COMMENTARY

Lipid sorting and the activity of Arf signaling complexes

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Efficient signal transduction by lipid-anchored small GTPases requires assembly of specific complexes on cell membranes, comprising the small GTPase, and variably their guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and effectors. In this context, the lipid membrane plays a key role in regulating the structural integrity and thus functional efficiency of the signaling complex. How small GTPases interact with lipid membranes is currently generating a great deal of attention. Karandur et al. (1), in the article "Multiple interactions between an Arf/GEF complex and charged lipids determine activation kinetics on the membrane" in PNAS systematically explore how the ADP ribosylation factor (Arf) small GTPase associates with Brag2, an ArfGEF, on lipid bilayers. The study uses X-ray crystallography, coarse-grained molecular dynamics (CG-MD), and experimental reconstitution of the myristoylated Arf and Brag2 complex on synthetic model liposomes.

Brag2 Specifically Sorts PIP_2

Arf is a member of the Ras superfamily small GTPases that oscillates between an active GTP- and inactive GDP-bound state to regulate vesicular trafficking of membrane constituents (2). Arf contains a myristoylated N-terminal helical region, which anchors Arf to cell membranes. GTP-loading leads to recruitment of downstream effectors to membranes and signal transmission (3). Binding of an ArfGEF to Arf on cell membranes is an essential step in Arf activation. The Cherfils group first solved the crystal structure of Brag2, an ArfGEF, and found that negatively charged phosphoinositides contribute to Brag2 function in vitro (4). In the current study, Karandur et al. (1) first determined the crystal structures of the Sec7-PH module of Brag2 in the presence and absence of bound ArfGTP. The Sec7 and PH domains of Brag2 maintain the same intramolecular interface and expose the Arf-binding domain and membrane-binding domain with or without ArfGTP bound. This suggests that Brag2 is constitutively active, unlike other ArfGEFs, such as cytohesin. The Brag2 PH domain also prominently displays multiple positively charged residues, suggesting efficient association with negatively charged membranes. Indeed, subsequent CG-MD simulations show that both unbound Brag2 and the Arf/Brag2 complex robustly associate with multivalent phosphoinositol 4,5-bisphosphate (PIP_2) in a bilayer containing 15% phosphatidylserine (PS) and 2% PIP_2, mimicking the relative abundance of PS and PIP_2 in a typical mammalian cell plasma membrane. Anchoring of unbound Brag2 or the Arf/Brag2 complex to the bilayer induces local aggregation of PIP_2 without affecting PS, consistent with the idea that Brag2 specifically sorts PIP_2. In unbound Brag2, the PIP_2-associating sites are located not only in the canonical lipid-binding domain (Arg654, Lys667 and Arg681), and PH domain (Lys645, Lys648, Lys671, Lys672, Lys673), but also in the linker region (Lys610 and Lys611) found only in Brag2. The presence of Arf in the Arf/Brag2 complex causes some small changes in lipid association. Specifically, the loop connecting the Sec7 domain and the linker region of Brag2 no longer associates with PIP_2. On the other hand the Arf interswitch region, which is key to conformational orientation changes during GDP/GTP exchange, associates extensively with PIP_2 in the bilayer, suggesting that lipid binding is correlated with guanine nucleotide exchange.

In further in vitro experiments using a reconstituted complex of myristoylated Arf and Brag2 in model liposomes, Karandur et al. show that guanine nucleotide exchange is 4 times more efficient on liposomes containing acidic lipids, such as PS and PIP_2, than liposomes containing only neutral lipids, consistent with the view that PIP_2-mediated conformational orientation of Arf and Brag2 on the membrane is essential to Brag2 activity. Interestingly, Brag2 binds soluble PIP_2 only with weak affinity, yet increasing levels of PIP_2 in the liposomes dose-dependently enhances Brag2 recruitment. Taken together, these results suggest that Brag2 does not sort PIP_2 via simple electrostatics. There is clear lipid selectivity that potentially originates from distinct packing characteristics of PIP_2 lipids within bilayers, as well as the correlative conformational packing of the Brag2 Sec7-PH lipid binding domains on the bilayer that allows specific recognition of the PIP_2 head group. Further, the presence of monovalent PS enhances

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Brag2 binding to liposome bilayers but negatively impacts Brag2 GEF activity. This is also consistent with the view that appropriate sorting of distinct lipid species is important to Brag2 activity.

Karandur et al. then examined association of Brag2 Sec7 domain with lipid bilayers. CG-MD simulations predict that Lys549 and Arg552 in the α8-α9 loop of the Sec7 domain associate with acidic lipids extensively. In reconstitution experiments, Brag2 wild-type and Brag2 with a double mutation in the Sec7 domain (K549A/R552A) maintain similar GEF activity on liposomes containing both PIP_2 and PS. On the other hand, the Brag2 double mutant loses most of its activity on liposomes containing PS alone, compared with wild-type Brag2. Further, removing PIP_2 from liposome membrane has minimal impact on the GEF activity of Brag2 wild-type. Taken together, the presence of PIP_2 likely compensates for the negative effects of the double mutation on the Brag2 Sec7 domain. Strikingly, the α8-α9 loop of the Sec7 domain seems to more selectively sort PS lipids.

Wider Implications

Findings in the Karandur study have some significant implications. Many proteins, such as small GTPases K-Ras, Rac1, Rap1, G protein coupled receptors, and growth factor receptors, anchor to membranes using polybasic sequences in addition to other elements that include lipid moieties, transmembrane domains and hydrophobic sequences. Polybasic sequences have traditionally been thought to simply mediate electrostatic interactions and to be sensitive to only global electronegativity on the membranes. Karandur et al., however, have revealed that more complex information can be coded by a polybasic sequence that allows selection or sorting of specific anionic lipids, PIP_2 rather than PS, in the case of Brag2. Similarly recent work has shown that the polybasic domain of K-Ras selectively binds and sorts highly specific species of PS in preference to PIP_2 (5). Well-defined conformational folding of regions enriched with lysine and arginine residues allow these domains to sample different lipid head groups and acyl chains with protein–lipid interactions driving specific lipid sorting (1, 5).

1 Karandur D, Nawrotek A, Kuriyan J, Cherfils J (2017) Multiple interactions between an Arf/GEF complex and charged lipids determine activation kinetics on the membrane. *Proc Natl Acad Sci USA* 114:11416–11421.

2 Donaldson JG, Jackson CL (2011) ARF family G proteins and their regulators: roles in membrane transport, development and disease. *Nat Rev Mol Cell Biol* 12:362–375.

3 Antonny B, Beraud-Dufour S, Chardin P, Chabre M (1997) N-terminal hydrophobic residues of the G-protein ADP-ribosylation factor-1 insert into membrane phospholipids upon GDP to GTP exchange. *Biochemistry* 36:4675–4684.

4 Aizel K, et al. (2013) Integrated conformational and lipid-sensing regulation of endosomal ArfGEF BRAG2. *PLoS Biol* 11:e1001652.

5 Zhou Y, et al. (2017) Lipid-Sorting Specificity Encoded in K-Ras Membrane Anchor Regulates Signal Output. *Cell* 168:239–251 e216.