

Meeting Report

Cell cycle regulation

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Drosophila researchers met in sunny San Diego for the 49th Annual Meeting of The Genetics Society of America. It was cold outside and even colder inside. Like last year, 'Mitosis, Meiosis and Cell Division' was no longer a session. Instead, we searched out and covered talks and posters in 'Cell Division and Growth Control', 'Gametogenesis', 'Cytoskeleton and Cell Biology' and 'Genome and Chromosome Structure'. We split up for maximal coverage and re-grouped later for the Workshop on Cell Cycle and Checkpoints. We apologize in advance for the brevity or omission of some reports.

Mitotic Spindle Orientation

The cell cycle portion of the meeting began on the first day with an excellent historical address by Antonio Garcia-Bellido (Universidad Autonoma de Madrid). Among the many accomplishments in his career is the work on how mitotic spindle orientation contributes to organ shape by determining the orientation of the daughter cells^{1,2} (Fig. 1). In the future wing pouch region of the wing imaginal disc, spindles orient along the long (proximal-distal) axis of the future wing. Clonal analysis showed that such preferential spindle orientation produced clones that are longer along the P-D axis than they are wide. The orientation appears to be dependent on the gradient of Vestigial (Vg) protein; uniform expression of Vg abolished preferential orientation of divisions and also made resulting wings more uniform in width along both P-D and A-P axis. Thus, a bias in spindle orientation ultimately gives the wing its oblong shape.

These lessons on how the orientation of the mitotic spindle, a basic cell biological phenomenon, can profoundly affect the shape of a multicellular structure (clones/wings) carries over to the work from Nick Baker's lab at Albert Einstein's College of Medicine that was presented in the Cell Cycle Workshop. The Baker lab has previously described the role of apoptosis and cell engulfment in the competition between clones of cells with different growth profiles, for example wild-type versus Minute mutants with half the dosage of a ribosomal protein gene, in wing imaginal discs.^{3,4} New work shows that during cell competition, dividing cells in the wild-type clones

tend to orient their mitotic spindles perpendicular to the clonal boundary with the Minute/+ cells. As a result, wild-type daughter cells that have growth advantage invade the territory of M/+ cells that are at a growth disadvantage. This could explain how M/+ clones appear to be broken up and infiltrated by wild-type cells, perhaps allowing for efficient engulfment. Spindle orientation during competition depends on some of the genes already implicated in normal spindle orientation that contributes to wing shape by the work of Garcia-Bellido's group.

The Spindle-Cortex Interaction

The orientation of the mitotic spindle is mediated through its interaction with the cortex of the cell. Three groups reported on proteins that mediate the interaction between the spindle microtubules and the cortical actin in S2 cells and in syncytial embryos.

In a talk in the Cell Cycle and Checkpoints workshop, Stephen Gregory from Rob Saint's group (Adelaide University) reported the detection of an interaction between the actin-binding protein Anillin and RacGAP50c in vivo using FRET. In similar experiments, Actin shows an interaction with Anillin as expected but not with RacGAP50c. Depletion of Anillin by RNAi still allows RacGAP50c to localize to the furrow but results in subsequent de-localization of the latter, coinciding with failure of cytokinesis. Likewise, RacGAP50c is needed for Anillin to localize to the furrow initially. Given that RacGAP50c is a known spindle associated protein, Anillin could bridge the two cytoskeletal systems by directly binding actin directly and binding microtubules via RacGAP.⁵

Sebastien Carreno (now at IRIC/University of Montreal) from Francois Payre's lab (CNRS/University of Toulouse) describes a new protein that interacts genetically and physically with Moesin (Moe), the only ERM protein in *Drosophila*. ERM bridge actin network to the plasma membrane; *Drosophila* Moe has been shown to be important for cortical stability and also for mitotic spindle positioning.⁶ In newer studies, the group identified a genetic interactor of Moe (GIM), and confirmed physical interaction between recombinant proteins in vitro. Depletion of GIM in S2 cells phenocopies the depletion of Moe; the defects include mitotic cell shape defects, loss of cortical integrity, spindle defects and metaphase delay. GIM contains a Calponin Homology domain, a protein motif that is also found in EB1 and implicated in binding actin filaments and microtubules. GIM colocalizes with phospho-Moe on the cell cortex and on the cleavage furrow, but GIM also shows localization at the centrosomes. Moe localizes normally in GIM-depleted cells and the role of Moe in GIM localization is currently under investigation. GIM proteins are

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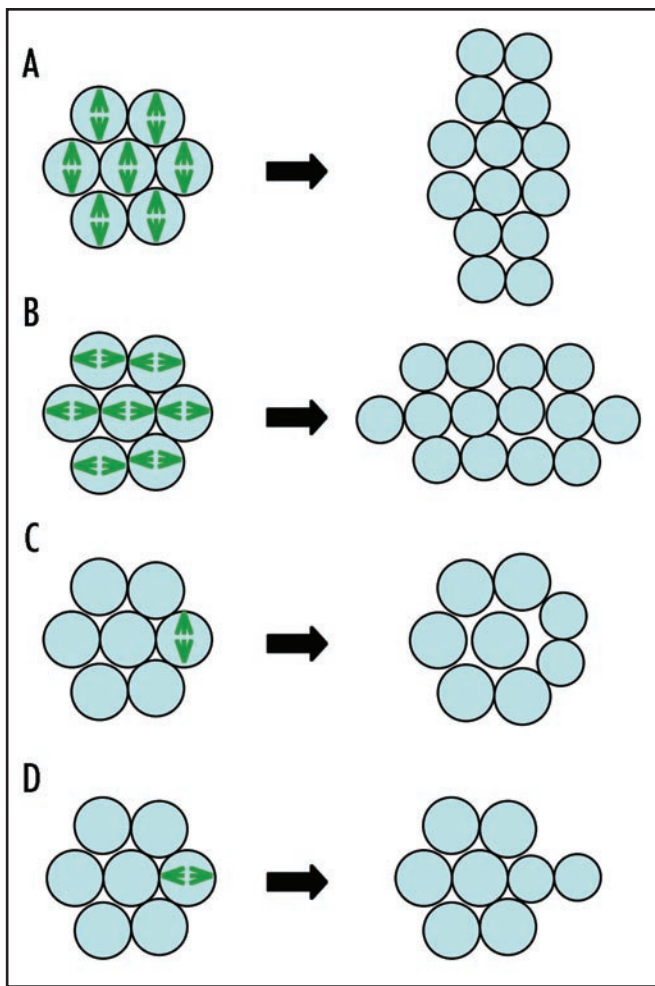


Figure 1. The orientation of the mitotic spindle (green) can determine the shape of the resulting tissue (A and B) and whether a daughter cell remains close to the clone (C) or ventures away from the clonal territory (D).

evolutionarily conserved; there are four GIM homologs in human, one of them has been cloned and has been shown to localize as its *Drosophila* ortholog does during cell division.

Centrosomin (Cnn) is an integral component of the centrosome that is necessary for the microtubule organizing center (MTOC) activity at mitosis. Tim Megraw (UT Southwestern) reported the role of two conserved peptide motifs in *Drosophila* Centrosomin (Cnn), in a talk in the Cell Cycle and Checkpoints workshop and on a poster. Motif 2 is conserved only in metazoan homologs and shows less sequence conservation than Motif 1 discussed below (29% identity between fly and human). Motif 2 appears to be needed for binding to the Myosin A Tail domain Interacting Protein (MTIP) and for the formation of actin furrows that form between mitotic figures during syncytial divisions.

Spindle Assembly

Before the orientation of the mitotic spindle can play a role in biological processes, the spindle first must be properly assembled. Microtubules that make up the mitotic spindle originate from both centrosomes and chromosomes. Three groups provided insight into spindle assembly.

Tim Megraw further reported that motif 1 of Cnn is more highly conserved than motif 2, with the *Drosophila* sequence showing 40% identity with the human sequence. The Megraw lab had found that the deletion of motif 1 from Cnn in syncytial embryos results in the failure to properly localize two components of the Peri-Centriolar Material (PCM), D-TACC and Msps, while another PCM component, Aurora A localizes to the centrosome normally.⁷ Consistent with this data, newer studies show that Cnn and Msps co-immunoprecipitate in a motif 1-dependent manner. In larval neuroblasts that are recovering from cold-induced microtubule de-polymerization, Cnn that lacks motif 1 can support the regrowth of kinetochore microtubules, albeit more slowly than did wild-type, but could not support the regrowth of centrosome-mediated microtubules. Thus two peptide motifs of Cnn could explain the dual role for this protein in centrosome function and in the interaction between the MTOC and cortical actin during syncytial divisions.

Robert Eisman from Thomas Kaufman's lab (Indiana University) reported that Cnn is subject to multiple modes of protein modification. Cnn is heavily phosphorylated in extracts of activated unfertilized eggs and in syncytial stage embryos. Cnn sequence shows potential phosphorylation sites for Polo, PKA, PKC and CKII. 2D gel electrophoresis of syncytial embryo extracts resolves ten spots that are reduced to one upon phosphatase treatment, while activated unfertilized eggs show six spots. Analysis of ovaries suggests alternate or additional modifications on Cnn as well as a possible product of an Ub- or SUMO-mediated degradation. Low abundance of Cnn in embryos led the group to turn to cell culture to gather enough material to map phosphorylation sites by mass spectrometry, which is in progress. Interestingly, while S2 cells show a single splice variant, two variants are found in KC cells, which also show multiple phosphorylated versions of Cnn. This group is taking advantage of the recently completed genome sequences in the *Drosophila* genus to identify conserved phospho-acceptor sites in multiple *Drosophila* species. Ten sites that may be to be substrates for polo, PKA and CKII are being subjected to targeted mutagenesis. Cnn is also known to exist as multiple splice variants. A smaller variant (Cnn-PA) is predominant in the egg and a larger variant that differs from Cnn-PA in the middle region is predominant in 0–2 hr-old embryos. Cnn-PA rescues the embryonic phenotypes that result from *cnn* mutations; the rescue ability of the larger variant remains to be tested.

Violaine Mottier from Silvia Bonaccorsi's group (University of Rome) reported on the role of Misato (Mst) in mitotic spindle assembly. Mst shares motifs with tubulin and myosin and previous work has shown that mutants die at the larval/pupal boundary and exhibit frequent polyploid cells in larval brains. Mottier and coworkers reported that *mst* neuroblasts exhibit dramatic spindle defects that include low microtubule (MT) density, diminished kinetochore fibers and monopolar spindles. In *mst* cells, which show normal localization of centrosomal proteins, centrosomes are separated at prophase but progressively collapse, giving rise to monopolar spindles. *mst* neuroblasts can re-grow MTs from the centrosomes but not from the chromosomes during recovery from cold-induced depolymerization. These results lead to the idea that Mst is needed for chromosome-based MT formation and that these MTs are essential for the assembly of a bipolar spindle.

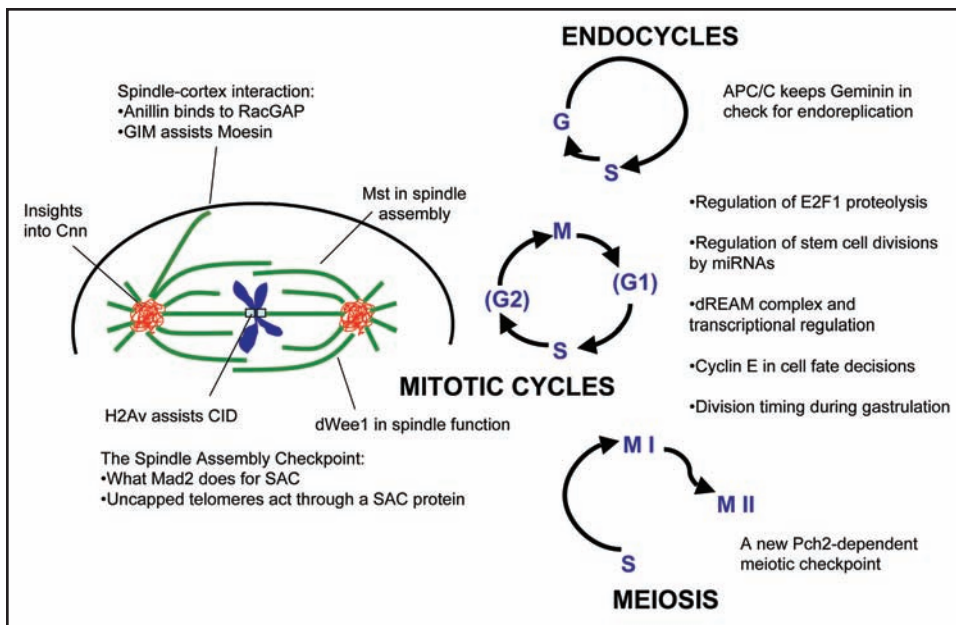


Figure 2. Three different types of cell cycles found during *Drosophila* development are depicted along with relevant presentations. For mitotic cycles, the spindle is shown along with relevant presentations. The mitotic spindle consists of microtubules (green) that nucleate from centrosomes that are the microtubule organizing centers (MTOC, red). Chromosomes (dark blue) align on the metaphase plate through microtubule interactions at the kinetochores (light blue). Some of the microtubules interact with the cell cortex (curved black line). G₁ and G₂ phases are in brackets because some of the mitotic cycles during *Drosophila* development lack one or both of these phases.

Spindle Assembly Checkpoint

Two talks from the Cell Cycle and Checkpoints workshop provide new insight into the spindle checkpoint that monitors the fidelity of spindle-chromosome attachment.

Claudio Sunkel (Universidade do Porto) reported an extension of a previously published remarkable finding that pharmacological lengthening of metaphase could fulfill the requirement for Mad2 in mitosis. Mad2-depleted S2 cells accelerate through mitosis, undergoes mitotic catastrophe and cannot activate the spindle checkpoint; transient incubation with a proteasome inhibitor rescues these phenotypes.⁸ In newer studies, depletion of the checkpoint protein ZW10 by RNAi prevented Mad2 from localization to the kinetochore. These cells still show a Mad2-dependent control of prometaphase length and could still be established but not properly maintain a spindle checkpoint arrest; thus cytoplasmic Mad2 can still provide most of the functions. BubR1 still accumulates at the kinetochore under these conditions, and double depletion of ZW10 and BubR1 or destruction of the kinetochore by depletion of CENP-C phenocopies the loss of Mad2 depletion. These data can be explained by a model in which Mad2 acts to allow time for accumulation of BubR1 at the kinetochore, a critical requirement for the spindle checkpoint to engage during the initial part of mitosis.

Giovanni Cenci (Univ of L'Aquila) reported the unexpected finding that uncapped telomeres activate the spindle checkpoint in *Drosophila*.⁹ Mutants in *caravaggio (cav)* that encodes *Drosophila* HOAP show low mitotic index but also low anaphase index indicating a delay in pre-anaphase stages of mitosis. Mutations in the spindle checkpoint genes BubR1 or ZW10 rescue the anaphase phase index while mutations in DNA checkpoint genes *grp/CHK1*, *mei-41/ATR*

and *mus304/ATRIP* (but not *tefil/ATM*) rescue both mitotic and anaphase indices. In *cav* mutants, BubR1, but not other spindle checkpoint proteins, is found at about 25% of telomeres, further suggesting that the spindle checkpoint is indeed activated by telomere defects. Mutations in *grp/CHK1*, *mei-41/ATR*, *mus304/ATRIP* and *tefil/ATM*, reduced the percent of telomeres that show BubR1 staining. These intriguing results suggest that uncapped telomeres are recognized by the DNA damage checkpoint that recruits the spindle checkpoint protein BubR1 to engage the spindle checkpoint. The DNA checkpoint appears to act to prevent both mitotic entry and anaphase entry, while the spindle checkpoint may act to prevent only the anaphase entry in response to uncapped telomeres.

The Chromosome Cycle

Spindle assembly requires a properly functioning kinetochore. The centromere-specific histone variant CENP-A (CID in *Drosophila*) is a structural and functional foundation for kinetochore formation. Weiguo Zhang

from Gary Karpen's lab (University of California, Berkeley) reported that histone variant H2Av localizes to the centromeres and has a role in the localization of centromeric histone variant CID. On stretched chromatin fibers, H2Av signal and CID signals occur at different places but also show some overlap; thus H2Av-containing nucleosomes and CID-containing nucleosomes are interspersed along centromeric chromatin although there may be nucleosomes that includes both. In addition H2Av signal also occurs outside of centromeric chromatin. Tagged CID can precipitate H2Av (and H2A) in micrococcal nuclease treated chromatin that represents a preparation of mononucleosomes. A previously described 'SNAP-quench' system allows the analysis of assembly of newly synthesized proteins.¹⁰ Using this system, the assembly of new CID onto existing centromeres was found to be defective in H2Av-depleted S2 cells.

Norman Zielke (currently at the Fred Hutchinson Cancer Research Center) from Frank Sprenger's lab (University of Cologne and University of Zuerich) described the regulation of endoreplication by the Anaphase Promoting Complex, APC. In the endoreplication cycles of salivary glands, Geminin, an inhibitor of S phase, was found to oscillate. This fluctuation is mediated by the APC/C-Fzr complex, which is periodically inhibited by CycE/Cdk2. Inhibition of APC/C activity by overexpression of Rca1 or CycE, or by RNAi-mediated depletion of an APC subunit, Cdc16, results in the accumulation of Geminin and a block in the endocycle.

Eric Joyce from Kim McKim's lab (Waksman Institute, Rutgers University) described in the Cell Cycle and Checkpoints workshop a new meiotic checkpoint that appears to allow additional crossover events during female meiosis. This checkpoint is mediated by Pch2, whose homologs also function in meiotic checkpoints in *C. elegans* and *S. cerevisiae*. The result of activating this checkpoint includes

a delayed oocyte specification and a delayed response to double strand breaks (DSBs). The Pch2-dependent checkpoint is activated in mutants that are deficient in repair of DSBs into crossovers. Curiously, the Pch2-dependent checkpoint is not suppressed in mutants that do not even initiate DSBs. These results suggest that DNA repair genes have functions independent of their ability to repair DSBs. A defect in this DSB-independent function would lead to checkpoint activation.

Cell Cycle Regulation

Jenn-Yah Yu and Steven Reynolds from the Ruohola-Baker lab (University of Washington) described the role of microRNAs in the regulation of the cell cycle in the female germline stem cells (GSC). Previous work from this lab has shown that mutants in *dicer-1*, which is needed for miRNA biogenesis, show increased levels of the Cdk2 inhibitor Dacapo (Dap) and a delay in G₁-S transition.¹¹ The *dap* transcript includes potential sites for regulation by miRNA *bantam* (*ban*), *mir-7* and *mir-278*. In the germline cells in Stage 1 germlaria, GFP sensors that have *ban* or *mir-7* target sequences in the 3'UTR show low levels of GFP (reflection high *ban* and *mir-7* activity). *ban* mutant germline clones show a decrease in cell number, consistent with the idea that *ban* is needed to repress Dap and allow cell cycle progression. *mir-278* and *mir-7* mutant clones showed smaller defects in GSC division, suggesting that regulation of Dap by these miRNAs may be subtle and possibly combinatorial.

Jean Davidson from the Duronio lab (University of North Carolina, Chapel Hill) reported on the analysis of E2f1 destruction that occurs in the S phase of the cell cycle in cellular stage embryos. Previous work from the Duronio lab has shown that E2f1 is rapidly destabilized as cells enter S phase and re-accumulates later during interphase in the embryo.¹² This is similar to the case of Cdt1, a protein that loads MCM replication factors onto DNA and is essential for S phase. Previous work on Cdt degradation showed that PCNA binds a PIP (PCNA interacting) motif in the amino-terminus of Cdt1, and this interaction is thought to recruit a Cul4 E3 ubiquitin ligase that targets Cdt1 for degradation.¹³⁻¹⁶ E2f1 is found to also contain a PIP motif, and in S2 cells E2f1 mutants with altered PIP sequences accumulate in S phase while wild-type protein does not. Thus, the S phase-specific destruction of E2f1 may resemble that of Cdt1.

New Tricks for Old Dogs; Cell Cycle Regulators with New Functions

Rb and E2F homologs have established roles in regulating the entry into S phase in different systems. In a talk in the Cell Cycle and Checkpoints workshop, Hong Wen from Joe Lipsick's lab (Stanford University) reported that the dREAM protein complex that includes *Drosophila* RBF, E2F2 and Myb has a role in transcriptional regulation of genes needed for G₂/M transition and the spindle assembly checkpoint (SAC) such as *polo*.¹⁷ In larval imaginal discs, loss of G₂/M and SAC transcripts in *Myb* null mutants can be rescued in the posterior half of the disc by expression of Myb via *engrailed*-GAL4. Interestingly, mutations in another member of the dREAM complex, Mip130, rescue the lethality and loss of Polo expression seen in *Myb* mutants. Similarly, mutations in *E2F2* also rescued Polo expression in *Myb* mutants. These results suggest that Mip130 and E2F2 proteins act in opposition to Myb protein in regulating these target

genes. In support of this hypothesis, dREAM complex members bind to the *polo* promoter in chromatin immunoprecipitation assays. Polo expression displays variegation in *Myb* and *mip130*, or *Myb* and *E2F2* double mutant imaginal discs. The variegated expression of Polo can be suppressed by restoration of *Myb*, and enhanced by the restoration of *mip130*. These results demonstrate the epigenetic regulation of gene expression by the dREAM complex in vivo. A genetic screen for modulators of *polo* expression in a *Myb* mutant background is yielding additional regulators of this process.

Christian Berger from the Technau lab (University of Mainz) expanded on their previous findings that Cyclin E has a role in cell-fate determination in embryonic neuroblasts (NBs) that is independent of its role in regulation of S phase (reviewed in ref. 18), in a talk in the Cell Cycle and Checkpoints workshop. In this system, zygotic CycE is normally expressed in the thoracic neuroblast (NB6-4), which generates neurons and glial cells, separating these two fates in the first asymmetric division. The abdominal variant of these NBs generates only two glial cells and has no zygotic CycE expression. Zygotic mutations in cyclin E (*cycE*^{AR95}) transform asymmetric thoracic divisions into symmetric divisions such that NB6-4 now produces only glial cells. Overexpression of CycE in the wild-type background transforms symmetric abdominal divisions into asymmetric divisions like those in thoracic NB6-4. The role in NB fate appears to be specific to CycE because mutations in other G₁/S regulators tested (*cycA*, *cdk1*, *e2f1* and *Dp*) did not produce a similar phenotype. Newer studies separated the two functions of cyclin E. *CycE*^{dom1} allele can provide the cell cycle function, at least partially, but could only generate glial fate. Mutations in *Cdc25^{stg}* arrest the cell cycle in G₂, two cycles prior to neuroblast divisions. *stg* mutant embryos do not show differentiation of neuronal or glial cells. Mutating *cycE* in the *stg* mutant background restored glial fate to G₂-arrested NB6-4, supporting the idea that the function of cyclin E in repressing glial fate occurs independent of its established role in G₁-S transition. Expression of Prospero was also sufficient to restore the glial fate in *stg* mutants. These data lead to the model that the function of cyclin E in preventing glial fate occurs by repression of Pros function.

In a talk in the Cell Cycle and Checkpoints workshop, Kristin Garcia from our own group reported a new role for dWee1 kinase. Wee1 homologs have an established role in phosphorylation and inhibition of Cdk1 and regulating the entry into mitosis. We have found that dWee1 also interacts with the *Drosophila* Kinesin-5, Klp61F, in embryo extracts. Recombinant dWee1 phosphorylates recombinant Klp61F in vitro and three tyrosines in the head domain are necessary for this phosphorylation event. Klp61F that carries mutations in the three tyrosines is unable to rescue the phenotypes of *klp61f* mutants, suggesting that the phospho-acceptor residues on Klp61F recognized by dWee1 are important for Klp61F function.

In vivo Live Imaging of Mesoderm Invagination Reveal Two Timed Divisions

We end this meeting review with a report on the power of in vivo live imaging in *Drosophila* embryos that was presented by Amy McMahon in the Stathopoulos lab (Caltech). Two-photon microscopy allowed the imaging of deep tissue layers with reduced phototoxicity and better spatial and temporal resolution. The analysis focused on cell movements during mesoderm migration using

H2A-GFP. The embryos were imaged at 50-second intervals for 2 hr, but all survived to hatching. Imaris software was used to mark and follow each nucleus and the results plotted after translating Cartesian coordinates into cylindrical coordinates so that so that each nucleus, and thus each cell, may be tracked with respect to the major body axis. This study showed that cells are spatially organized and the migration is directed; cells that originate in the leading position, stay in position. The ventral midline acts as an axis of symmetry, with little to no mixing of cells on one side of the ventral midline with those on the other side. The ability to track each cell makes it possible to detect every cell division. This analysis revealed that every cell divides during mesoderm migration and does so in two temporal waves of mitoses. There are distinct groups of cells that comprise each “wave”—the two groups are defined by the position of each cell within the initial ventral furrow. A tight regulation of when and where cell divisions occur throughout the migrating population may be necessary to ensure that this process does not negatively impact the migration.

Summary

We hope this review illustrates not only the exciting nature of ongoing cell cycle research but also the power of the *Drosophila* model to tackle diverse questions, spanning from the role of spindle orientation in shaping an appendage to the question of how a chromosome is built. We look forward to seeing what this bug has to tell us about the cell cycle next year.

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