Clathrin assembly defines the onset and geometry of cortical patterning

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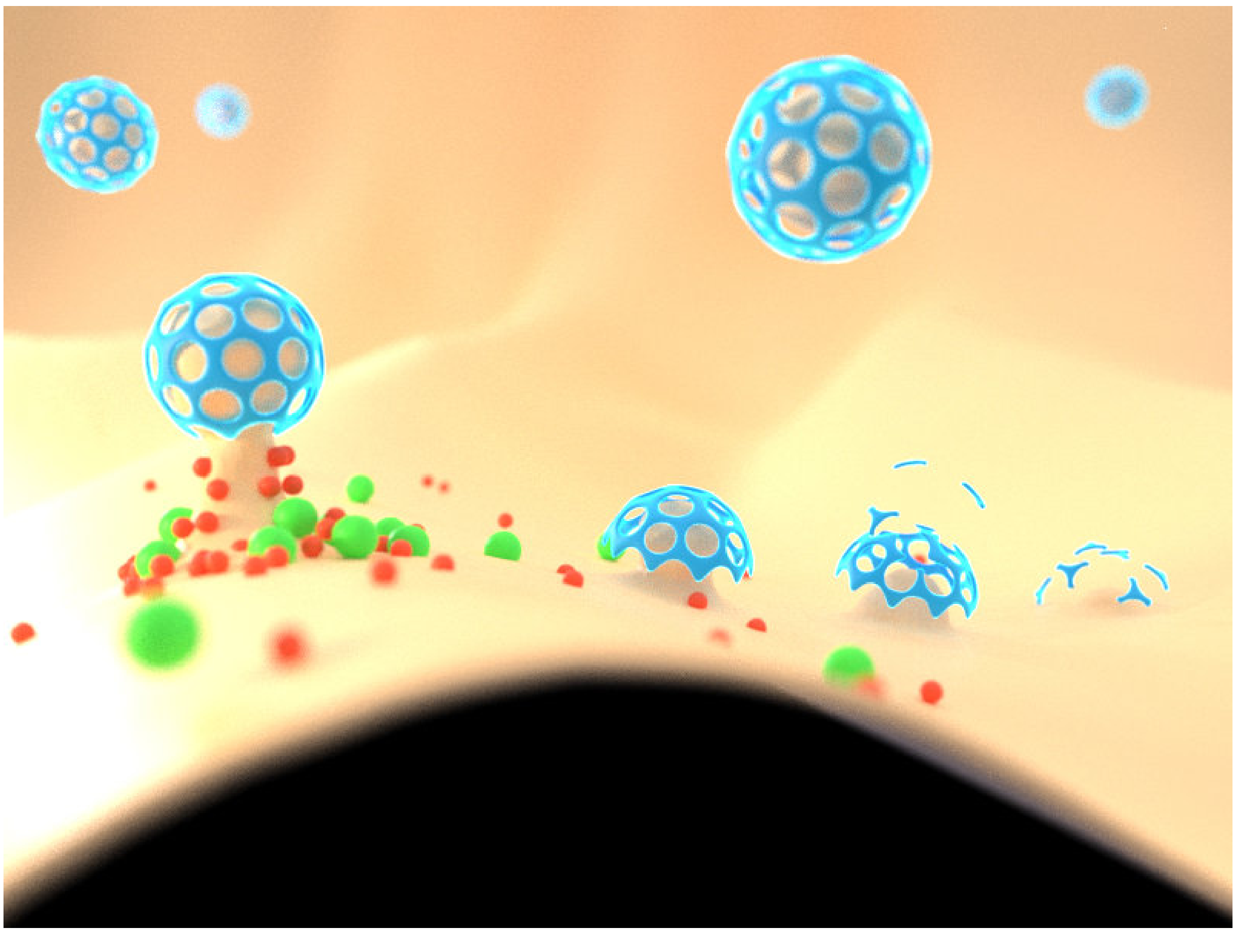

An artist's impression of Clathrin-Mediated Endocytosis (CME) occurring as periodic traveling waves on the membrane. Clathin coated vesicles (blue) bud off from wave peaks. Clathrin waves require intermediate PIP3 levels (red) and also feedback from downstream components of cortical wave machinery such as FBP17 (green). Credit: National University of Singapore

Researchers from the Mechanobiology Institute, Singapore (MBI) at the National University of Singapore, report that endocytosis, which was previously thought to be a random process, actually occurs in a coordinated manner through collective dynamics. The work, led by Assistant Professor Wu Min, showed how a major endocytic pathway mediated by the protein clathrin, was found to commence with periodic traveling waves of clathrin, which were coupled temporally and spatially to downstream cortical actin waves. Clathrin endocytic waves were identified here as the upstream initiator of cortical actin waves. This work was published in *Developmental Cell* on 20 November 2017.

"Birds of a feather flock together" is an old proverb that uses the collective dynamics of nature to describe grouping tendencies of living beings. Not only do birds flock together but so too do countless examples throughout life, from the macroscopic to the microscopic, including schools of fish, colonies of bacteria, groups of migrating cells and even some proteins. Collective dynamics is therefore a natural phenomenon which occurs when the dynamics of each individual within the group becomes synchronised with each other, lending a collective visual and functional effect.

Within cells, collective dynamics of the cytoskeletal protein actin is well known. Actin is a filamentous protein, which together with other cytoskeletal elements forms a dynamic network of filaments that provide both structural support, as well as critical functional capabilities, to the cell. The coordinated assembly and disassembly of actin in the cell cortex, which lies just beneath the plasma membrane, generates cortical actin waves that are crucial for important cell functions including migration and cell polarity.

Although proteins that participate in the formation of cortical waves have been identified, the factors/processes that trigger their onset, or establish the directions in which they will propagate, were not known.

Role of clathrin in initiating cortical actin waves

As endocytic proteins such as F-BAR have been found to play a role in cortical actin waves, the researchers sought out to understand the link between endocytosis and cortical actin waves. Endocytosis is a fundamental membrane trafficking process by which cells uptake external factors such as proteins and pathogens. There are multiple modes of endocytosis, of which Clathrin-Mediated Endocytosis (CME) is a major example. CME is initiated by the binding of a ligand to a receptor, followed by membrane invagination, coating of the invagination by the triskelion shaped protein clathrin, and budding off of the clathrin coated vesicle.

Using sensitive live cell imaging, the team noted that endocytic waves initiated by clathrin emerge in some cell populations. These clathrin waves were temporally related to and coordinated with the downstream waves of F-BAR and other actin regulating proteins such as Cdc42 and N-WASP. The researchers observed that for such coordination between the endocytic and cortical waves, a feedback between downstream F-BAR/Cdc42/N-WASP and clathrin was required.

The feedback, which couples the initiation of endocytic waves by clathrin to cortical actin waves is analogous to a group of people walking on a wobbly bridge. As one individual steps on the bridge, a small movement of the bridge will be generated. This movement will increase when another person walks in sync with the first. The resulting swaying motion of the bridge forces others on the bridge to walk in synchrony. However, once they are off the bridge, the feedback coupling is lost and people can again walk at their own pace.

A similar situation can be envisaged for CME. Under normal conditions there is no coordination between clathrin assembly and cortical actin waves and therefore CME occurs as a random process. However, endocytic waves may be initiated by clathrin through a positive feedback mechanism between clathrin and downstream proteins which includes actin regulating proteins as well as membrane proteins such as PIP3. This, in effect, sees clathrin regulate the onset of cortical actin waves.

Finding the link between endocytosis and cortical actin waves

This study reports for the first time the occurrence of collective dynamics of endocytosis and shows how this regulates the onset of cortical actin waves. The link between endocytosis and generation of cortical actin waves may provide deeper insight into how cells maintain the composition and dynamics of the plasma membrane.

More information: Yang Yang et al. Clathrin Assembly Defines the Onset and Geometry of Cortical Patterning, *Developmental Cell* (2017). DOI: 10.1016/j.devcel.2017.10.028

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