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Complexity Revealed: A Hierarchy of Clustered Membrane Proteins

Ken Jacobson[1,2,*] and Ping Liu[1]
[1]Department of Cell Biology & Physiology, and [2]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

One relatively unexplored aspect of membranes, dating back to the Singer Nicolson Fluid-Mosaic model (1), is the lateral heterogeneity that exists in the plane of the membrane. This is an intrinsic feature of membranes and therefore must be a key to functionality. Indeed, the plasma membrane is generally accepted as being compartmentalized, permitting lipids and proteins to be organized in specific domains whose size and composition vary (2,3). Such compartmentalization may rely predominantly on the membrane-apposed cytoskeleton, termed the "membrane skeleton fence model" (4). Another prominent type of compartmentalization is the lipid raft (5), in which certain types of lipids are associated with compatible proteins. However, in general, such domains must be small and transient, considering the amount of protein in the membrane (6), and the results of superresolution fluorescence correlation spectroscopy (FCS) studies show that raft lipids are confined on short spatial and temporal scales (7). What is surprising is that a number of recent studies have shown that at least a portion of many mammalian plasma membrane proteins exist in nanoclusters, and that these clusters may be organized into larger domains that support a number of functions including cell adhesion, pathogen binding, and immune cell recognition (8).

In this issue of *Biophysical Journal*, Mocsár et al. (9) performed a needed study on how the clustering behavior for one particular protein and its partners depends on its expression level. They accomplished a comprehensive study of coclusters of MHC-1 and interleukin receptors (IL-2, IL-15) in T lymphoma cells in which the level of MHC-1 was diminished by siRNA treatment. A battery of techniques was employed, including FCS, to study the lateral diffusion of the mobile fraction of membrane proteins, flow cytometric- and image-based Förster resonance energy transfer for molecular scale interactions, and stimulated emission depletion superresolution microscopy for superclustering behavior. A large portion of MHC-1 proteins and the interleukin receptors were found to be organized into overlapping superclusters of dimension ~500 nm, with the remainder of these molecules residing outside of these domains. Within superclusters, nanoclusters containing both MHC-1 and the interleukin receptors, exist as inferred from heterogeneity evident in the superresolution images and from the FCS data, which suggest a number (on the order of 10) of small aggregates within the supercluster. All superclusters colocalized appreciably with the ganglioside, GM1, a classical lipid raft marker. The authors find that cluster properties (i.e., size, lateral mobility, number of molecular entities in a cluster, intermolecular associations) are dependent on the level of membrane protein expression (i.e., MHC-1). Specifically, MHC-1 knockdown markedly reduced the number of MHC-1 proteins per supercluster and substantially decreased their size (but not that of the interleukin receptor superclusters). This suggests that the law of mass action and apparent thermodynamic equilibrium were in effect in contrast to the nonequilibrium behavior of nanoclusters of lipid-linked GPIAPs (10) and the Ras proteins (11) in which the ratio of nanoclusters to monomers/dimers was independent of expression levels. In addition, the lateral diffusion of the small aggregates of proteins within the superclusters and those outside the domains, was increased and the close association of MHC-1 with the interleukin receptors in coclusters was diminished when MHC-1 expression level was decreased.

Hierarchical organization of plasma membrane components appears to be an emerging organizational theme (8) for which the full functional consequences must be determined. Mocsár et al. (9) provide an example of how the properties of an array of apparent nanoclusters arranged as superclusters

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*Correspondence: frap@med.unc.edu

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can be modulated by expression levels. This study will undoubtedly have general implications for how diverse species of nano- and superclusters are both assembled and maintained. The precise mix of intrinsic membrane protein, cytoskeletal, and pericellular matrix factors needed for clustering behavior on all levels remains to be elucidated.

Moreover, the impact of clustering behavior on signal transduction and related plasma membrane functions is an open challenge. For example, Hancock and co-workers (11,12) have hypothesized that short-lived, switch-like plasma membrane nanoclusters may proportionally digitize input analog signals by increasing the number of nanoclusters, the signals from which can then be integrated into the cytoplasm to provide a high-fidelity response. As another example, the dendritic cell pathogen receptor, DC-SIGN, exists in single nanoclusters capable of binding the tiny dengue virus (13) before internalization, and in superclusters (8) composed of nanoclusters that can bind pathogens as large as yeasts (14) and presumably direct signal transduction at the phagocytic synapse. Overall, the studies of Mocsár et al. (9) both raise significant scientific questions and provide a paradigm, including the attendant interpretational limitations, for further investigation of lateral heterogeneity in the plane of the plasma membrane.

REFERENCES

1. Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science.* 175:720–731.
2. Maxfield, F. R. 2002. Plasma membrane microdomains. *Curr. Opin. Cell Biol.* 14: 483–487.
3. Nicolson, G. L. 2014. The fluid-mosaic model of membrane structure: still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years. *Biochim. Biophys. Acta-Biomembr.* 1838:1451–1466.
4. Kusumi, A., C. Nakada, …, T. Fujiwara. 2005. Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: high-speed single-molecule tracking of membrane molecules. *In* Annual Review of Biophysics and Biomolecular Structure. Annual Reviews, Palo Alto, CA, pp. 351–354.
5. Lingwood, D., and K. Simons. 2010. Lipid rafts as a membrane-organizing principle. *Science.* 327:46–50.
6. Jacobson, K., O. G. Mouritsen, and R. G. W. Anderson. 2007. Lipid rafts: at a crossroad between cell biology and physics. *Nat. Cell Biol.* 9:7–14.
7. Eggeling, C., C. Ringemann, …, S. W. Hell. 2009. Direct observation of the nanoscale dynamics of membrane lipids in a living cell. *Nature.* 457:1159–1162.
8. Garcia-Parajo, M. F., A. Cambi, …, K. Jacobson. 2014. Nanoclustering as a dominant feature of plasma membrane organization. *J. Cell Sci.* 127:4995–5005.
9. Mocsár, G., J. Volkó, …, G. Vámosi. 2016. MHC I expression regulates coclustering and mobility of interleukin-2 and -15 receptors in T cells. *Biophys. J.* 111:this issue, 100–112.
10. Mayor, S., and M. Rao. 2004. Rafts: scale-dependent, active lipid organization at the cell surface. *Traffic.* 5:231–240.
11. Zhou, Y., and J. F. Hancock. 2015. Ras nanoclusters: versatile lipid-based signaling platforms. *Biochim. Biophys. Acta.-Mol. Cell. Res.* 1853:841–849.
12. Harding, A. S., and J. F. Hancock. 2008. Using plasma membrane nanoclusters to build better signaling circuits. *Trends Cell Biol.* 18:364–371.
13. Liu, P., X. Wang, …, N. L. Thompson. 2014. Low copy numbers of DC-SIGN in cell membrane microdomains: implications for structure and function. *Traffic.* 15:179–196.
14. Itano, M. S., M. S. Graus, …, A. K. Neumann. 2014. Super-resolution imaging of C-type lectin spatial rearrangement within the dendritic cell plasma membrane at fungal microbe contact sites. *Front. Phys.* 2:2.