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RESEARCH NEWS

Cholesterol helps PIEZO1 use the force

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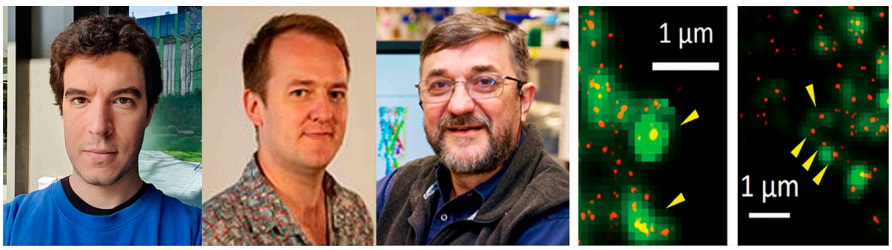
***JGP* study shows that disruption of membrane cholesterol alters the organization and activity of mechanosensitive PIEZO1 channels.**

PIEZO1 is a nonselective cation channel that can be activated by membrane stretch. It plays key roles in a number of physiological processes, including vascular development and the regulation of red blood cell volume, and gain-of-function mutations in the *PIEZO1* gene cause xerocytosis, a form of hereditary anemia. In this issue of *JGP*, Ridone et al. reveal that PIEZO1 activity is regulated by membrane cholesterol, which controls the organization of PIEZO1 clusters within the plasma membrane (1).

Like most, if not all, mechanosensitive ion channels, PIEZO1 is activated via a force-from-lipids mechanism (2,3), a phenomenon first described in bacterial mechanosensitive channels by Boris Martinac and colleagues 30 yr ago (4). Though the details remain uncertain, forces within the lipid bilayer are sufficient to drive conformational changes in PIEZO1 that trigger channel gating. PIEZO1 activity is therefore likely to be influenced by the lipid content of the plasma membrane. "We decided to take a look at the role of cholesterol, which stiffens and thickens the membrane," explains Martinac, a professor at the Victor Chang Cardiac Research Institute in Sydney, Australia.

Cholesterol might specifically regulate PIEZO1 activity by binding to a PIEZO1-associated protein called STOML3 (5), but Martinac and colleagues, including cofirst authors Pietro Ridone and Elvis Pandzic, focused on the general, physical effects of membrane cholesterol by stably expressing GFP-tagged PIEZO1 in HEK293T cells, which do not express STOML3.

The researchers found that depleting cholesterol using methyl-β-cyclodextrin (MBCD) or disrupting cholesterol organization using dynasore slowed both the activation and inactivation of PIEZO1 in response to membrane stretch. MBCD treatment also reduced the channel's pressure sensitivity. The researchers saw similar effects on native PIEZO1 in neuronal cells.



Left to right: Pietro Ridone, Elvis Pandzic, Boris Martinac, and colleagues reveal that membrane cholesterol regulates the organization and activity of mechanosensitive PIEZO1 channels. Superresolution STORM TIRF imaging of cells expressing PIEZO1-GFP (green) and stained with an anti-GFP antibody (red) shows that PIEZO1 forms clusters in the plasma membrane (second from right), but the size and density of these clusters is reduced upon cholesterol depletion (far right).

Intriguingly, *PIEZO1* mutations that cause xerocytosis also delay channel inactivation. "So, simply removing cholesterol from the membrane generates the gain-of-function phenotype associated with disease," Martinac says. Indeed, Ridone et al. found that cholesterol depletion exacerbates the slow-inactivating phenotype of mutant PIEZO1 channels (1).

The researchers then examined the effect of manipulating cholesterol on PIEZO1's membrane organization. Superresolution STORM imaging revealed that PIEZO1-GFP forms clusters within the plasma membrane, and that cholesterol depletion or disruption breaks up these clusters, reducing their size and decreasing their density in the membrane. PIEZO1 clusters were also more mobile in the absence of cholesterol, suggesting that cholesterol "fences" might connect neighboring clusters and limit their diffusion away from one another. Martinac thinks these fences could transmit forces between PIEZO1 clusters, synchronizing their rapid opening and closing in response to membrane stretch. "When we remove or disrupt cholesterol, we change the viscoelastic properties of the membrane, and this delays channel activation and inactivation," he explains.

Martinac and colleagues are now using acoustic force spectroscopy to measure directly how cholesterol depletion and disruption alters the plasma membrane's viscoelastic properties, and are also investigating whether PIEZO1 activity is affected by local membrane curvature. The researchers are also interested in the other member of the PIEZO channel family, PIEZO2, which is not thought to be regulated by membrane stretch due to the inability to gate this channel by stretch in classical cell-attached patches, and was recently suggested to be curvature sensitive (6).

References

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