

Research Roundup

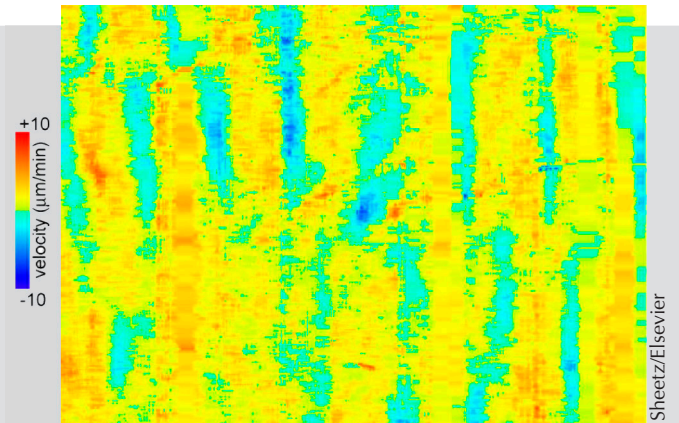
Backward waves move cells forward

Advancing cells periodically pull on their substrates to test the environment, say Grégory Giannone, Michael Sheetz (Columbia University, New York, NY), and colleagues. The actomyosin contractions both strengthen attachments to more solid substrates, and break off part of a signaling complex so that it can be carried into the cell. Once that retrograde signal reaches myosin, which is some distance from the cell edge, a new contraction is initiated.

“This cements the idea that what the cell is doing with its actin machinery is testing its environment,” says Sheetz. And, he says, it provides a justification for why cells allow actin to flow backward, away from the cell front, even as they use forward protrusion of actin to drive cell movement.

The key to the probing, and one reason that it took some time to spot, is that its periodicity is localized and not synchronized over the whole cell. Each part of the cell surface is advancing and contracting (i.e., stopping) on its own schedule, although the time between contractions is the same all over the cell (24 s for the cell type under study). The 24 s matches the time taken for signaling complexes—F-actin and associated α -actinin and myosin light chain kinase (MLCK)—to traverse the protruding lamellipodia. The time increases or decreases after treatments that expand or shrink the lamellipodia.

The contractions may be triggered by arrival of the myosin-activating MLCK at the base of the lamellipodium. Each periodic contraction then results in a row of transient integrin–paxillin



Waves of contraction (blue) slow down forward movement but allow adhesion.

clusters being laid down near the cell front. Formation of these links to the extracellular matrix, and thus the occurrence of effective protrusion, is only supported by rigid substrates. This requirement for rigidity may reflect the matrix dependence that keeps nontransformed cells from growing in places where they are not wanted.

The contractions are needed to test the environment, but they must be periodic so that the cell doesn't spend its whole time going backward. The periodicity is enforced by the distance traveled by the contraction signal. And directionality of the signal is maintained in transit by restricting the signal to travel along actin filaments. Similar direction-conserving signaling may operate in growth cones of neurons. ■

Reference: Giannone, G., et al. 2004. *Cell*. 116:431–443.

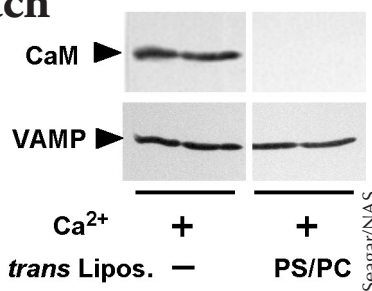
Flipping the fusion switch

Calcium and calmodulin transfer a critical region of the vesicle protein VAMP from donor to target membrane, suggest Luc de Haro, Michael Seagar (INSERM, Marseille, France), and colleagues. The transfer may help to fuse the two membranes together.

VAMP is a v-SNARE: a helical protein sticking out of donor vesicles that zippers up with t-SNAREs on target membranes. Seagar has previously found that a membrane-proximal section of VAMP shows mutually exclusive binding to calmodulin or acidic phospholipids. Interaction with lipids in the donor vesicle membrane may inhibit fusion.

Now, Seagar finds that Ca^{2+} /calmodulin alleviates this inhibition, at least as far as the binding step. Ca^{2+} /calmodulin binds to VAMP-loaded vesicles, and is needed for target membranes to bind to the VAMP on VAMP-loaded vesicles.

The group suggests that the VAMP association with cis membranes is disfavored



Calmodulin binding (left) displaces part of VAMP from cis membranes, but trans membranes then displace calmodulin (right).

by a kinking back of the protein, allowing displacement by Ca^{2+} /calmodulin. This interaction (and calmodulin itself) is then displaced by the more straightforward insertion of the VAMP residues into the target membrane.

Thus, Ca^{2+} /calmodulin can unidirectionally transfer a protein between two lipid domains of identical composition. But how important is this for fusion? Ca^{2+} /calmodulin was an early favorite of

those looking for a fusion trigger but later fell out of favor when it became clear that inhibitors had pleiotropic effects on numerous kinases and channels. Now, says Seagar, “to say that calmodulin is doing something important in membrane fusion is still to some people saying something heretical.”

Some of those people subscribe to the theory that SNARE proteins can do fusion by themselves. But Seagar points out that several groups have now observed the inhibitory property of the source membrane on SNARE activity. The new results show how Ca^{2+} /calmodulin can overcome this inhibition and perhaps convert it into fusion.

Ca^{2+} /calmodulin is not the whole story. Seagar suggests that it may be an archaic fusion trigger used in yeast and some intracellular trafficking events in mammals. But in the nervous system, where a faster trigger was needed, it may have been superseded by synaptotagmin. ■

Reference: de Haro, L., et al. 2004. *Proc. Natl. Acad. Sci. USA*. 101:1578–1583.