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Battle of the actin junctions

Thomas Stossel is a fervent promoter of his favorite molecule, filamin. “For 25 years I’ve been saying that this protein is important for making orthogonal actin networks at the leading edge of the cell,” he says. “However, recent work has focused attention on a similar structural role for the Arp2/3 complex, which can nucleate actin filaments to form branched structures.”

Stossel claims that branching is not sufficient for the formation of a strong actin network capable of pushing out the front of a migrating cell. A highly branched structure can still give way, like a bush that cannot support any significant weight. The cell needs to cross-link the branches together so that they no longer bend under pressure. This, says Stossel, is where filamin comes into the picture.

On page 511, Stossel and colleagues take a closer look at actin filament structure in cells lacking filamin. These cells cannot migrate, and the authors find that they have a dense mat of actin filaments that are almost parallel to each other. The addition of filamin to these cells results in a more open, delicate, and three-dimensional actin network. By immunogold microscopy, many junctions between actin filaments contain filamin, some have both filamin and Arp2/3, and a few have only Arp2/3. With filamin back in the spotlight, Stossel is hoping that he can determine how filamin cross-linking and Arp2/3 nucleation might be coordinated.

Cleaving and migrating

A glycoprotein, more often thought of as an immobilized substrate for crawling over, can also be cleaved to form a soluble signal that promotes cell migration, according to Mechtersheimer et al. (page 661).

The glycoprotein L1 mediates axon guidance and cell migration in the nervous system. Mechtersheimer et al. find that L1 in newborn mouse brain and in certain tumor cells is cleaved. The soluble fragment promotes migration over several substrates, and transfection of CHO cells with an L1 construct enhances migration.

Based on inhibition studies and experiments with mutants, the authors suggest that the metalloprotease ADAM10 cleaves L1, and the L1 fragment then binds integrins on the same or a nearby cell. Signaling downstream of the integrin probably acts as a general stimulus for migration. ADAM10 can also cleave certain growth factors, thus activating their signaling pathways, which may converge with the L1/integrin pathway. As L1 and ADAM10 are widely expressed, the temporal and spatial regulation of L1 cleavage may involve as yet uncharacterized molecules.

Toxic apoptosis

Bantel et al. report that α-toxin from *Staphylococcus aureus* can induce apoptosis in immune cells (page 637). This may help the bacterium to immunosuppress the victim and continue proliferating.

*S. aureus* was known to induce apoptosis, but the underlying mechanism was unknown. Bantel et al. find that the inducer is soluble. Experiments with antibodies and purified proteins indicate that the relevant soluble factor is α-toxin, which forms pores in the target cell membrane.

The α-toxin activates intracellular caspases independently of transmembrane death receptor proteins, and can induce release of cytochrome c from isolated mitochondria. In both cases, however, the mechanism is likely to be indirect. Pores formed by α-toxin are unlikely to be big enough to allow either entry of α-toxin into the cell or exit of cytochrome c from mitochondria. Thus, apoptosis may be triggered by loss of monovalent ions through the plasma membrane pores. In addition, if the bacterium gains access to the inside of the cell, the intracellular α-toxin may form pores in mitochondria that activate a process leading to cytochrome c release.
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**Myosin V: tight grip and big steps**

Moving a muscle and moving an organelle are two very different propositions. Myosin II in muscle, safely held in place in the highly structured sarcomere, can give an occasional shove between lengthy rests. But myosin V is diffusely distributed on organelles, and therefore must maintain a tight grip on an actin cable to prevent random diffusion of the organelle. On page 625, Moore et al. first provide evidence that a single myosin V molecule maintains that grip at least half of the time (compared with less than 5% of the time for myosin II), and then give a measure of the large power stroke that drives myosin V along the actin filament.

The power stroke has been the subject of much contention in the myosin field. Toshio Yanagida (Osaka University, Osaka, Japan) contends that ATP hydrolysis biases myosin to bobble along a variable number of steps (the loose-coupling model), whereas a number of other researchers favor a tight-coupling model in which ATP hydrolysis powers the swinging of a lever arm and a single well-defined step. Moore et al. adhere to the latter model, and have in the past shown that longer levers result in bigger steps.

In the new work, Moore et al. use a laser trap to show that the double-headed myosin V undergoes an initial, tightly tethered step of 19 nm, and a second, looser step of another 18 nm. The initial 19-nm step fits well with the previous correlation of lever length and step size—in the case of myosin V, both parameters are large. But, the 19 nm contrasts with the 40-nm “stride” detected by Jim Spudich (Stanford University, Stanford, CA) and colleagues using tissue-isolated myosin V.

Moore et al. suggest that their two, smaller steps arise as follows. The myosin V starts with one head tethered and the other free. As the second head collapses toward its destination on the actin filament, this gives rise to the first 19-nm step. This leading head then goes through a power stroke, yanking the lagging head free, and bringing the whole structure forward another 18 nm. Thus, the 40-nm stride seen by Spudich may have been broken down into two components.

Hoffmann et al. use a clever trick to get around the complexity of the surface of an apoptotic cell. Instead of apoptotic cells they use a novel target: red blood cells coated with a sandwich of biotin, avidin, and a single biotinylated protein. This allows them to test the function of a single protein in phagocyte recognition, rather than having to interpret the results of blocking experiments, which are complicated by redundancy and an inability to tell which step is being blocked.

Attachment is mediated by targets of any one of the many proposed receptors. But only added PS results in engulfment. PS is exposed on the outer leaflet of cells when they become apoptotic, and thus it acts as a specific identifier of apoptotic cells. As for attachment to the phagocyte, it seems that pretty much anything will do.

When it comes to receptors for the phagocytosis of apoptotic cells, many have been proposed, but none has been proven. Now, Hoffmann et al. (page 649) report that a receptor for phosphatidylserine (PS) is uniquely required for engulfment of apoptotic cells, whereas a large number of other proposed receptors may function only in attachment of the apoptotic cell to the phagocytic cell. PS is exposed on the outer leaflet of cells when they become apoptotic, and thus it acts as a specific identifier of apoptotic cells. As for attachment to the phagocyte, it seems that pretty much anything will do.

A marker for munching

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Attachment is mediated by targets of any one of the many proposed receptors. But only added PS results in engulfment. The PS stimulates macropinocytosis, a process in which the cell takes large gulps from its surroundings by forming membrane ruffles that fold over and fuse to each other. In contrast, classic, receptor-mediated endocytosis involves the extension of a single pseudopod via sequential receptor engagement.

Macropinocytosis can also be stimulated by several growth factors, and this stimulation plus attachment is also sufficient for phagocytosis. This promiscuous phagocytic behavior is consistent with the finding that many, if not all, cells can act as phagocytes. Hoffmann et al. suggest that one of the things that distinguish classic phagocytes, such as macrophages, is the plethora of receptor molecules that are available on their surface for the initial tethering stage.