Molecular Biology of the Cell, Sixth Edition
Chapter 10: Membrane Structure
Journal Club

Paper

Rocks O, Gerauer M, Vartak N et al. (2010) The palmitoylation machinery is a spatially organizing system for peripheral membrane proteins. *Cell* 141, 458–471.

Readings from *Molecular Biology of the Cell* (pp. 576–579)

- Membrane Proteins Can Be Associated with the Lipid Bilayer in Various Ways
- Lipid Anchors Control the Membrane Localization of Some Signaling Proteins

Relevant Techniques

- Fluorescence microscopy (pp. 536–539)
- Photoactivation/fluorescence recovery after photobleaching (FRAP; pp. 542–546)
- RNAi (pp. 429–433, 499–501)
- Confocal microscopy (pp. 540–542)

Questions

1. What is palmitoylation, and what is it used for in the cell?

2. What is/are the primary question(s) that the authors were trying to address in their study?

3. Palmitoylated proteins typically contain additional lipid modifications nearby (such as prenylation or myristoylation). The authors wanted to determine whether proteins with single lipid modifications are preferentially found at certain membrane domains in the cell. Explain how photoactivatable GFP (PA-GFP) was used to show that monolipidated proteins display a random distribution. What is the advantage/disadvantage of using a PA-GFP? What did this suggest to them about the presence of receptors for monolipidated proteins?

4. Explain how FRAP was used to show a difference in the behavior of CysFar (a substrate that resembles depalmitoylated NRas) and SerFar (a substrate that cannot be palmitoylated) at the Golgi (Figure 2). What is the difference between these two semisynthetic lipoproteins, and how do the authors interpret this difference?

5. What is the main conclusion from Figure 3? How was this shown?

6. What did PalFar and HDFar demonstrate about depalmitoylation?

7. How did the authors demonstrate that there is not a strong or specific structure involved in recognition of palmitoylation sites?

8. What is the mechanism for the shRNA-mediated knockdown shown in Figure 7? What did the authors conclude about the role of DHHC9 in HRas palmitoylation?

9. What additional experiments would you perform if you were going to follow up on this work?