

SPOTLIGHT

Clathrin: Bender or bystander?

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Cell biologists have long debated the role of clathrin in curving membranes during endocytosis. New findings from Cail et al. (2022. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202109013>) take an innovative approach to directly demonstrate the indispensable functions of both clathrin and its adaptor network in shaping endocytic vesicles.

The clathrin coat, which was first characterized more than 40 years ago, consists of a curved lattice of hexagons and pentagons (1). The rigidity and inherent curvature of the clathrin lattice has led to the long-standing assumption that assembly of clathrin provides a major driving force for the curvature of endocytic vesicles. However, a fundamental problem has dogged this hypothesis since its inception: namely, that clathrin does not bind directly to membrane surfaces. Instead, it is recruited to membranes by a family of adaptor and accessory proteins that contain membrane-binding domains (2). Over the decades since clathrin's discovery, many of these proteins have been found to drive curvature themselves through diverse mechanisms including insertion of amphipathic helices (3), scaffolding by intrinsically curved domains (4), and steric pressure at crowded membrane surfaces (5). These discoveries have fueled a debate about clathrin's role in bending membranes. Does clathrin drive curvature directly or is it simply organizing curvature-driving adaptors? In short, is clathrin a bender or a bystander?

Over the past decade, several groups have presented new data on clathrin dynamics, which have alternately strengthened and weakened the case for clathrin's role in generating membrane curvature. In 2015, Avinoam et al. (6) made two important findings. First, they reported the dynamic exchange of clathrin during assembly of endocytic sites in mammalian cells.

Additionally, using correlative light-electron microscopy, they found that clathrin initially assembled as a flat lattice at endocytic sites, making an abrupt transition to high curvature, while largely conserving the area of the lattice. If a flat and rapidly exchanging clathrin lattice is recruited to endocytic sites, how can clathrin be a strong driver of membrane bending? However, electron microscopy cannot follow the morphological changes that occur during development of individual clathrin-coated vesicles. This gap was partially addressed by Willy and Kural who, in a 2021 paper, used super-resolution microscopy to analyze changes in the shape and area of the clathrin coat during vesicle biogenesis (7). In contrast to the work of Avinoam et al. (6), data from these studies suggested that clathrin is recruited to lattices of high, constant curvature.

Against the backdrop of this debate, Cail et al. (8) take a novel approach to provide exciting new insight into clathrin's role in creating membrane curvature during endocytosis. Specifically, they use the curvature of the substrate on which cells are plated to probe clathrin's role in bending the membrane. Using nano-fabricated substrates with highly curved ridges, the research team examined the impact of substrate curvature on the dynamics and cargo content of clathrin-coated vesicles. They find a striking colocalization of clathrin-coated pits with the highly curved regions of their substrates. Remarkably, substrate curvature is found to rescue

endocytosis locally when clathrin heavy chain is strongly knocked down. Specifically, in the near absence of clathrin heavy chain, endocytosis proceeds robustly in regions of the cell attached to a highly curved substrate but is halted almost entirely in regions of the cell that are attached to a flat substrate.

The results suggest at least two conclusions. First, and most obviously, clathrin heavy chain appears to be essential for budding of coated vesicles from flat membranes. However, a second and somewhat contradictory conclusion is also supported by the data—when a highly curved substrate is available, clathrin may be expendable. Specifically, it is important to note that the authors' nano-fabricated ridges had curvatures substantially lower than endocytic structures. So, the adaptor network, in the absence of clathrin, must have driven a substantial increase in membrane curvature to complete the budding of the resulting "un-coated" vesicles. Taken together, the authors' findings suggest that both clathrin and the adaptor network contribute to membrane curvature in ways that are essential to endocytosis in the absence of pre-imposed curvature.

Interestingly, a second study, which has recently appeared on bioRxiv (9), takes an entirely different experimental approach to produce data that ultimately supports a similar set of conclusions. The investigative team led by Küey and Royle recruited clathrin artificially to the surfaces of mitochondria, which have high curvatures,

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similar to the ridges used in the study by Cail et al. (8). Here, recruitment of clathrin was accomplished using a pair of engineered proteins, one in the mitochondrial membrane and the other in the cytosol, which bound to one another upon addition of rapamycin, leading to strong recruitment of the cytosolic protein to the surface of mitochondria. The cytosolic protein contained the hinge and appendage domains of the $\beta 2$ subunit of the major clathrin adaptor, AP-2, and was therefore capable of recruiting clathrin to the outer surface of mitochondria upon rapamycin addition. When clathrin was recruited, the authors observed budding and scission of fully coated vesicles from the outer mitochondrial membrane. Neither dynamin nor its known mitochondrial analogs were found to participate in the process. Some clathrin adaptor proteins normally found at the plasma membrane were recruited to clathrin-coated buds of the mitochondria, but their knockdown did not appear to inhibit the process. These observations suggest that when clathrin is recruited to a highly curved membrane surface, it is sufficient to drive membrane budding and fission. Notably, the protein chimera used to recruit clathrin to the mitochondrial surface contained a bulky disordered domain, suggesting that crowding

of bulky domains beneath the clathrin coat could have played a significant role in curvature generation and membrane fission, as previously reported (10).

Collectively, the studies by Cail et al. (8) and Küey et al. (9) suggest that both clathrin and its adaptor network play important roles in generating membrane curvature. When clathrin-coated vesicles bud from the relatively flat plasma membrane, both the adaptors and clathrin are required to complete the process. In contrast, when vesicles are derived from a curved substrate, either the adaptor network, in the case of Cail et al. (8), or the clathrin lattice, in the case of Küey et al. (9), will suffice. The unified picture that emerges from these disparate studies shows clearly that clathrin in an important driver of membrane bending, bringing us closer to resolving a controversy that has existed since the early days of the field. However, the finding that the adaptor network plays an essential role in generating curvature is equally compelling. The heterogeneity and dynamic assembly of the adaptors makes it inherently more difficult to identify the underlying mechanisms. Further, the well-established robustness of endocytosis, despite the knockdown of almost any individual adaptor, suggests that the capacity to generate membrane curvature is likely to

arise from the properties of the adaptor network rather than the function of individual domains. Therefore, while the role of clathrin in driving membrane curvature appears increasingly clear, the equally essential contribution of the adaptor network provides a puzzle that will continue to fascinate us and fuel our debates for the foreseeable future.

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