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Endocytosis takes occludin for a ride

Researchers show how cells remove a tight junction protein to tweak small intestine permeability.

The lining of the small intestine locks out bacteria and toxins while soaking up nutrients and water. By observing intestinal cells of live mice through the microscope, Marchiando et al. (1) reveal the mechanism that regulates this barrier. The study shows the importance of the tight junction protein occludin for the process and identifies the endocytic route that removes occludin to adjust permeability.

An intestinal leak is a potential disaster. "The gut is a cesspool," says senior author Jerry Turner. "You will die from an overwhelming infection if your barrier fails." The epithelial cells that line the intestine establish the barrier. Adherens junctions fasten adjacent cells in the lining together (2), but tight junctions seal the gaps, functioning like a gasket between cells. Unlike a rubber gasket, a tight junction can alter its permeability. Tumor necrosis factor (TNF), which underlies some symptoms of Crohn's disease, spurs the tight junctions to relax and allows water to leak into the intestine, resulting in diarrhea (3). The junction appears to remain intact, and the structural changes that produce this altered permeability have remained elusive.

The researchers first ruled out one group of tight junction proteins, the claudins, which are crucial for maintaining the intestinal barrier. Turner's group then turned to occludin. Although the protein isn't essential for junction formation, a previous study from Turner's lab (3) indicated that when TNF induces intestinal leakiness, occludin exits the membrane of the epithelial cells. However, researchers hadn't resolved whether occludin relocation alters the lining's permeability or whether it is a marker of other events.

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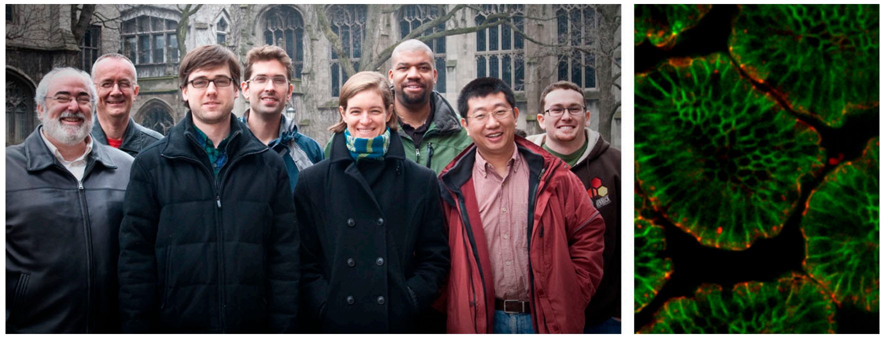
Marchiando et al. tracked occludin dynamics in transgenic mice that produced a GFP-tagged version of the protein. After anesthetizing an animal, the researchers cut open the abdomen, popped out a loop of small intestine, and sliced it open. During the procedure the organ retains normal blood flow and nerve connections. With the animal resting on a confocal microscope stage, the researchers could lay the intestinal lining over a cover slip and watch how occludin's location shifted after a dose of TNF. There was no visible effect until nearly 90 minutes after the cytokine's injection. Then occludins in the junction began to clump. Shortly afterward, vesicles containing the protein started streaming away from the cell membrane. Occludin "looked like it was internalizing directly from tight junctions," says Turner.

The researchers also took advantage of the transgenic mice to nail down occludin's role in setting the barrier's permeability. The engineered mice manufacture almost twice as much occludin as normal, and their tight junctions appear to be extra tight. Unlike in normal mice, TNF did not cause diarrhea in the transgenic animals.

Marchiando et al. then asked what abducts occludin and spirits it to the cell interior. Earlier in vitro studies by Turner's group and by other scientists (4, 5) identified three suspects. The team found that blocking macropinocytosis, a method by which cells engulf fluid, didn't affect the number of vesicles harboring occludin, casting doubt on one potential mechanism. A second possibility—removal by clathrin-coated pits—also clashed with the evidence. A drug that prevents endocytosis via these pits had no effect on occludin endocytosis. Instead, the team's experiments pointed to a role for cholesterol-rich membrane rafts that are similar to caveolae. For instance, occludin often internalized with caveolin-1, a marker of this endocytic route. And in mice lacking caveolin-1, TNF doesn't spur endocytosis of occludin or diarrhea.

"The data show that even though it is not required for basal function, occludin does play a really important role in tight junction regulation," says Turner. Removal of the protein seems to make the junctions more porous. This increased permeability can be detrimental, as in Crohn's disease. But it could also be helpful, possibly allowing cells to quickly absorb a surge of nutrients in the intestine. What researchers now need to figure out, Turner says, is how addition and removal of occludin change tight junction structure.

Focal Point



Amanda Marchiando (second from right, front row), Jerry Turner (far left, front row), and colleagues are interested in how the intestinal lining fine-tunes its permeability by moving the tight junction protein occludin. In this cross-section of intestinal villi, occludin (green) concentrates at tight junctions.

1. Marchiando, A.M., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200902153.
2. Marchiando, A.M., et al. 2010. *Annu. Rev. Pathol.* 5:119–144.
3. Clayburgh, D.R., et al. 2005. *J. Clin. Invest.* 115:2702–2715.
4. Bruewer, M., et al. 2005. *FASEB J.* 19:923-933.
5. Shen, L., and J.R. Turner. 2005. *Mol. Biol. Cell.* 16:3919–3936.