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RESEARCH HIGHLIGHT

Self-eating with your fingers

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BECN1 plays a central role in determining cell fate, acting as a balance point in the decision to undergo autophagy or apoptosis. Three distinct structural domains allow BECN1 to interact with several other proteins. Recent crystallographic data provide insight into the nature of the coiled-coil and evolutionarily conserved domains.

Macroautophagy (hereafter autophagy) is a primarily degradative pathway that plays critical roles in cellular homeostasis [1]. The morphological and functional hallmark of autophagy is the double-membrane autophagosome, a sequestering compartment that is derived from the phagophore. One of the key components of the protein machinery that drives autophagy is BECN1, the product of the *BECN1* gene and the homolog of yeast Vps30/Atg6. There are two phosphatidylinositol 3-kinase (PtdIns3K) complexes in yeast, both of which contain the PtdIns3K Vps34, the presumed regulatory subunit Vps15, and Vps30/Atg6 [2]. Complex I also includes Atg14, and is specific to autophagy, whereas this component is replaced in complex II by Vps38, which functions in endosomal trafficking.

BECN1 is part of at least three class III PtdIns3K complexes in mammals [3] that also include the PtdIns3K PIK3C3/VPS34, PIK3R4/VPS15, and different combinations of ATG14/ATG14L/BARKOR, UVRAG, AMBRA1 and/or KIAA0226/RUBICON. The ATG14-containing complex acts at an early stage of autophagosome formation, whereas UVRAG (which binds BECN1 at the same domain) directs the complex to act at a later stage of autophagosome maturation and also participates in endocytic trafficking. KIAA0226/RUBICON localizes the PtdIns3K complex to late endosomes/lysosomes and acts to inhibit autophagy. The PtdIns3K complex plays a critical role in autophagy by synthesizing PtdIns3P, a phosphoinositide that recruits certain autophagy-related (ATG) proteins to the site of phagophore formation. BECN1 in particular has been the subject of much research, stemming in part from its identification as a tumor suppressor that is mutated in several human cancers. In addition to its autophagic function as a component of the PtdIns3K complex, BECN1 is connected to the apoptosis pathways via its ability to bind to antiapoptotic factors such as BCL2, BCL2L1/Bcl-XL and related proteins. A critical point regarding its function is that BECN1, along with AMBRA1 [4], may act as part of a balance point that controls the cellular response to stress, dictating whether the outcome is cytoprotective or results in cell death; for example, the BCL2-BECN1 complex inhibits autophagy and favors apoptosis. Accordingly, several regulatory molecules modulate autophagy through posttranslational modification of BECN1 or BCL2, including DAPK1 [5] and MAPK8/JNK1 [6]. Furthermore, BECN1 may also contribute to apoptosis following caspase cleavage and mitochondrial translocation [7], whereas the endoplasmic reticulum-localized population of BECN1 is involved in promoting autophagy [8].

Despite the identification of over 30 Atg proteins in yeast, at least half of which are conserved in higher eukaryotes, relatively little structural information is available. Furthermore, almost all of the crystallography data has centered on the components of the ubiquitin-like protein conjugation systems [9]. The structure of the short BCL2-homology-3 (BH3, amino acids 105-125) domain bound to BCL2L1/Bcl-XL was determined five years ago [10]. BECN1, however, interacts with a large number of other proteins [11, 12] and these interactions are mediated through additional domains. Now, two new studies bring a much larger portion of BECN1 into structural view. Li *et al*. [13] focused on the coiled-coil domain (CCD, amino acids 174-266 (the crystal structure is for residues 172-264, rat BECN1 numbering)), whereas Huang *et al*. [14] focused on an extended version

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of the evolutionarily conserved domain (ECD, originally defined as amino acids 267-337; actually human BECN1 residues 248-450). The extended ECD is a stable, folded minimal unit on its own, and so should now be considered the relevant structural and functional unit. Collectively, these structures span more than half of the molecule. Indeed, the crystallized fragments contain nearly 20 overlapping residues, making it possible to generate a composite model of nearly the entire ordered portion of BECN1 (Figure 1). The functional studies carried out in parallel with the crystallography have begun to assign functions to these various subregions of BECN1. The coiled coil seems to be a major locus for the assembly of BECN1 into the autophagy- and endosome-specific PtdIns3K complexes, whereas the ECD may have an unexpected role in membrane targeting.

The function of BECN1 in autophagy depends on its incorporation into the two major forms of the PIK3C3 complex, which contain either ATG14 or UVRAG. ATG14 and UVRAG both contain coiled coils responsible for their complex formation with the rest of PIK3C3. The coiled-coil regions of ATG14 and UVRAG target the BECN1 coiled coil, and together form a putative heterodimeric coil. The heterodimeric BECN1-ATG14 and BECN1-UVRAG coils have not been crystallized, but the homodimeric assembly of BECN1 with itself is now reported. This heterodimer is probably not functional in autophagy, since its assembly competes with ATG14 and UVRAG binding. Indeed, the full homodimeric assembly is sterically incompatible with the fold of the ECD, suggesting that when full-length BECN1 homodimerizes in solution, at least the first 6-7 turns of one coil would have to unravel. The homodimeric coiled coil contains charged residues and bulky amino acids at unfavorable positions that destabilize the structure, thus favoring assembly of BECN1-ATG14 and BECN1-UVRAG heterodimers instead. The homodimeric structure serves for now as a surrogate for the functional heterodimers, as shown in Figure 1. The metastable nature of the BECN1 CCD thus appears to be critical for the correct localization and function of BECN1 in autophagy.

The ECD forms a compact structure consisting of four α-helices, and three antiparallel β-sheets. It is now clear that the first helix of the ECD is actually the C terminus of the CCD, and the remainder of the ECD has an elegant pseudo three-fold symmetry. Loop 4, which connects the second β-sheet to helix 3, contains three aromatic amino acids—an aromatic finger—whose side-chains protrude away from the remainder of the protein. The ECD associates with membranes *in vitro*, and displays a preference for acidic lipids such as phosphadtidylserine, phosphatidylinositol, and cardiolipin,
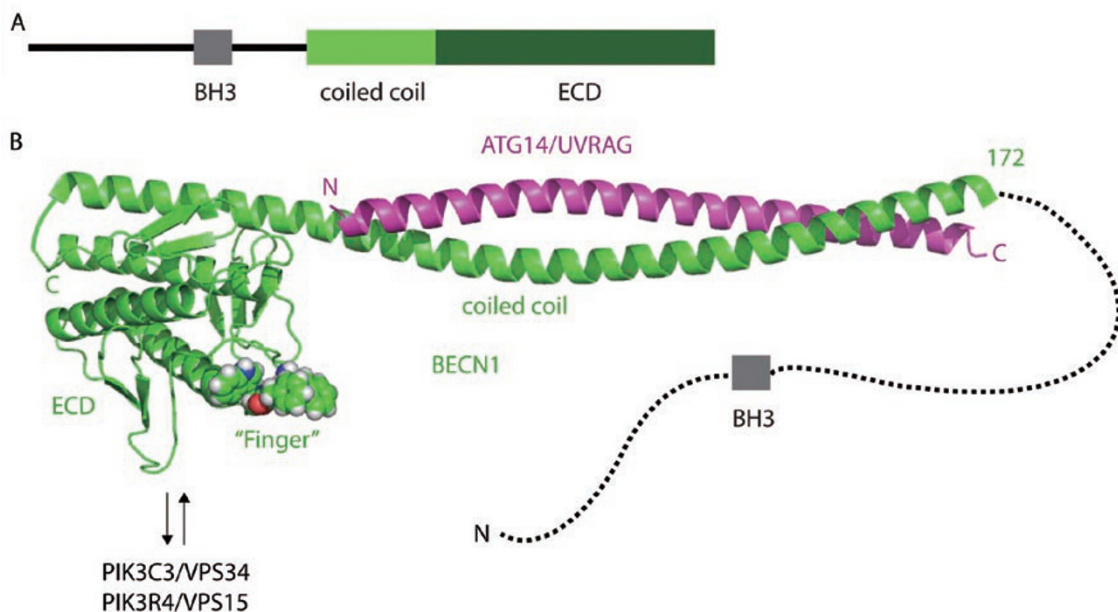

Figure 1 Composite model of the ordered portion of BECN1. **(A)** Schematic of the domain structure of BECN1. The black line denotes regions thought to be intrinsically disordered and flexible. **(B)** Structures of BECN1 ordered domains. The CCD and ECD structures were combined by superposition of the 19 overlapping residues in the two structures. In addition to the membrane binding role of the finger, the ECD was previously implicated in binding to the VPS34 and VPS15 subunits [20]. The second of the two coils of the CCD (magenta) is highlighted as a surrogate for the likely mode of binding of ATG14 or UVRAG. The second coil was truncated prior to residue 200 to avoid steric overlap with the ECD. The finger residues of the ECD are shown in a space-filling model. The black dotted line corresponds to the disordered regions in **A**.

suggesting that it may play a role in targeting BECN1 to the site of phagophore formation. In support of this hypothesis, mutation of the residues in the aromatic finger reduces membrane association *in vitro* and localization *in vivo*. The human BECN1 ECD joins at least two other recently identified domains, KA1 and MABP, that combine hydrophobic protrusions with basic surfaces in order to interact with acidic phospholipids [15, 16]. Some of these other domains are thought to interact physiologically with the widely distributed monoanionic lipid phosphatidylserine. BECN1-ECD is proposed to interact with the bianionic mitochondrial lipid cardiolipin. It seems likely that the ECD finger will have additional functions beyond cardiolipin binding, given that the finger residues are not conserved in yeast and that PIK3C3 has activity at many cell membranes that are not enriched in cardiolipin.

One of the intriguing questions in the autophagy field concerns the mechanism that induces curvature of the phagophore membrane; there is no evidence for a protein coat similar to those that act in this manner throughout the secretory pathway. Electron microscopy data suggest that the ECD may cause liposomes to undergo deformation. The induction and sensing of membrane curvature is a complex topic [17] that with respect to the ECD has so far been addressed in a preliminary fashion, and will require further investigation. There are several other candidates for a membrane curvature-sensing element in the PIK3C3 interactome. For example, SH3GLB1 interacts with BECN1 via UVRAG; the former has a BAR domain that may be critical in membrane deformation for autophagy [18]. In addition, there are components that detect membrane curvature such as the BARKOR autophagosome targeting sequence (BATS) domain of ATG14 [19], which may be critical in the membrane recruitment of the PtdIns3K. Resolution

of this question is just one facet of the world-class puzzles in molecular membrane biology: how is the phagophore nucleated, expanded and closed?

Acknowledgments

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