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RESEARCH NEWS

Single molecule imaging reveals a slice of life

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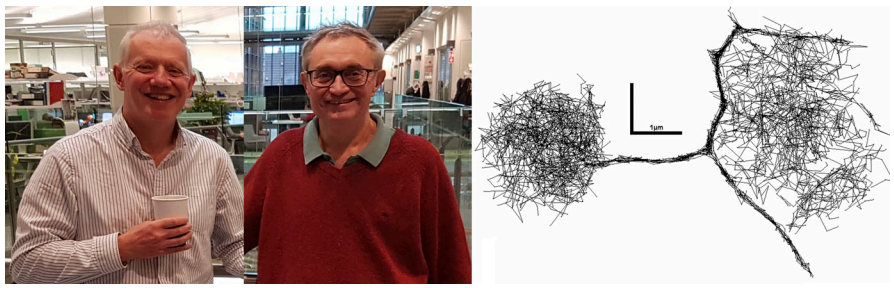
***JGP* study describes method to trace the real-time movements of individual membrane proteins in live tissue slices.**

Directly observing the movements of single, fluorescently labeled molecules can provide crucial information about a molecule's interactions in living cells. Plasma membrane proteins, for example, may freely diffuse around the lipid bilayer, pausing only when they collide and interact with other proteins. These movements can be followed relatively easily in single-cell organisms or cultured mammalian cells but are much more challenging to observe in multicellular organisms, where cell–cell interactions can dramatically alter the properties of the plasma membrane. In this issue of *JGP*, Mashanov et al. describe a new method to image and track individual plasma membrane proteins in living tissue slices (1).

Justin Molloy's group at The Francis Crick Institute in London are interested in how the M_2 muscarinic acetylcholine receptor regulates the heartbeat. This G protein–coupled receptor diffuses through the plasma membrane and, in response to acetylcholine, alters the resting potential of cardiomyocytes via a $G_{\beta\gamma}$-mediated interaction with inwardly rectifying potassium GIRK channels (2, 3).

"It's a diffusion-limited signaling cascade, so it's important to look at the movement of the molecules within the membrane," Molloy explains. "We've tracked the movements of single M_2 receptors in cultured cardiomyocytes, but we wanted to do it in tissues where the cells are in their native environment."

Molloy and colleagues, led by Gregory Mashanov, developed a technique to image single M_2 receptors in cardiac tissue slices (1). Freshly extracted mouse hearts are quickly placed in a custom-made, 3-D–printed cutting block, then sectioned by a multi-blade assembly into 1-mm-thick slices. These slices are treated with a fluorescently labeled ligand that tightly binds to M_2 receptors, before being transferred to coverslips for TIRF video microscopy.



Justin Molloy (left), Gregory Mashanov (right), and colleagues describe a method to image single plasma membrane proteins in live tissue slices. By tracking individual M_2 muscarinic acetylcholine receptors in cardiac tissue over time, the researchers can construct a super-resolution map of the tissue, encompassing both the round cardiomyocytes and the ultrathin nerve fibers that innervate them.

Mashanov immediately noticed that cardiomyocytes in living tissue are much more rounded than they are in cell culture. More remarkable still, however, were the differences Mashanov observed when he compared the movements of single M_2 receptors in cells and tissues. "The M_2 receptors move around the membrane around four times faster in tissue than they do in cultured cells," Mashanov says.

The reason for this increased mobility in tissues remains unclear, but Mashanov et al. saw a similarly rapid movement of M_2 receptors in zebrafish hearts, which the researchers were also able to dissect and prepare for TIRF microscopy with their new technique, even though these organs measure just ∼0.5 mm in length.

In addition, the researchers discovered that they could use their single-molecule tracking data to create super-resolution images of the cardiac tissue slices. "When we average our tracking data over time, the paths of individual M_2 receptors combine to delineate the cellular structure of the tissue," Molloy explains.

Because neurons also express M_2 receptors, these super-resolution tissue maps include not only the cardiomyocytes but also the nerve fibers that innervate them. "These nerve fibers are only ∼0.2 µM in diameter and they aren't really visible by light microscopy," Mashanov says. "But we could see hundreds of them. Every cardiomyocyte has a nerve fiber associated with it."

Mashanov et al.'s technique should be easily adapted for other tissues and membrane proteins and may even facilitate single-molecule imaging in entire model organisms like zebrafish or fruit flies. For Molloy's laboratory, though, the next step is to develop dual-color labeling of M_2 receptors and the downstream proteins in the pathway, $G_{\beta\gamma}$ and GIRK, so that the kinetics of the molecules' interactions can be studied in living tissues.

References

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