In This Issue

STALLing for signaling

Extracellular receptors anchored in the plasma membrane outer leaflet somehow entice intracellular proteins to the cytosolic side of the membrane for activation. Companion studies by Suzuki et al. (pages 717 and 731) give a peek at these dynamics at the level of single molecules.

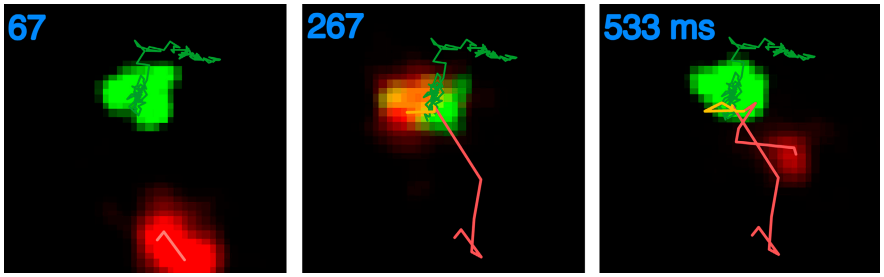
The team shows that liganded clusters of the GPI-anchored receptor (GPI-AR) CD59 undergo temporary immobilizations, called STALLs (stimulation-induced temporary arrest of lateral diffusion), which serve as fleeting platforms for activating a signaling cascade. How a signal gets from the outside in without a transmembrane stretch has been intensely investigated.

When GPI-ARs come together, a slew of events take place inside the cell: the cluster can associate with Gα proteins, activate Src-family kinases (such as Lyn), and trigger the IP3/calcium signaling cascade. Previous studies based on large aggregates of GPI-ARs pointed to the involvement of raft microdomains for regulating the interactions.

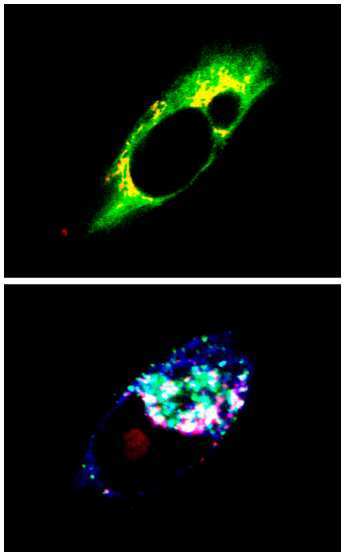
To show how these events occur over space and time, the authors chose a single-molecule approach that used a more physiological clustering of just three to nine CD59 receptors. The CD59 clusters recruited both Lyn and Gαi2 frequently and transiently (100–200 ms). The resulting meeting between Gαi2 and Lyn activated Lyn and led to a CD59 STALL, which lasted for about half a second.

The STALL may be the result of Lyn's phosphorylating an unknown protein that hooks the CD59 receptors to actin filaments. However it happens, the STALL created a temporary landing platform for PLCγ, which converts PIP2 to IP3 and thereby releases calcium from the ER. Treatments that interfered with STALLs also blocked the calcium signal.

Each PLCγ molecule hovered at the membrane for only ~0.25 s, but the total IP3 signal has been measured to last for ~15 min. The authors suggest that the bulk signal is produced by the summation of thousands of digital bursts from individual PLCγ molecules. A transmembrane mutant of CD59 also induced Lyn recruitment, albeit at lower levels, leading this group to conclude that both protein- and lipid raft–based interactions are at work. Exactly how a raft microdomain might draw this molecular crowd remains up for grabs. JCB



A CD59 cluster (green) transiently recruits (left to right) a Lyn-GFP molecule (red).



Killer protein Bax (green) only moves from the cytosol to mitochondria (red) when its pro-death teammate Bim (blue, bottom) is present.

Bim plays apoptosis offense

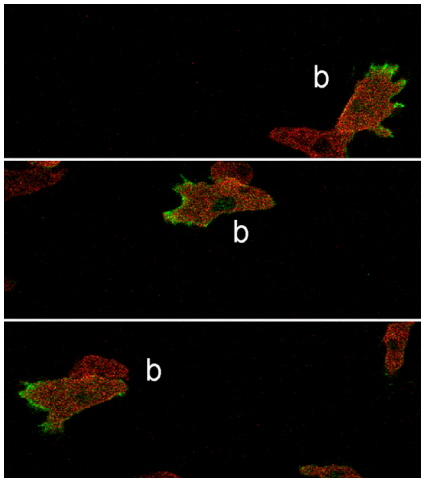
A revision of competing ideas about programmed cell death is in order, according to results from Weber et al. (page 625). The work gets a step closer to nailing down the controversial role of the BH3-only family of proteins as playing offense on the pro-death team of apoptotic proteins, rather than defense against the opposing protective agents.

Three groups of apoptosis proteins control a cell's life-or-death fate: Bax/Bak (the executioners) and BH3-only proteins are pro-apoptotic, whereas a subset of Bcl-2 proteins are anti-apopototic. But researchers have had a hard time deciding how the BH3-only proteins, a group that includes Bim, factor into the equation. One side argues that BH3-only proteins bind to Bax/Bak directly to turn on these killing machines. Another line of thinking has the BH3-only proteins running interference for the killers by soaking up and neutralizing the protective Bcl-2 members.

Here, Weber and colleagues introduce clues that suggest a bit of rethinking may be in order. An inducible version of Bim showed that high levels of Bim caused death without coming into contact with Bcl-2 protectors. In yeast that lacked any Bcl-2 proteins, Bim still enhanced the death action of Bax. This enhancement appeared to work by helping Bax insert into the mitochondrial membrane—but without a direct interaction.

The authors speculate that the translocation of Bim and other BH3-only proteins into the mitochondrial membrane sets up their pro-death teammate Bax to join them there. In any event, neither the direct-binding model or the displacement model appears to explain entirely how these proteins play the game. JCB

Text by Kendall Powell
kendall2@nasw.org



A repellent on the right causes a *Dictyostelium* cell (b) to move to the left by raising PIP3 levels on the opposite side.

Chemotaxis in reverse

A cell polarity switch controls whether cells rush toward an attractive chemical or shift into reverse when they encounter something repulsive, according to Keizer-Gunnink et al. on page 579. They show that the status of phospholipase C (PLC) sets up the polarity axis for directional movement.

As professional crawlers, *Dictyostelium* cells had previously revealed that chemoattractants cause a PIP3 build-up on that side of the cell, which in turn induces the actomyosin motility machinery. Now, the authors show that the same crawlers hold the keys to chemorepulsion.

When faced with a repellent, the cells built up PIP3 on the opposite side of the cell, inducing the motility machinery to reverse direction. Attractants and repellents caused PIP3 levels to rise on opposite sides of the cell using a symmetry break established by the mutually exclusive locations of the enzymes that produce and degrade PIP3.

The producing enzyme, PI3 kinase, hangs out at sites of PIP3-induced actin filaments, whereas the degrading enzyme, PTEN, binds to its product, PIP2. The two therefore do not cross paths in the cell.

To back up, cells increased PIP2 levels at the leading edge. Repellents inactivated the PIP2 destroyer, PLC, at the near edge, thus allowing PIP2 to accumulate there. The PIP2 then recruited PTEN to the front, thereby concentrating most PIP3 in the rear of the cell for a getaway. Chemoattractants, in contrast, activated PLC. Whether repulsion works the same in nature—by mammalian cells during development or by *Dictyostelium* cells warding off hungry neighbors during starvation—remains unknown. JCB

Zinc messenger

Zinc has always had its fingers in the structure and activity of enzymes, transcription factors, and signaling molecules. Now, Yamasaki et al. (page 637) find zinc also working as a second messenger capable of converting an external signal into internal events.

When the authors created an external signal in mast cells by cross-linking IgE receptors, they observed an internal wave of free zinc released from the vicinity of the ER. Calcium and activated MAP kinase were necessary for the wave, which took several minutes to occur, in contrast to rapid calcium releases.

Cranking up the levels of free zinc prolonged the expression of cytokines IL-6 and TNFα—late stages of IgE signaling—and vice versa. Higher zinc also enhanced tyrosine phosphorylation levels, so zinc may boost signaling efficiency by inhibiting phosphatase activity.

The precise origin of the wave and how zinc stores are released remain a mystery, as do the downstream targets of the free zinc. Another important step will be to determine whether zinc wave signaling is a general phenomenon or is specific to mast cells. The zinc wave could be a potent addition to the cell's limited second messenger repertoire, as 2–3% of total cell proteins have zinc-binding domains. JCB

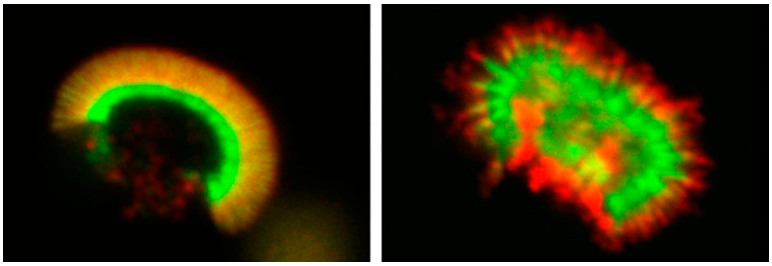
Motoring microvillar membrane

Just as intestines constantly shed gut cells, so too may gut cells shed their brush border membranes. On page 671, McConnell and Tyska show that microvillar myosin-1a pulls membrane for shedding along the core actin bundle.

Myosin-1a was first seen in micrographs 30 years ago bridging the actin bundle and the overlying membrane in microvilli. But it was not until 1989 that myosin-1a was characterized as an actin plus end–directed motor. Its potential as an active motor in the microvillus, rather than mere structural element, was overlooked until now.

A motor is useful in isolated brush borders, which dramatically shear off their membranes from the base to the tip of the microvillar stalks when fed ATP. McConnell and Tyska find that this actin plus end–directed shearing results in vesicles' being shed from the microvilli tips. The ATP activated shedding by turning on myosin-1a: brush borders from myosin-1a knock-out mice shed membrane at only five percent of the wild-type level.

How myosin-1a gains traction on the membrane surface is an open question. But the discovery promotes the brush border from merely a static scaffold of boosted surface area for nutrient breakdown to an active zone for shedding vesicles—tiny packets that may speed nutrient breakdown, shed invading microbes, send messages, and improve membrane remodeling. JCB



Brush border apical membrane (red) accumulates at the tips of actin bundles (green) upon addition of ATP (right) and myosin-1a activation.