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Following the fat trail to death

Monitoring the changing lipid composition of phagosome membranes reveals where these vesicles go and how they might get there.

After being swallowed alive by an immune cell, most pathogens meet a grisly death-by-digestion in the cell's equivalent of a stomach—the lysosomes. Some sneaky bugs, however, have learned to avoid this fate. They are still swallowed—into membrane pouches called phagosomes—but never make it to the deadly lysosomes. Instead, they carry on living inside other membrane-enclosed compartments within the cell.

The trafficking of phagosomes and other vesicles around the cell is dependent upon interactions of their membranes with organelles such as endosomes and with the cell's transport machinery, particularly microtubules. Membrane composition, in terms of both the proteins and lipids that are present, thus affects cellular destination. Yeung et al. are interested in how the membrane composition of phagosomes changes after these vesicles are gulped down from the plasma membrane, until they merge with lysosomes or get diverted by resident bugs.

Certain lipids called polyphosphoinositides disappear from phagosomes' surface membranes shortly after they pinch off from the plasma membrane (1–3). Polyphosphoinositides carry multiple negative charges, so their loss leads to reduced negativity, but not a complete reduction. Yeung et al. now show that phagosomes maintain a significant negative charge, thanks to another type of membrane lipid called phosphatidylserine. However, if phagosomes are diverted from their lysosome by distinctive pathogens, they lose their phosphatidylserine and virtually all of their negative charge (4).

The researchers had looked for phosphatidylserine in phagosomes before, and didn't find it. But Sergio Grinstein, the team leader, decided to keep looking. "We had tried to measure phosphatidylserine in phagocytosis with the tools that were available at the time," he says, "but we reached the wrong conclusion because the tools were not accurate."

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The old technique required that the cells be fixed and permeabilized. Permeabilization, however, can dislocate membrane lipids, a fact that had been nagging Grinstein. Now, his group has designed a shiny new tool for detecting phosphatidylserine. And it literally does shine.

It's a C2 protein domain, which binds phosphatidylserine with high specificity, fused to a fluorescent protein, which allows for visualization in living cells. To avoid having to permeabilize the cell, says Grinstein, "We make a cDNA construct and ask the cell to manufacture the protein for us, from within."

The new improved probe revealed that, unlike phosphoinositides, phosphatidylserine levels persisted in phagosomes. To maintain phosphatidylserine, the phagosomes went through regular fusion and fission events with endosomes and lysosomes. This endosome/lysosome fusion did not occur if the phagosomes contained *Legionella pneumophila* or *Chlamydia trachomatis*. These bugs inject microbial proteins into the host cell that highjack the cellular machinery and redirect phagosome trafficking.

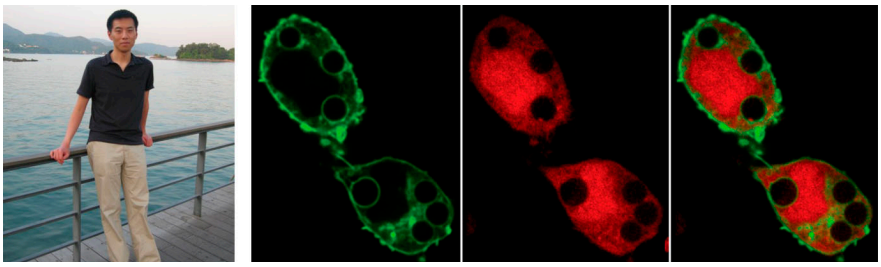
So, what makes the team think that the presence of phosphatidylserines in phagosome membranes is functional? Could their presence be simply a sign of the vesicle's trafficking history?

Yeung et al. think not. For starters, the phagosome's negative charge attracted a positively charged protein called Src that might promote phago–lysosome fusion. Src did not associate with *Legionella*- or *Chlamydia*-containing phagosomes.

Definitive functional proof, however, could only come from removing phosphatidylserine and watching where phagosomes go. "The problem with mammalian cells is that you cannot have a viable cell without phosphatidylserine," says Grinstein. "But, there is always a yeast mutant for everything." Yeast that were unable to make phosphatidylserine showed defects in their endocytic traffic, the team observed.

The authors believe the functional importance of phosphatidylserine is conserved in mammals. "Now, what exactly the mechanism is…," Grinstein postures, "give us a couple of years and hopefully we'll know more."

Focal Point



(L-R) Tony Yeung, lead author. Phagosomes (circles) maintain a significant negative charge on their surface membranes (green) despite loss of phosphoinositide (red). This persistence of charge is due to the presence of phosphatidylserine. Phagosomes lose their phosphatidylserine and thus their charge, however, if they contain pathogens such as *Legionella* and *Chlamydia*. These bugs direct phagosomes away from their normal lysosome fate to alternative safe cellular destinations.

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