

RhoA controls furrow formation

Evidence that the cytokinetic apparatus is dependent on spindle microtubules comes primarily from classical experiments in which the spindle was manually moved or disrupted. On page 91, Bement et al. reveal the physiology that underlies these findings. Active RhoA localizes to a narrow zone at the site of future furrow formation in a microtubule-dependent manner, and the RhoA zone moves in response to spindle movement.

RhoA, a GTPase that positively regulates actin–myosin contraction, has been detected before at the site of furrow formation. However, it wasn't clear if or how RhoA functioned in furrow formation, especially because both guanine nucleotide exchange factors, which activate RhoA, and GTPase-activating proteins, which inhibit are also in the furrow region.

Bement et al. injected sea urchin and *Xenopus* embryos with a GFP reporter for RhoA activity. Using 4D imaging, they saw active RhoA accumulate in a tight band at the site of the future furrow. Nocodazole, which disrupts microtubules, prevented RhoA localization, but drugs that depolymerize actin did not.

A new mode of moving

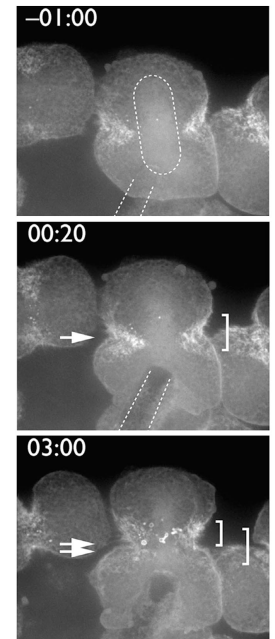
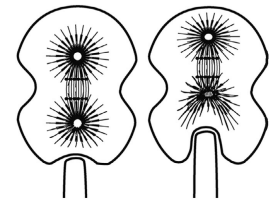
The well-described mechanism of movement by fibroblasts and other flat cells is characterized by outstretched lamellipodia, which are attached through focal adhesions, and retraction of a trailing edge. But how lymphocytes move up to 100-times faster has been unclear. On page 141, Smith et al. report that T cells have a zone of clustered high-affinity LFA-1 integrin ($\alpha_1\beta_2$) at the midzone of the cell, and that disruption of the pattern by removal of talin reduces the speed of cell migration.

When T cells contact a monolayer of cells expressing ICAM-1, activated LFA-1 concentrates in the midzone of the cells, a region that the researchers referred to as the “focal zone.” The concentrated active LFA-1 in this focal zone colocalized with bound ICAM-1 in the supporting cells. Furthermore, the LFA-1 was unable to diffuse freely and was found to interact with the cytoskeleton via the

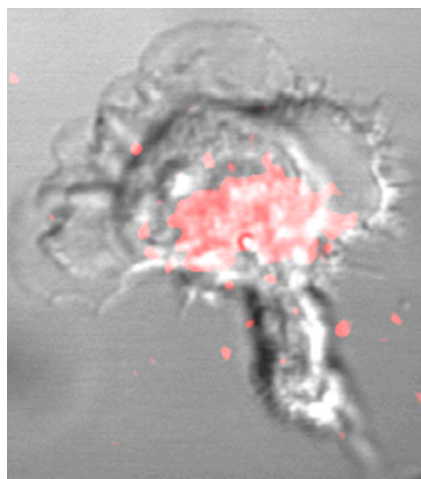
The RhoA zone preceded furrow formation in both symmetric and asymmetric divisions.

When the team repeated classical experiments that altered the location or structure of the spindle, the RhoA zone relocated in a manner similar to the spindle movement, but took several minutes to do so. The delay in RhoA movement suggests that it is not directly tethered to the microtubules but is concentrated by them.

Microtubules thus appear to direct specification of the cytokinetic apparatus by establishing a local zone of RhoA activity, which then promotes the concentration of actin filaments and myosin-2. The team hypothesizes that RhoA regulatory proteins move along the microtubules toward the site of the future zone, as suggested by previous experiments. They think the presence of both RhoA activators and inhibitors increases the rate at which RhoA moves through the GTPase cycle, driving up the rate of activity in a narrow region without causing zone spreading. Although this hypothesis remains to be tested, the team's approach does bridge the gap between classical manipulation experiments and modern molecular genetic methods. **JCB**



Pushing the spindle upwards with a needle (dotted lines, top) moves the RhoA zone (white) upwards (bottom).



A band of ICAM-1 (red) bound to the integrin LFA-1 helps T cells move quickly.

cytoskeletal linker protein talin. An siRNA knockdown of talin destabilized the LFA-1 focal zone and slowed T cell migration.

By contrast, interference reflection microscopy showed that the lamellipodia

made intermittent contacts with ICAM-1, but did not contain high-affinity, clustered LFA-1. The trailing edge uropod, which was not in contact with the underlying cells but was rather held in the air above them, also contained significant quantities of LFA-1, but this LFA-1 was not active.

Based on these data, the researchers conclude that T cells use a novel mode of migration that depends on an interaction between the localized integrin LFA-1 and ICAM ligand expressed by neighboring cells. The focal zone type of movement lacks stable lamellipodial or uropodial attachments. Smith et al. speculate that without these constraints—or the time it takes to make or break them—lymphocytes can move more rapidly than firmly attached cells. If that is true, then one might expect other rapidly migrating cells, such as neural crest cells, to display a concentrated band of ligand-binding integrin at their midsection. **JCB**

Sun and survival with Hif1 α

Stabilized Hif1 α helps cells to survive in the face of low oxygen. Now, Busca et al. (page 49) report that elevated cAMP, which occurs in melanocytes downstream of UV irradiation from the sun, also leads to higher Hif1 α . This should help cells survive the sun's insults, but may also aid in melanoma development.

Oxygen-dependent Hif1 α regulation occurs primarily at the posttranslational level. But Busca et al. report that cAMP acts as an independent stimulant of Hif1 α expression by increasing transcription of the Hif1a gene (which encodes Hif1 α) in melanocytes.

The elevated cAMP up-regulates expression of MITF, a key melanocyte-specific transcription factor. When the team performed a series of reporter gene experiments using MITF and Hif1a constructs, they saw that MITF was necessary and sufficient to turn on Hif1 α expression. Furthermore, MITF bound directly to Hif1a promoter DNA, based on ChIP analysis. Significantly, cells that had elevated cAMP were resistant to cell death triggers, but only when Hif1 α was present.

Although cAMP-MITF activation of Hif1 α was not detected in other cell types—presumably due to the absence of MITF—the scientists think similar transcriptional regulation of the Hif1a gene likely occurs elsewhere. If that is true, researchers may have one more tool to turn off Hif1 α 's pro-survival role in tumor cells. **JCB**

The degranulation two-step

Degranulation of mast cells involves a two-step process, report Nishida et al. on page 115. First, antigen stimulation triggers microtubule polymerization and granule translocation to the cell surface in a calcium-independent process. Second, the granules fuse with the plasma membrane in a well-characterized calcium-dependent process.

Mast cells are so full of granules that degranulation was thought to occur through granule-to-membrane fusion and granule-to-granule fusion, without the need for granule transport. However, inhibition of microtubule polymerization blocked degranulation.

In response to antigen stimulation, Nishida et al. found that tubulin staining increased and fluorescently tagged granules translocated to the cell surface before exocytosis. Removal of calcium from the culture medium prevented granule fusion to the membrane but had no effect on microtubule polymerization or granule movement, suggesting that the steps are distinct.

When the team stimulated mast cells deficient for Fyn or Lyn tyrosine kinase signaling proteins, the cells had reduced degranulation, as previously reported. However, only the

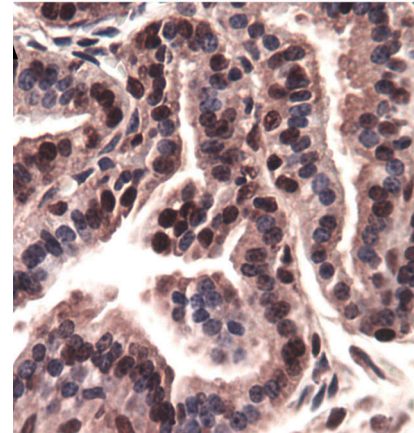
Regulating the prostate

Prostate cancer and benign prostatic hyperplasia result from excessive proliferation of cells in this organ, likely due to deregulation of stem cells. Salm et al. (page 81) find that cells in the proximal region of the prostate, where the stem cells reside, respond differently to TGF- β relative to distal cells in mice. A regulatory teeter-totter between TGF- β expression, which inhibits cell proliferation and pro-growth cytokines, maintains stem cell quiescence in the healthy organ.

The researchers looked first at androgen-expressing animals. They found that cells in the proximal region of the prostate had more TGF- β -mediated signaling than did cells in the distal region. This should keep the stem cells quiescent, but the cells still survive, probably because of the high expression of anti-apoptotic Bcl-2 in the region.

The distal region normally has lower TGF- β signaling, but androgen withdrawal reversed the pattern. Now the high TGF- β in the distal region induced apoptosis, whereas the lowered TGF- β signaling in the proximal region allowed stem cells in this area to respond to pro-growth cytokines.

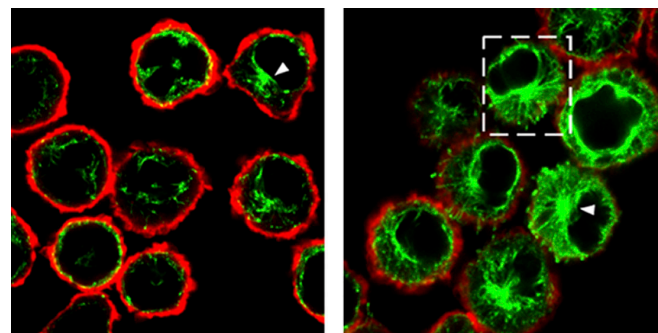
The balance between TGF- β and pro-growth cytokines likely maintains the quiescent state of stem cells in a healthy prostate, and deregulation may lead to prostate disease. A similar balancing act may also be present in the epidermal and hematopoietic stem cells already known to be regulated by TGF- β . **JCB**



TGF- β signaling (brown) keeps stem cells dormant but alive in the proximal prostate.

Fyn mutant cells showed a disruption in microtubule polymerization and granule movement, suggesting that Fyn signaling directs microtubule polymerization.

The scientists are now looking for proteins that link granules to microtubules. They reason that such molecules might provide a relatively specific target for drugs aimed at blocking unwanted histamine release from mast cells. **JCB**



Degranulation relies on transport by an induced microtubule network (green, right).