In This Issue

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Keeping DNA replication below PAR

Deconvolution and threshholding of an image of a GFP::PCN-1-expressing worm embryo reveals that the nucleus of the posterior blastomere (right) contains fewer sites of active DNA replication than its anterior sister (left).

The polarity proteins PAR-4 and PAR-1 delay cell division in early *C. elegans* embryos by inhibiting DNA replication, Benkemoun et al. reveal.

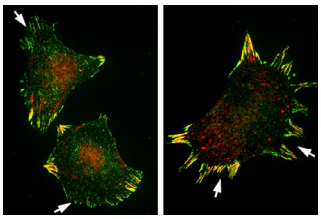
Cells must divide at the right time during development. This is true from the get-go in *C. elegans* embryos where, at the two-cell stage, the anterior blastomere divides while its smaller, posterior sister is still in S phase. This cell cycle asynchrony is promoted by proteins that regulate embryonic polarity, such as the kinases PAR-4 and PAR-1. In embryos lacking these proteins, the posterior blastomere speeds through S phase so that both cells divide at the same time. But how PAR-4 (a homologue of human LKB1) regulates the cell cycle is unclear.

By imaging the replication protein PCN-1, the *C. elegans* orthologue of PCNA, Benkemoun et al. found that the posterior blastomere in wild-type embryos took longer to complete S phase than the anterior cell because, at every time point, it contained fewer sites of active DNA replication. But in the absence of PAR-4, or its downstream target PAR-1, the posterior blastomere formed just as many replication foci as its anterior sister. Knocking down proteins that initiate DNA replication increased the duration of S phase in the posterior blastomere of PAR-4–deficient embryos, restoring division asynchrony and enhancing embryonic viability.

Senior author Jean-Claude Labbé now wants to investigate how PAR-4 and PAR-1 inhibit replication initiation in the posterior blastomere.

Benkemoun, L., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201312029.

Late endosomes uproot focal adhesions



Compared with control cells (left), p14-deficient fibroblasts (right) form elongated focal adhesions containing paxillin (green) and zyxin (red).

Schiefermeier et al. reveal that late endosomes promote cell migration by transporting a signal transduction scaffold complex that stimulates focal adhesion turnover.

p14 and MP1 are two components of an adaptor complex that regulates MAP kinase and mTORC signaling on late endosomes. Schiefermeier et al. noticed that, instead of accumulating in the center of cells like other late endosomes, MP1-positive organelles moved along microtubules toward peripheral focal adhesions attaching the cell to its underlying substrate. In particular, the endosomes targeted the dynamic regions of mature focal adhesions, where adhesion components turn over in order to support cell migration.

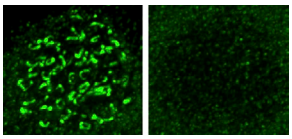
The researchers therefore examined fibroblasts lacking p14, in which MP1 is degraded instead of being recruited to late endosomes. These cells developed large, stable focal adhesions and migrated more slowly than control fibroblasts. Knocking down the small GTPase Arl8b, which recruits the motor protein kinesin-1 to late endosomes, abolished the delivery of late endosomes to focal adhesions and caused a similar decrease in adhesion turnover and cell migration.

Schiefermeier et al. found that MP1 binds to the cytoskeletal regulator IQGAP1, a small pool of which localizes to focal adhesions. Excess IQGAP1 accumulated at the enlarged adhesions of fibroblasts lacking p14 or Arl8b. Knocking down IQGAP1 returned adhesions to their normal size and rescued the migration defects of p14-deficient cells.

Late endosomal p14 and MP1 therefore stimulate focal adhesion turnover and cell migration by promoting the removal of IQGAP1 from mature adhesions. Senior author Lukas A. Huber now wants to investigate how this pathway contributes to cancer cell metastasis and wound healing.

Schiefermeier, N., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201310043.

The spindle checkpoint's on-off switch



Mad1 (green) localizes to the kinetochores of control cells treated with nocodazole (left) but is absent from the kinetochores of cells that lack both CENP-I and Aurora B activity (right).

Matson and Stukenberg describe how the centromeric protein CENP-I cooperates with the Aurora B kinase to control the kinetochore localization of spindle checkpoint proteins.

Mad1 and the RZZ complex are critical components of the spindle assembly checkpoint that prevent anaphase onset by binding to kinetochores that aren't attached to the mitotic spindle correctly. Once spindle microtubules are properly attached, the motor protein dynein strips Mad1 and the RZZ complex away from kinetochores and allows mitosis to proceed. Aurora B helps recruit RZZ and Mad1 to kinetochores in early mitosis, but cells treated with Aurora B inhibitors and the microtubule-depolymerizing drug nocodazole can still activate the spindle checkpoint as long as they express a group of centromeric proteins that includes CENP-I. How CENP-I supports checkpoint activation is unknown, however.

Matson and Stukenberg found that CENP-I stabilized RZZ and Mad1's interaction with kinetochores, limiting their dissociation and preventing dynein from stripping them away prematurely. Thus, when Aurora B activity is lowered by inhibitors, CENP-I helps retain enough RZZ and Mad1 at unattached kinetochores to activate the spindle checkpoint.

On the other hand, Aurora B promoted RZZ and Mad1's association with kinetochores. The kinase's activity was enhanced by so-called PreK-fibers, microtubule bundles nucleated by the kinetochores themselves. In prometaphase cells lacking the stabilizing influence of CENP-I, checkpoint proteins only accumulated at kinetochores with PreK-fibers and high levels of Aurora B activity. Under normal circumstances, however, Aurora B and CENP-I combine to regulate checkpoint signaling at individual kinetochores according to their microtubule attachment status.

Matson, D.R., and P.T. Stukenberg. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201307137.