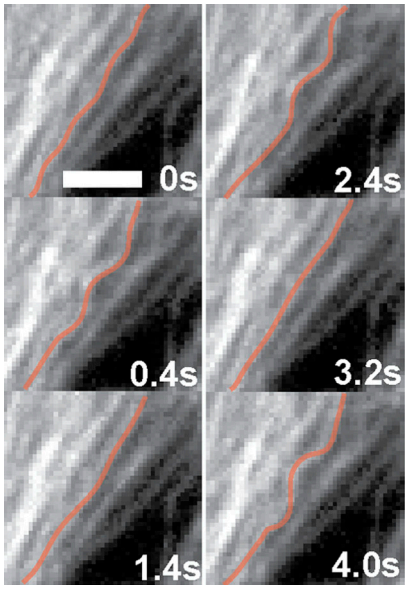
# In This Issue




**Heart contractions cause periodic wavy buckling of microtubles.**

## Reinforced microtubules

Much of the mechanical strength of microtubules comes from the cytoskeleton surrounding them, report Brangwynne et al. on page 733.

When compressive forces push on a microtubule in a cell, such as when a growing polymer butts up against the cell edge, the fiber bends in multiple short wavelength curves like a snake. By contrast, when the end of an isolated microtubule is pushed with even small forces, the fiber compresses and bends in a single large arch. The minor forces necessary to bend isolated microtubules call into question the importance of the fibers in determining cell shape and strength.

Brangwynne et al. found that if they pressed on the end of a microtubule inside a cell with a microneedle, short wavelength bending occurred. Moreover, contraction of the actin–myosin cytoskeleton induced such buckling in rhythmically contracting cardiac myocytes, and neighboring microtubules bent in a coordinated pattern.

To find out if an elastic medium surrounding a fiber could cause a shift from long to short wavelength bending, the team compressed a thin plastic rod first in aqueous solution and then in a gelatin matrix. In water, the rod formed a long arc, but when constrained by gelatin, which the rod had to push out of the way, it bent in a shorter sinusoidal pattern. Mathematical modeling showed that the wavelength of bending in response to compression resulted from the combined strength of the fiber and the resistance of the medium.

When the team disrupted the actin–myosin matrix with cytochalasin and then compressed microtubules with a microneedle, they saw that the microtubule now bent in a longer arc than occurred when the actin fibers were intact.

The team concluded that the surrounding network adds substantial strength to the microtubules. Furthermore, by increasing the reinforcement in particular regions, the cell can hold one part of a microtubule straight while allowing small wavelength bends in other regions. Thus, microtubules can withstand and generate the forces necessary to support motility and tissue development. JCB

## An unused license to fire

Not all available origins of replication fire during a normal S-phase. But when replication is perturbed, otherwise dormant origins go to work, Woodward et al. show on page 673.

Cells initially respond to slowed replication by turning on the ATR-dependent checkpoint, which prevents other origins from firing and thus getting into trouble too. But if the cell decides it is time to recover from that checkpoint, the mechanism discovered by Woodward et al. may ensure that there are enough origins to get the job done.

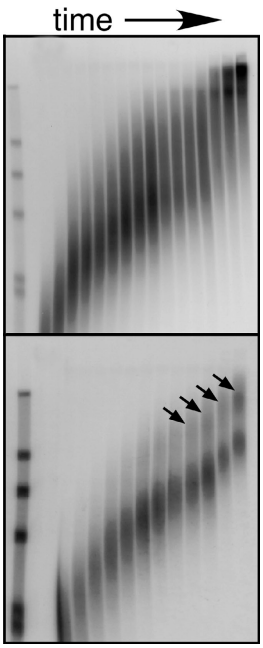
The excess supply of origins arises from an excess of sites that have the minichromosome maintenance protein complexes, Mcm2-7. These complexes are loaded onto chromatin before S-phase and are required to license replication origins for use. However, the number of complexes loaded is much higher than the number normally used.

Working in *Xenopus* egg extract, Woodward et al. found that replication speed, origin spacing, and the slowing in response to the DNA polymerase inhibitor aphidicolin were normal with either the full complement of Mcm2-7 or a minimal amount.

When aphidicolin-treated cells were supplemented with caffeine, which inhibits the ATR-dependent DNA replication checkpoint, the cells' DNA replication was completely rescued if and only if there was the normal excess of Mcm2-7. The rescue correlated with a large increase in the number of forks fired.

In worms, reducing MCM7 levels with siRNA had little or no effect in the absence of replicative stress. But when DNA replication was inhibited, wild-type worms were fine but siRNA-treated worms died.

Woodward et al. think that a replication origin is actually a cluster of Mcm2-7–licensed sites spread around a primary origin. Once one of the Mcm2-7 sites fires, the ATR checkpoint protein blocks activation of neighboring complexes, but if it stalls, the checkpoint is relieved and other complexes can fire. Just how that system might work though is not yet clear. Given that not all origins in the genome fire at the same time, ATR must be able to exert local control. JCB




**Low Mcm2-7 levels (bottom) slow recovery from DNA replication inhibition.**

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Text by Rabiya S. Tuma
rabiya@nasw.org

## Tunneling through cells

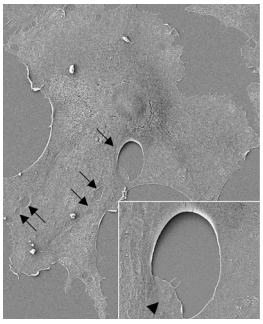
*Staphylococcus aureus* inactivates RhoA to open tunnels through endothelial cells, report Boyer et al. (page 809). These "macroapertures" may allow the pathogenic bacteria easy access to the endothelial basement membranes for tissue invasion and colonization.

Numerous bacterial virulence factors, including EDIN, target proteins in the Rho GTPase family, deregulating the actin cytoskeleton and changing cell shape and adhesion. EDIN is an ADP–ribosyltransferase that locks RhoA in an inactive state.

Boyer et al. found that when endothelial cells in culture or in rat arteries were exposed to *S. aureus* expressing EDIN or to recombinant EDIN protein, macroapertures formed in a dose- and time-dependent manner. The timing and number of macroapertures correlated with RhoA–ADP–ribosylation.

Without active RhoA, the actin cytoskeleton was rearranged with a loss of stress fibers. The macroapertures appeared as a result of retraction of the membrane and were not associated with tears or wounds in the membrane.

Once a macroaperture formed, the cell appeared to detect the problem. A dense meshwork of actin encircled the opening and lamellipodium-like structures formed at the edges leading to closure. Therefore, the openings only lasted a few minutes, but that is more than enough time for bacteria to access the basement membrane.

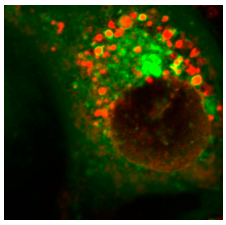


**A bacterial protein induces holes for migration.**

Researchers reported recently that as leukocytes move from the bloodstream to surrounding tissues they can induce similar openings in endothelial cells in a Rho-dependent manner. Pathogenic bacteria may have co-opted this system for more nefarious purposes. JCB

## Rab proteins move integrins

Proper trafficking of integrins is important for cell adhesion and migration, but the machinery involved has been unknown. On page 767, Pellinen et al. report that small GTPase Rab proteins that are known to be important for endocytosis and exocytosis associate with integrins and facilitate their internalization and recycling.



**Rab21 (green) brings β1 integrins into vesicles.**

In epithelial cancer cells, β1-containing integrin heterodimers associated with Rab21. Rab21 expression triggered localization of active β1 integrin and Rab21 to large vesicles, consistent with Rab21 being an early endosomal protein. A large fraction of the integrin rapidly returned to the cell surface.

Cells that overexpressed Rab21 attached to the substrate more efficiently than did wild-type cells, whereas cells treated with Rab21 siRNAs had less affinity for the substrate and migrated less efficiently in a wounding assay.

As Rab 21 did not alter the amount of integrins in the cell, the team hypothesizes that it affects attachment and migration by increasing integrin recycling to newly formed sites of attachment. The researchers hypothesize that the integrins are stripped of their ligands in the vesicle and thus readied to return to the surface to bind new substrate. JCB

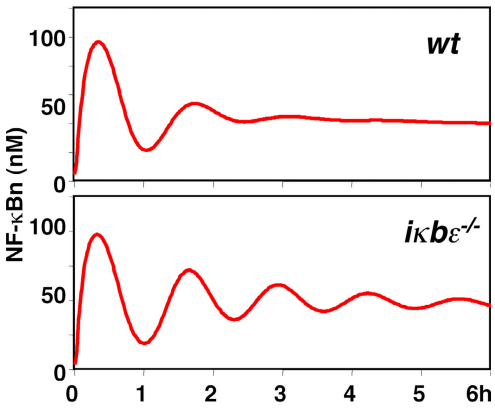
## It takes two to regulate

Two isoforms of the IκB inhibitor of NF-κB are required to turn oscillation into steady regulation during chronic stimulation, according to Kearns et al. (page 659). The use of two out-of-phase regulators may be a common means to control signaling pathways.

NF-κB activation triggers expression of IκBα, which leads to down-regulation of the signaling pathway and a decrease in IκBα transcription. However, under chronic stimulation the NF-κB signaling pathway becomes reactivated as soon as the amount of IκBα drops below a certain level. Thus, in cells engineered so that IκBα is the only IκB isoform present, NF-κB activity oscillates over many cycles. In unmodified cells, however, NF-κB activity is steady, and computational modeling suggested the existence of an active damping mechanism that limits fluctuation.

Kearns et al. found that IκBε expression was also induced by NF-κB. There was, though, a significant delay in its expression relative to IκBα. Mathematical modeling and cell experiments showed that, with the two regulators out of phase due to IκBε's lag, NF-κB expression was dampened to a steady half-maximal level in chronically stimulated cells after an initial peak.

A recent report showed that two signals that trigger NF-κB activity also induce oscillation individually but lead to an even activity level when combined (Covert et al. 2005 *Science*. 309:1854-7). Thus, Kearns et al. speculate that this sort of regulatory mechanism may be a way for cells to modulate the level of activity of a signaling pathway, rather than being limited to simple on/off switches. JCB




**NF-κB levels settle to steady-state unless IκBε is missing (bottom).**

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