JCB Journal of Cell Biology

SPOTLIGHT

Clathrin coated pits as signaling platforms for Akt signaling

Elizabeth Smythe

Signaling by the activated epidermal growth factor receptor (EGFR) results in diverse cell fates. In this issue, Cabral-Dias et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.201808181) demonstrate how plasma membrane clathrin coated pits can act as a signaling platform for one branch of EGFR downstream signaling.

One of the most fascinating elements of mitogenic and developmental signaling pathways is how one signal can give rise to a plethora of cellular outcomes during development and in adult homeostasis. In the case of epidermal growth factor receptors (EGFRs), these responses include proliferation, cell division, and apoptosis. It is additionally well established that defects in EGFR signaling give rise to a variety of cancers. For these reasons, the context-dependent regulation of EGFR signaling is the focus of significant research. Endocytic uptake of activated EGFR, specifically its rate of flux through the endocytic pathway, is one mechanism which regulates downstream cell behaviors because the activated receptor is known to signal from the plasma membrane as well as endosomes, often with different cellular outcomes. Such differential signaling is facilitated by specialized membrane microdomains, signalosomes, which provide platforms that allow optimal assembly of the correct complement of signaling molecules for distinct cell behaviors (1). A key question therefore is understanding the temporal and spatial regulation of signalosome assembly.

Clathrin coated pits (CCPs) are specialized areas of the plasma membrane which participate in the uptake of cargo such as EGFR. Following engagement with ligand, EGFRs undergo dimerization and transphosphorylation which results in clustering into CCPs which bud off as clathrin coated vesicles (CCVs) and are targeted to endosomes. Clathrin is a peripheral membrane protein which assembles to form a morphologically defined coat, with adaptor proteins within the coat linking the cargo to the assembled clathrin lattice (2). Classically it was thought that the main function of CCPs was to remove phosphorylated EGFR from the cell surface, allowing delivery to recycling endosomes for re-presentation at the cell surface, or to lysosomes which will ultimately result in signal attenuation.

The paper by Antonescu and colleagues unpicks the molecular mechanisms by which CCPs act as a signaling platform following EGFR activation (3). EGF binding to EGFR results in activation of Akt where there are three isoforms, Akt1, Akt2 and Akt3. Akt1 and Akt2 are are ubiquitously exprerssed with Akt3 expressed in neurons and testes. This study demonstrates how signaling to Akt2, but not Akt1, can be specifically regulated within CCPs. Building on previous intriguing results showing that clathrin, but not internalization, is key for EGF-dependent Akt activation (4), the current work shows that there is a subpopulation of CCPs that is enriched in the Src kinase, Fyn, as well as TOM1L1, the latter acting as a bivalent adaptor protein to link Fyn to the clathrin lattice. siRNA-mediated depletion of either protein has a selectively inhibitory effect on EGF-stimulated Akt2 phosphorylation while Akt1 phosphorylation, surface levels of EGFR, and levels of phosphorylated EGFR are unaffected. Fyn is not normally resident in CCPs but is recruited in response to treating cells with EGF. Through direct interaction with clathrin, TOM1L1 is already present in a subset of CCPs which is longer lived than CCPs lacking Fyn and TOM1L1, consistent with the observation that activated EGFR prolongs CCPs lifetimes. SHIP2, a lipid phosphatase that generates PI(3,4)P_2, which is a specific activator of Akt2 and not Akt1 (5), is recruited to CCPs in a TOM1L1- and Fyn-dependent manner, thus further ensuring segregation of a subset of Akt signaling. Importantly, analysis of CCP dynamics eliminated the possibility that Fyn and TOM1L1 are global regulators of CCP initiation and maturation, further emphasizing specific links with EGFR activation.

The composition and dynamics of CCPs make them ideally suited to act as signaling platforms (Fig. 1). Within the clathrin lattice there is enrichment of proteins and phospholipids where many different adaptor and regulatory proteins can be accommodated via low affinity interactions. This means that CCPs are very plastic and can accommodate diverse cargo/adaptor combinations. The existence of compositionally distinct CCPs has been demonstrated (6), which begs the question as to how CCP subpopulations emerge with a given

School of Biosciences, University of Sheffield, Sheffield, UK.

Correspondence to Elizabeth Smythe: e.smythe@sheffield.ac.uk.

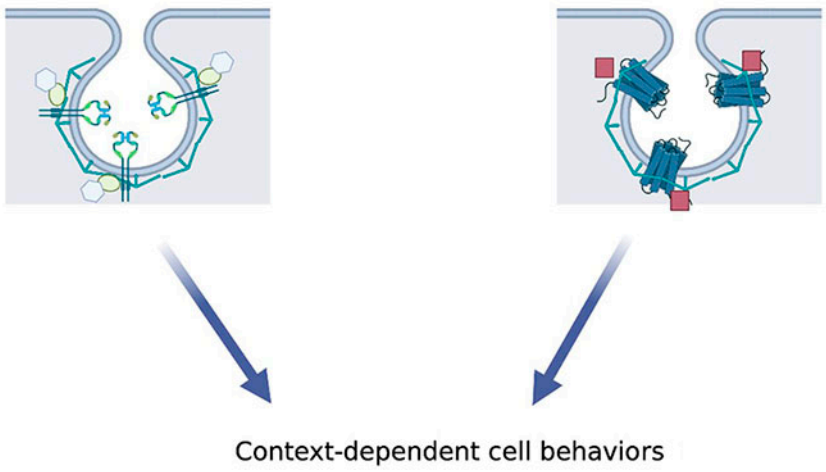

Figure 1. **CCPs can act as signaling platforms.** CCPs represent membrane microdomains that can be specialized for signaling. Enrichment of particular receptors, receptor tyrosine kinases (left-hand image) or G protein–coupled receptors (right-hand image), along with specific adaptors (blue hexagon, green circle, and red square), allows signaling to specific effectors to be segregated in an optimal environment. Image created with https://biorender.com/.

complement of adaptors appropriate for the uptake of individual cargo. Signaling cargoes can modify the endocytic machinery to facilitate their transit through the endocytic pathway, e.g., reference 7. Cells in tissues and organisms are exposed to many different external cues which likely results in alterations in affinity of adaptors for clathrin through posttranslational modifications such as phosphorylation and ubiquitination. Additionally, activation of receptors such as EGFR which dimerize, and G protein-coupled receptors which oligomerize, following ligand engagement, may well compete out other receptors resulting in feedforward mechanisms which increase local concentration of signaling molecules. The subset of CCPs containing TOM1L1 in this study could be considered as being "primed" for EGFR signaling to Akt2.

CCPs demonstrate heterogenous lifetimes which can be modulated by cargo (8, 9). Thus CCP dynamics allow quite sophisticated temporal control of signaling where a relatively small change in CCP lifetime may amplify a signaling intermediate, leading to significant effects on downstream cell behavior. For example, short-lived CCPs can lead to non-productive signaling (10), while this study shows continuous recruitment of Fyn in response to EGF through the lifetime of a subset of CCPs, presumably contributing to ongoing signal strength. It is worth noting that the effects of depletion of both TOM1L1 and Fyn lead to a reduction as opposed to an abrogation of Akt2 activation, suggesting that they function to regulate the magnitude of signaling rather than acting as on/off switches.

For precise signal regulation, signalosome disassembly is as important as assembly and CCPs are again an ideal platform, losing their coat of peripheral membrane proteins following scission which may promote signal attenuation. Signaling outcome is affected also by the onward destination of subclasses of CCVs to endosomal populations that are specialized for signaling (7). Understanding how this is achieved will require the targeting machinery, including the complement of SNAREs in each CCV subpopulation, to be determined. Previous in vivo studies have demonstrated that the Rab5 effector, APPL1, drives the formation of a signalosome on endosomes specific for Akt-dependent cell survival, which is independent of Akt-driven growth and proliferation (11). Such spatial segregation is especially relevant for Akt isoforms which play non-overlapping and sometimes opposing roles in tumorigenesis (12). Understanding context-dependent EGFR signaling is thus essential if we are to understand what goes wrong in diseases associated with dysregulation of EGFR and its downstream targets.

By dissecting the molecular details of specific EGFR-driven signalosomes, we can begin to think of identifying novel targets to use in combination with existing therapies. Elucidation of some of the mechanisms by which CCPs recruit effectors of EGFR signaling to Akt2 is an important step in this journey.

Acknowledgments

E. Smythe acknowledges support of a Leverhulme Trust Research Fellowship (RF-2020-620).

The author declares no competing financial interests.

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