Blastoderm Formation and Cellularisation in *Drosophila melanogaster*

Introductory article

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Immediately following fertilisation in *Drosophila* and many other arthropods, the embryo undergoes a series of rapid syncytial nuclear divisions. These divisions are driven by maternally supplied components and occur in the absence of zygotic transcription. Unlike typical cell divisions, these divisions alternate between S and M phases, resulting in cell cycles that last only from 10 to 25 min. After four rounds of division, the nuclei undergo axial expansion, a process that relies on microfilaments. Subsequently migration of the nuclei to the cortex relies on microtubules. Once at the cortex, the nuclear divisions occur on a single plane and rely on partial cleavage furrows to maintain an even distribution. The cortical nuclear divisions continue until the mid-blastula transition (MBT), at which time cellularisation results in the formation of a multicellular embryo.

Drosophila's Unusual Syncytial Blastoderm

Overview

Like in many other insects, early embryonic development in *Drosophila melanogaster* is rapid and occurs in a syncytium. The first 13 nuclear divisions are completed in just over 3 h and occur in the absence of cytokinesis, producing an embryo of some 6000 nuclei in a common cytoplasm (Foe and Alberts, 1983; Zalokar and Erk, 1976). At interphase of nuclear cycle 14, these nuclei are packaged into individual cells in a process known as cellularisation. This rapid development is achieved by several attributes of the early embryo. First, the embryo is endowed with an abundance of maternally supplied products, which allows it to bypass zygotic transcription during the first several divisions. Second, the nuclear division cycles alternate between M and S phases. Lastly, nuclear division is uncoupled from cytokinesis. Thus, the initial division cycles proceed rapidly, ranging from 10 to 25 min, compared to the typical cell cycle duration of 24 h. Once fertilisation and fusion of the male and female pronuclei occur, the syncytial cycles are initiated. During these divisions, the nuclei undergo a precisely orchestrated pattern of migration and movements. During the first three divisions, the nuclei remain clustered in a ball at the anterior third of the embryo. During nuclear cycles 4–6, in a process called axial expansion, the nuclei become evenly distributed along the length of the embryo. Nuclear migration to the cortex begins during nuclear cycle 8 and the first nuclei arrive at the posterior end of the cortex at nuclear cycle 9. These nuclei are the germline precursors and protrude through the plasma membrane to form pole cells during nuclear cycle 10. Most of the remaining nuclei arrive synchronously at the cortex during interphase of nuclear cycle 10. These cortical nuclei undergo four more divisions and finally cellularise during interphase of nuclear cycle 14. Space is limited during the syncytial divisions. This problem is particularly acute during the cortical divisions when thousands of nuclei are rapidly dividing in a confined monolayer. In spite of the crowding, embryogenesis is an extremely precise process. This article highlights classic and recent studies that illuminate the mechanisms driving these events.

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Fertilisation and Preblastoderm Cycles

Fertilisation and the first mitotic cycle

After mating, a *Drosophila* female typically stores 300–500 sperms in specialised sperm storage organs called the

spermathecae and seminal receptacle. This supply of sperm enables the female to fertilise mature oocytes in the uterus for up to 2 weeks without remating. Sperms are released from the storage organs and enter the egg through a special opening at the anterior end called the micropyle. Although egg activation and fertilisation occur simultaneously, the former does not depend on sperm entry but rather on hydration of the egg during passage through the oviduct (Horner and Wolfner, 2008). Egg activation induces a number of events including release from the metaphase meiosis I arrest (**Figure 1**). Completion of meiosis produces four haploid meiotic products arranged perpendicularly with respect to the plasma membrane. The innermost nucleus migrates towards the interior while the remaining three nuclei cluster to form polar bodies enveloped by microtubules. Immediately following fertilisation, the nuclear envelope of the sperm nucleus is removed and the chromatin undergoes extensive remodelling replacing protamines with maternally supplied histones (Jayaramaiah Raja and Renkawitz-Pohl, 2005). This is followed by nuclear envelope reformation, S phase and fusion with the female pronucleus (Callaini and Riparbelli, 1996; Longo, 1985).

As in many other species, germline centrosome inheritance in *Drosophila* is asymmetric. During oogenesis, centrioles are lost but the surrounding pericentriolar material (PCM) is retained (Riparbelli and Callaini, 1996). Conversely during spermiogenesis, the sperm retains core centriolar components in its basal body but lacks PCM (Wilson *et al*., 1997). Upon fertilisation, the basal body relies on maternally supplied components for transformation into a centriole and reformation of the PCM. Although the sperm is normally required for centriole formation, recent work demonstrates that all the components necessary for centriole formation are present in the egg, as overexpression of core centriolar proteins induces centriole formation in the absence of fertilisation (Peel *et al*., 2007; Rodrigues-Martins *et al*., 2007).

The first mitotic division in *Drosophila* is known as a gonomeric division because the maternal and paternal

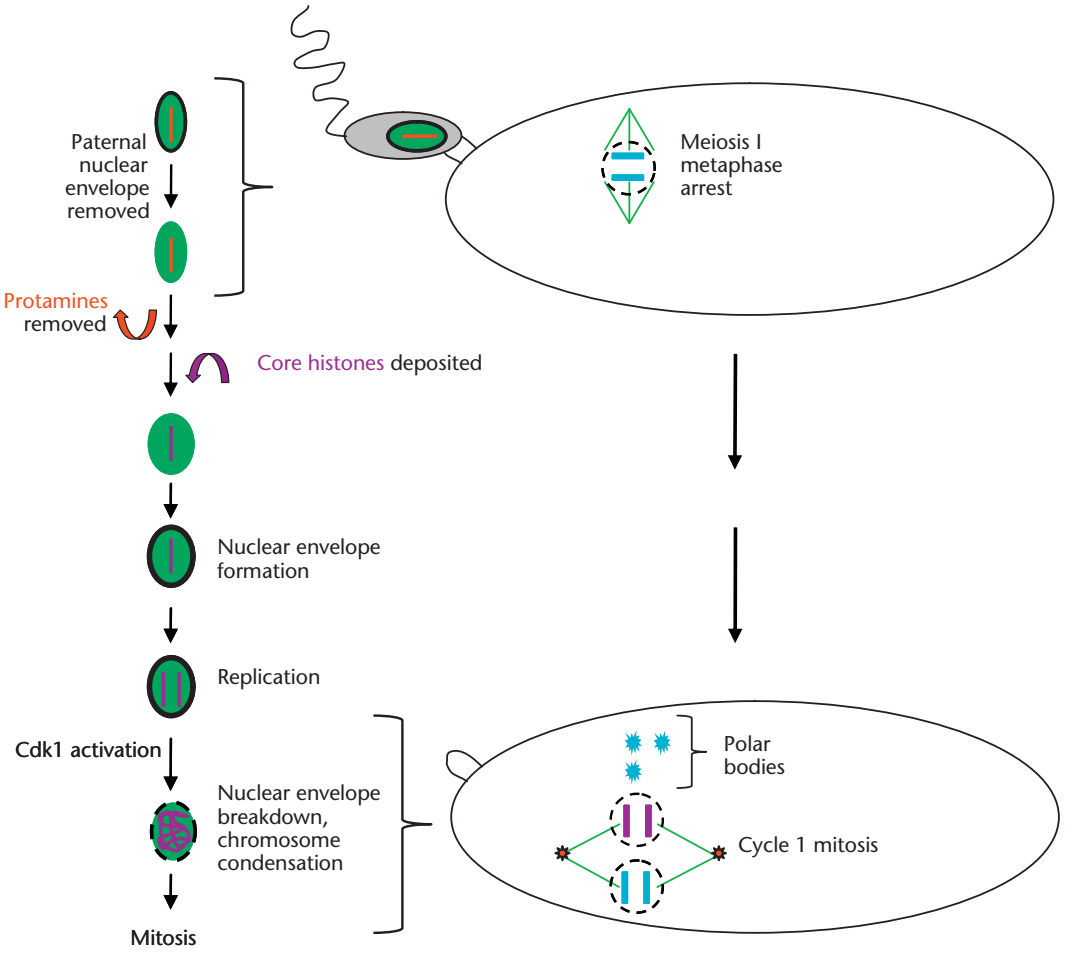

Figure 1 Fertilisation and pronuclear fusion. Sperm fertilises the meiosis I arrested egg. The egg then undergoes two meiotic divisions to generate three polar bodies and one pronucleus (blue). During this process, the male nucleus sheds its protamines (red) and deposits histones (purple) to become replication competent. Following this, chromosomes condense, the nuclear envelope breaks down and fusion with the female pronucleus occurs. (This figure was modelled after **Figure 5** in Landmann *et al.* (2009).)

genomes remain separated on the metaphase plate (Callaini and Riparbelli, 1996). Once the male and female pronuclei reside next to each other, the newly assembled sister centrosomes separate to form the poles of the spindle. This is immediately followed by nuclear envelope breakdown and entry into metaphase. Large sections of the nuclear envelope remain intact, however, maintaining the separation of maternal and paternal chromosome complements through anaphase. Subsequently, in telophase, the male and female nuclei join to form two diploid zygotic nuclei that proceed to the syncytial embryonic divisions.

Mitotic cycling

In *Drosophila*, as well as in many other insects, the first embryonic divisions after fertilisation are syncytial. That is, nuclear divisions occur in the absence of cytokinesis. *Drosophila* has 13 syncytial mitotic cycles, the first 9 of which occur in the centre of the embryo and are referred to as the preblastoderm cycles. These cycles consist of only S and M phases, and each lasts approximately 10 min. This rapid, but faithful, division is achieved by an abundance of maternally supplied factors.

Early cycles and axial expansion

During the first three divisions, the nuclei remain clustered in a ball at the anterior third of the embryo (**Figure 2**). During nuclear cycles 4–6, in a process called axial expansion, the nuclei become evenly distributed along the length of the embryo. This process is sensitive to cytochalasin but not colcemid, indicating that the underlying mechanism requires microfilaments, but is independent of microtubules (von Dassow and Schubiger, 1994). Mutational analysis demonstrates that cell cycle-regulated rounds of actin–myosin based cortical contraction are required for driving axial expansion. It is thought that the cortical contractions drive cytoplasmic streaming, which in turn drives axial expansion. **See also**: Actin and Actin Filaments

During these early cycles, the centrosome plays a key role in generating astral microtubules that orient and prevent collisions between neighbouring syncytial nuclei (Rothwell and Sullivan, 2000). Embryos containing defective centrosomes that fail to produce the robust astral microtubule arrays develop functional mitotic spindles and undergo several rounds of division but eventually large numbers of fused nuclei are often observed.

Cortical migration and yolk nuclei formation

Axial expansion distributes the nuclei evenly throughout the interior of the embryo. During nuclear cycles 7–10, the nuclei form an ellipsoid and migrate to the cortex in a stepwise fashion. Maximal cortical migration occurs during telophase of cycle 9 when extensive centrosome-based astral microtubule arrays are present. In contrast to axial expansion, microtubule inhibitors, but not microfilament

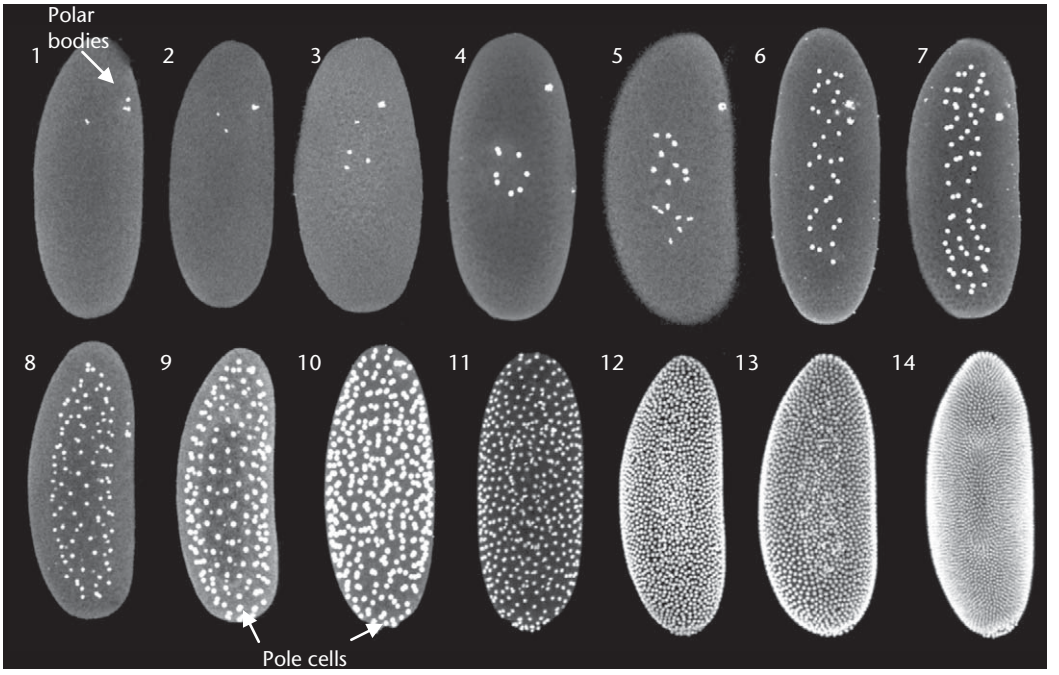

Figure 2 Early nuclear divisions and migration during *Drosophila* embryogenesis. Cycle 1 is initiated after fusion of the male and female pronuclei. During divisions 1–3, nuclei divide in a sphere at the anterior of the embryo. During divisions 4–6, nuclei divide and spread out along the anterior–posterior axis (axial expansion). Nuclei migrate to the cortex of the embryo during divisions 8–10 (cortical migration). Pole cells form at the posterior end of the embryo (cycle 9), whereas yolk nuclei remain in the interior. After four rounds of cortical syncytial divisions, during interphase of nuclear cycle 14, invagination of the plasma membrane around each nucleus produces a cellularised embryo.

inhibitors, disrupt this process (von Dassow and Schubiger, 1994; Zalokar and Erk, 1976). These findings suggest a model in which the expansion force is generated through plus-end-directed motors acting on neighbouring arrays of overlapping centrosome-based astral microtubules (Baker *et al*., 1993; Foe and Alberts, 1983). Support for this model is provided by the observation that centrosomes unassociated with nuclei migrate to the cortex. **See also**: Tubulin and Microtubules

Establishment of the yolk nuclei occurs during cortical nuclear migration. A fraction of the nuclei 'fall' back into the interior. These are destined to become the yolk nuclei and initially divide in synchrony with the migrating nuclei (Foe and Alberts, 1983). However, mitosis of nuclear cycle 10 becomes disrupted and centrosomes fail to fully separate and many disassociate from the nuclear envelope. Around nuclear cycle 11, the yolk nuclei cease dividing and become polyploid and their number remains at approximately 200 from nuclear cycles 11 through 14. Although yolk nuclei are a conserved feature of insect development, little is known concerning their function. Recent studies demonstrate that the *Drosophila sisterlessA*, a gene involved in sex determination, is required in yolk nuclei for proper midgut formation (Walker *et al*., 2000).

Pole cell formation

Because the ellipsoid of migrating nuclei is skewed towards the posterior, the first nuclei arrive at the posterior cortical region during nuclear cycle 9. These nuclei exert an outward force on the plasma membrane, producing localised protrusions known as pole buds. During telophase of this cycle, the buds pinch off and form a cluster of posteriorly localised pole cells, the germline precursors. Special properties of the posterior cytoplasm limit these events to the posterior pole, and interestingly, transplantation of polar cytoplasm is sufficient to generate ectopic pole cell formation (Illmensee and Mahowald, 1974; Okada *et al*., 1974). The cytoplasm at the posterior pole contains morphologically distinct polar granules consisting of ribonucleic acid (RNA) and protein. Essential maternal RNAs and protein localised to the future germ cells include Nanos, Oskar, Vasa and Tudor (Jin and Xie, 2006). Oskar localisation requires functional Vasa and Tudor and is anchored to the posterior pole (Markussen *et al*., 1995; Thomson and Lasko, 2004). Recently, PIWI (P-element induced wimpy testis), a class of proteins that bind small RNAs, through its microRNA pathway, was found to localise Oskar and Vasa to the posterior pole (Megosh *et al*., 2006). However, unlike Oskar protein, ectopic PIWI cannot induce pole cell formation when anteriorly expressed in the embryo (Ephrussi and Lehmann, 1992). The posterior pole also contains extra plasma membrane in the form of extensive microvillar projections and high levels of contractile components such as myosin. This suggests that the posterior region of the cortex is primed to form cells requiring only the proper stimulus to do so. **See also**: *Drosophila* Oogenesis

Blastoderm Cycles

Cortical divisions and metaphase furrows

After nine divisions in the interior of the embryo, syncytial divisions 10–13 occur at the actin-rich cortex just beneath the plasma membrane. Before the arrival of the nuclei and their tightly associated centrosomes, the actin is homogeneously distributed. Upon reaching the cortex, the centrosomes induce a dramatic redistribution in the actin (**Figure 3** and **Figure 4**). During interphase, actin concentrates into apical caps centred above each cortical nucleus and its centrosomes. In *centrosomin* mutants, which lack astral microtubules, cortical actin is dispersed throughout the embryo, demonstrating that asters are necessary for proper actin dynamics (Kao and Megraw, 2009). As the nuclei progress into prophase, the centrosomes migrate away from each other, approaching the approximate equator of the nucleus. This movement coincides with, and

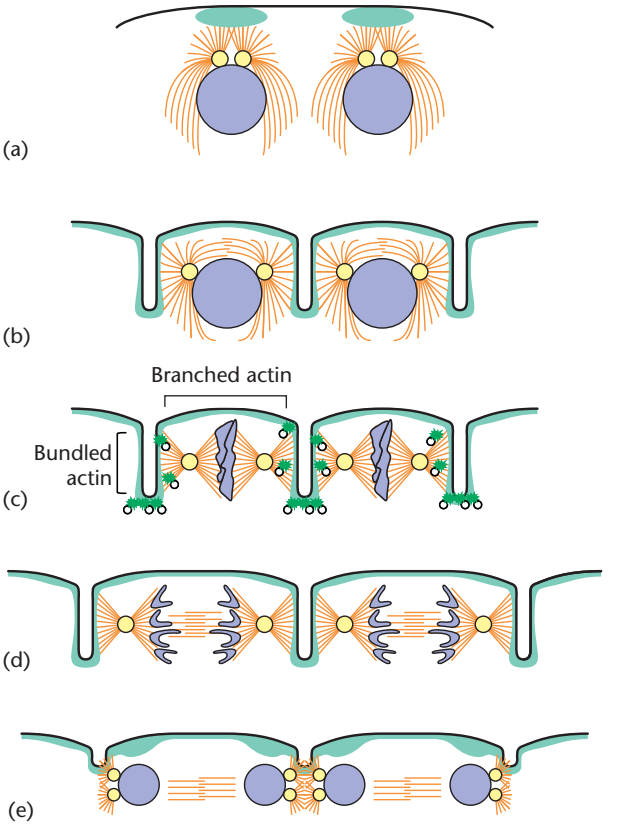

Figure 3 Metaphase furrow formation. (a) During interphase, the actin (green) concentrates into apical caps centred above each cortical nucleus (blue) and its apically positioned centrosomes (yellow). (b) As the nuclei progress into prophase, the centrosomes migrate towards opposite poles and the actin caps undergo a dramatic redistribution to outline each nucleus and its associated separated centrosome pair. (c) At metaphase, the furrows invaginate to a depth of approximately 10 μm to surround each spindle both apically and laterally, but not basally. Vesicles (black circles) transport actin puncta (green) that fuse with the growing furrow. (d) and (e) During late anaphase and telophase, the metaphase furrows rapidly regress.

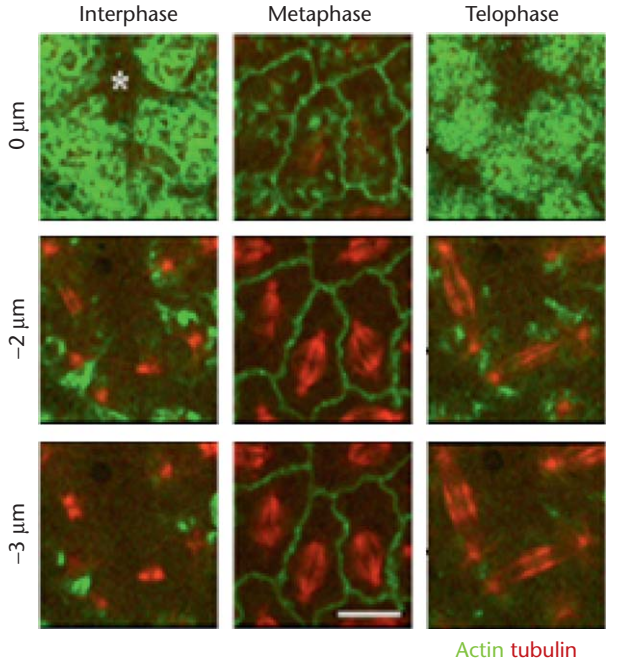

Figure 4 Live imaging of metaphase furrow formation. Surface confocal sections highlight the reorganisation of interphase actin caps above each nucleus into metaphase furrows. Actin caps are distinct from one another during interphase, where gaps (*) can be observed. By metaphase, actin caps have expanded laterally to fill these gaps and invagination into metaphase furrows has reached maximum length (~10 µm). Metaphase furrows retract by telophase, reforming apical caps. Actin (green) and microtubules (red). Scale bar=10 µm.

requires, the lateral expansion of the apical actin caps. More specifically, highly dynamic actin branching in the expanding caps contributes significantly to the early separation of centrosomes (Cao *et al*., 2010).

During prophase, lateral expansion of the actin caps cease and the cortical actin undergoes a dramatic reorganisation. Plasma membrane and actin invaginate to form structures known as metaphase or pseudocleavage furrows that encompass each nucleus and its associated centrosome pair. These furrows are equivalent in structure and composition to conventional cytokinesis furrows and include actin, myosin II, spectrins, anillin, septins and formins (Miller and Kiehart, 1995; Stevenson *et al*., 2002). Key differences between metaphase furrows and more conventional cytokinesis furrows are that the former encompass rather than bisect the spindle, and furrow formation occurs during prophase/metaphase rather than anaphase/telophase. In addition, although myosin is present in the metaphase furrows, it is not required for furrow invagination. Instead, furrow invagination is primarily driven by fusion of vesicles originating from the centrosome-associated recycling endosome (Lecuit and Wieschaus, 2000; Riggs *et al*., 2003). The vesicles provide a source of membrane, as well as potent actin remodellers. Furrow formation begins at prophase and continues through metaphase, with the furrows reaching a depth of approximately 8 µm. **See also**: Actin and Actin Filaments; Cytoskeleton

The primary function of the metaphase furrows is to maintain separation between neighbouring spindles. This is especially critical during the cortical divisions, in which the nuclei divide in a tightly packed monolayer. Fused spindles and abnormal nuclear divisions are routinely observed in mutants that disrupt furrow formation (Sullivan *et al*., 1993).

During late anaphase and telophase, the metaphase furrows are dismantled and actin caps form above each nucleus. Centrosome duplication occurs during late anaphase and the newly formed centrosome pairs are again located apically, reforming a focus for the assembly of actin caps in the next interphase. This alteration between interphase actin caps and metaphase furrows occurs up until interphase of nuclear cycle 14.

Cellularisation

By nuclear cycle 14, the nuclei are arranged in a closely packed monolayer on the cortex and enter a prolonged interphase during which cellularisation occurs. During the hour-long process of cellularisation, plasma membrane invaginates around each nuclei and constricts basally to form a multicellular embryo (**Figure 5**). An early step in cellularisation is the formation of an inverted basket of microtubules extending from a pair of apically localised centrosomes. Once this basket is formed, the nuclei elongate within its confines. The microtubules guide invagination of the cellularisation furrow through an initial slow phase followed by a second fast phase. The furrows reach a depth of approximately 35 µm and form distinct apical lateral and basal membrane domains. Neighbouring furrows are connected through adherens junctions. Once furrow elongation is complete, the furrows constrict laterally in a process known as basal closure.

The forces driving furrow formation remain unclear. The leading edge of the ingressing furrow is occupied by an actin–myosin ring that encompasses and moves basally along the microtubule basket. Surprisingly, myosin disruption has only minor effects on furrow ingression, indicating other forces can drive furrow progression (Royou *et al*., 2004). Mutational analysis and inhibitor studies demonstrate that Golgi- and recycling endosome vesicle-based membrane addition plays an important role in driving furrow elongation (Lecuit and Wieschaus, 2000; Riggs *et al*., 2003; Sisson *et al*., 2000). **See also**: Actin and Actin Filaments; Cytoskeleton

Cell cycle regulation

In eukaryotes, a highly conserved family of Cyclin-dependent kinases (Cdks) drive the major transitions in the cell cycle (Sullivan and Morgan, 2007). Cdk activity is regulated through phosphorylation, as well as associating with specific members of the Cyclin family of proteins. Activated Cdk1 promotes entry into mitosis by targeting a

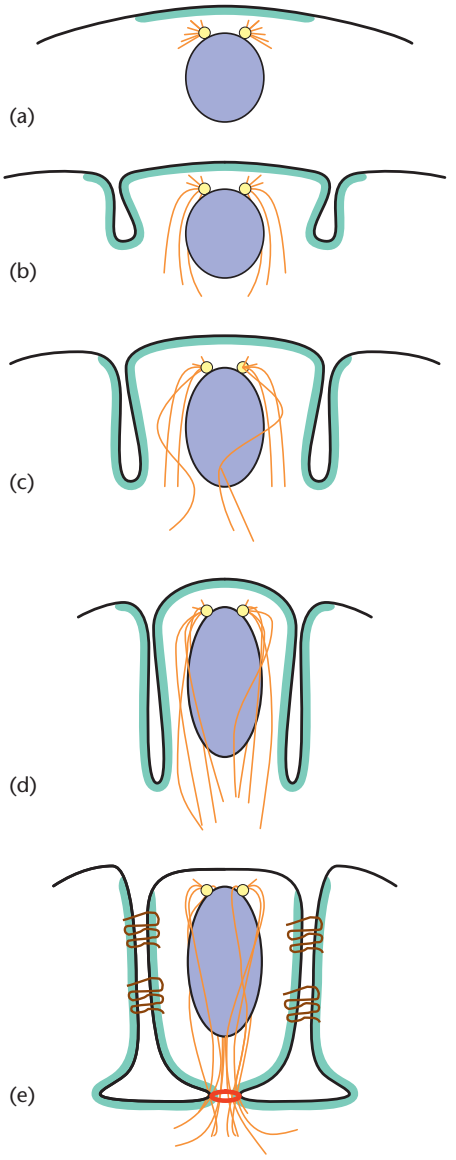

Figure 5 Formation of the cellularisation furrows. (a) Cellularisation begins with actin (green) concentrated at the cortex above each nucleus and apical centrosome pair (yellow). Astral microtubules extend basally and form a cage within which nuclear elongation occurs. (b) The plasma membrane initiates invagination and actin is concentrated at the cortex and the leading edge of the furrow. (c) The slow phase of furrow invagination is initiated. Golgi- and recycling endosome-derived vesicles drive furrow elongation. (d) Furrows invaginate rapidly once they have passed the fully elongated nuclei. (e) Once furrows have reached a depth of 35 μm, the leading edge relies on actin–myosin-based contraction (red) to pinch off basally. Adherens junctions (brown) connect neighbouring cellular membranes.

diverse array of nuclear and cytoplasmic substrates. Activated Cdk1 also drives exit from mitosis by activating APC, a ubiquitin ligase responsible for degradation of Cyclin as well as cohesin, a protein that holds sister chromosomes together. Thus, activated Cdk1 promotes both entry and exit from mitosis. **See also**: Cell Cycle; Mitosis

An unusual feature of nuclear cycles 2–7 is that the bulk of Cdk1 lacks inhibitory phosphorylations, raising the possibility that these are primarily driven by Cyclin levels (Edgar *et al*., 1994). This is supported by the finding that inhibiting protein synthesis prevents entry into mitosis and purified Cyclin B protein overcomes this inhibition (Royou *et al*., 2008). Another distinctive feature of these cycles is that Cyclin B is not fully degraded upon exit from mitosis. This suggests mitotic exit is achieved by local degradation of Cyclin B. Cycles 8–13 reflect a more traditional cell cycle regulation, consisting of Cdk1 phosphorylation, checkpoints and dramatic oscillations in cyclin level, yet these divisions still lack gap phases (Edgar *et al*., 1994).

The fact that the syncytial divisions lack gap phases, a time when many checkpoints function, led to the idea that the role of checkpoints was limited during these cycles. In addition, because checkpoints induce cell cycle delays, it was thought that this would disrupt division synchrony necessary for normal development. However, it is now clear that the major checkpoints, deoxyribonucleic acid (DNA) damage, S phase and spindle assembly, are present and operational during these late syncytial divisions. For example, disrupting spindle formation results in a metaphase arrest and damaged or unreplicated DNA prolongs interphase (Crest *et al*., 2007; Dawson *et al*., 1993; Fogarty *et al*., 1997; Sibon *et al*., 1997). Additionally, it is known that pools of nuclear and cytoplasmic Cyclin B communicate with each other through the S-phase checkpoint (Royou *et al*., 2008). Collectively, the checkpoint-induced delays result in the elimination of abnormal nuclei from the cortex (Fogarty *et al*., 1997; Takada *et al*., 2003). Significantly, mutants in *Drosophila* homologues of conserved checkpoint genes eliminate these delays.

The mid-blastula transition

The mid-blastula transition (MBT) occurs during nuclear cycle 14 and is characterised by chromatin reorganisation, S-phase lengthening, cellularisation, degradation of maternal RNAs and a dramatic activation of zygotic transcription. The latter two events signal a maternal-to-zygotic transfer of developmental control (Tadros and Lipshitz, 2009). Before the MBT, maternally supplied RNA and proteins primarily drive embryogenesis. In fact, very little zygotic transcription occurs during the initial divisions following fertilisation. During interphase of cycle 14, maternal RNAs are rapidly degraded and zygotic transcription increases approximately sixfold. Several molecular pathways have been implicated in maternal RNA degradation, including maternally supplied RNA-binding proteins such as Smaug, which recruits enzymes to an mRNA that then processes it for degradation, and zygotically transcribed microRNAs, which directly target maternal mRNAs for degradation. **See also**: Cleavage and Gastrulation in Leech Embryos; Cleavage and Gastrulation in *Xenopus laevis* Embryos

The mechanisms timing the MBT remain unresolved, although studies examining haploid embryos indicate that

two distinct mechanisms drive specific MBT events. In haploid embryos, S-phase lengthening and cellularisation occur in cycle 15 instead of cycle 14 (Lu *et al*., 2009). However, zygotic transcription of a majority of genes in these embryos occurred at the normal time after fertilisation ($\sim$3 h). In addition, a minority of zygotic genes are transcribed only during the prolonged S phase of nuclear cycle 15. This demonstrates that while cell cycle timing is controlled by the nuclear-cytoplasmic (N/C) ratio, zygotic activation relies both on the N/C ratio and on the absolute developmental time.

Insight into the mechanism by which absolute developmental time is controlled comes from analysis of Smaug, a protein translated immediately following fertilisation and reaching a peak accumulation by the end of cycle 13, regardless of DNA content (Benoit *et al*., 2009). At early cycle 14, Smaug levels rapidly decline coinciding with maximal zygotic transcription. This suggests that Smaug levels are not regulated by nuclear cycles but instead depend on its rate of translation. In addition, this suggests that Smaug levels need to be developmentally regulated to completely diminish maternal factors that serve to inhibit zygotic transcription.

The mechanisms by which the N/C ratio is read are unclear. It is assumed that they involve the titration of specific factors, but the specific factors have yet to be identified. Twine, a maternally supplied Cdc25 homologue, may be involved as its abundance rapidly declines after 13 divisions. Significantly, reduction of Twine leads to a premature MBT whereas increasing Twine levels delays the MBT (Lu *et al*., 2009). Therefore, Twine may be an essential factor that becomes limiting after successive nuclear divisions, leading to a prolonged interphase allowing time for zygotic transcription.

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