Lumen cues calcium release

Calcium release from lumenal stores is sensitive to the redox state of the ER lumen, as shown by Takayasu Higo, Katsuhiko Mikoshiba (University of Tokyo, Japan), and colleagues. The group has identified the first ER lumenal protein that controls IP$_3$-regulated calcium channels (IP$_3$Rs).

The opening of IP$_3$Rs is tightly controlled by cytosolic conditions. Most scientists assume that conditions in the ER also regulate channel opening, but the evidence was minimal. To find any relevant regulators in the ER, the authors used a lumenal domain of IP$_3$R1 to fish out binding proteins. They discovered ERp44, whose association with IP$_3$R1 blocked IP$_3$-induced calcium release.

But the binding of ERp44 and IP$_3$R1, and subsequent channel blockage, depended on several conditions. First, the association occurred at low calcium levels, as would be seen after ER calcium stores are depleted. Second, it required that the lumenal domain of the channel be in its reduced state, with free thiol groups.

That ERp44 is sensitive to the redox state is not surprising—ERp44 is a member of the thioredoxin family and can either donate or accept electrons from other proteins. But this is the first demonstration that oxidative stress, as occurs during inflammation, may deplete calcium stores.

“If the channel is oxidized,” says Mikoshiba, “it’s open, and letting out calcium,” resulting in calcium depletion. “Then ERp44 comes along…changes [IP$_3$R1] to the reduced state [by donating electrons], binds, and blocks the channel.” Strong reducing conditions, which are unfavorable for protein folding, would also keep the channel closed, thus maintaining the high calcium levels that ER chaperones need.

Calcium oscillations resulting from calcium release from the store are important in fertility and neuronal activity. The new findings suggest that these processes may be disrupted by oxidative stress. JCB

Reference: Higo, T., et al. 2005. Cell. 120:85–98.

Supersensitive phospholipid sensing

A lysine-laden sensor makes an actin regulator ultrasensitive to phospholipid levels, according to Venizelos Papayannopoulos, Wendell Lim (University of California, San Francisco, CA), and colleagues.

Phospholipids, particularly PIP$_2$, are activators of N-WASP—an Arp2/3 regulator that turns on actin polymerization. None of the common PIP-binding domains (e.g., Pleckstrin Homology or Phox domains) are found in N-WASP. Instead, a region of 10 basic residues (the B motif) binds to the phospholipids. The new research reveals that this conglomerate of positive charges turns N-WASP into an all-or-nothing switch in response to changing PIP$_2$ density.

At 10% PIP$_2$, N-WASP bound tenfold more strongly to vesicles than it did at 2% PIP$_2$. Even sharper effects from lipid density were seen in actin polymerization assays. Cholesterol, which recruits PIP$_2$ to lipid rafts, lowered the activation threshold, suggesting that local density, not overall concentration, of the phospholipid is key. “Polymerization must be spatially precise,” says Lim. “Having N-WASP respond to the spatial organization of input molecules adds a high level of precision.”

Another activator of N-WASP is Cdc42, which the team found significantly lowered the level of PIP$_2$ required for N-WASP activity. This ability may allow N-WASP to be turned on without altering cellular PIP$_2$ levels.

The activation threshold is determined by the number of basic residues in the B motif—more lysines allowed N-WASP to be activated at a lower PIP$_2$ density. N-WASP with extra lysines also bypassed the usual activation mechanisms (i.e., PI5K activation and PIP$_2$ synthesis) required for vesicle motility in vivo. Says Lim, “this implies that the native protein threshold is set just above the native density of PIP$_2$,” which is several-fold higher than that of other phospholipids. “It’s tuned to optimize sensitivity,” he says, “but also suppress noise under basal conditions.” JCB

Reference: Papayannopoulos, V., et al. 2005. Mol. Cell. 17:181–191.