Cellular sorting and trafficking mediated by membrane microdomains

Joseph Lorent[1], Barbara B. Diaz-Rohrer[1], Kandice R. Levental[1], and Ilya Levental [1]
1 McGovern Medical School at the University of Texas Health Science Center at Houston

***Short Abstract* — Ordered membrane domains known as rafts are believed to serve crucial functional roles in mammalian cells; however, their investigation has been hampered by poor methodologies. We have developed a quantitative framework for investigating raft affinity of transmembrane proteins and used it to define the structural determinants of transmembrane protein partitioning between coexisting membrane phases. We relate this partitioning to subcellular trafficking, establishing a direct, quantitative role for raft domains in membrane protein sorting. We are establishing the molecular mechanisms of this domain-mediated traffic using systems approaches and super-resolution quantitative imaging.**

I. Introduction

Eukaryotic cells are organized into spatially and functionally distinct membrane-bound organelles, whose functions are defined by their lipid and protein composition. Accurate and robust sorting of membrane components between these compartments is necessary for the maintenance of organelle identity. For most membrane proteins, the determinants of their steady-state subcellular localization remain unknown (1).

Lateral membrane domains known as lipid rafts provide an ideal platform for membrane sorting processes, and have been widely implicated in post-Golgi sorting and endocytosis/recycling. However, the structural determinants of protein association with such domains are almost entirely unknown. We have developed and characterized a robust experimental system for direct, quantitative measurements of raft affinity in intact plasma membranes and used it to explore the determinants of transmembrane protein recruitment into raft domains and the consequences of this recruitment on subcellular traffic.

II. Results

A. Structural determinants of raft affinity

Using our quantitative platform, we quantified ordered domain affinity for >100 transmembrane proteins and identified three physical features – transmembrane domain surface area, length, and palmitoylation – that independently affect raft partitioning (2). Specifically, long, palmitoylated TMDs with smaller surface areas partition efficiently to the more ordered raft domains. These findings were rationalized with a mechanistic, physical model wherein raft affinity is determined by the interfacial energy between a protein TMD and the surrounding lipid matrix. This model was shown to be capable of correctly predicting raft affinity solely from protein sequence. Using bioinformatics, we generated proteome-wide predictions of raft affinity and observed that PM proteins have higher predicted raft affinity than those of intracellular membranes.

B. Functional consequences of raft affinity

We established a quantitative and functional relationship between raft association and subcellular protein localization. Specifically, we observed that raft association is fully sufficient for plasma membrane recycling of certain proteins, and that abrogation of raft partitioning for these proteins led to their degradation in the lysosomes. These findings support the conclusion that ordered membrane domains mediate recycling of specific membrane components from the endosomal compartments to the PM. We have proceeded to define the molecular machinery that mediates raft lipid and protein sorting and recycling to the PM. Using a set of orthogonal transmembrane proteins as probes of raft and non-raft domains, we developed a high throughput siRNA screen to dissect the molecular machinery and dynamics for raft-mediated sorting. We identified a number of validated hits including known players of the early endocytic traffic, but also novel players that appear to define a distinct class of trafficking mediators specific for raft-associated proteins. This pathway is not dependent on the classical recycling pathways, rather defining a novel route for PM recycling of raft-preferring cargo.

III. Conclusion

Our findings define the structural features that determine protein association with ordered membrane microdomains, and validate the key role of these sub-microscopic domain in sub-cellular sorting and trafficking. However, vital questions remain unanswered regarding the underlying mechanistic principles and molecular mechanisms by which these domains serve as cellular sorting hubs. These questions can only be answered by quantitative microscopy and mechanistic modeling approaches.

[1] Diaz-Rohrer B, Levental KR, Levental I. (2014) Rafting through traffic: Membrane domains in cellular logistics. *BBA-Biomem*.
[2] Lorent JH, Diaz-Rohrer B, Lin X, Spring K, Gorfe AA, Levental KR, Levental I. (2017) Structural determinants and functional consequences of protein affinity for membrane rafts. *Nat Commun.* 8(1):1219.
[3] Diaz-Rohrer BB, Levental KR, Simons K, Levental I. (2014) Membrane raft association is a determinant of plasma membrane localization. *PNAS* 111(23):8500-5.