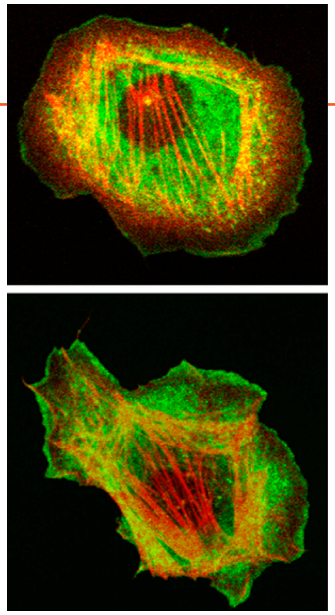
In This Issue



The mDia1 formin (green) creates protruding actin (red) bundles in the periphery when N-WASP and WAVE2 are inhibited (bottom).

Lamellipodia fight back formin

The actin-organizing activities in a reaching lamellipod fight back the trailing lamella, say Sarmiento et al. The feedback might coordinate inchworm-like migration in normal and cancerous cells.

Tumor cells such as breast cancer carcinomas become dangerously motile because they have hyperactive actin-polymerizing pathways that respond to epidermal growth factor (EGF). On flat surfaces, migrating cells extend separate actin-filled structures such as filopodia, invadopodia, and lamellipodia, which in 3D tumors combine into a single structure at the leading edge. The group wondered how these individual compartments are initiated and maintained and how they interact.

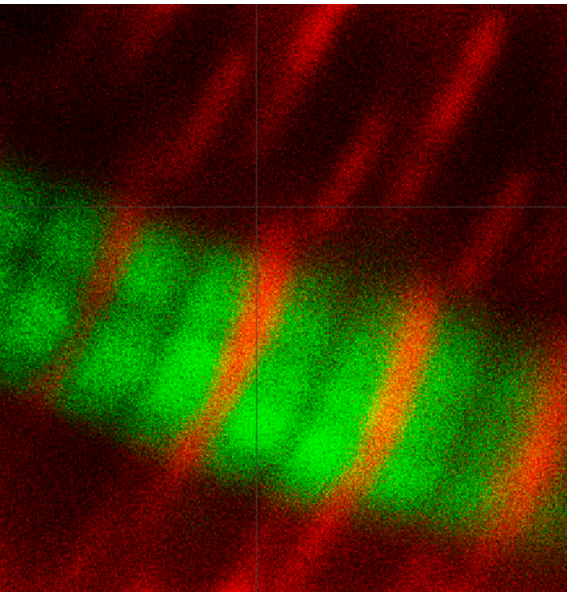
They now identify the main actin-polymerizing activity that creates and maintains lamellipodia as WAVE2, a member of the WASP family that works with Arp2/3 to form branched actin networks. The only other prominent WASP member in the carcinoma cells was N-WASP, which was not needed for lamellipod formation.

When both WAVE2 and N-WASP were depleted from the carcinoma line, the group noticed an explosion of jagged protrusions with filopod-like tips. The filopodia seemed to stem from merging and bundling lamellar actin, as they colocalized with the lamellar marker, tropomyosin.

Filopodia are often associated with actin-bundling formins. The authors found that the mDia1 formin created the odd filopod protrusions in these deletion lines. Loss of mDia1 blocked the jagged extensions, whereas an overactive version replicated the effect of WAVE2 and N-WASP inhibition. The WASP activities seem to prevent filopodia by somehow blocking one of mDia1 activators, RhoA GTPase.

The group suggests that the combination of WAVE2 and WASP in extending lamellipodia keeps back the trailing lamella, which contains the machinery to stabilize the new cell boundary. This inhibition would allow lamellipodia to search the environment and select the correct direction (toward an EGF source) before shutting down and permitting lamella to inch forward as well. JCB

Sarmiento, C., et al. 2008. *J. Cell Biol.* 180:1245–1260.



Myosin's chaperone, Unc45b (green), relocates from the Z-line (red) to the A-band when muscle cells are stressed.

Myosin chaperones: from A to Z and back

Like overprotective parents, muscle myosin's chaperones don't stray far from their charge, according to images from Etard et al.

Muscle contraction depends on the proper arrangement of the myosin motor within the basic repeating muscle subunit, known as the sarcomere. The steps involved in constructing the sarcomere are not well-understood. As yet, an in vitro assembly system has not been devised, so researchers rely on the zebrafish system to unravel vertebrate muscle assembly.

As zebrafish muscles develop, their myosin is folded into a part of the sarcomere known as the A-band. Folding occurs with the help of two chaperones, called Unc45b and Hsp90a. Evidence in worm suggests that these chaperones remain with myosin in the A-band even after the muscle is fully formed. But Etard et al. found that their association with the A-band in zebrafish was much more short-lived: only by slowing down muscle development could the authors catch a glimpse of the chaperones in the forming A-band.

In normally developing fish, the chaperones made a swift relocation to a nearby sarcomere structure called the Z-line, where the large protein titin anchors myosin to actin filaments. This location was not permanent, however. The chaperones returned to the A-band, the group found, when muscles were stressed. Stresses that mobilized the chaperones included heat and cold shocks and damage to the muscle cell membrane.

The group proposes that these sorts of challenges somehow lead to myosin unfolding. In its unfolded form, they suggest, myosin's affinity for the chaperones is high enough to draw them away from their Z-line residence. FRAP measurements revealed that the two chaperones were highly dynamic at the Z-line, constantly shuttling between the sarcomere and the cytoplasm. Given the close proximity of the Z-line and the A-band, this dynamic behavior would allow the chaperones to probe myosin's condition repeatedly and help correct any folding errors. JCB

Etard, C., et al. 2008. *J. Cell Biol.* 180:1163–1175.

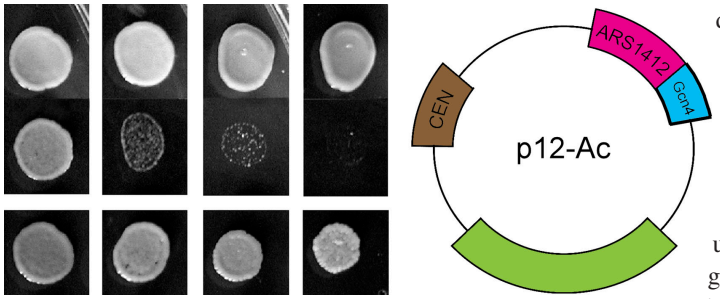
Text by Nicole LeBrasseur
lebrasn@rockefeller.edu

Early origins sound S phase alarm

Early risers turn on the alarm that prevents genomic instability stemming from problems with DNA replication, say Caldwell et al. The findings show that those DNA replication origins that are activated earliest are needed to trigger the S phase checkpoint.

The S phase checkpoint is turned on during every cell cycle when DNA polymerases stall upon encountering lesions in the DNA template. The checkpoint keeps polymerases on the replication fork, prevents the fork's intricate DNA structure from collapsing, and delays S phase progression into mitosis. In the meantime, the cell has time to find ways around the lesion, for instance by using different polymerases or switching template strands.

In the new work, the authors sought to determine how this checkpoint is activated. They hypothesized that early replication origins might be needed, based on evidence that early origins fire even if cells are not fully ready for replication—if dNTP levels are too low, for example.



In the absence of the DNA damage checkpoint, treatments that activate the S phase checkpoint kill off (left to right) yeast that lack early firing replication origins (middle row). Adding episomes with early firing origins (p12-Ac) rescues the mutants (bottom row).

Those early origins do not fire in a yeast mutant that lacks a kinase called Dun1 (the basis for the misfiring is so far unknown). The group now shows that, when exposed to treatments that normally activate the S phase checkpoint, *dun1* mutants rely on the DNA damage checkpoint for survival. The dependence belies the cells' inability to activate the S phase checkpoint and the resulting damage to the genome that must be repaired before mitosis.

The addition of just one or two functional early origins restored the mutants' S phase checkpoint. The working origins, which were introduced into the yeast cells on episomes, fired normally in the *dun1* mutants. The group hypothesizes that these episomal origins might fire because they lack a chromatin structure that somehow cripples endogenous *dun1* origins.

Forcing the extra origins to fire later blocked their ability to turn on the S phase checkpoint. So did deletion of their centromeres, suggesting that the checkpoint can be activated by proteins that participate in fork pausing, as occur when forks encounter large protein complexes on centromeres or actively transcribed regions. JCB

Caldwell, J.M., et al. 2008. *J. Cell Biol.* 180:1073–1086.

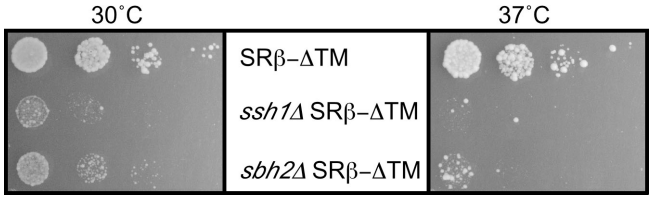
The SR finds the translocon

Results by Jiang et al. reveal an elusive interaction that leads to protein translocation into the ER.

As proteins destined to enter the secretory pathway are translated, they are brought to ER channels known as translocons. The process begins when the signal sequence that tags secreted proteins first emerges from the ribosome. This peptide is bound by the signal recognition particle (SRP), which must then find its receptor (SR). Models dating back a decade propose that empty translocons are identified via interactions with the SR.

This SR–translocon interaction has not been definitely shown, however. Because both components lie in the ER membrane, experiments designed to identify the interaction have been difficult. The transient nature of the interaction—once the ribosome docks on the translocon, the SR departs—has not helped matters. But the group's new genetic evidence backs the prevailing model.

A genetic trick was provided by a yeast mutation that results in a soluble version of SR, via deletion of its transmembrane segment. Translocation efficiency was reduced in the mutants, suggesting that SR was finding the translocon at a slower rate, because it must search in three dimensions rather than just in the plane of the ER membrane.



Yeast with a soluble SR mutant are still viable (top), but the mutation becomes deadly when combined with the loss of the entire Ssh1 translocon (middle) or just its β subunit (bottom).

When the Ssh1 secondary translocon, or just its β subnunit, was deleted from the soluble SR strain, the cells were severely crippled in translocation and growth. This genetic interaction suggests that soluble SR locates translocons through interactions with translocon β subunits. Deletion of the β subunit of either the primary or secondary translocon alone does not impair translocation in cells with wild-type SR, but loss of both causes a severe defect. JCB

Jiang, Y., et al. 2008. *J. Cell Biol.* 180:1149–1161.