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Journal
Autophagy, 15(6)

ISSN
1554-8627

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Publication Date
2019-06-03

DOI
10.1080/15548627.2019.1596501

Peer reviewed

AUTOPHAGY
2019, VOL. 15, NO. 6, 1122–1123
https://doi.org/10.1080/15548627.2019.1596501

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COMMENTARY

The BARA necessities of PtdIns 3-kinase activation in autophagy

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ABSTRACT

Macroautophagy/autophagy is an evolutionarily conserved degradation system with fundamental biological functions. The activation of the class III phosphatidylinositol 3-kinase (PtdIns3K) complexes and the subsequent production of phosphatidylinositol 3-phosphate (PtdIns3P) are pivotal to autophagy. Using a combination of structural biology, biochemistry, and biophysics, we revealed how the non-catalytic subunit BECN1 serves as a membrane-binding switch in the regulation of PtdIns3K complexes and autophagy.

ARTICLE HISTORY
Received 15 February 2019
Revised 8 March 2019
Accepted 14 March 2019

KEYWORDS
Cryoelectron microscopy; hydrogen-deuterium exchange; HIV; human immunodeficiency virus; Nef; Rubicon; Tat-BECN1 peptide

Autophagy is an essential cellular catabolic process, which is characterized by a transient double-membrane structure called the phagophore, which sequesters cytoplasmic cargoes. The phagophore matures into an autophagosome, which delivers the cargo to the lysosome for degradation. Autophagy maintains cellular homeostasis, and plays important roles in protecting organisms from a variety of diseases, including neurodegenerative disorders, autoimmune diseases, muscle, bone and heart disorders, and cancer. A critical signaling lipid, PtdIns3P, is required for autophagosome formation and maturation. The PtdIns3K phosphorylates the 3′-position of the inositol head group of PtdIns to generate PtdIns3P, which is essential for the initiation and progression of autophagy.

In mammalian cells, there are 2 heterotetrameric PtdIns3K complexes known as complex I (PtdIns3K-C1) and complex II (PtdIns3K-C2). PtdIns3K-C1 is composed of PIK3C3/VPS34, PIK3R4/VPS15, BECN1, and ATG14, whereas ATG14 is substituted by UVRAG (UV radiation resistance associated) in PtdIns3K-C2. The difference of one subunit determines the specific localization and function of these 2 complexes. PtdIns3K-C1 is critical for autophagy initiation, whereas PtdIns3K-C2 functions in autophagosome maturation, endocytosis, and LC3-associated phagocytosis (LAP). Structurally, PtdIns3K-C1 and -C2 have essentially the same overall V-shaped architecture, with PIK3C3/VPS34 and PIK3R4/VPS15 forming the catalytic 'right' arm of the V, while BECN1 and ATG14-UVRAG form the regulatory 'left' arm. Although BECN1 is the smallest subunit within the complex, its C-terminal BARA domain (formerly known as the 'ECD') is indispensable for targeting the entire complex to membrane. The BARA domain binds to membranes, in part, by inserting an aromatic finger loop into the membrane. BECN1 is a central node in autophagy regulation. In addition, different accessory proteins such as NRBF2 (nuclear receptor binding factor 2), RUBCN/Rubicon, and AMBRA1 bind to the PtdIns3K complexes and positively or negatively regulate their activity. These known PtdIns3K activating and inhibiting proteins provide tools for understanding at a structural-mechanistic level how these complexes are switched on and off.

To understand how RUBCN regulates PtdIns3K-C2, we mapped a minimal inhibitory region of RUBCN, which we named the 'PIKBD' (PtdIns3K-C2 binding domain) [1]. We found that the RUBCN PIKBD binds to the first β-sheet of the BECN1 BARA domain and UVRAG BARA2 domain based on hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) and cryo-electron microscopy. We also probed PtdIns3K-C2 activity using a giant unilamellar vesicle (GUV) assay, and the data showed that both the full-length RUBCN and PIKBD significantly inhibit PtdIns3K-C2 by blocking membrane binding. These data revealed a completely unexpected role for the β-sheet 1 of the BECN1 BARA domain in binding to membranes (Figure 1). We confirmed this by carrying out molecular dynamics (MD) simulations of the BECN1 BARA domain bound to a phospholipid membrane. Combined with GUV-based assays of β-sheet 1 mutants, these data showed that 2 conserved Phe residues within β-sheet 1 contribute to membrane anchoring, working together with the known aromatic finger to form an expanded membrane docking region.

RUBCN is a member of a small family of autophagy regulatory proteins that is characterized by the C terminal RUBCN homology (RH) domain, which also includes PLEKHM1 (pleckstrin homology and RUN domain containing M1) and RUBCNL/Pacer. RUBCNL positively regulates autophagosome maturation, and it contains a region similar to RUBCN PIKBD that contributes to its interaction with PtdIns3K-C2. Our data suggest that RUBCN and RUBCNL are mutually exclusive in binding to PtdIns3K-C2 through a common site on the BECN1 BARA domain. Because RUBCNL activates PtdIns3K-C2, whereas PIKBD binding alone inhibits, we speculate that full-length

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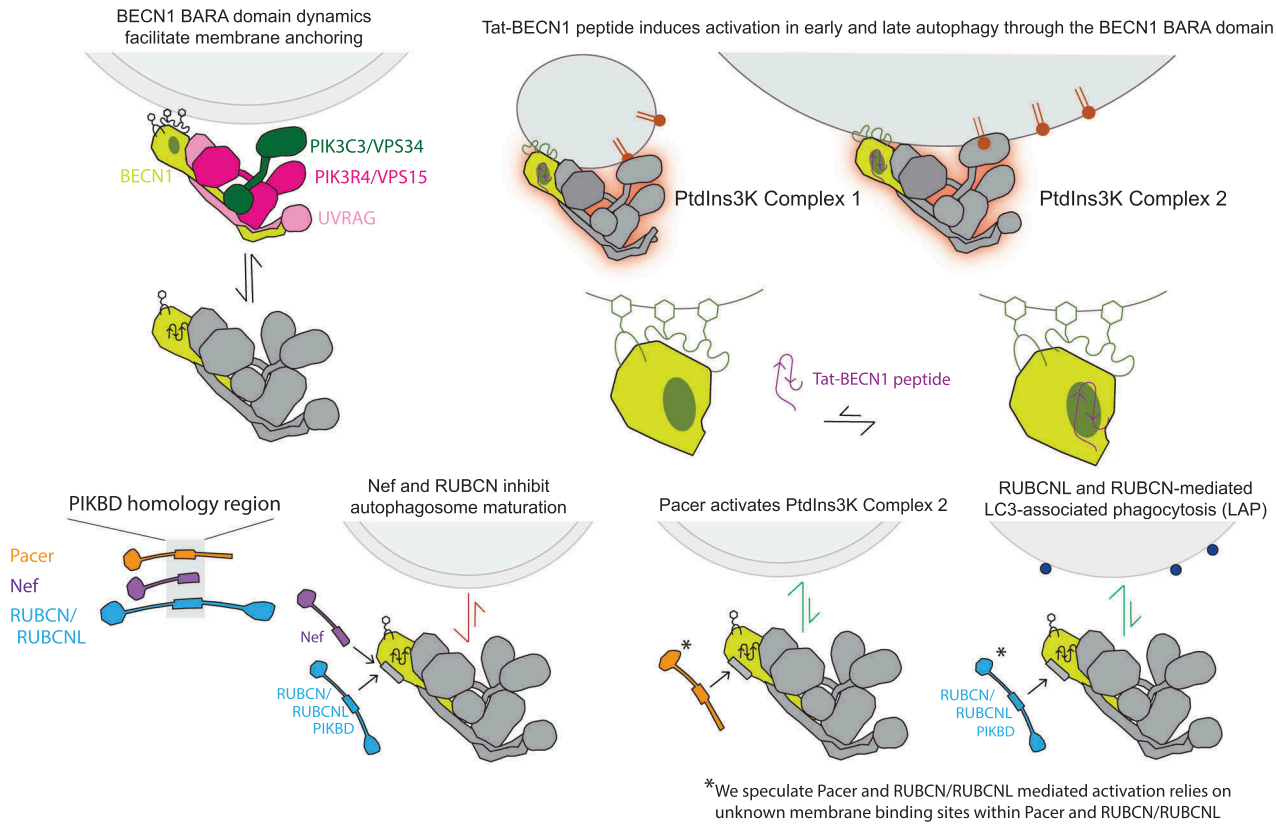

Figure 1. Model for regulation of PtdIns3K via the BECN1 BARA domain. The concepts for the activation of PtdIns3K-C2 by RUBCNL, and by RUBCN in LAP, are based on the known presence of the PIKBD in RUBCNL and RUBCN, and the known cellular functions of these proteins. However, the presence of additional membrane targeting domains in RUBCNL and RUBCN is speculative, as is the putative ability of these proteins to target PtdIns3K-C2 to membranes under some conditions.

RUBCNL may contain an additional membrane-binding site that retargets the complex to membrane after blocking the membrane binding of the BECN1 BARA domain. In effect, this provides a mechanism to redirect the localization of the complex or to alter the specificity of its regulation. RUBCN itself can be a positive effector of the autophagy-related process LAP. One possible scenario, by analogy to the model proposed for RUBCNL, in that in the special context of LAP, other domains of full-length RUBCN form a new, LAP-specific, membrane-targeting region.

It is striking that the sequence of β-sheet 1 of the BECN1 BARA domain corresponds almost exactly to a cell-permeable BECN1 peptide (T-BP) that induces autophagy in cells. Our data show that the peptide directly acts on BECN1 and activates both PtdIns3K-C1 and -C2 in vitro. The structural hypothesis is that the T-BP competes with β-sheet 1 in a domain-swapping reaction and thus drives the complexes onto membranes. These findings suggest that the β-sheet 1 within the BECN1 BARA could be a general membrane-targeting site for both PtdIns3K complexes.

It has been reported that human immunodeficiency virus (HIV) can be targeted for elimination by autophagy, which is countered by the HIV-1 Nef protein. We found that HIV-1 Nef contains a sequence that is similar to critical residues of RUBCN PIKBD, and confirmed that HIV-1 Nef specifically inhibits PtdIns3K-C2 in vitro. These results are in agreement with the known inhibitory effects of Nef on autophagy maturation. It should be noted that the N terminus of Nef is myristoylated during HIV infection. The myristoylation anchors Nef to the membrane, which is essential for its activity. The effect of myristoylated Nef on PtdIns3K activity and autophagy remain to be investigated.

Altogether, the new findings of the centrality of membrane docking by the BECN1 BARA domain and the bidirectional regulation by a number of autophagy regulators including RUBCN, T-BP, and Nef deepen our understanding of how autophagy is switched on and off in physiology and pathophysiology. The BECN1 BARA domain is therefore an attractive target for the creation of novel autophagy-inducing agents.

Disclosure statement

J. H. H. is a founder of Casma Therapeutics.

Funding

This work was supported by the National Cancer Institute [CA223029]; National Institute of General Medical Sciences [GM111730].

Reference

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