ENDOCYTOSIS

Unlocking AP2 activity

> an autoinhibitory mechanism, and no other proteins, prevents AP2 from binding to clathrin

The assembly polypeptide 2 (AP2) complex is a key player in clathrin-mediated endocytosis (CME); it recruits clathrin to membranes, promotes the polymerization of clathrin into clathrin-coated pits and binds cargo destined for CME. AP2 is known to exist in a 'locked', inactive state in the cytoplasm and to become 'open' and permissive to cargo and clathrin binding at membranes containing phosphatidylinositol-3,4-bisphosphate (PtdIns(4,5)P_2). Kelly *et al*. now show that cytoplasmic AP2 is inactive owing to an autoinhibitory mechanism that prevents AP2–clathrin interactions.

AP2 predominantly binds to clathrin via a flexible hinge between its β2 appendage and β2 trunk that contains a canonical clathrin box. The authors generated a recombinant form of AP2 — FLβ.AP2 — which comprises full-length β2, μ2 and σ2 subunits and the α-trunk domain. In solution or when attached to membranes lacking PtdIns(4,5)P_2, FLβ.AP2 was unable to strongly bind clathrin or stimulate clathrin polymerization, despite the presence of the clathrin box, although the isolated β2 hinge–appendage region could. However, FLβ.AP2 could recruit and polymerize clathrin when the AP2 was bound to liposomes containing PtdIns(4,5)P_2 and the endocytic motif that is present in CME cargo. Thus, an autoinhibitory mechanism, and no other proteins, prevents AP2 from binding to clathrin, and this is relieved when AP2 binds to PtdIns(4,5)P_2 and cargo.

To gain insight into the mechanism behind AP2 autoinhibition, the authors solved the crystal structure of a form of AP2 harbouring the clathrin box-containing hinge. Analysis of this structure, and others containing single methionine substitutions in the hinge region, showed that the clathrin box is buried in the centre of the AP2 core as a result of two key intramolecular interactions. This ensures that the clathrin box is inaccessible to clathrin when AP2 is in its locked form. Interfering with the residues involved in these intramolecular interactions enabled AP2 to bind to clathrin.

In short, this study reveals that AP2 binding to PtdIns(4,5)P_2 and CME cargo at plasma membranes relieves autoinhibitory interactions to expose the clathrin box and permit AP2–clathrin interactions. The authors also showed that, once in its open conformation, AP2 is sufficient to drive the formation of clathrin-coated buds *in vitro*.

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ORIGINAL RESEARCH PAPER Kelly, B. T. *et al.* AP2 controls clathrin polymerization with a membrane-activated switch. *Science*, **345**, 459–463 (2014)