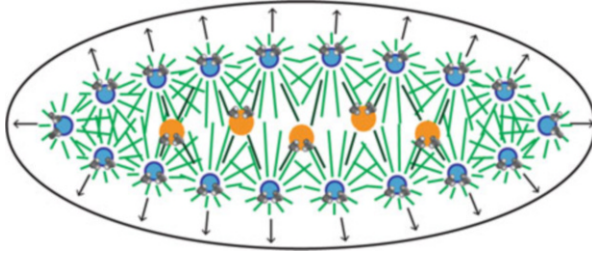## 12.7   Cortical Nuclear Migration Positions the Nuclei at the Cortex

During cleavage cycles 7–9, the majority of nuclei migrate from the interior of the embryo to the cortex (Fig. 12.1c) (Foe and Alberts 1983). A subset of nuclei, the yolk nuclei, fall back into the interior of the embryo between cleavage cycles 8 and 9 (Foe et al. 1993). Little is known about the function of these nuclei, but they are involved in yolk digestion (Bownes 1982) as well as the future development of the midgut (Walker et al. 2000).

The yolk nuclei asynchronously divide twice, then complete two rounds of DNA replication without divisions to become polyploid (Zalokar and Erk 1976; Foe and Alberts 1983). During these divisions, the centrosomes display defects in mitotic spindle organization that ultimately leads to centrosome loss at the yolk nuclei. During the first asynchronous division, a majority of the nuclei display defective centrosome separation, resulting in "V"-shaped spindles that resemble monopolar spindles. These aberrant spindles result in defective DNA segregation, yet the centrosomes continue to replicate, and a second round of abnormal divisions occurs. The replicated centrosomes also do not separate, as mother and daughter centrosomes remain close to one another (Callaini and Dallai 1991; Riparbelli and Callaini 2003). By the second yolk nuclei division, most of the centrosomes have detached from the spindles. The centrosomes appear normal, as CP190, Asp, Pavarotti, and γ-Tubulin all remain localized there. However, Cyclin B localization is disrupted as it does not associate with the aberrant spindles but weakly localizes to the centrosomes during anaphase and telophase. It does not localize to the entire spindle pole but rather the inner core of the centrosomes (Callaini and Dallai 1991; Riparbelli and Callaini 2003).

In contrast with axial nuclear migration, cortical nuclear migration relies on microtubules as colchicine treatment inhibits cortical nuclear migration, and does not rely on actin, as cytochalasin treatment does not inhibit cortical nuclear migration (Zalokar and Erk 1976). Centrosomes appear to carry nuclei during this migration, as centrosomes dissociated from their respective nuclei continue to migrate to the cortex (Raff and Glover 1989).

It was initially hypothesized that the centrosomes nucleate microtubules that connect to the cortex to pull the nuclei by forces produced by the microtubules (Wolf 1980). However, analysis of the microtubule arrays during cortical nuclear migration has disputed this theory. The majority of microtubules project inwards, rather than to the cortex, and interact with the microtubules of the yolk nuclei (Fig. 12.5). Shorter microtubules project toward the cortex, but do not reach the membrane (Fig. 12.5). Microtubules of neighboring nuclei, as well as nuclei at the opposite side of the embryo, also interact with one another (Fig. 12.5) (Baker et al. 1993).

The density of astral microtubules is far greater in the cortical nuclear migration stages compared to those during the axial nuclear migration stages (Baker et al. 1993). In *tacc* mutants, the astral microtubules are reduced or absent, and the nuclei

Fig. 12.5 Cortical nuclear migration relies on microtubules for proper migration. Cross-sectional view of a stage 7 embryo where the nuclei (blue) are migrating toward the cortex. The centrosomes (gray) emanate small astral microtubules (green) that stretch toward the cortex and long microtubules that stretch toward the yolk nuclei (orange). The yolk nuclei emanate microtubules (dark green) that interact with the migrating nuclei

do not migrate to the cortex (Gergely et al. 2000b). The current hypothesis is that antiparallel microtubule interactions provide a pushing force that directs the centrosomes, with their nuclei as passengers, toward the cortex (Fig. 12.5) (Baker et al. 1993; Raff and Glover 1989).

Very little is known about the molecular regulators of cortical nuclear migration, but it is coordinated with the cleavage cycle. The nuclear velocity is greatest during telophase when astral microtubules are most abundant, further supporting their necessity during this migration (Foe and Alberts 1983; Baker et al. 1993). As mentioned earlier, higher levels of cyclin B reduce the speed of nuclear migration, but this could be due to the decrease in microtubule length caused by the increased levels (Stiffler et al. 1999). In *oosy* mutants, the cleavage divisions are not synchronous and cortical migration is defective, suggesting that this synchrony is also important for proper migration (Yohn et al. 2003).

Overall, while little is understood about how cortical nuclear migration is regulated, it appears to be driven by astral microtubules assembled at centrosomes that grow toward the center of the embryo and which push the nuclei outward toward the cortex as they grow.

12.8 Pole Cells Cellularize Before the Other Nuclei

During cortical nuclear migration, a subset of nuclei reaches the posterior cortex and pole plasm during cleavage cycle 9. These nuclei will cellularize at cycle 10, becoming pole cells, while the majority of nuclei at the cortex continue to divide (Fig. 12.1d) (Foe and Alberts 1983). Pole cells will develop into the germ cells of the fly and contain germ cell-specific components. The germ plasm is localized to the posterior end of the oocyte during oogenesis and is contained in polar granules in the early embryo that are anchored by the actin cytoskeleton (Fig. 12.6a) (Lantz et al. 1999; Mahowald 2001).
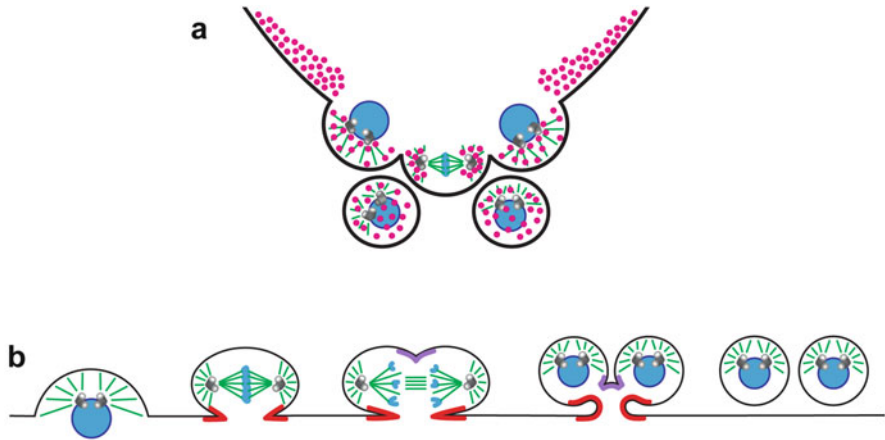
Fig. 12.6 Formation for the pole cells at the posterior cortex relies on membrane invaginations to cleave the cells as the nuclei divide. (**a**) The pole plasm is contained in polar granules (pink) that transport along the astral microtubules (green) toward the nuclei (blue) located at the cortex. (**b**) During prophase, the nuclei reach the posterior cortex and the centrosomes (gray) impinge on the plasma membrane to form the pole bud. (**c**) During metaphase, the BF (red) constricts beneath the furrow. (**d**) During anaphase, the BF remains and the AF (purple) forms above the chromosomes. (**e**) The two newly formed nuclei are cleaved from the plasma membrane at the bud furrow and anaphase furrow. (**f**) Two pole cells are formed after cleavage and remain localized at the posterior cortex

The pole plasm is sufficient to initiate pole cell formation, as transplantation of the pole plasm to the anterior cortex stimulates abnormal pole cell formation at the anterior pole (Illmensee and Mahowald 1974). Oskar (Osk), a germ cell-specific protein required to localize other germ cell-specific components, is also sufficient to cause abnormal pole cell formation when transplanted to the anterior pole (Ephrussi and Lehmann 1992). Germ-cell less (Gcl) is necessary for pole cell formation, but is not sufficient for the formation of pole cells when transplanted to the anterior pole (Jongens et al. 1992). However, when transplanted together with Anillin (encoded by *scraps*), which regulates actomyosin contractile rings and is also necessary for pole cell formation, they can stimulate abnormal pole cell formation at the anterior pole (Cinalli and Lehmann 2013; Field et al. 2005).

Gcl also plays a role in limiting pole cell formation to the posterior pole. Gcl promotes actomyosin organization downstream of the Rho1 pathway to constrict the plasma membrane and form the pole cells at the posterior pole (Cinalli and Lehmann 2013). The Arf-GEF Stepkke (Step) inhibits the Rho1-actomyosin pathway, and during pole cell formation stages, it is equally distributed around the cortical membrane of the embryo, preventing any cellular formation. Loss of Step activity results in abnormal pole cell formation at the anterior cortex due to loss of Rho1 inhibition. However, the posterior pole is distinct from the remainder of the embryonic membrane due to the presence of Gcl, which locally inhibits Step activity to

allow for Rho1-mediated actomyosin membrane constriction. Loss of Step in a *gcl* mutant background allows for proper pole cell formation (Lee et al. 2015).

Another determinant of pole cell formation is proper axial nuclear migration. During interphase of cleavage cycle 9, the first nuclei reach the posterior pole in the proper time window for pole cell formation to begin. If axial nuclear migration is defective and the nuclei do not reach the posterior pole until a time point after cleavage cycle 10, pole cell formation is inhibited (Niki and Okada 1981; Niki 1984; Okada 1982; Hatanaka and Okada 1991). This is likely due to the degradation of germ plasm components before the nuclei can reach the posterior pole. When pole cell formation fails in *gs(1)N26* or *gs(1)N441* mutants, which display defective axial nuclear migration, the localized pole plasm components either degrade or delocalize from the posterior pole (Iida and Kobayashi 2000). In *shkl* or *oosy* mutant embryos, which also display defective axial expansion, pole cell numbers are lower than wildtype, further supporting the necessity of axial nuclear migration and timely arrival of nuclei at the posterior pole plasm for pole cell formation (Yohn et al. 2003).

When the nuclei reach the posterior membrane during cleavage cycle 9, they enter prophase and plasma membrane protrusions, called pole buds, form above each nuclei (Fig. 12.6b) (Foe et al. 2000; Warn et al. 1985; Cinalli and Lehmann 2013). As the nuclei begin to divide, the pole buds protrude farther from the membrane to surround the metaphase spindles (Fig. 12.6b). Membrane furrows, termed the bud furrows (BFs), form at the edge of the pole buds and constrict beneath the chromosomes at the basal membrane (Fig. 12.6b). Once the nuclei progress into anaphase, a second furrow, the anaphase furrow (AF), forms above the spindle opposite to the BF. Similar to the cytokinetic furrow, it forms in between the dividing nuclei (Fig. 12.6b). Once mitosis is complete, the AF constricts to separate the two nuclei into separate cells, while the BF constricts, liberating the nascent pole cells from the embryo (Fig. 12.6b) (Cinalli and Lehmann 2013).

Known cytokinetic factors Anillin, MyoII, Peanut (Pnut), and Rho guanine nucleotide exchange factor 2 (RhoGEF2) all localize to the actin-rich BFs in preparation for cleavage of the plasma membrane (Padash Barmchi et al. 2005; Warn et al. 1985; Field and Alberts 1995; Young et al. 1991). Anillin and MyoII also localize to the actin-rich AFs, but further analysis is required to determine other components of the AF. Rho1 functions upstream of cytokinetic components, and inhibiting Rho1 or Rok prevents pole bud formation, specifically diminishing Anillin localization at the BFs (Cinalli and Lehmann 2013). Anillin is necessary for cleavage of the pole cells, as *scraps* mutants display BFs that retract and never form pole cells (Field et al. 2005). *diaphanous (dia)* (a Formin downstream of Rho1) or *rhogef2* mutants display defective BF cleavage due to the disruption of the actomyosin contractile ring, as actin and MyoII are absent at the BFs (Afshar et al. 2000; Padash Barmchi et al. 2005).

The rate of BF constriction is regulated by Gcl, as overexpressing Gcl causes over-constriction of the BFs, resulting in the displacement of somatic nuclei and increased pole cell numbers (Cinalli and Lehmann 2013; Jongens et al. 1994). *gcl* mutants display under-constriction of the BFs, resulting in decreased pole cell

numbers (Robertson et al. 1999; Cinalli and Lehmann 2013). In *gcl* mutants, the AF constricts to separate the cells but the BF never constricts, even though Anillin is present at both, preventing the formation of the pole cells. Therefore, Gcl is necessary for BF cleavage, but not AF cleavage (Cinalli and Lehmann 2013).

Both actin and microtubules are necessary for proper pole cell formation as injection of either Colcemid or cytochalasin inhibits pole cell formation (Raff and Glover 1989; Cinalli and Lehmann 2013). Colcemid treatment prevents AF cleavage but not BF cleavage and the nuclei arrest in metaphase, resulting in large pole cell-like cells with inappropriate DNA content (Cinalli and Lehmann 2013). Cytochalasin injection after pole cell formation causes the cells to collapse, indicating actin is necessary for pole cell stabilization (Raff and Glover 1989).

Centrosomes alone are sufficient to produce pole cells. Active centrosomes dissociated from nuclei during aphidicolin injection still migrate to the posterior pole and produce pole buds and reorganize actin. Pole cells form that are indistinguishable from normal pole cells except that they lack nuclei (Raff and Glover 1989). The centrosomal protein CP110 regulates pole cell formation. Neuralized E3 ubiquitin protein ligase 4 (Neurl4) localizes to the centrosome and downregulates CP110 levels in mammalian cells (Al-Hakim et al. 2012; Li et al. 2012). In *neurl4* mutant embryos, CP110 levels are elevated at centrosomes compared to wildtype as well as enriched at foci distinct from centrosomes. *neurl4* mutants display reduced pole cell numbers with abnormal morphologies due to CP110 overexpression, as the phenotype was partially rescued in *neurl4* mutants with the addition of one mutant copy of *cp110*, resulting in less abnormal pole cells (Jones and Macdonald 2015). How CP110 impacts the centrosome and affects pole cell formation is unclear.

Centrosome separation during mitosis regulates the formation and size of the BFs. *gcl* mutants display disrupted centrosome separation specifically in the pole cells and not somatic cells, which leads to shallow BFs (Lerit et al. 2017). These smaller BFs often retract, preventing their cellularization which leads to lower numbers of pole cells (Cinalli and Lehmann 2013). The percentage of pole buds with defective centrosome separation in *gcl* mutants correlates with the number of *gcl* mutant embryos that lack pole cells (Lerit et al. 2017; Robertson et al. 1999). Some nuclei overcome this BF defect and form pole cells, but they contain an abnormal number of centrosomes and multipolar spindles (Lerit et al. 2017). The CM1 domain of Cnn and also the Kinesin-5 motor protein (Klp61F), a plus-end-directed motor, are necessary for centrosome separation (Heck et al. 1993; Zhang and Megraw 2007), and *cnn*$^{\Delta 1}$ mutants or RNAi-mediated knockdown of Klp61F results in shallow, abnormally shaped BFs and reduced pole cell numbers (Lerit et al. 2017).

Defective centrosome separation also disrupts astral microtubule organization, which assists in the transportation of the polar granules that contain germ cell-specific mRNAs and proteins toward the cortical nuclei (Fig. 12.6a) (Lerit and Gavis 2011; Lerit et al. 2017). Polar granules migrate along the astral microtubules toward the centrosomes during interphase and remain localized around the centrosomes during mitosis until they segregate into separate cells during membrane cleavage (Fig. 12.6a) (Lerit and Gavis 2011). In *gcl* or *Klp61f* mutants where centrosomes do not properly separate and microtubules are not properly organized, polar granules still migrate

from the posterior cortex, but their distribution around the nuclei is aberrant resulting in a reduced number of granules in pole cells (Lerit et al. 2017).

Microtubules are necessary for polar granule transport, as colcemid treatment prevents their movement, but actin is not as cytochalasin or latrunculin does not disrupt their transport (Lerit and Gavis 2011). In *png* mutants, active centrosomes travel to the posterior end without their respective nuclei (Shamanski and Orr-Weaver 1991), and polar granules are still trafficked along their astral microtubules, indicating centrosomes and astral microtubules are sufficient for not only pole cell formation but also transport of the polar granules. Cnn, Tacc, and AurA all regulate astral microtubule length and stability (Gergely et al. 2000b; Giet et al. 2002; Megraw et al. 1999), and loss of any of these three proteins results in impaired polar granules transport. Additionally, fewer pole cells are formed, and those that do form contain either a reduced level of polar granules or none at all (Lerit and Gavis 2011).

Polar granules rely on Dynein for trafficking along the astral microtubules in a minus-end-directed manner toward the centrosomes. In *dhc64c* mutants, astral microtubules remain intact, but the directed movement of polar granules is inhibited, resulting in a reduction of pole plasm in pole cells and reduced pole cell numbers (Lerit and Gavis 2011). Overexpression of Dynactin 2, p150 subunit (DCTN2-p150) inhibits dynein function (Burkhardt et al. 1997), resulting in cessation of polar granule motility. Conversely, the plus-end-directed motor kinesin does not function in trafficking polar granules, as *kinesin heavy chain* (*khc*) mutants display proper polar granule movement (Lerit and Gavis 2011). Therefore, the transport of polar granules relies on minus-end motors but not plus-end.

12.9 The Cortical Cleavage Cycles

After cortical nuclear migration, four final cleavage divisions (cycles 10–13) occur at the cortex before cellularization in interphase of cycle 14. These divisions form actin-rich furrows that resemble pole cell BFs, but the nuclei do not cleave off to form separate cells (Fig. 12.7a) (Raff and Glover 1988; Ede and Counce 1956; Turner and Mahowald 1976; Foe and Alberts 1983). Instead, these actin furrows are called pseudo-cleavage furrows (also known as cortical cleavage or Rappaport furrows). They are analogous to the furrows that form in-between adjacent nuclei from overlapping astral microtubules discovered by Rappaport in sand dollar embryos (Rappaport 1961). The centrosome regulates the formation of these furrows.

When nuclei reach the cortex during interphase of cleavage cycle 10, the centrosomes organize the plasma membrane to form a cortical bud, similar to the pole bud but not as protruding (Fig. 12.7a) (Raff and Glover 1988; Ede and Counce 1956; Turner and Mahowald 1976; Foe and Alberts 1983). Filamentous actin is rearranged from an evenly distributed layer along the cortical membrane to highly localized pockets directly over the centrosomes, termed actin caps (Fig. 12.7b) (Karr and Alberts 1986; Kellogg et al. 1988; Warn et al. 1984). Actin caps are necessary for even nuclear distribution along the cortex as disrupted cap formation due to
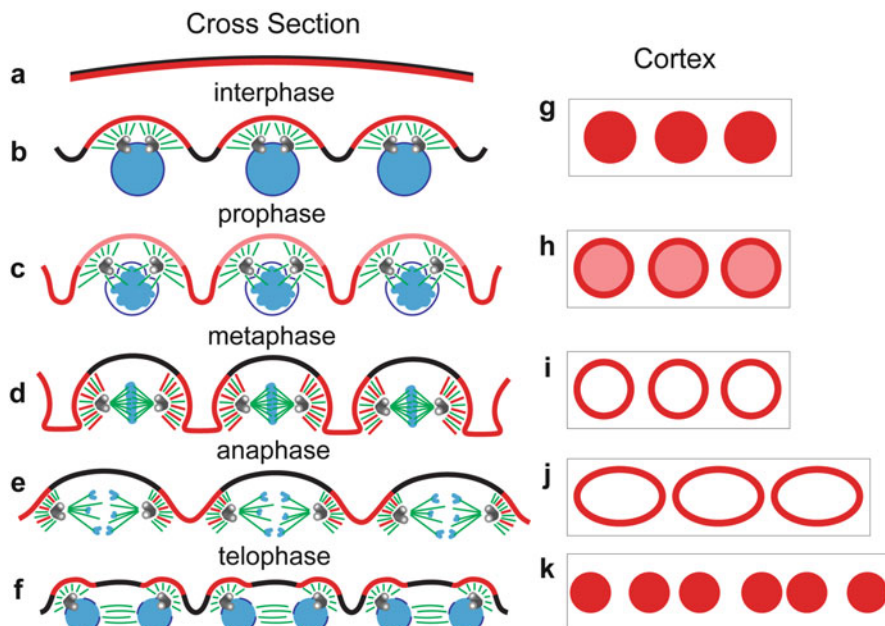
Fig. 12.7 The cortical cleavage cycles (**a**) During the cortical cleavage cycles, actin (red) is rearranged from an even distributed layer at the cortex, to pseudo-cleavage furrows that surround the dividing nuclei (blue). The centrosomes (gray) nucleate microtubules (thinner green lines) that assist in this actin redistribution. Astral actin filaments (thicker red lines) also surround the centrosomes. (**b**) At the cortex, actin is rearranged to form a cap above the nuclei (dark red) during interphase. As the nuclei divide, the actin cap expands (light red) into to surround the nuclei as they divide. During telophase, the separated chromosomes begin to form new actin caps

cytochalasin treatment or mutants that affect actin organization leads to abnormal clustering of nuclei (Stevenson et al. 2001; Zalokar and Erk 1976; Callaini et al. 1992). As the nuclei enter mitosis and the centrosomes separate, the actin caps expand and distribute into furrows of invaginated membrane (Fig. 12.7a–b). As mitosis proceeds, the furrows surround the spindles and then begin to recede during telophase to form actin caps over the centrosomes of the newly divided nuclei as they proceed into the next cleavage cycle (Fig. 12.7a–b) (Karr and Alberts 1986; Kellogg et al. 1988; Warn et al. 1984).

Actin cap expansion is necessary for furrow formation and relies on a number of actin nucleators to organize actin at the furrows. The Arp2/3 complex aids in actin nucleation to create branched actin filaments through the regulation of SCAR/WAVE proteins (Pollitt and Insall 2009). Actin-related protein 3 (Arp3), a component of the Arp2/3 complex, localizes to the furrows and between actin caps dependent on the centrosomal protein Scrambled (Sced) (Stevenson et al. 2002). Scrambled localizes to the same locations as Arp3, as well as the centrosomes, independent of microtubules. In *sced* mutants, the actin caps do not expand preventing furrow formation, which leads to spindle fusion and chromosome

segregation errors (Stevenson et al. 2001). Loss of Actin-related protein 2/3 complex, subunit 1 (Arpc1), another component of the Arp2/3 complex, displays the same phenotype as *sced* mutants, suggesting Sced may recruit Arp2/3 to actin cap margins to aid in actin polymerization at the furrows (Stevenson et al. 2002; Zallen et al. 2002). It is unclear how Sced may recruit Arp2/3, but further studies have revealed that SCAR also localizes to the furrows and is required for furrow assembly (Zallen et al. 2002). Therefore, Sced may interact with SCAR to recruit Arp2/3 to the furrows, implicating a role for the centrosome in actin polymerization at the furrows.

Rho1 inhibition also prevents actin cap expansion and is an upstream regulator of the formin Dia that nucleates actin filaments (Cao et al. 2010; Watanabe et al. 1997). Dia localizes between the actin caps and furrows, specifically the tips of the advancing furrows (Afshar et al. 2000), and *dia* mutants do not form furrows due to defective actin cap expansion (Cao et al. 2010; Afshar et al. 2000; Webb et al. 2009). Dia is also required for the localization of cytokinetic components such as MyoII, Anillin, Pnut, and Adenomatous polyposis coli 2 (Apc2) to the invaginated furrows, and between the actin caps, as they weakly localize between the caps, but are absent from furrows (Afshar et al. 2000; Webb et al. 2009).

As the cleavage furrows form, new membrane and actin is supplied through recycling endosome (RE)-derived vesicles that are localized at the centrosome and transported along astral microtubules to the growing furrow (Swanson and Poodry 1981; Mermall et al. 1994; Mermall and Miller 1995; Rothwell et al. 1999; Riggs et al. 2003). The recruitment of these vesicles relies on Nuclear fallout (Nuf), which is an adaptor protein that links Rab11 to microtubule-based motors (dynein and Kinesin-1) for trafficking on microtubules (Riggs et al. 2003). Loss of either Nuf or Rab11 disrupts vesicle-based membrane recruitment and transport of furrow components, leading to disrupted actin furrows (Riggs et al. 2003; Rothwell et al. 1999). Nuf/Rab11 complexes localize Discontinuous actin hexagon (Dah), which is required for furrow formation, to the furrows, as *nuf* or *rab11* mutants display abnormal localization of Dah (Riggs et al. 2003; Rothwell et al. 1999; Zhang et al. 2000). Both *nuf* and *rab11* also display disruption in RhoGEF2 localization at the furrow, and injection of active RhoA (the mammalian ortholog of Rho1) in *nuf* mutants rescues the furrow phenotype. However, in *nuf* mutants, Rho1 and Dia localization is normal, suggesting their localization does not rely on RE vesicle transport, but RhoGEF2 localization does (Cao et al. 2008).

Endocytosis at the membrane occurs from interphase until metaphase, where it is inhibited to distribute membrane from RE-derived vesicles to the furrows as they are growing. Endocytosis once again occurs during telophase as the furrows are regressing (Sokac and Wieschaus 2008a; Rikhy et al. 2015). Dynamin, encoded by *shibire* (*shi*), and Clathrin are involved in endocytic vesicle formation and localize to the cleavage furrows and between actin caps at times of invagination, while localizing to the spindles during metaphase. In *shi* mutants, furrow formation is disrupted, and vesicles cannot sever from the plasma membrane, while Dia, Anillin, Pnut, involved in actin remodeling, are no longer localized to the furrows (Rikhy et al. 2015).

The cleavage furrows contain similar components to cytokinetic furrows (Miller and Kiehart 1995), including the centralspindlin complex, and yet cytokinesis does not occur (Crest et al. 2012; Minestrini et al. 2003). This is due to the absence of the cytokinetic regulator of Rho1, RhoGEF Pebble (Pbl), at the furrows and central spindle in the syncytial embryo. Instead, RhoGEF2 activates Rho1 locally at the cleavage furrows. Introducing ectopic active RhoA into the embryo during the cortical cleavage stages induces cytokinetic furrow formation over the central spindle, indicating that the machinery is in place but that spatial Rho1 is deterministic of the site of furrow formation (Crest et al. 2012). Rho1 and another major actin regulator Cdc42 play an antagonistic role in furrow formation. Constitutively active Cdc42 or dominant negative Rho1 both disrupt actin furrows and MyoII localization, but the microtubules remain intact (Crawford et al. 1998).

Centrosomes are sufficient for actin rearrangement and furrow ingression. In aphidicolin-injected embryos where only the centrosomes reach the cortex, cortical buds are formed, and actin is rearranged in a cell cycle-dependent manner (Raff and Glover 1989; Yasuda et al. 1991). Microtubules are required for actin reorganization in a specific time window. Colchicine injection during anaphase disrupts actin rearrangement to the furrows, but injection during interphase or telophase does not disrupt actin rearrangement. During telophase, robust astral microtubules of neighboring centrosomes overlap, possibly defining the furrow position for the subsequent cleavage cycle (Riggs et al. 2007). The polar bodies lack centrosomes but form microtubule projections similar to the astral microtubules of the centrosome and when the polar bodies lie close to the cortex, they rearrange the cortical actin (Foe et al. 2000).

There is also an accumulation of actin at the centrosomes that form aster-like filaments as the nuclei divide, appearing to emanate from the centrosome similar to astral microtubules (Fig. 12.7a). These actin asters appear to be reliant on proper centrosome activity and microtubules, as *cnn* mutants that disrupt the PCM and astral microtubules, or colchicine treatment also disrupts actin aster formation (Riparbelli et al. 2007). However, the role of actin asters in cortical cleavage divisions remains unclear.

The furrows provide a physical barrier between the dividing nuclei that prevents centrosomes from interacting with the spindles of neighboring nuclei (Kotadia et al. 2010; Sullivan et al. 1993b). When furrows are disrupted, mitotic spindles of neighboring nuclei aberrantly interact, which causes centrosomes to dissociate from their respective nuclei. The nuclei recede into the embryo leaving a patch devoid of a nucleus at the cortex (Sullivan et al. 1993a; Sullivan et al. 1993b). This process, which occurs in multiple mutant backgrounds when cleavage furrows are disrupted, is known as nuclear fallout. *cnn*, *arpc1*, *scar*, and *dia* mutants all display fused spindles and nuclear fallout due to the absence of furrow formation (Stevenson et al. 2002; Zallen et al. 2002; Afshar et al. 2000; Megraw et al. 1999).

The mechanism of nuclear fallout is a protective checkpoint that prevents damaged nuclei from multiplying and integrating into the future embryo by removing them from the cortex (O'Farrell et al. 2004). Due to the absence of gap phases and decrease in transcription, the syncytial embryo has an altered response to cell cycle

checkpoint mechanisms. For example, DNA replication arrest due to aphidicolin injection does not block nuclei from entering mitosis (Raff and Glover 1988; Foe and Alberts 1983). This is especially important during the cortical cleavage cycles, due to the increase in cell cycle length and the MZT.

During cleavage cycles 10–13, the cell cycle increases in length from roughly 8 to 21 min (Foe and Alberts 1983). The increase in transcription that starts during cleavage cycle 10 requires a longer cell cycle, especially in S phase (Lamb and Laird 1976; McKnight et al. 1977; Zalokar 1976; Edgar and Schubiger 1986; Shermoen et al. 2010). The increase in length during these cycles is reliant on *grapes* (*grp*, Chk1 homolog) and meiotic 41 (*mei-41*, ATR homolog) as *grp* or *mei-41* mutants the lengthening of the cleavage cycles fails (Sibon et al. 1997; Sibon et al. 1999). As a result, *grp* or *mei-41* mutants also arrest during the cleavage division cycles with damaged nuclei and nuclear fallout (Fogarty et al. 1994; Fogarty et al. 1997). Centrosomes lose their function indicated by the loss of γ-Tubulin and γTuRC components from the centrosomes and the inability to separate chromosomes during mitosis (Sibon et al. 2000). Centrosome inactivation and nuclear fallout also occur during aphidicolin injection as well as a treatment with a variety of DNA-damaging agents (Raff and Glover 1988; Sibon et al. 2000; Takada et al. 2003).

This pathway of centrosome inactivation is regulated by *loki* (Lok, also known *mnk*, a Chk2 homolog), which localizes to centrosomes and spindles. During DNA damage, this localization increases, and Lok also accumulates at the nuclei (Takada et al. 2003). Lok causes mRNA nuclear retention after DNA damage, including mRNAs that encode for centrosomal proteins, such as γ-Tubulin ring protein 91 (Grip91); this mRNA nuclear retention causes nuclear fallout (Iampietro et al. 2014). In *lok* mutants, when DNA is damaged through either Bleomycin (induces DNA damage) injection or in a *grp* mutant background, centrosomes do not inactivate (Takada et al. 2003).

Multiple PCM proteins also play an important role in regulating cleavage furrow formation, and their dysfunction can result in fused spindles, aneuploid nuclei, and nuclear fallout. *cnn* mutants are maternal-effect lethal, arresting in the cleavage furrow stages and displaying a failure in furrow ingression that leads to fused metaphase spindles and colliding nuclei in telophase and inevitable nuclear fallout (Megraw et al. 1999; Vaizel-Ohayon and Schejter 1999). In $cnn^{\Delta 1}$ mutants where astral microtubules can still form, some furrows still ingress (Zhang and Megraw 2007), but in CM2 domain *cnn* mutants (cnn^{b4}), astral microtubules are present and furrow ingression was severely impaired. The CM2 domain of Cnn interacts with Centrocortin (Cen), and *cen* mutants display aberrant actin organization at the furrows as well as spindle fusions, indicating it interacts with *cnn* to regulate actin organization at the cleavage furrow (Kao and Megraw 2009).

sponge (*spg,* a Rho GEF family protein) mutants have defective actin caps and furrows leading to aberrant spindle interactions (Postner et al. 1992; Riparbelli et al. 2007). Loss of Eb1, which binds the plus ends of microtubules, does not result in defective actin localization at the furrows. Instead, furrows are partially invaginated, resulting in severe spindle defects and loss of nuclei due to nuclear fallout (Rogers

et al. 2002; Webb et al. 2009). Nuf, which supplies membrane and other components to the invaginating furrows, localizes to the centrosome during prophase dependent on microtubules (Rothwell et al. 1998; Riggs et al. 2003). Because REs display a pericentriolar accumulation, this localization of Nuf is important for vesicle transport. *nuf* mutants display incomplete actin furrows, which results in spindle fusions (Rothwell et al. 1998).

12.10   Centrosome Separation During the Cortical Cleavage Cycles

As nuclei enter mitosis, the centrosomes begin to separate at prophase to form bipolar spindles. Centrosome separation is especially important in the cortical cleavage cycles due to the membrane invagination and spreading of actin that is orchestrated in conjunction with centrosome movement. It was hypothesized that centrosome separation guides actin cap expansion and furrow invagination as multiple centrosomal components are necessary for proper actin reorganization. Free centrosomes are sufficient to reorganize actin at the membrane (Raff and Glover 1989; Yasuda et al. 1991), and the cycling of actin structures parallels centrosomes' movements (Karr and Alberts 1986). However, in colchicine-treated embryos, centrosome separation fails, but actin caps still expand (Stevenson et al. 2001; Cao et al. 2010). Therefore, it appears that actin spreading may guide the centrosomes, as either latrunculin, cytochalasin, or jasplakinolide (actin filament stabilizer) injection both inhibit centrosome separation (Cao et al. 2010; Stevenson et al. 2001). Jasplakinolide also shrinks the actin caps, indicating actin turnover is required for centrosome separation (Cao et al. 2010).

It is hypothesized that the astral microtubules of centrosomes interact with actin to allow for proper centrosome separation. In support of this, Apc2, which stabilizes microtubules with Eb1 through Dia (Wen et al. 2004) and interacts with actin at actin-microtubule interaction sites (McCartney and Peifer 2000), plays a role in centrosome separation. Apc2 localizes to the actin caps and furrows where astral microtubules are interacting with the actin furrows (McCartney et al. 2001; Webb et al. 2009). *apc2* mutants display centrosome separation defects as well as defective furrow formation (Webb et al. 2009; Buttrick et al. 2008). Apc2 localization is reliant on Akt1 and Shaggy (Sgg, a Zw3 homolog), which is upstream of Akt1 (Shaw et al. 1997). *Akt1* mutants have normal actin and microtubule organization, yet they display centrosome separation defects and nuclear fallout due to disruption in Apc2 localization. Introduction of one copy of a *sgg* mutant allele into *akt1* mutants rescues this phenotype. Therefore, Apc2 regulates centrosome separation through microtubule-actin interactions, dependent on Akt1 and Sgg (Buttrick et al. 2008).

αTub67C is enriched at the interpolar microtubules that embrace the nuclear envelope, and *αTub67C* mutants display shorter microtubules. αTub67C mutants arrest in mitosis due to defective centrosome separation. These interpolar

microtubules must rapidly assemble, and αTub67C appears to be necessary for this rapid growth as cells depleted of αTub67C will eventually grow long microtubules from other isoforms of α-Tubulin. Therefore, αTub67C is hypothesized to quickly nucleate these interpolar microtubules to push the centrosomes apart during centrosome separation (Venkei et al. 2006).

Centrosome separation also relies on multiple microtubule-based motor proteins to provide the force that moves them apart. Dynein is required for proper centrosome separation, as *dhc64c* mutant embryos display centrosomes that do not fully separate (Robinson et al. 1999). Klp61f is required to keep the centrosomes separated during metaphase but is not required for their initial separation. *klp61f* mutants display normal centrosome separation in prometaphase, but the centrosomes slide back together before the metaphase spindles can form. However, in *ncd klp61f* double mutant embryos, the centrosomes separate and remain separated until telophase indicating an opposing force on the minus- and plus-end-directed motors, respectively, that keeps the metaphase centrosomes separated. However, in these double mutant backgrounds, the distance between daughter nuclei is abnormally short, indicating a role for these motors in internuclear spacing as well as centrosome separation (Sharp et al. 1999).

During centrosome separation, Cnn fibers, hypothesized to be intercentrosomal microtubules, connect the centrosomes and persist into late anaphase, while $cnn^{\Delta 1}$ mutants display defects in centrosome separation (Zhang and Megraw 2007). In *sced* or *sponge* mutants, where furrows do not form, centrosome separation is also delayed (Stevenson et al. 2001; Postner et al. 1992). *arpc1* or *dia* mutants as well as Rho1 inhibition by C3 exotransferase, which display abnormal furrow formation, all result in defective centrosome separation (Cao et al. 2010). Loss of Daughterless-like (Dal), a protein of unknown function, also results in defective centrosome separation resulting in spindle fusion and nuclear fallout, but only during the cortical cleavage cycles (Sullivan et al. 1990).

In summary, centrosome separation in the early embryo is necessary for proper cleavage furrow formation during the cortical cleavage cycles. Centrosome separation is reliant on actin, microtubules, microtubule motors, and a variety of proteins involved in cleavage furrow formation. The mechanisms that force the centrosomes apart during mitosis still remain unclear, but the variety of factors involved suggest it is a complicated process that requires further investigation.

12.11 Cellularization Transitions the Syncytial Embryo to the Cellular Blastoderm

After 13 successive cleavage divisions, the cortical nuclei remain in interphase for roughly 70 min as cellular membranes surround each nucleus to form the multicellular embryo. The process of cellularization begins identically to the cortical cleavage cycles: the membrane protrudes forming a cortical bud and actin cap above

each nucleus and its associated pair of centrosomes (Fig. 12.8a) (Foe and Alberts 1983). The actin cap is localized to microvillar projections that densely decorate the cortical buds at the onset of cellularization (Fig. 12.8a) (Turner and Mahowald 1979; Fullilove and Jacobson 1971). Furrows begin to form, and the hairpin-shaped tip of each furrow, the furrow canal (FC), is enriched in components necessary for cellularization (Fig. 12.8b) (Fullilove and Jacobson 1971; Warn and Robert-Nicoud 1990; Young et al. 1991). Immediately after furrow ingression, basal cell junctions assemble below the FC composed of E-cadherin, α-catenin, and β-catenin in preparation for cell formation (Hunter and Wieschaus 2000; Müller and Wieschaus 1996).

As the furrow ingresses, the nuclei elongate and extend into the embryo, resulting in oblong nuclei (Lecuit and Wieschaus 2000; Knoblich 2000). This change in nuclear shape is due to a basally extending microtubule basket formed by the astral microtubules (Fig. 12.8b–e) (Callaini and Anselmi 1988; Kellogg et al. 1991). Nocodazole treatment prevents nuclear shape change, supporting a role for these microtubule baskets in nuclear elongation during furrow ingression (Brandt et al. 2006). These microtubule baskets also rely on actin, as cytochalasin treatment disrupts microtubule basket organization (Edgar et al. 1987).

Unlike the cortical cleavage cycles, cellularization requires the nuclei to be present as aphidicolin-injected embryos do not cellularize (Raff and Glover 1989). This is most likely due to the requirement of zygotic transcription that starts during the cortical cleavage cycles and decreases at the end of cellularization (Lamb and Laird 1976; McKnight et al. 1977; Zalokar 1976; Edgar and Schubiger 1986) as α-amanitin injection (a transcription inhibitor) blocks cellularization (Edgar et al. 1986). Most gene products required for cellularization are maternally supplied but some necessary proteins are zygotically transcribed, indicating they have a specific role in this process (Mazumdar and Mazumdar 2002).

The furrows slowly progress until they reach the basal end of the nuclei, where progression then rapidly increases with furrows ultimately reaching a depth of approximately 35 µm (Lecuit and Wieschaus 2000; Foe and Alberts 1983). An actomyosin contractile ring forms at the FC as it invaginates and begins to contract once the furrows have passed the nuclei. This results in a change from the hexagonal array of actin that surrounds the nuclei to a ring shape localization in preparation for cell closure (Fig. 12.8f–g) (Theurkauf 1994). The contractile rings then pinch off the membranes at the end of cellularization to form single cells (Fig. 12.8e).

Because furrow invagination is so extensive and demanding of membrane resources, the membrane present at the cortex does not provide a sufficient supply needed for cellularization, and new sources of membrane are required (Figard et al. 2013). Two different deposits of membrane are supplied to the furrows during different time points of cellularization.

The first deposit is derived from the microvillar actin projections on the surface of the cortex above each nucleus (Fig. 12.8a–d). These microvillar projections assemble before cellularization, and are depleted by its completion (Fabrowski et al. 2013; Fullilove and Jacobson 1971). It is estimated that the microvilli contain about half of the membrane necessary for furrow formation (Figard et al. 2013). New membrane is
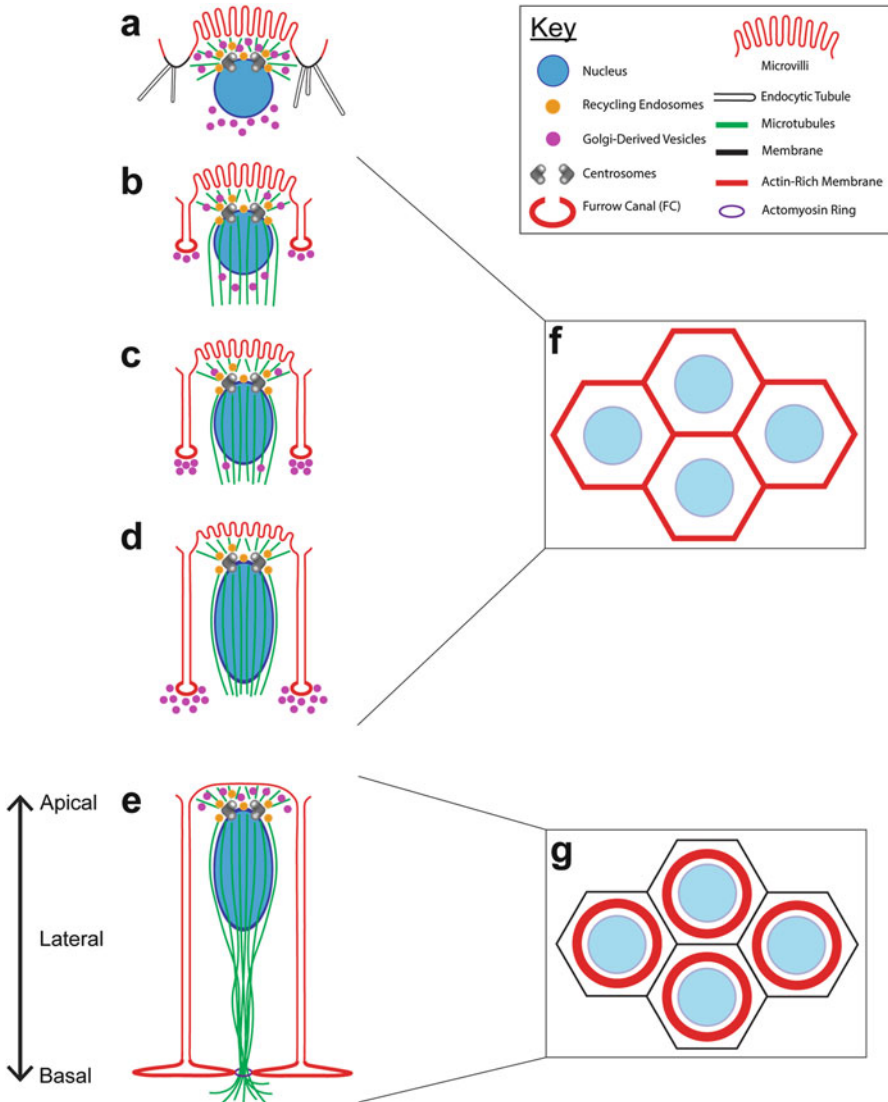
Fig. 12.8 Cellularization of the nuclei requires a deep invagination of the membrane which eventually cleaves to form separate cells. (**a**) During prophase, the centrosomes impinge on the plasma membrane to form a cortical bud over the nucleus. The REs remains apical to the nucleus near the centrosomes and Golgi-derived vesicles localize at the basal end of the nucleus. Long microvillar projections rich in actin form above each nucleus. (**b**) The furrow canal forms, slowly invaginating away from the cortex. The microtubules begin to form around the nucleus as it elongates. Golgi-derived vesicles localize to the furrow canal. The microvilli slowly regress into the furrows. (**c**) The furrow continues to grow, and the microtubules surround the nucleus as it continues to elongate. More Golgi-derived vesicles begin to localize at the furrow canal and the microvilli continue to recede as the membrane invaginates. (**d**) The nucleus is fully elongated, and the microtubules surround it in a basket shape. The Golgi-derived vesicles are all localized at the furrow canal. The microvilli are almost depleted as the furrow canal reaches the basal end of the nucleus. (**e**) The furrow canal spreads to form a ring at the bottom of the nucleus that pinches off the

added at the apical end of the furrows and old membrane is pushed basally into the furrows, rather than the addition of membrane to the FC, supporting the role for microvillar membrane addition (Lecuit and Wieschaus 2000). Microvillar depletion also mimics the kinetics of furrow ingression, starting slow as the furrows reach the bottom of the nuclei and speeding up toward the end of cellularization. Therefore, the microvilli are unfolded and pulled directly into the invaginating furrows (Figard et al. 2013).

Abl tyrosine kinase (Abl) is involved in the regulation of microvillar density and length as *abl* mutants display longer microvilli than wildtype at the onset of cellularization which do not diminish over time as in wildtype. Actin also abnormally accumulates at the apical end of the furrows due to enrichment of the actin nucleators, Arp3 and Dia, resulting in excessive F-action nucleation and abnormal furrow formation. These defects are due to abnormal localization of Enabled (Ena) at the apical cortex and abnormal actin accumulation, which is normally disperse during cellularization, as *ena abl* mutants have less cortical actin accumulation. *abl* mutants also display disrupted microtubule baskets, a further indication that actin regulation plays a role in their formation (Grevengoed et al. 2003).

The second source of membrane is supplied by Golgi-derived vesicles and REs. Centrosomes assist in trafficking these vesicles which contain the proteins and membrane needed for furrow invagination along the microtubules (Mazumdar and Mazumdar 2002). Centrosomes are necessary for furrow invagination and loss of astral microtubules disrupts furrow invagination (Zalokar and Erk 1976; Foe and Alberts 1983). During early cleavage cycles, the Golgi is localized in disperse puncta at the cortex of the embryo in close proximity to the ER (Ripoche et al. 1994; Stanley et al. 1997). Once the nuclei reach the cortex and transcription increases, separate ER and Golgi structures segregate to single nuclei, with each nucleus containing a single ER/Golgi system. This segregation is dependent on microtubules, as nocodazole treatment disrupts ER/Golgi compartmentalization, indicating a role for the centrosome (Frescas et al. 2006). Golgi-derived vesicles either fuse to the apical membrane and endocytose to be sorted by the RE for transport, or traffic directly to the membrane (LaLonde et al. 2006; Lee and Harris 2014). Golgi-derived vesicle and RE transport rely on separate trafficking pathways to transport different components to the invaginating furrows.

Golgi-derived vesicles supply membrane and components that are required for the rapid extension of the furrows. Brefeldin A, which inhibits Golgi vesicle transport, inhibits furrow progression in the final, fast stage of cellularization (Sisson et al. 2000; Frescas et al. 2006). Transport of these vesicles is dependent on microtubules, as colcemid or colchicine treatment during the slow cellularization

---

Fig. 12.8 (continued) membrane to form a cell. The Golgi-derived vesicles move away from the furrow canal to the apical side of the nucleus. (**f**) Cortical view of cellularization during **b–d**. Actin surrounds the nuclei forming a hexagonal pattern at neighboring junctions. (**g**) Cortical view of cellularization during e. The actin surrounding each nucleus begins to constrict into rings

stage stalls vesicle movements at the basal end of the nuclei, preventing furrow invagination (Lecuit and Wieschaus 2000; Sisson et al. 2000; Foe and Alberts 1983; Zalokar and Erk 1976). Golgi vesicle transport is not dependent on actin as cytochalasin injection does not disrupt Golgi vesicle movement during cellularization (Sisson et al. 2000).

The dynein-dynactin complex in association with Cytoplasmic linker protein 190 (CLIP190), which links vesicles to microtubules (Lantz and Miller 1998), assists in transporting Golgi-derived vesicles along the microtubules. *dhc64C* mutants do not form furrows and Golgi-derived vesicle movement is blocked, while the microtubules remain intact (Papoulas et al. 2005). Dhc64C, DCTN2-p150, and CLIP190 all associate at the Golgi and specifically bind the Golgi-associated protein Lava Lamp (Lva), which acts as an adaptor for Dynein-Dynactin vesicular trafficking (Sisson et al. 2000; Papoulas et al. 2005). In support of this, *lva* mutants that cannot bind Dhc64C, DCTN2-p150, or CLIP190 display impaired furrow progression and inhibition of Golgi vesicle movement. Lva is necessary for CLIP190-dependent microtubule-vesicle attachment, as *lva* mutants that cannot bind dynein inhibit CLIP190 localization to the Golgi and FC, resulting in impaired furrow formation (Papoulas et al. 2005).

The second class of vesicles, REs, are necessary for all stages of cellularization. REs localize intermediately near the centrosomes as the vesicles are being sorted to transport membrane and protein to the furrows (van Ijzendoorn 2006). Rab5 and Dynamin are necessary for the initial endocytosis and budding of REs from the apical membrane, as overexpressing a dominant negative variant of Rab5 or *shi* mutant backgrounds display impaired furrow ingression (Pelissier et al. 2003). An intermediate stage of the RE pathway displays tubular membrane projections from the FC, but only at the onset of cellularization (Fig. 12.8a) (Sokac and Wieschaus 2008a). In *shi* mutants, these endocytic tubules are longer than normal, indicating stalled trafficking of REs (Sokac and Wieschaus 2008a; Su et al. 2013; Sherlekar and Rikhy 2016).

The regulation of the REs follows similar dynamics to RE trafficking during the cortical cleavage cycles. Rab11, Dynamin, and Nuf are required for vesicle trafficking and dominant negative Rab11, *shi*, and *nuf* mutants all display impaired furrow invagination, while *shi* mutants display Rab11 vesicles halted at the centrosome (Pelissier et al. 2003; Rothwell et al. 1998). Rab11 and Nuf also localize RhoGEF2 to the furrows to drive furrow invagination through the same mechanisms outlined in the cortical cleavage cycles section. (Riggs et al. 2003; Cao et al. 2008).

Rab11, Nuf, and Dynamin regulate the localization of Slow as molasses (Slam), which is both maternally supplied and zygotically transcribed, to the FCs (Acharya et al. 2014). Slam is necessary for Rho1, RhoGEF2, and MyoII localization to the furrows and *slam* mutants display disrupted furrow invagination (Wenzl et al. 2010; Acharya et al. 2014). Centrosomes are also necessary for Slam localization to the furrows as nuclei-less centrosomes still localize Slam, and ablating centrosomes disrupts Slam localization (Acharya et al. 2014). Therefore, Slam utilizes centrosome-dependent RE vesicle transport for localization at the FCs.

Slam plays a redundant role with Nullo, another zygotically transcribed gene product that is necessary for furrow invagination and stabilization, in Dia localization (Sokac and Wieschaus 2008a, b; Hunter and Wieschaus 2000). *slam nullo* double mutants, but not single mutants of either, display disruption of Dia localization at the FCs (Acharya et al. 2014). Dia localization is also dependent on RhoGEF2, both of which localize to the FCs before invagination, suggesting they aid in FC formation. Mutants for *rhogef2* and *dia* display areas absent of furrows and misshapen FCs. When either *rhogef2* or *dia* mutants are combined with *nullo* mutants, the furrow defects are stronger, suggesting Nullo acts in a pathway separate from RhoGEF2 and Dia (Großhans et al. 2005). Therefore, Nullo may coordinate furrow invagination through F-actin regulation by Slam, RhoGEF2, and Dia.

Rho1, Rok, and RhoGEF2 are all required for the final stage of cellularization. RhoGEF2 localizes Rho1 to the furrows for localized Rok activation (Padash Barmchi et al. 2005). *rhogef2* and *rok* mutants display similar defects in cellularization, defective furrow invagination (Dawes-Hoang et al. 2005), and dominant negative *rho1* mutants do not cellularize (Crawford et al. 1998). The Rho1 pathway regulates MyoII activation, which is necessary for contractile ring constriction (Xue and Sokac 2016). Anillin is known to link Rho1, actin, and MyoII during cytokinesis (Piekny and Glotzer 2008) and is necessary for MyoII localization as well as Pnut localization to the FCs. In *anillin* mutants, FC morphology is abnormal, contractile rings fail to form, and cellularization does not occur, indicating it may link Rho1, actin, and MyoII during cellularization (Field et al. 2005; Thomas and Wieschaus 2004).

Bottleneck (Bnk), a zygotically transcribed gene product, regulates the constriction of the contractile ring toward the end of cellularization. Bnk localizes to the FCs during the slow phases of cellularization and disappears during the fast stage when constriction begins. In *bnk* mutants, the hexagonal actin rings constrict before the furrows are past the nuclei resulting in bottle-shaped nuclei (Schejter and Wieschaus 1993; Theurkauf 1994). Therefore, bnk is a negative regulator of actomyosin constriction during cellularization.

For more on *Drosophila* cellularization, see How one becomes many: blastoderm cellularization in *Drosophila melanogaster* (Mazumdar and Mazumdar 2002).

12.12 Summary

The centrosome plays an important role in regulating the dynamics of early development in the *Drosophila* syncytial embryo at multiple stages. The centrosome regulates the cytoskeletal elements, microtubules and actin, in varying contexts to allow for each step of embryogenesis to properly occur. The first three centrosome-dependent stages of embryogenesis are nuclear migrations that utilize cytoskeletal components. The remaining three stages utilize centrosome-dependent actin regulation to form furrows that separate the nuclei as they divide and eventually form cellular membranes. Defective centrosomes can disrupt the cell cycle, mitotic

cleavage divisions, and nuclear positioning, resulting in morphogenic defects and embryonic lethality. The syncytial embryonic cell cycle is modified to address the rapid nuclear divisions, resulting in changes to the regulation of the centrosome. The central roles of the centrosome in the early embryo contrast with the ability of zygotic development to be accomplished successfully without functional centrosomes. All of the processes controlled by the centrosome in the early embryo are not well understood, so much remains for investigators to discover.

References

Acharya S, Laupsien P, Wenzl C, Yan S, Großhans J (2014) Function and dynamics of *slam* in furrow formation in early Drosophila embryo. Dev Biol 386(2):371–384

Afshar K, Stuart B, Wasserman SA (2000) Functional analysis of the *Drosophila* diaphanous FH protein in early embryonic development. Development 127(9):1887–1897

Al-Hakim AK, Bashkurov M, Gingras AC, Durocher D, Pelletier L (2012) Interaction proteomics identify NEURL4 and the HECT E3 ligase HERC2 as novel modulators of centrosome architecture. Mol Cell Proteomics 11(6):M111.014233

Archambault V, Pinson X (2010) Free centrosomes: where do they all come from? Fly 4 (2):172–177

Archambault V, Zhao X, White-Cooper H, Carpenter AT, Glover DM (2007) Mutations in *Drosophila Greatwall/Scant* reveal its roles in mitosis and meiosis and interdependence with polo kinase. PLoS Genet 3(11):e200

Archambault V, D'Avino PP, Deery MJ, Lilley KS, Glover DM (2008) Sequestration of polo kinase to microtubules by phosphopriming-independent binding to map 205 is relieved by phosphorylation at a CDK site in mitosis. Genes Dev 22(19):2707–2720

Baker J, Theurkauf WE, Schubiger G (1993) Dynamic changes in microtubule configuration correlate with nuclear migration in the preblastoderm *Drosophila* embryo. J Cell Biol 122 (1):113–121

Barros TP, Kinoshita K, Hyman AA, Raff JW (2005) Aurora a activates D-TACC-Msps complexes exclusively at centrosomes to stabilize centrosomal microtubules. J Cell Biol 170(7):1039–1046

Belecz I, Gonzalez C, Puro J, Szabad J (2001) Dominant-negative mutant dynein allows spontaneous centrosome assembly, uncouples chromosome and centrosome cycles. Curr Biol 11 (2):136–140

Blachon S, Cai X, Roberts KA, Yang K, Polyanovsky A, Church A, Avidor-Reiss T (2009) A proximal centriole-like structure is present in *Drosophila* spermatids and can serve as a model to study centriole duplication. Genetics 182(1):133–144

Blachon S, Khire A, Avidor-Reiss T (2014) The origin of the second centriole in the zygote of Drosophila melanogaster. Genetics 197(1):199–205

Bownes M (1982) Embryogenesis. A handbook of *Drosophila*. Elsevier, Amsterdam

Brandt A, Papagiannouli F, Wagner N, Wilsch-Bräuninger M, Braun M, Furlong EE, Loserth S, Wenzl C, Pilot F, Vogt N, Lecuit T, Krohne G, Grosshans J (2006) Developmental control of nuclear size and shape by Kugelkern and Kurzkern. Curr Biol 16(6):543–552

Brent AE, MacQueen A, Hazelrigg T (2000) The *Drosophila wispy* gene is required for RNA localization and other microtubule-based events of meiosis and early embryogenesis. Genetics 154(4):1649–1662

Brunk K, Vernay B, Griffith E, Reynolds NL, Strutt D, Ingham PW, Jackson AP (2007) Microcephalin coordinates mitosis in the syncytial *Drosophila* embryo. J Cell Sci 120. (Pt 20:3578–3588

Burkhardt JK, Echeverri CJ, Nilsson T, Vallee RB (1997) Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. J Cell Biol 139(2):469–484

Buttrick GJ, Beaumont LM, Leitch J, Yau C, Hughes JR, Wakefield JG (2008) Akt regulates centrosome migration and spindle orientation in the early *Drosophila melanogaster* embryo. J Cell Biol 180(3):537–548

Callaini G, Anselmi F (1988) Centrosome splitting during nuclear elongation in the *Drosophila* embryo. Exp Cell Res 178(2):415–425

Callaini G, Dallai R (1991) Abnormal behavior of the yolk centrosomes during early embryogenesis of *Drosophila melanogaster*. Exp Cell Res 192(1):16–21

Callaini G, Riparbelli MG (1996) Fertilization in *Drosophila melanogaster*: centrosome inheritance and organization of the first mitotic spindle. Dev Biol 176(2):199–208

Callaini G, Dallai R, Riparbelli MG (1992) Cytochalasin induces spindle fusion in the syncytial blastoderm of the early *Drosophila* embryo. Biol Cell 74(3):249–254

Callaini G, Whitfield WG, Riparbelli MG (1997) Centriole and centrosome dynamics during the embryonic cell cycles that follow the formation of the cellular blastoderm in *Drosophila*. Exp Cell Res 234(1):183–190

Callaini G, Riparbelli MG, Dallai R (1999) Centrosome inheritance in insects: fertilization and parthenogenesis. Biol Cell 91(4–5):355–366

Cao J, Albertson R, Riggs B, Field CM, Sullivan W (2008) Nuf, a Rab11 effector, maintains cytokinetic furrow integrity by promoting local actin polymerization. J Cell Biol 182 (2):301–313

Cao J, Crest J, Fasulo B, Sullivan W (2010) Cortical actin dynamics facilitate early-stage centrosome separation. Curr Biol 20(8):770–776

Cesario JM, Jang JK, Redding B, Shah N, Rahman T, McKim KS (2006) Kinesin 6 family member Subito participates in mitotic spindle assembly and interacts with mitotic regulators. J Cell Sci 119(Pt 22):4770–4780

Chen JV, Buchwalter RA, Kao LR, Megraw TL (2017) A splice variant of centrosomin converts mitochondria to microtubule-organizing centers. Curr Biol 27(13):1928–1940.e1926

Chodagam S, Royou A, Whitfield W, Karess R, Raff JW (2005) The centrosomal protein CP190 regulates myosin function during early *Drosophila* development. Curr Biol 15(14):1308–1313

Cinalli RM, Lehmann R (2013) A spindle-independent cleavage pathway controls germ cell formation in *Drosophila*. Nat Cell Biol 15(7):839–845

Crawford JM, Harden N, Leung T, Lim L, Kiehart DP (1998) Cellularization in *Drosophila melanogaster* is disrupted by the inhibition of rho activity and the activation of Cdc42 function. Dev Biol 204(1):151–164

Crest J, Concha-Moore K, Sullivan W (2012) RhoGEF and positioning of rappaport-like furrows in the early *Drosophila* embryo. Curr Biol 22(21):2037–2041

Cullen CF, Ohkura H (2001) Msps protein is localized to acentrosomal poles to ensure bipolarity of *Drosophila* meiotic spindles. Nat Cell Biol 3(7):637–642

Dävring L, Sunner M (1973) Female meiosis and embryonic mitosis in *Drosophila melanogaster*. I. Meiosis and fertilization. Hereditas 73(1):51–64

Dawes-Hoang RE, Parmar KM, Christiansen AE, Phelps CB, Brand AH, Wieschaus EF (2005) *folded gastrulation*, cell shape change and the control of myosin localization. Development 132 (18):4165–4178

Debec A, Kalpin RF, Daily DR, McCallum PD, Rothwell WF, Sullivan W (1996) Live analysis of free centrosomes in normal and aphidicolin-treated *Drosophila* embryos. J Cell Biol 134 (1):103–115

Debec A, Marcaillou C, Bobinnec Y, Borot C (1999) The centrosome cycle in syncytial *Drosophila* embryos analyzed by energy filtering transmission electron microscopy. Biol Cell 91 (4–5):379–391

Debec A, Grammont M, Berson G, Dastugue B, Sullivan W, Couderc JL (2001) Toucan protein is essential for the assembly of syncytial mitotic spindles in *Drosophila melanogaster*. Genesis 31 (4):167–175

Deneke VE, Melbinger A, Vergassola M, Di Talia S (2016) Waves of Cdk1 activity in S phase synchronize the cell cycle in *Drosophila* embryos. Dev Cell 38(4):399–412

Dix CI, Raff JW (2007) *Drosophila* Spd-2 recruits PCM to the sperm centriole, but is dispensable for centriole duplication. Curr Biol 17(20):1759–1764

Ede DA, Counce SJ (1956) A cinematographic study of the embryology of *Drosophila melanogaster*. Roux Arch Dev Biol 148(4):402–415

Edgar BA, Schubiger G (1986) Parameters controlling transcriptional activation during early *Drosophila* development. Cell 44(6):871–877

Edgar BA, Kiehle CP, Schubiger G (1986) Cell cycle control by the nucleo-cytoplasmic ratio in early *Drosophila* development. Cell 44(2):365–372

Edgar BA, Odell GM, Schubiger G (1987) Cytoarchitecture and the patterning of fushi tarazu expression in the *Drosophila* blastoderm. Genes Dev 1(10):1226–1237

Edgar BA, Sprenger F, Duronio RJ, Leopold P, O'Farrell PH (1994) Distinct molecular mechanism regulate cell cycle timing at successive stages of *Drosophila* embryogenesis. Genes Dev 8 (4):440–452

Elfring LK, Axton JM, Fenger DD, Page AW, Carminati JL, Orr-Weaver TL (1997) *Drosophila* PLUTONIUM protein is a specialized cell cycle regulator required at the onset of embryogenesis. Mol Biol Cell 8(4):583–593

Ephrussi A, Lehmann R (1992) Induction of germ cell formation by oskar. Nature 358 (6385):387–392

Fabrowski P, Necakov AS, Mumbauer S, Loeser E, Reversi A, Streichan S, Briggs JA, De Renzis S (2013) Tubular endocytosis drives remodelling of the apical surface during epithelial morphogenesis in *Drosophila*. Nat Commun 4:2244

Farache D, Emorine L, Haren L, Merdes A (2018) Assembly and regulation of γ-tubulin complexes. Open Biol 8(3)

Farina F, Gaillard J, Guérin C, Couté Y, Sillibourne J, Blanchoin L, Théry M (2016) The centrosome is an actin-organizing centre. Nat Cell Biol 18(1):65–75

Fenger DD, Carminati JL, Burney-Sigman DL, Kashevsky H, Dines JL, Elfring LK, Orr-Weaver TL (2000) PAN GU: a protein kinase that inhibits S phase and promotes mitosis in early *Drosophila* development. Development 127(22):4763–4774

Field CM, Alberts BM (1995) Anillin, a contractile ring protein that cycles from the nucleus to the cell cortex. J Cell Biol 131(1):165–178

Field CM, Coughlin M, Doberstein S, Marty T, Sullivan W (2005) Characterization of anillin mutants reveals essential roles in septin localization and plasma membrane integrity. Development 132(12):2849–2860

Figard L, Xu H, Garcia HG, Golding I, Sokac AM (2013) The plasma membrane flattens out to fuel cell-surface growth during *Drosophila* cellularization. Dev Cell 27(6):648–655

Flor-Parra I, Iglesias-Romero AB, Chang F (2018) The XMAP215 ortholog Alp14 promotes microtubule nucleation in fission yeast. Curr Biol 28(11):1681–1691.e1684

Foe VE, Alberts BM (1983) Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. J Cell Sci 61:31–70

Foe VE, Odell GM, Edgar BA (1993) Mitosis and morphogenesis in the *Drosophila* embryo: point and counterpoint. In: Bate M, Martinez Arias A (eds) The Development of *Drosophila melanogaster*, vol 1. Cold Spring Harbor Press, Cold Spring Harbor, NY

Foe VE, Field CM, Odell GM (2000) Microtubules and mitotic cycle phase modulate spatiotemporal distributions of F-actin and myosin II in *Drosophila* syncytial blastoderm embryos. Development 127(9):1767–1787

Fogarty P, Kalpin RF, Sullivan W (1994) The *Drosophila* maternal-effect mutation *grapes* causes a metaphase arrest at nuclear cycle 13. Development 120(8):2131–2142

Fogarty P, Campbell SD, Abu-Shumays R, Phalle BS, Yu KR, Uy GL, Goldberg ML, Sullivan W (1997) The *Drosophila grapes* gene is related to checkpoint gene *chk1/rad27* and is required for late syncytial division fidelity. Curr Biol 7(6):418–426

Franz A, Roque H, Saurya S, Dobbelaere J, Raff JW (2013) CP110 exhibits novel regulatory activities during centriole assembly in *Drosophila*. J Cell Biol 203(5):785–799

Freeman M, Glover DM (1987) The *gnu* mutation of *Drosophila* causes inappropriate DNA synthesis in unfertilized and fertilized eggs. Genes Dev 1(9):924–930

Freeman M, Nüsslein-Volhard C, Glover DM (1986) The dissociation of nuclear and centrosomal division in *gnu*, a mutation causing giant nuclei in *Drosophila*. Cell 46(3):457–468

Frescas D, Mavrakis M, Lorenz H, Delotto R, Lippincott-Schwartz J (2006) The secretory membrane system in the *Drosophila* syncytial blastoderm embryo exists as functionally compartmentalized units around individual nuclei. J Cell Biol 173(2):219–230

Fullilove SL, Jacobson AG (1971) Nuclear elongation and cytokinesis in Drosophila montana. Dev Biol 26(4):560–577

Gergely F, Karlsson C, Still I, Cowell J, Kilmartin J, Raff JW (2000a) The TACC domain identifies a family of centrosomal proteins that can interact with microtubules. Proc Natl Acad Sci U S A 97(26):14352–14357

Gergely F, Kidd D, Jeffers K, Wakefield JG, Raff JW (2000b) D-TACC: a novel centrosomal protein required for normal spindle function in the early *Drosophila* embryo. EMBO J 19 (2):241–252

Giansanti MG, 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(4):303–309

Giet R, McLean D, Descamps S, Lee MJ, Raff JW, Prigent C, Glover DM (2002) *Drosophila* Aurora a kinase is required to localize D-TACC to centrosomes and to regulate astral microtubules. J Cell Biol 156(3):437–451

Giunta KL, Jang JK, Manheim EA, Subramanian G, McKim KS (2002) *subito* encodes a kinesin-like protein required for meiotic spindle pole formation in *Drosophila melanogaster*. Genetics 160(4):1489–1501

Glover DM (2005) Polo kinase and progression through M phase in *Drosophila*: a perspective from the spindle poles. Oncogene 24(2):230–237

Glover DM, Alphey L, Axton JM, Cheshire A, Dalby B, Freeman M, Girdham C, Gonzalez C, Karess RE, Leibowitz MH (1989) Mitosis in *Drosophila* development. J Cell Sci Suppl 12:277–291

González C, Saunders RD, Casal J, Molina I, Carmena M, Ripoll P, Glover DM (1990) Mutations at the asp locus of *Drosophila* lead to multiple free centrosomes in syncytial embryos, but restrict centrosome duplication in larval neuroblasts. J Cell Sci 96(Pt 4):605–616

González C, Tavosanis G, Mollinari C (1998) Centrosomes and microtubule organisation during *Drosophila* development. J Cell Sci 111(Pt 18):2697–2706

Gottardo M, Callaini G, Riparbelli MG (2015) The *Drosophila* centriole—conversion of doublets into triplets within the stem cell niche. J Cell Sci 128(14):2437–2442

Grevengoed EE, Fox DT, Gates J, Peifer M (2003) Balancing different types of actin polymerization at distinct sites: roles for Abelson kinase and enabled. J Cell Biol 163(6):1267–1279

Großhans J, Wenzl C, Herz HM, Bartoszewski S, Schnorrer F, Vogt N, Schwarz H, Müller HA (2005) RhoGEF2 and the formin Dia control the formation of the furrow canal by directed actin assembly during *Drosophila* cellularisation. Development 132(5):1009–1020

Gunawardane RN, Lizarraga SB, Wiese C, Wilde A, Zheng Y (2000) γ-tubulin complexes and their role in microtubule nucleation. Curr Top Dev Biol 49:55–73

Gunzelmann J, Rüthnick D, Lin TC, Zhang W, Neuner A, Jäkle U, Schiebel E (2018) The microtubule polymerase Stu2 promotes oligomerization of the γ-TuSC for cytoplasmic microtubule nucleation. elife 7

Hatanaka K, Okada M (1991) Retarded nuclear migration in *Drosophila* embryos with aberrant F-actin reorganization caused by maternal mutations and by cytochalasin treatment. Development 111(4):909–920

Heck MM, Pereira A, Pesavento P, Yannoni Y, Spradling AC, Goldstein LS (1993) The kinesin-like protein KLP61F is essential for mitosis in *Drosophila*. J Cell Biol 123(3):665–679

Hieda M (2017) Implications for diverse functions of the LINC complexes based on the structure. Cell 6(1)

Huang J, Raff JW (1999) The disappearance of cyclin B at the end of mitosis is regulated spatially in *Drosophila* cells. EMBO J 18(8):2184–2195

Hunter C, Wieschaus E (2000) Regulated expression of nullo is required for the formation of distinct apical and basal adherens junctions in the *Drosophila* blastoderm. J Cell Biol 150 (2):391–401

Iampietro C, Bergalet J, Wang X, Cody NA, Chin A, Lefebvre FA, Douziech M, Krause HM, Lécuyer E (2014) Developmentally regulated elimination of damaged nuclei involves a Chk2-dependent mechanism of mRNA nuclear retention. Dev Cell 29(4):468–481

Iida T, Kobayashi S (2000) Delocalization of polar plasm components caused by *grandchildless* mutations, *gs(1)N26* and *gs(1)N441*, in *Drosophila melanogaster*. Develop Growth Differ 42 (1):53–60

Illmensee K, Mahowald AP (1974) Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. Proc Natl Acad Sci U S A 71 (4):1016–1020

Jones J, Macdonald PM (2015) Neurl4 contributes to germ cell formation and integrity in *Drosophila*. Biol Open 4(8):937–946

Jongens TA, Hay B, Jan LY, Jan YN (1992) The *germ cell-less* gene product: a posteriorly localized component necessary for germ cell development in *Drosophila*. Cell 70(4):569–584

Jongens TA, Ackerman LD, Swedlow JR, Jan LY, Jan YN (1994) *Germ cell-less* encodes a cell type-specific nuclear pore-associated protein and functions early in the germ-cell specification pathway of *Drosophila*. Genes Dev 8(18):2123–2136

Jordan P, Karess R (1997) Myosin light chain-activating phosphorylation sites are required for oogenesis in *Drosophila*. J Cell Biol 139(7):1805–1819

Kao LR, Megraw TL (2009) Centrocortin cooperates with centrosomin to organize *Drosophila* embryonic cleavage furrows. Curr Biol 19(11):937–942

Karess RE, Chang XJ, Edwards KA, Kulkarni S, Aguilera I, Kiehart DP (1991) The regulatory light chain of nonmuscle myosin is encoded by *spaghetti-squash*, a gene required for cytokinesis in *Drosophila*. Cell 65(7):1177–1189

Karr TL (1991) Intracellular sperm/egg interactions in *Drosophila*: a three-dimensional structural analysis of a paternal product in the developing egg. Mech Dev 34(2–3):101–111

Karr TL, Alberts BM (1986) Organization of the cytoskeleton in early *Drosophila* embryos. J Cell Biol 102(4):1494–1509

Kellogg DR, Mitchison TJ, Alberts BM (1988) Behaviour of microtubules and actin filaments in living *Drosophila* embryos. Development 103(4):675–686

Kellogg DR, Field CM, Alberts BM (1989) Identification of microtubule-associated proteins in the centrosome, spindle, and kinetochore of the early *Drosophila* embryo. J Cell Biol 109(6 Pt 1):2977–2991

Kellogg DR, Sullivan W, Theurkauf W, Oegema K, Raff JW, Alberts BM (1991) Studies on the centrosome and cytoplasmic organization in the early *Drosophila* embryo. Cold Spring Harb Symp Quant Biol 56:649–662

Khire A, Jo KH, Kong D, Akhshi T, Blachon S, Cekic AR, Hynek S, Ha A, Loncarek J, Mennella V, Avidor-Reiss T (2016) Centriole remodeling during spermiogenesis in *Drosophila*. Curr Biol 26(23):3183–3189

Kiehart DP, Ketchum A, Young P, Lutz D, Alfenito MR, Chang XJ, Awobuluyi M, Pesacreta TC, Inoué S, Stewart CT (1990) Contractile proteins in *Drosophila* development. Ann N Y Acad Sci 582:233–251

Knoblich JA (2000) Epithelial polarity: the ins and outs of the fly epidermis. Curr Biol 10(21): R791–R794

Kollman JM, Greenberg CH, Li S, Moritz M, Zelter A, Fong KK, Fernandez JJ, Sali A, Kilmartin J, Davis TN, Agard DA (2015) Ring closure activates yeast γTuRC for species-specific microtubule nucleation. Nat Struct Mol Biol 22(2):132–137

Komma DJ, Endow SA (1997) Enhancement of the ncdD microtubule motor mutant by mutants of αTub67C. J Cell Sci 110(Pt 2):229–237

Kotadia S, Crest J, Tram U, Riggs B, Sullivan W (2010) Blastoderm formation and cellularisation in *Drosophila melanogaster*. eLS

LaLonde M, Janssens H, Yun S, Crosby J, Redina O, Olive V, Altshuller YM, Choi SY, Du G, Gergen JP, Frohman MA (2006) A role for phospholipase D in *Drosophila* embryonic cellularization. BMC Dev Biol 6:60

Lamb MM, Laird CD (1976) Increase in nuclear poly(A)-containing RNA at syncytial blastoderm in *Drosophila melanogaster* embryos. Dev Biol 52(1):31–42

Lantz VA, Miller KG (1998) A class VI unconventional myosin is associated with a homologue of a microtubule-binding protein, cytoplasmic linker protein-170, in neurons and at the posterior pole of *Drosophila* embryos. J Cell Biol 140(4):897–910

Lantz VA, Clemens SE, Miller KG (1999) The actin cytoskeleton is required for maintenance of posterior pole plasm components in the *Drosophila* embryo. Mech Dev 85(1–2):111–122

Lasko P (2012) mRNA localization and translational control in *Drosophila* oogenesis. Cold Spring Harb Perspect Biol 4(10)

Lattao R, Kovács L, Glover DM (2017) The centrioles, centrosomes, basal bodies, and cilia of *Drosophila melanogaster*. Genetics 206(1):33–53

Lecuit T, Wieschaus E (2000) Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the *Drosophila* embryo. J Cell Biol 150(4):849–860

Lee DM, Harris TJ (2014) Coordinating the cytoskeleton and endocytosis for regulated plasma membrane growth in the early *Drosophila* embryo. BioArchitecture 4(2):68–74

Lee MJ, Gergely F, Jeffers K, Peak-Chew SY, Raff JW (2001) Msps/XMAP215 interacts with the centrosomal protein D-TACC to regulate microtubule behaviour. Nat Cell Biol 3(7):643–649

Lee DM, Wilk R, Hu J, Krause HM, Harris TJ (2015) Germ cell segregation from the *Drosophila* Soma is controlled by an inhibitory threshold set by the Arf-GEF Steppke. Genetics 200 (3):863–872

Lerit DA, Gavis ER (2011) Transport of germ plasm on astral microtubules directs germ cell development in *Drosophila*. Curr Biol 21(6):439–448

Lerit DA, Shebelut CW, Lawlor KJ, Rusan NM, Gavis ER, Schedl P, Deshpande G (2017) Germ cell-less promotes centrosome segregation to induce germ cell formation. Cell Rep 18 (4):831–839

Li J, Kim S, Kobayashi T, Liang FX, Korzeniewski N, Duensing S, Dynlacht BD (2012) Neurl4, a novel daughter centriole protein, prevents formation of ectopic microtubule organizing centres. EMBO Rep 13(6):547–553

Lin TC, Neuner A, Schiebel E (2015) Targeting of γ-tubulin complexes to microtubule organizing centers: conservation and divergence. Trends Cell Biol 25(5):296–307

Lindeman RE, Pelegri F (2012) Localized products of *futile cycle/lrmp* promote centrosome-nucleus attachment in the zebrafish zygote. Curr Biol 22(10):843–851

Llamazares S, Tavosanis G, Gonzalez C (1999) Cytological characterisation of the mutant phenotypes produced during early embryogenesis by null and loss-of-function alleles of the γTub37C gene in *Drosophila*. J Cell Sci 112(Pt 5):659–667

Loppin B, Dubruille R, Horard B (2015) The intimate genetics of *Drosophila* fertilization. Open Biol 5(8)

Lucas EP, Raff JW (2007) Maintaining the proper connection between the centrioles and the pericentriolar matrix requires *Drosophila* centrosomin. J Cell Biol 178(5):725–732

Mahoney NM, Goshima G, Douglass AD, Vale RD (2006) Making microtubules and mitotic spindles in cells without functional centrosomes. Curr Biol 16(6):564–569

Mahowald AP (2001) Assembly of the *Drosophila* germ plasm. Int Rev Cytol 203:187–213

Malone CJ, Misner L, Le Bot N, Tsai MC, Campbell JM, Ahringer J, White JG (2003) The *C. elegans* hook protein, ZYG-12, mediates the essential attachment between the centrosome and nucleus. Cell 115(7):825–836

Mazumdar A, Mazumdar M (2002) How one becomes many: blastoderm cellularization in *Drosophila melanogaster*. BioEssays 24(11):1012–1022

McCartney BM, Peifer M (2000) Teaching tumour suppressors new tricks. Nat Cell Biol 2(4):E58–E60

McCartney BM, McEwen DG, Grevengoed E, Maddox P, Bejsovec A, Peifer M (2001) *Drosophila* APC2 and armadillo participate in tethering mitotic spindles to cortical actin. Nat Cell Biol 3 (10):933–938

McKnight SL, Bustin M, Miller OL (1977) Electron microscope analysis of chromosome metabolism in *Drosophila melanogaster embryo*. Cold Spring Harb Symp Quant Biol 41:741–754

Megraw TL, Li K, Kao LR, Kaufman TC (1999) The centrosomin protein is required for centrosome assembly and function during cleavage in Drosophila. Development 126(13):2829–2839

Megraw TL, Kao LR, Kaufman TC (2001) Zygotic development without functional mitotic centrosomes. Curr Biol 11(2):116–120

Mermall V, Miller KG (1995) The 95F unconventional myosin is required for proper organization of the *Drosophila* syncytial blastoderm. J Cell Biol 129(6):1575–1588

Mermall V, McNally JG, Miller KG (1994) Transport of cytoplasmic particles catalysed by an unconventional myosin in living *Drosophila* embryos. Nature 369(6481):560–562

Meyerzon M, Gao Z, Liu J, Wu JC, Malone CJ, Starr DA (2009) Centrosome attachment to the *C. elegans* male pronucleus is dependent on the surface area of the nuclear envelope. Dev Biol 327(2):433–446

Miller KG, Kiehart DP (1995) Fly division. J Cell Biol 131(1):1–5

Minestrini G, Harley AS, Glover DM (2003) Localization of Pavarotti-KLP in living *Drosophila* embryos suggests roles in reorganizing the cortical cytoskeleton during the mitotic cycle. Mol Biol Cell 14(10):4028–4038

Mirouse V, Dastugue B, Couderc JL (2005) The *Drosophila* toucan protein is a new mitotic microtubule-associated protein required for spindle microtubule stability. Genes Cells 10 (1):37–46

Mogensen MM, Tucker JB, Baggaley TB (1993) Multiple plasma membrane-associated MTOC systems in the acentrosomal cone cells of *Drosophila* ommatidia. Eur J Cell Biol 60(1):67–75

Moritz M, Braunfeld MB, Fung JC, Sedat JW, Alberts BM, Agard DA (1995) Three-dimensional structural characterization of centrosomes from early *Drosophila* embryos. J Cell Biol 130 (5):1149–1159

Müller HA, Wieschaus E (1996) *armadillo, bazooka,* and *stardust* are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in *Drosophila*. J Cell Biol 134(1):149–163

Niki Y (1984) Developmental analysis of the *grandchildless (gs(1)N26)* mutation in *Drosophila melanogaster*: abnormal cleavage patterns and defects in pole cell formation. Dev Biol 103 (1):182–189

Niki Y, Okada M (1981) Isolation and characterization of *grandchildless-like* mutants in *Drosophila melanogaster*. Roux Arch Dev Biol 190(1):1–10

Nithianantham S, Cook BD, Beans M, Guo F, Chang F, Al-Bassam J (2018) Structural basis of tubulin recruitment and assembly by microtubule polymerases with tumor overexpressed gene (TOG) domain arrays. elife 7

O'Farrell PH (2015) Growing an embryo from a single cell: a hurdle in animal life. Cold Spring Harb Perspect Biol 7(11)

O'Farrell PH, Stumpff J, Su TT (2004) Embryonic cleavage cycles: how is a mouse like a fly? Curr Biol 14(1):R35–R45

Oakley BR (2000) γ-Tubulin. Curr Top Dev Biol 49:27–54

Oakley BR, Paolillo V, Zheng Y (2015) γ-Tubulin complexes in microtubule nucleation and beyond. Mol Biol Cell 26(17):2957–2962

Okada M (1982) Loss of the ability to form pole cells in *Drosophila* embryos with artificially delayed nuclear arrival at the posterior pole. Prog Clin Biol Res 85(Pt A):363–372

Padash Barmchi M, Rogers S, Häcker U (2005) DRhoGEF2 regulates actin organization and contractility in the *Drosophila* blastoderm embryo. J Cell Biol 168(4):575–585

Paddy MR, Saumweber H, Agard DA, Sedat JW (1996) Time-resolved, in vivo studies of mitotic spindle formation and nuclear lamina breakdown in *Drosophila* early embryos. J Cell Sci 109 (Pt 3):591–607

Pai CY, Lei EP, Ghosh D, Corces VG (2004) The centrosomal protein CP190 is a component of the *gypsy* chromatin insulator. Mol Cell 16(5):737–748

Papoulas O, Hays TS, Sisson JC (2005) The golgin lava lamp mediates dynein-based Golgi movements during *Drosophila* cellularization. Nat Cell Biol 7(6):612–618

Peel N, Stevens NR, Basto R, Raff JW (2007) Overexpressing centriole-replication proteins in vivo induces centriole overduplication and de novo formation. Curr Biol 17(10):834–843

Pelissier A, Chauvin JP, Lecuit T (2003) Trafficking through Rab11 endosomes is required for cellularization during *Drosophila* embryogenesis. Curr Biol 13(21):1848–1857

Piekny AJ, Glotzer M (2008) Anillin is a scaffold protein that links RhoA, actin, and myosin during cytokinesis. Curr Biol 18(1):30–36

Pollitt AY, Insall RH (2009) WASP and SCAR/WAVE proteins: the drivers of actin assembly. J Cell Sci 122(Pt 15):2575–2578

Postner MA, Miller KG, Wieschaus EF (1992) Maternal effect mutations of the *sponge* locus affect actin cytoskeletal rearrangements in *Drosophila melanogaster* embryos. J Cell Biol 119 (5):1205–1218

Raff JW, Glover DM (1988) Nuclear and cytoplasmic mitotic cycles continue in *Drosophila* embryos in which DNA synthesis is inhibited with aphidicolin. J Cell Biol 107(6 Pt 1):2009–2019

Raff JW, Glover DM (1989) Centrosomes, and not nuclei, initiate pole cell formation in *Drosophila* embryos. Cell 57(4):611–619

Raff JW, Jeffers K, Huang JY (2002) The roles of Fzy/Cdc20 and Fzr/Cdh1 in regulating the destruction of cyclin B in space and time. J Cell Biol 157(7):1139–1149

Rappaport R (1961) Experiments concerning the cleavage stimulus in sand dollar eggs. J Exp Zool 148:81–89

Riggs B, Rothwell W, Mische S, Hickson GR, Matheson J, Hays TS, Gould GW, Sullivan W (2003) Actin cytoskeleton remodeling during early *Drosophila* furrow formation requires recycling endosomal components nuclear-fallout and Rab11. J Cell Biol 163(1):143–154

Riggs B, Fasulo B, Royou A, Mische S, Cao J, Hays TS, Sullivan W (2007) The concentration of Nuf, a Rab11 effector, at the microtubule-organizing center is cell cycle regulated, dynein-dependent, and coincides with furrow formation. Mol Biol Cell 18(9):3313–3322

Rikhy R, Mavrakis M, Lippincott-Schwartz J (2015) Dynamin regulates metaphase furrow formation and plasma membrane compartmentalization in the syncytial *Drosophila* embryo. Biol Open 4(3):301–311

Riparbelli MG, Callaini G (2003) Assembly of yolk spindles in the early *Drosophila* embryo. Mech Dev 120(4):441–454

Riparbelli MG, Callaini G, Glover DM (2000) Failure of pronuclear migration and repeated divisions of polar body nuclei associated with MTOC defects in *polo* eggs of *Drosophila*. J Cell Sci 113(Pt 18):3341–3350

Riparbelli MG, Callaini G, Glover DM, Avides MC (2002) A requirement for the abnormal spindle protein to organise microtubules of the central spindle for cytokinesis in *Drosophila*. J Cell Sci 115(Pt 5):913–922

Riparbelli MG, Callaini G, Schejter ED (2007) Microtubule-dependent organization of subcortical microfilaments in the early *Drosophila* embryo. Dev Dyn 236(3):662–670

Ripoche J, Link B, Yucel JK, Tokuyasu K, Malhotra V (1994) Location of Golgi membranes with reference to dividing nuclei in syncytial *Drosophila* embryos. Proc Natl Acad Sci U S A 91 (5):1878–1882

Robertson SE, Dockendorff TC, Leatherman JL, Faulkner DL, Jongens TA (1999) *germ cell-less* is required only during the establishment of the germ cell lineage of *Drosophila* and has activities which are dependent and independent of its localization to the nuclear envelope. Dev Biol 215 (2):288–297

Robinson JT, Wojcik EJ, Sanders MA, McGrail M, Hays TS (1999) Cytoplasmic dynein is required for the nuclear attachment and migration of centrosomes during mitosis in *Drosophila*. J Cell Biol 146(3):597–608

Rodrigues-Martins A, Bettencourt-Dias M, Riparbelli M, Ferreira C, Ferreira I, Callaini G, Glover DM (2007a) DSAS-6 organizes a tube-like centriole precursor, and its absence suggests modularity in centriole assembly. Curr Biol 17(17):1465–1472

Rodrigues-Martins A, Riparbelli M, Callaini G, Glover DM, Bettencourt-Dias M (2007b) Revisiting the role of the mother centriole in centriole biogenesis. Science 316 (5827):1046–1050

Rodrigues-Martins A, Riparbelli M, Callaini G, Glover DM, Bettencourt-Dias M (2008) From centriole biogenesis to cellular function: centrioles are essential for cell division at critical developmental stages. Cell Cycle 7(1):11–16

Rogers SL, Rogers GC, Sharp DJ, Vale RD (2002) *Drosophila* EB1 is important for proper assembly, dynamics, and positioning of the mitotic spindle. J Cell Biol 158(5):873–884

Rothwell WF, Fogarty P, Field CM, Sullivan W (1998) Nuclear-fallout, a drosophila protein that cycles from the cytoplasm to the centrosomes, regulates cortical microfilament organization. Development 125(7):1295–1303

Rothwell WF, Sullivan W (2000) The centrosome in early *Drosophila* embryogenesis. Curr Top Dev Biol 49:409–447

Rothwell WF, Zhang CX, Zelano C, Hsieh TS, Sullivan W (1999) The *Drosophila* centrosomal protein Nuf is required for recruiting Dah, a membrane associated protein, to furrows in the early embryo. J Cell Sci 112(Pt 17):2885–2893

Royou A, Sullivan W, Karess R (2002) Cortical recruitment of nonmuscle myosin II in early syncytial *Drosophila* embryos: its role in nuclear axial expansion and its regulation by Cdc2 activity. J Cell Biol 158(1):127–137

Schejter ED, Wieschaus E (1993) *bottleneck* acts as a regulator of the microfilament network governing cellularization of the *Drosophila* embryo. Cell 75(2):373–385

Shamanski FL, Orr-Weaver TL (1991) The *Drosophila plutonium* and *pan gu* genes regulate entry into S phase at fertilization. Cell 66(6):1289–1300

Sharp DJ, Yu KR, Sisson JC, Sullivan W, Scholey JM (1999) Antagonistic microtubule-sliding motors position mitotic centrosomes in *Drosophila* early embryos. Nat Cell Biol 1(1):51–54

Shaw M, Cohen P, Alessi DR (1997) Further evidence that the inhibition of glycogen synthase kinase-3beta by IGF-1 is mediated by PDK1/PKB-induced phosphorylation of Ser-9 and not by dephosphorylation of Tyr-216. FEBS Lett 416(3):307–311

Sherlekar A, Rikhy R (2016) Syndapin promotes pseudocleavage furrow formation by actin organization in the syncytial *Drosophila* embryo. Mol Biol Cell 27(13):2064–2079

Shermoen AW, McCleland ML, O'Farrell PH (2010) Developmental control of late replication and S phase length. Curr Biol 20(23):2067–2077

Sibon OC, Stevenson VA, Theurkauf WE (1997) DNA-replication checkpoint control at the *Drosophila* midblastula transition. Nature 388(6637):93–97

Sibon OC, Laurençon A, Hawley R, Theurkauf WE (1999) The *Drosophila* ATM homologue Mei-41 has an essential checkpoint function at the midblastula transition. Curr Biol 9 (6):302–312

Sibon OC, Kelkar A, Lemstra W, Theurkauf WE (2000) DNA-replication/DNA-damage-dependent centrosome inactivation in *Drosophila* embryos. Nat Cell Biol 2(2):90–95

Sigrist S, Jacobs H, Stratmann R, Lehner CF (1995) Exit from mitosis is regulated by *Drosophila fizzy* and the sequential destruction of cyclins A, B and B3. EMBO J 14(19):4827–4838

Sisson JC, Field C, Ventura R, Royou A, Sullivan W (2000) Lava lamp, a novel peripheral golgi protein, is required for *Drosophila melanogaster* cellularization. J Cell Biol 151(4):905–918

Sokac AM, Wieschaus E (2008a) Local actin-dependent endocytosis is zygotically controlled to initiate *Drosophila* cellularization. Dev Cell 14(5):775–786

Sokac AM, Wieschaus E (2008b) Zygotically controlled F-actin establishes cortical compartments to stabilize furrows during *Drosophila* cellularization. J Cell Sci 121(11):1815–1824

Stafstrom JP, Staehelin LA (1984) Dynamics of the nuclear envelope and of nuclear pore complexes during mitosis in the *Drosophila* embryo. Eur J Cell Biol 34(1):179–189

Stanley H, Botas J, Malhotra V (1997) The mechanism of Golgi segregation during mitosis is cell type-specific. Proc Natl Acad Sci U S A 94(26):14467–14470

Stevens NR, Raposo AA, Basto R, St Johnston D, Raff JW (2007) From stem cell to embryo without centrioles. Curr Biol 17(17):1498–1503

Stevens NR, Dobbelaere J, Brunk K, Franz A, Raff JW (2010) *Drosophila* Ana2 is a conserved centriole duplication factor. J Cell Biol 188(3):313–323

Stevenson VA, Kramer J, Kuhn J, Theurkauf WE (2001) Centrosomes and the scrambled protein coordinate microtubule-independent actin reorganization. Nat Cell Biol 3(1):68–75

Stevenson V, Hudson A, Cooley L, Theurkauf WE (2002) Arp2/3-dependent pseudocleavage [correction of psuedocleavage] furrow assembly in syncytial *Drosophila* embryos. Curr Biol 12(9):705–711

Stiffler LA, Ji JY, Trautmann S, Trusty C, Schubiger G (1999) Cyclin A and B functions in the early *Drosophila* embryo. Development 126(23):5505–5513

Su TT, Sprenger F, DiGregorio PJ, Campbell SD, O'Farrell PH (1998) Exit from mitosis in *Drosophila* syncytial embryos requires proteolysis and cyclin degradation, and is associated with localized dephosphorylation. Genes Dev 12(10):1495–1503

Su J, Chow B, Boulianne GL, Wilde A (2013) The BAR domain of amphiphysin is required for cleavage furrow tip-tubule formation during cellularization in *Drosophila* embryos. Mol Biol Cell 24(9):1444–1453

Sullivan W, Minden JS, Alberts BM (1990) *daughterless-abo-like*, a *Drosophila* maternal-effect mutation that exhibits abnormal centrosome separation during the late blastoderm divisions. Development 110(2):311–323

Sullivan W, Daily DR, Fogarty P, Yook KJ, Pimpinelli S (1993a) Delays in anaphase initiation occur in individual nuclei of the syncytial *Drosophila* embryo. Mol Biol Cell 4(9):885–896

Sullivan W, Fogarty P, Theurkauf W (1993b) Mutations affecting the cytoskeletal organization of syncytial *Drosophila* embryos. Development 118(4):1245–1254

Swanson MM, Poodry CA (1981) The *shibire(ts)* mutant of *Drosophila*: a probe for the study of embryonic development. Dev Biol 84(2):465–470

Takada S, Kelkar A, Theurkauf WE (2003) *Drosophila* checkpoint kinase 2 couples centrosome function and spindle assembly to genomic integrity. Cell 113(1):87–99

Tavosanis G, Llamazares S, Goulielmos G, Gonzalez C (1997) Essential role for γ-tubulin in the acentriolar female meiotic spindle of *Drosophila*. EMBO J 16(8):1809–1819

Technau M, Roth S (2008) The *Drosophila* KASH domain proteins Msp-300 and Klarsicht and the SUN domain protein Klaroid have no essential function during oogenesis. Fly (Austin) 2(2):82–91

Theurkauf WE (1994) Actin cytoskeleton. Through the bottleneck. Curr Biol 4(1):76–78

Thomas JH, Wieschaus E (2004) *src64* and *tec29* are required for microfilament contraction during *Drosophila* cellularization. Development 131(4):863–871

Tillery MML, Blake-Hedges C, Zheng Y, Buchwalter RA, Megraw TL (2018) Centrosomal and non-centrosomal microtubule-organizing centers (MTOCs) in *Drosophila melanogaster*. Cell 7(9)

Tucker JB, Milner MJ, Currie DA, Muir JW, Forrest DA, Spencer MJ (1986) Centrosomal microtubule-organizing centers and a switch in the control of protofilament number for cell

surfacing-associated microtubules during *Drosophila* wing morphogenesis. Eur J Cell Biol 41:279–289

Turner FR, Mahowald AP (1976) Scanning electron microscopy of *Drosophila* embryogenesis. 1. The structure of the egg envelopes and the formation of the cellular blastoderm. Dev Biol 50 (1):95–108

Turner FR, Mahowald AP (1979) Scanning electron microscopy of *Drosophila* embryogenesis. III. Formation of the head and caudal segments. Dev Biol 68(1):96–109

Vaizel-Ohayon D, Schejter ED (1999) Mutations in centrosomin reveal requirements for centrosomal function during early *Drosophila* embryogenesis. Curr Biol 9(16):889–898

van Ijzendoorn SC (2006) Recycling endosomes. J Cell Sci 119(Pt 9):1679–1681

Varmark H, Llamazares S, Rebollo E, Lange B, Reina J, Schwarz H, Gonzalez C (2007) Asterless is a centriolar protein required for centrosome function and embryo development in *Drosophila*. Curr Biol 17(20):1735–1745

Venkei Z, Gáspár I, Tóth G, Szabad J (2006) alpha4-tubulin is involved in rapid formation of long microtubules to push apart the daughter centrosomes during early *Drosophila* embryogenesis. J Cell Sci 119(Pt 15):3238–3248

von Dassow G, Schubiger G (1994) How an actin network might cause fountain streaming and nuclear migration in the syncytial *Drosophila* embryo. J Cell Biol 127(6 Pt 1):1637–1653

Von Stetina JR, Orr-Weaver TL (2011) Developmental control of oocyte maturation and egg activation in metazoan models. Cold Spring Harb Perspect Biol 3(10):a005553

Wakefield JG, Huang JY, Raff JW (2000) Centrosomes have a role in regulating the destruction of cyclin B in early *Drosophila* embryos. Curr Biol 10(21):1367–1370

Walker JJ, Lee KK, Desai RN, Erickson JW (2000) The *Drosophila melanogaster* sex determination gene *sisA* is required in yolk nuclei for midgut formation. Genetics 155(1):191–202

Wang P, Pinson X, Archambault V (2011) PP2A-twins is antagonized by greatwall and collaborates with polo for cell cycle progression and centrosome attachment to nuclei in *Drosophila* embryos. PLoS Genet 7(8):e1002227

Warn RM, Robert-Nicoud M (1990) F-actin organization during the cellularization of the *Drosophila* embryo as revealed with a confocal laser scanning microscope. J Cell Sci 96 (Pt 1):35–42

Warn RM, Magrath R, Webb S (1984) Distribution of F-actin during cleavage of the *Drosophila* syncytial blastoderm. J Cell Biol 98(1):156–162

Warn RM, Smith L, Warn A (1985) Three distinct distributions of F-actin occur during the divisions of polar surface caps to produce pole cells in *Drosophila* embryos. J Cell Biol 100 (4):1010–1015

Watanabe N, Madaule P, Reid T, Ishizaki T, Watanabe G, Kakizuka A, Saito Y, Nakao K, Jockusch BM, Narumiya S (1997) p140mDia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. EMBO J 16(11):3044–3056

Webb RL, Zhou MN, McCartney BM (2009) A novel role for an APC2-diaphanous complex in regulating actin organization in *Drosophila*. Development 136(8):1283–1293

Wen Y, Eng CH, Schmoranzer J, Cabrera-Poch N, Morris EJ, Chen M, Wallar BJ, Alberts AS, Gundersen GG (2004) EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. Nat Cell Biol 6(9):820–830

Wenzl C, Yan S, Laupsien P, Grosshans J (2010) Localization of RhoGEF2 during *Drosophila* cellularization is developmentally controlled by Slam. Mech Dev 127(7–8):371–384

Wheatley S, Kulkarni S, Karess R (1995) Drosophila nonmuscle myosin II is required for rapid cytoplasmic transport during oogenesis and for axial nuclear migration in early embryos. Development 121(6):1937–1946

Williams BC, Dernburg AF, Puro J, Nokkala S, Goldberg ML (1997) The *Drosophila* kinesin-like protein KLP3A is required for proper behavior of male and female pronuclei at fertilization. Development 124(12):2365–2376

Wilson PG, Borisy GG (1998) Maternally expressed γTub37CD in *Drosophila* is differentially required for female meiosis and embryonic mitosis. Dev Biol 199(2):273–290

Wilson PG, Zheng Y, Oakley CE, Oakley BR, Borisy GG, Fuller MT (1997) Differential expression of two gamma-tubulin isoforms during gametogenesis and development in Drosophila. Dev Biol 184(2):207–221

Wolf R (1980) Migration and division of cleavage nuclei in the gall midge, *Wachtliella persicariae*: II. Origin and ultrastructure of the migration cytaster. Roux Arch Dev Biol 188(1):65–73

Xue Z, Sokac AM (2016) Back-to-back mechanisms drive actomyosin ring closure during *Drosophila* embryo cleavage. J Cell Biol 215(3):335–344

Yasuda GK, Baker J, Schubiger G (1991) Independent roles of centrosomes and DNA in organizing the *Drosophila* cytoskeleton. Development 111(2):379–391

Yohn CB, Pusateri L, Barbosa V, Lehmann R (2003) *l(3)malignant* brain tumor and three novel genes are required for *Drosophila* germ-cell formation. Genetics 165(4):1889–1900

Young PE, Pesacreta TC, Kiehart DP (1991) Dynamic changes in the distribution of cytoplasmic myosin during *Drosophila* embryogenesis. Development 111(1):1–14

Zallen JA, Cohen Y, Hudson AM, Cooley L, Wieschaus E, Schejter ED (2002) SCAR is a primary regulator of Arp2/3-dependent morphological events in *Drosophila*. J Cell Biol 156(4):689–701

Zalokar M (1976) Autoradiographic study of protein and RNA formation during early development of *Drosophila* eggs. Dev Biol 49(2):425–437

Zalokar M, Erk I (1976) Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster*. J Microsc Biol Cell 25:97–106

Zhang J, Megraw TL (2007) Proper recruitment of γ-tubulin and D-TACC/Msps to embryonic *Drosophila* centrosomes requires Centrosomin Motif 1. Mol Biol Cell 18(10):4037–4049

Zhang CX, Rothwell WF, Sullivan W, Hsieh TS (2000) Discontinuous actin hexagon, a protein essential for cortical furrow formation in *Drosophila*, is membrane associated and hyperphosphorylated. Mol Biol Cell 11(3):1011–1022

Zhang G, Breuer M, Förster A, Egger-Adam D, Wodarz A (2009) Mars, a *Drosophila* protein related to vertebrate HURP, is required for the attachment of centrosomes to the mitotic spindle during syncytial nuclear divisions. J Cell Sci 122(Pt 4):535–545