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The Beclin 1 interactome

Congcong He[1,2] and **Beth Levine**[1,2,3]

[1]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

[2]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

[3]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

Abstract

The mammalian ortholog of yeast Atg6/Vps30, Beclin 1, is an essential autophagy protein that has been linked to diverse biological processes, including immunity, development, tumor suppression, lifespan extension, and protection against certain cardiac and neurodegenerative diseases. In recent years, major advances have been made in identifying components of functionally distinct Beclin 1/class III phosphatidylinositol 3-kinase complexes, in characterizing the molecular regulation of interactions between Beclin 1 and the autophagy inhibitors, Bcl-2/BcL-X_L, and in uncovering a role for viral antagonists of Beclin 1 in viral pathogenesis. The rapidly growing list of components of the 'Beclin 1 interactome' supports a model in which autophagy, and potentially other membrane trafficking functions of Beclin 1, are governed by differential interactions with different binding partners in different physiological or pathophysiological contexts.

Beclin 1: a conserved autophagy protein

Autophagy is a lysosomal degradation pathway that functions in a variety of stress conditions, in which long-lived or aggregated proteins, damaged organelles and pathogens are transported in double-membraned autophagosomes to lysosomes for destruction. So far, genetic screens have identified approximately 32 autophagy-related genes (known as '*ATG*' genes) in the yeast *S. cerevisiae*, of which approximately 18 are essential for autophagy, and many of these are found in mammals and other higher eukaryotes [1]. Human Beclin 1 (coiled-coil, myosin-like BCL2-interacting protein) shares 24% sequence homology with yeast Atg6/Vps30 and restores autophagic activities in *atg6* null yeast mutants, demonstrating that it is a functional homolog of Atg6/Vps30 [2••]. Like yeast Atg6/Vps30, mammalian Beclin 1 interacts with the class III phosphatidylinositol 3-kinase (PI3K), Vps34, and is involved in autophagic vesicle nucleation [3••]. Gene knockout/knockdown studies indicate a conserved requirement for *ATG6*/*beclin* 1 in autophagy in plants, slime molds, nematodes, fruit flies, mice, and human cells [4]. Decreases in Beclin 1 expression and/or functional activity have been linked to increased susceptibility to cancer, Alzheimer's disease, Huntington's disease, and desmin-related cardiomyopathy; alterations in microbial pathogenesis; defects in apoptotic corpse clearance and development; and aging [5]. An open question is whether these phenotypes are a direct consequence of deficient autophagy, or as-of-yet unidentified alternate functions of Beclin 1.

Corresponding author: Levine, Beth (beth.levine@utsouthwestern.edu).
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Beclin 1/class III PI3K complexes

The yeast ortholog of Beclin 1, Atg6/Vps30, was independently discovered in two different genetic screens, including one for proteins required for autophagy and one for proteins required for vacuolar protein sorting, a pathway that sorts hydrolases from the trans-Golgi network (TGN) to the yeast vacuole. Subsequently, two distinct Atg6/Vps30–class III PI3K complexes were described in yeast [6]. Atg6/Vps30, the class III PI3K Vps34, and the regulatory myristoylated kinase Vps15 are common elements of each complex, but Atg14 is uniquely present in the complex involved in autophagy and Vps38 is uniquely present in the complex involved in vacuolar protein sorting. Since the discovery of mammalian Beclin 1, an important question has been whether, Beclin 1, like yeast Atg6/Vps30, functions in distinct class III PI3K complexes that mediate different membrane trafficking events.

Three lines of evidence in early studies suggested that Beclin 1 may function specifically in autophagy, and not in vacuolar protein sorting. First, Beclin 1 rescued autophagy, but not vacuolar protein sorting, in *atg6*/*vps30* null yeast [2••]. Second, the proteolytic processing of cathepsin D, which requires intact Vps34-dependent vacuolar protein sorting function, was found to be normal in autophagy-deficient, low Beclin 1-expressing mammalian cells [7]. Third, siRNA-mediated silencing of human Beclin 1 suppressed autophagy, but not other PI3K-dependent trafficking pathways such as the post-endocytic sorting of the epidermal growth factor (EGF) receptor or cathepsin D maturation [8]. Despite these negative data, suspicions remained that Beclin 1 may function in other membrane trafficking events given (1) the role of yeast Atg6/Vps30 in both autophagy and vacuolar protein sorting and (2) the different phenotype of *beclin 1* null mouse embryos (which are early embryonically lethal) versus *atg5* or *atg7* null mouse embryos (which die during the early neonatal period) [5].

Recent findings provide strong biochemical evidence that mammalian Beclin 1 exists in distinct class III PI3K complexes. Like in yeast, each complex seems to consist of Beclin 1, Vps34, and Vps15 [9•,10•,11•], as well as possibly, a mammalian specific Beclin 1-interacting WD40 domain protein, Ambra1 [12••] (and personal communication, Francesco Cecconi); for purposes of this review, we designate Beclin 1, Vps34, Vps15, and Ambra1 as the core complex (Figure 1). Within the past year, four independent laboratories isolated Beclin 1-binding proteins that are part of biochemically distinct Beclin 1/class III PI3K core complexes and postulated to have distinct functions in membrane trafficking events, including human Atg14 (also known as Atg14L (Atg14-like protein) or Barkor (Beclin 1-associated autophagy-related key regulator)), UVRAG, and Rubicon [9•,10•,11•,13] (Figure 1).

These findings underscore certain common themes as well as unanswered questions regarding Beclin 1/class III PI3K complexes. One unequivocal finding is that human Atg14, which only has limited structural homology with yeast Atg14 (18% identity, 32% similarity), has functional parallels to yeast Atg14 [9•,10•,11•,13]. Human Atg14 is reported to localize to isolation membranes [9•,10•] as well as to the endoplasmic reticulum [9•] (which may be a source of autophagosomal membranes) and genetic deletion or siRNA silencing of *Atg14* decreases Vps34 lipid kinase activity, autophagosome formation, and autophagic flux [9•, 10•,11•,13]. The independent discovery of a similar function for human Atg14 in autophagosome formation by four independent laboratories provides strong evidence that it is a functional homolog of yeast Atg14 in a Beclin 1/class III PI3K autophagy-inducing complex (Figure 1).

The interrelationship between UVRAG (UV irradiation resistance-associated gene), Beclin 1/ class III PI3K complexes, and different steps of autophagy is less clear. Three recent studies indicate that UVRAG and Atg14 are present in mutually exclusive Beclin 1/class III PI3K complexes [9•,10•,11•], and Itakura *et al.* provide evidence to support the hypothesis that

UVRAG (which shares weak homology with yeast Vps38) is not involved in autophagosome formation [10•]. They showed that, at least in HeLa cells, UVRAG was primarily associated with Rab9-positive endosomes and UVRAG silencing did not suppress autophagosome formation or flux. It is not yet clear how to reconcile these data, and the biochemical evidence that Atg14 (which is required for autophagosome formation) and UVRAG exist in distinct biochemical complexes [9•,10•,11•], with previous reports that UVRAG and Bif-1/endophilin B1 are involved in the activation of Beclin 1/Vps34 complexes and autophagosome formation [14,15]. Perhaps cell type-specific differences exist in the composition and/or function of Beclin 1/UVRAG-containing complexes and in some contexts, UVRAG can function like Atg14 in autophagosome formation.

In addition to autophagosome formation, UVRAG has also been reported to function in autophagosome maturation and endocytic trafficking [16•] (Figure 1). The interaction between UVRAG and the class C Vps complex, which is known to be involved in endosomal fusion, triggers Rab7 GTPase activity and promotes the fusion of autophagosomes with late endosomes/lysosomes. UVRAG also accelerates endocytic trafficking of EGF to lysosomes and EGF-induced lysosomal degradation of EGF receptors. Of note, the function of UVRAG in autophagosome maturation does not require the Beclin 1-interacting coiled-coil domain of UVRAG, suggesting that it may be genetically separable from its interaction with Beclin 1. However, similar analyses have not been performed to evaluate whether UVRAG-dependent acceleration of the trafficking of endocytic cargo to late endosomes is also Beclin 1-independent. Thus, at present, a role for Beclin 1/class III PI3K complexes in UVRAG-dependent autophagosome maturation or endocytic trafficking is not established.

In contrast to Atg14, UVRAG and Bif-1, which are reported to be either positive regulators of autophagosome formation (e.g. Atg14 and more controversially, UVRAG and Bif-1), autophagosome maturation (e.g. UVRAG), or endosome maturation (e.g. UVRAG), another newly identified component of Beclin 1/class III PI3K complexes, Rubicon (a RUN domain and cysteine-rich domain containing, Beclin 1-interacting protein), probably plays an important inhibitory role in some (or all) of these membrane trafficking events [9•,13]. Rubicon localizes to endosomes and lysosomes and its knockdown leads to an increase in numbers of autophagosomes and autolysosomes and an acceleration of the lysosomal degradation of endocytosed EGF receptor, demonstrating an inhibitory effect on autophagosome formation, autophagosomal maturation, and endosomal maturation. Although it is clearly established that there is a distinct Rubicon-containing Beclin 1/class III PI3K-containing complex that contains UVRAG (as well as a Rubicon-independent UVRAG Beclin 1/class III PI3K complex) [9•], it is not yet known whether Rubicon exerts its inhibitory effects on autophagosome maturation and endocytic trafficking through its interaction with the Beclin 1/class III PI3K core complex, or through Beclin 1-independent mechanisms. The ability of overexpressed Rubicon to decrease Vps34 kinase activity [13] raises the possibility that Rubicon may indeed interfere with autophagosome formation through inhibition of the Beclin 1/class III PI3K core complex. Similarly, Matsunaga *et al*. postulated that the Beclin 1/UVRAG/Rubicon complex may work in an opposing manner to the Beclin 1/UVRAG complex in the regulation of autophagosome maturation and endocytosis [9•]. However, as noted above, it is not yet clear that Beclin 1 is involved in UVRAG-dependent autophagosome or endocytic maturation.

Further studies are urgently needed to sort out the precise functions of Beclin 1-interacting partners, especially as they relate to the regulation of Beclin 1/class III PI3K complexes in mediating different membrane trafficking processes. Presently, we know that distinct Beclin 1/PI3K complexes exist; that Atg14 and other components of the Beclin 1/PI3K core complex (e.g. Beclin 1, Vps34, Ambra1) are involved in autophagosome formation; that UVRAG, a Beclin 1-interacting protein, may function in autophagosomal maturation, endosomal maturation, and autophagosome formation (at least in certain contexts); and that Rubicon,

another Beclin 1-interacting protein, negatively regulates these three processes. However, aside from the Atg14/core complex that functions in autophagosome formation, the roles of Beclin 1/class III PI3K complexes in other membrane trafficking events (such as autophagosome maturation and endosome maturation) remain undefined, and it is not yet known whether UVRAG and Rubicon regulate these processes through their interactions with the Beclin 1/class III PI3K complex or through other mechanisms. While Atg14 and UVRAG seem to interact with Beclin 1 in a mutually exclusive manner through their coiled-coil domains, it is unclear which interactions in the core complex and which interactions between members of the core complex and other Beclin 1-binding partners are direct or indirect. Another crucial unanswered question is whether Beclin 1-binding partners alter the composition, subcellular localization, and/or function of the Beclin 1/class III PI3K complexes. Along these lines, there is growing research indicating that Atg14 and Ambra1 may help localize Beclin 1 to autophagosomes or the endoplasmic reticulum, which in the case of Ambra1 may involve interactions with the dynein motor complex regulated by autophagy-inducing stimuli [13] (and personal communication, Francesco Cecconi). These findings probably represent 'the tip of the iceberg' in understanding how the biochemical identification of distinct Beclin 1/class III PI3K complexes relates to the cellular processes of autophagosome formation, autophagosome maturation, and endocytic trafficking.

At a physiological level, it is noteworthy that Beclin 1, and three of its interacting partners, Ambra1, UVRAG, and Bif-1, play a role in negative growth control and/or tumor suppression. Loss of *Ambra1* results in uncontrolled neural cell proliferation during embryogenesis [12••] and heterozygous deletion of *Ambra1* results in a spectrum of spontaneous tumors (personal communication, Francesco Cecconi) similar to those observed in mice with monoallelic loss of *beclin 1* [17,18]. *UVRAG* is frequently monoallelically deleted in human colon cancer, and its overexpression suppresses human colon cancer cell proliferation and tumorigenicity in nude mice [14]. Similarly, *Bif-1* knockout mice display an increased incidence of spontaneous tumors [15]. Thus, while there are conflicting *in vitro* data regarding the role of UVRAG in autophagy (and by extension Bif-1, which functions through its interaction with UVRAG), it is tempting to speculate that the common roles of these different Beclin 1-interacting proteins in suppressing tumorigenesis may indicate common underlying functions in cell biology. The determination of whether Atg14, a Beclin 1-interacting partner that acts specifically in autophagosome formation, also has tumor suppressor function will be crucial to dissect whether the common underlying cell biology function is autophagy or other Beclin 1/class III PI3K complex-dependent processes.

Beclin 1–Bcl-2/Bcl-X_L interactions

Beclin 1 was initially identified as a Bcl-2-interacting protein in a yeast two-hybrid screen [19], and subsequently, it was shown that Bcl-2 and Bcl-X_L function as anti-autophagy proteins through their interactions with Beclin 1 [20••]. Structural and biochemical studies indicate that Beclin 1 contains a BH3 (Bcl-2-homology-3) domain that is necessary and sufficient to bind cellular and virally-encoded, anti-apoptotic Bcl-2 family members, such as Bcl-2, Bcl-X_L, and murine γ-herpesvirus M11 [21,22,23•,24••,25]. Unlike other known BH3-only proteins, Beclin 1 does not function as a pro-apoptotic molecule [23•,26], suggesting that the BH3 domain may be a common structural motif by which Bcl-2 family members recognize and dually regulate both apoptosis and autophagy molecules rather than an invariant signature of a death-inducing protein [27]. Bcl-2 decreases Beclin 1 interactions with Vps34 and Beclin 1-associated Vps34 kinase activity [20••] and may therefore sequester Beclin 1 away from the autophagy-inducing class III PI3K 'core complex'. Endoplasmic reticulum-localized Bcl-2, but not mitochondrial-localized Bcl-2, inhibits autophagy [20••], which is consistent with the growing evidence that ER-associated class III PI3K activity may be crucial in the initiation of autophagosome membrane formation. It has also been proposed that Bcl-X_L inhibits Beclin 1 activity by

stabilizing Beclin 1 homo-dimerization, which can be disrupted by UVRAG [28]. Further research is required to more fully elucidate the precise mechanisms by which Bcl-2/Bcl-X_L inhibit autophagy.

In the past few years, there has been increasing evidence that the regulation of Bcl-2/Bcl-X_L interactions with Beclin 1 represents a central mechanism by which autophagy is turned on or off in response to diverse cellular stimuli [29]. There have also been numerous advances made in understanding the molecular mechanisms that govern such interactions: post-translational modifications of either Bcl-2 or Beclin 1 can alter Bcl-2/Bcl-X_L–Beclin 1 interactions; BH3-only peptides or proteins can competitively disrupt Bcl-2/Bcl-X_L–Beclin 1 interactions; and different membrane-anchored receptors or their adaptor proteins may also be able to regulate the interaction between Bcl-2 and Beclin 1 (Figure 2). Together, these and other regulatory mechanisms that have yet to be identified probably fine tune autophagy activities, at least partly, through the regulation of Bcl-2/Bcl-X_L–Beclin 1 interactions. The disruption of Bcl-2/Bcl-X_L–Beclin 1 interactions may be a mechanism that cells use to promote autophagy and cell survival during stress conditions, such as nutrient deprivation, hypoxia, or detachment from the extracellular matrix [30,31].

The most potent known physiological inducer of autophagy is starvation, and Pattingre *et al.* originally showed that nutrient status regulates the interaction of endogenous Bcl-2 and Beclin 1 [20••]. During nutrient rich conditions when autophagy is suppressed, Bcl-2 binding to Beclin 1 is maximal, whereas during nutrient deprivation when autophagy is stimulated, Bcl-2 binding to Beclin 1 is minimal. Wei *et al.* discovered that the underlying mechanism by which nutrient starvation disrupts the Bcl-2/Beclin 1 complex, leading to autophagy stimulation, is through JNK1-mediated multisite phosphorylation of residues Thr69, Ser70, and Ser97 of the non-structured loop of Bcl-2 [32•]. A similar mechanism has also been shown to be involved in ceramide-induced autophagy [33]. This mechanism of autophagy induction is likely to be physiologically relevant, as starvation fails to induce disruption of the Bcl-2/Beclin 1 complex and autophagy in MEFs that either lack JNK1 or that contain non-phosphorylatable knock-in mutations in Bcl-2 Thr69, Ser70, and Ser97 [32•].

The disruption of Bcl-2/Bcl-X_L–Beclin 1 interactions (and subsequent autophagy activation) by phosphorylation is bidirectional. Not only does Bcl-2 phosphorylation disrupt Bcl-2/Beclin 1 interactions, the phosphorylation of Thr119 in the BH3 domain of Beclin 1 by enforced expression of the death-inducing kinase, DAP-kinase (DAPk), also promotes the dissociation of Beclin 1 from Bcl-X_L (and Bcl-2) and induction of autophagy [34,35•]. It is not yet known whether endogenous DAPk is required for disruption of the Bcl-X_L (or Bcl-2)-Beclin 1 complex, and if so, under which settings. It is also unknown whether this activity of DAPk contributes to its role in cell death and tumor suppression. For both Bcl-2 and Beclin 1 phosphorylation-mediated regulation of the Bcl-2/Bcl-X_L–Beclin 1 complex, it is unknown whether adding a phosphate group sterically blocks the binding sites or induces conformational changes in Beclin 1 and Bcl-2/Bcl-X_L proteins that prevent their interactions with each other. Another unanswered question is whether phosphorylation events on Bcl-2 proteins and Beclin 1 occur synergistically or independently under autophagy-inducing conditions.

A third mechanism for regulating the interaction between Bcl-2/Bcl-X_L and activating autophagy involves competitive disruption by either BH3-only proteins (e.g. Bad in mammalian cells and EGL-1 in *C. elegans*) or pharmacological BH3 peptidomimetic agents (e.g. ABT737) [23•] (Figure 2) or, by a less-defined mechanism, the binding of the ARF tumor suppressor to mitochondrial Bcl-X_L [36]. Bcl-2 and Bcl-X_L bind to Beclin 1 with a relatively low affinity; therefore, this interaction can be readily disrupted by BH3 proteins or peptides that have higher affinity interactions with Bcl-2/Bcl-X_L. This mechanism is likely to be physiologically relevant, since the knockdown of Bad or deletion of EGL-1 impairs starvation-

induced autophagy in mammalian cells and *C. elegans*, respectively [23•]. However, virtually nothing is known about the relative contributions of BH3-only proteins versus post-translational modifications of Bcl-2/Bcl-X_L and Beclin 1 in the regulation of Bcl-2/Bcl-X_L–Beclin 1 interactions and autophagy. Another important question is to what extent BH3 mimetics exert their anti-cancer effects through autophagy induction (in addition to their established role in apoptosis induction [37–40]) and whether more selective BH3 mimetics can be designed that inhibit the anti-autophagy activity of Bcl-2 family members without inhibiting the anti-apoptosis activity of Bcl-2 family members. Such reagents could be useful in the treatment of conditions other than cancer where selective autophagy activation may be beneficial, such as infectious diseases, neurodegenerative diseases, and aging.

A newly emerging area of research relates to the effects of different membrane-anchored proteins or their adaptor proteins on Bcl-2/Bcl-X_L–Beclin 1 interactions. For example, the endoplasmic reticulum-localized inositol-1,4,5-triphosphate receptor (IP_3R), which is a major regulator of apoptotic signaling, may also regulate autophagy through its effects on Bcl-2/Beclin 1 interactions [41]. Treatment with the IP_3R antagonist or physiological induction of autophagy by nutrient starvation disrupts the interaction between IP_3R and Beclin 1, which is an interaction that is abolished by Bcl-2 knockdown or enhanced by Bcl-2 overexpression. Another study suggested that Toll-like receptor (TLR) signaling may indirectly regulate Bcl-2/Beclin 1 interactions and thereby, induce autophagy; the TLR adaptor proteins, MyD88 and Trif interact with Beclin 1, which results in the reduced binding of Beclin 1 to Bcl-2 [42]. However, for both the IP_3R antagonist and MyD88/Trif-dependent induction of autophagy, further studies are required to determine whether disruption of the Beclin 1/Bcl-2 interaction is mechanistically involved in autophagy induction, and if so, how the IP_3R and TLR adaptors regulate the interaction.

Although not yet related to Bcl-2/Bcl-X_L–Beclin 1 interactions, it is worth noting in this section that other membrane-anchored and nuclear receptors are also part of the ‘Beclin 1 interactome’. This includes the membrane-anchored delta 2 glutamate receptor that is aberrantly activated in mice with cerebellar degeneration and linked to Beclin 1 through an interaction with a PDZ domain-containing protein, nPIST [43], the pathogen receptor CD46 that is linked to Beclin 1 through an isoform of nPIST, GOPC (also known as PIST) [44], and the estrogen receptor, ERα [45]. It will be interesting to explore in future studies whether these receptors regulate autophagy either through altering Beclin 1/class III PI3K complexes and/or through altering Bcl-2/Bcl-X_L–Beclin 1 interactions.

The Beclin 1 viral protein interactome

Perhaps the best evidence that Beclin 1 is an important autophagy protein stems from the diversity of the expanding known components of its ‘viral protein interactome’. Autophagy plays a crucial role in antiviral host defense and functions to limit viral replication, activate Type I interferon signaling, and enhance MHC class II presentation of endogenously synthesized viral antigens [46,47]. Consequently, there is probably a strong selection pressure for viruses to evolve mechanisms to counteract host autophagy; the most common molecular strategy identified thus far by which viruses accomplish this goal is through encoding virulence proteins that bind to Beclin 1 and inhibit its autophagy function. The list of viral proteins that interact with Beclin 1 and modulate its autophagy activity includes viral Bcl-2-like proteins encoded by the oncogenic γ-herpesviruses (e.g. KSHV vBcl-2, murine γHV68 M11) [22, 24••,25,32•,48]; a neurovirulence protein, ICP34.5 encoded by the α-herpesvirus, HSV-1 [49•,50]; a pathogenic factor, Nef, encoded by HIV-1 [51•]; and possibly the M2 protein of influenza virus [52]. The herpesvirus-encoded Beclin 1 antagonists, including the viral Bcl-2 molecules and HSV-1 ICP34.5, inhibit autophagosome formation, whereas HIV-1 Nef and influenza M2 inhibit autophagosome maturation (Figure 3).

For both HSV-1 and murine γHV68, there is strong *in vitro* evidence that viral proteins inhibit autophagosome formation through their interaction with Beclin 1 and strong *in vivo* evidence that viral inhibition of Beclin 1 is important in viral pathogenesis. With respect to HSV-1, Orvedahl *et al.* originally showed that a mutant virus containing a small deletion in ICP34.5 that blocks its ability to interact with Beclin 1 is highly attenuated following intracerebral inoculation in mice, with significantly reduced CNS viral replication and animal lethality [49•]. In addition, a more recent study demonstrated that this mutant virus stimulates a significantly stronger CD4 T cell response and is more rapidly cleared in a corneal infection model [50]. With respect to murine γHV68, a virus that contains a deletion mutation in the α1 helix of the viral Bcl-2, which blocks its interaction with Beclin 1 and anti-autophagy activity but not its interaction with pro-apoptotic BH3 proteins or its anti-apoptotic activity, is deficient in the maintenance of viral latent infection [48].

The precise mechanisms by which herpesvirus-encoded viral proteins inhibit autophagosome formation are unknown. On the basis of crystallographic and NMR studies of the viral Bcl-2 bound to the BH3 domain of Beclin 1, the analysis of binding affinities, and mutational analyses [27], the structural determinants of viral Bcl-2/Beclin 1 interactions generally resemble those observed for cellular Bcl-2/Bcl-X_L interactions with Beclin 1. However, of note, it appears that viral Bcl-2s may have evolved mechanisms to be more successful than their cellular counterparts in inhibiting autophagy. The γHV68-encoded Bcl-2 molecule, M11, binds to Beclin 1 with significantly higher affinity than do cellular Bcl-2 or Bcl-X_L [22,25]. In addition, KSHV vBcl-2, unlike cellular Bcl-2, lacks potential JNK1 target phosphorylation sites, escapes physiological regulation of binding to Beclin 1, and constitutively inhibits autophagy [32•]. Given the importance of herpesvirus antagonism of Beclin 1 function in viral pathogenesis in mouse models, pharmacological agents that can disrupt HSV-1 ICP34.5/Beclin 1 or viral Bcl-2/Beclin 1 interactions may be beneficial in treating HSV-1 and KSHV infections in patients.

Recent studies also provide strong evidence that HIV-1 Nef and the influenza M2 proteins interact with Beclin 1 and block autophagosome maturation [51•,52]. Yet, it is not yet definitively known whether the mechanism by which these viral proteins inhibit autophagosome maturation is through their interactions with Beclin 1. Kyei *et al.* propose that this is the case for HIV-1 Nef, since they observed a correlation between the effects of mutations in HIV-1 Nef on Beclin 1-binding activity and the ability of HIV-1 Nef to block autophagosome maturation [51•]. However, the ^{174}DD^{175} → ^{174}AA^{175} mutation in the diacidic motif that blocks Nef's interaction with Beclin 1 and Nef's inhibition of autophagosome maturation also blocks its interaction with the V_1 domain of vacuolar H^+ ATPase. Thus, an interesting question is whether the interaction with the vacuolar H^+ ATPase may also contribute to Nef's ability to block autophagosome maturation. With respect to influenza, Gannage *et al.* demonstrate that the viral M2 protein, including a fragment containing the N-terminal 60 amino acids that are sufficient to block autophagosome fusion with lysosomes, also interacts with Beclin 1 [52]. They speculate that the N-terminal 60 amino acids of M2, independent of its proton channel function (which requires the C-terminal membrane spanning residues), may block autolysosome formation through interfering with Beclin 1 and UVRAG-containing PI3K complexes. However, further studies are required to test this hypothesis.

If HIV-1 Nef and influenza virus M2 inhibit autolysosome formation through their interaction with Beclin 1, this will be an important indication that Beclin 1 functions not only in autophagosome formation, but also in autophagosome maturation. Moreover, such a finding will underscore the crucial importance of viral antagonism of multiple stages of autophagy. Perhaps, a dual role of Beclin 1 in both autophagosome formation and autophagosome maturation renders it a prime target for viral inhibitors of autophagy, that is, the virus can 'kill two birds with one stone'.

Conclusion

In the past few years, the repertoire of the Beclin 1 interactome has vastly expanded, with the identification of new components of Beclin 1/PI3K complexes, the identification of new mechanisms for regulating Bcl-2/Bcl-X_L–Beclin 1 interactions, and the identification of new viral inhibitors of Beclin 1, and many of these interactions have been shown between endogenous proteins (Table 1). Together, these findings suggest that Beclin 1 may not only function in autophagosome formation, but also in autophagosome/endosome maturation, and that temporally-modulated or spatially-modulated interactions between Beclin 1 and its positive regulators and negative regulators may govern these activities. Within the next few years, there will probably be a dramatic further expansion of known proteins in the Beclin 1 interactome, as a result of ongoing genetic and proteomic screens in diverse laboratories, as well as enhanced efforts to detect proteins that form unstable or transient complexes with Beclin 1 under specific conditions. Dissecting the Beclin 1 interactome in further detail will help elucidate the mechanisms by which complex regulatory networks integrate diverse environmental cues with this core component of the autophagy machinery to fine tune autophagy levels, coordinate distinct steps in autophagy, and maintain cellular homeostasis.

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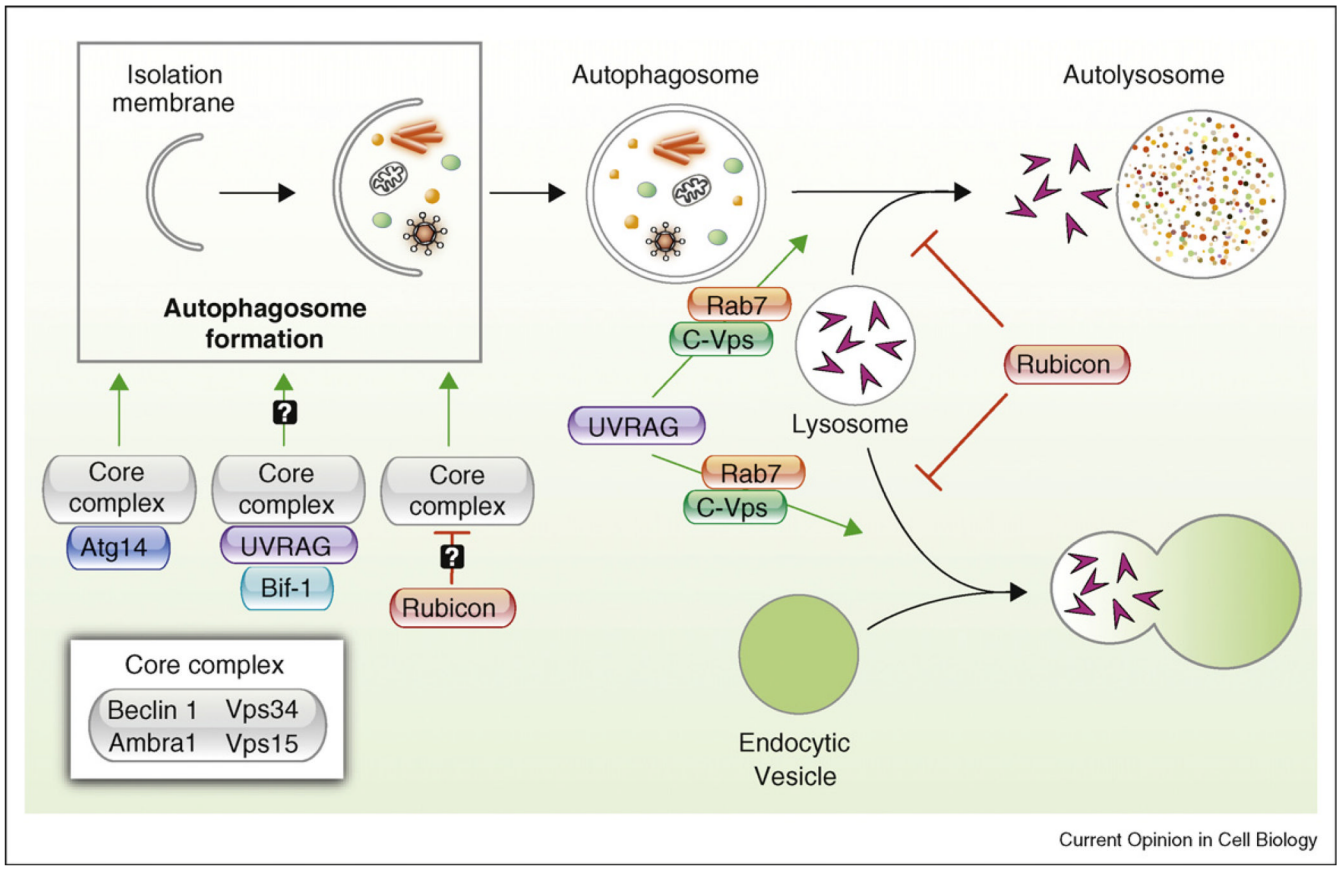

Figure 1.

Function of proteins that interact with Beclin 1/class III PI3K complexes in different steps of autophagy. Beclin 1, Vps34, Vps15, and possibly Ambra 1, compose a class III PI3K core complex that binds either Atg14 or UVRAG. Atg14 activates the core complex and biogenesis of autophagosomes; however, there are conflicting reports as to whether UVRAG/Bif-1 facilitates the core complex and upregulates autophagosome formation. A better studied function of UVRAG is promoting the maturation of autophagosomes and endocytic vesicles by recruiting class C Vps and the Rab7 GTPase and inducing vesicle fusion. As an important negative regulator of autophagy, Rubicon inhibits multiple steps in autophagy, including the formation of autophagosomes and the fusion between lysosomes and autophagosomes/endocytic vesicles. However, it is not yet known whether Rubicon inhibits autophagosome formation directly through an inhibitory interaction with the core complex or whether Beclin 1 is involved in the inhibitory effects of Rubicon on autophagosome/endosome maturation. Green arrows, stimulatory action; red bars, inhibitory action.

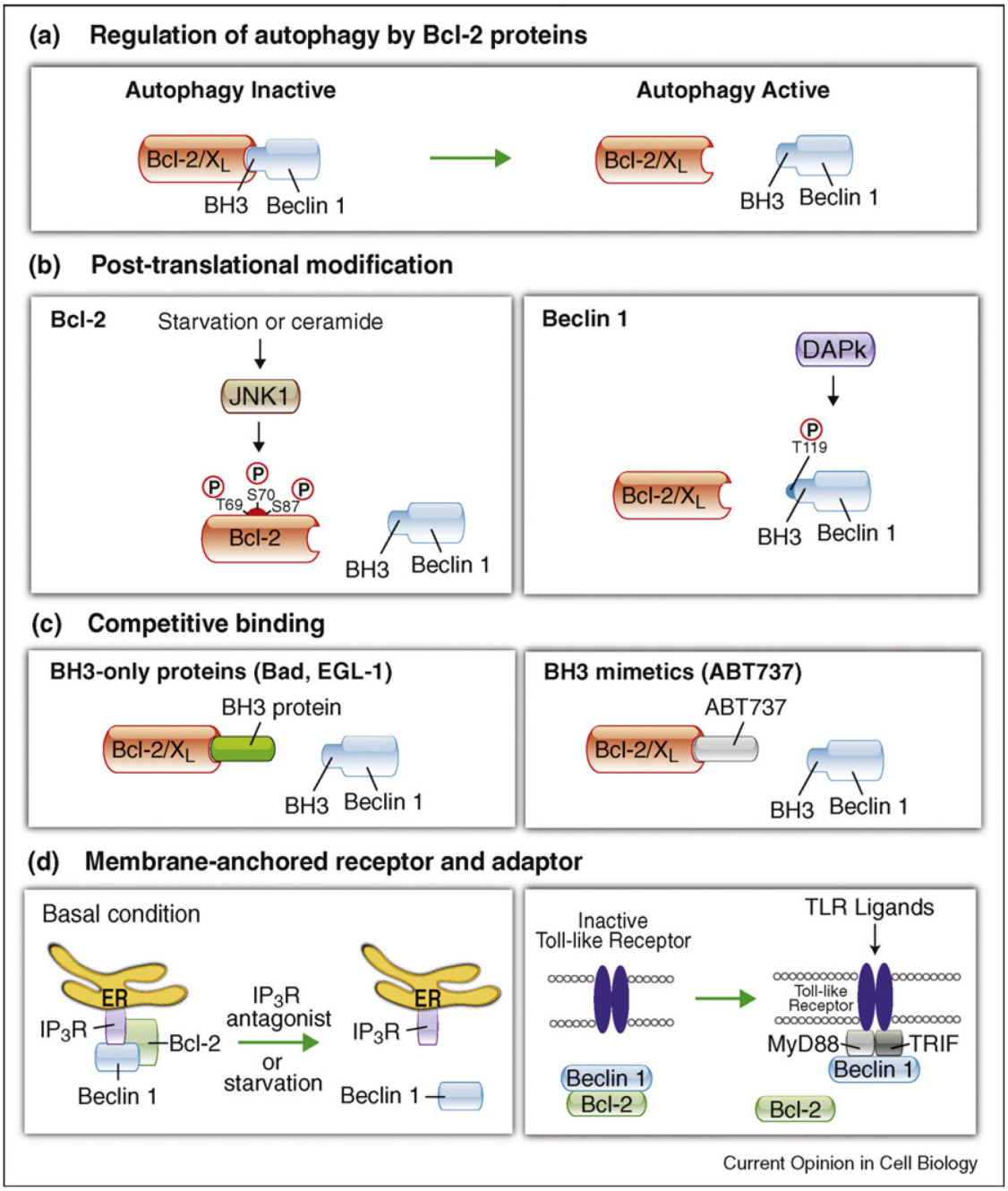

Figure 2.

Mechanisms underlying the regulation of Bcl-2/Bcl-X_L interactions with Beclin 1. (**a**) When Bcl-2/Bcl-X_L are bound to Beclin 1, they inhibit autophagy. The disruption of Bcl-2/Bcl-X_L–Beclin 1 interactions is essential for autophagy activation. Mechanisms by which this occurs include post-translational modifications of either Bcl-2/Bcl-X_L or Beclin 1 (**b**); competitive disruption of Bcl-2/Bcl-X_L binding to Beclin 1 by BH3-only proteins (e.g. Bad, EGL-1) or BH3 mimetics (e.g. ABT737) (**c**), or potentially, effects of membrane-anchored receptors or their adaptors on Bcl-2/Beclin 1 interactions (**d**). In (**b**), starvation induces JNK1-dependent phosphorylation of residues T69, S70, and S87 of Bcl-2, resulting in the disruption of Bcl-2 from Beclin 1. The death-associated kinase, DAPk, phosphorylates residue T119 in the BH3

domain of Beclin 1, leading to the disruption of Bcl-2 from Beclin 1. In (**d**), Beclin 1 is released from ER-localized inositol-1,4,5-trisphosphate receptor (IP_3R) and Bcl-2 after antagonist binding or during starvation, leading to activation of autophagy. The activation of Toll-like receptors recruits the adaptor proteins MyD88 and TRIF, which interact with Beclin 1 and decrease the inhibitory interaction of Bcl-2 with Beclin 1. Green arrows, autophagy induction; red bars, autophagy inhibition.

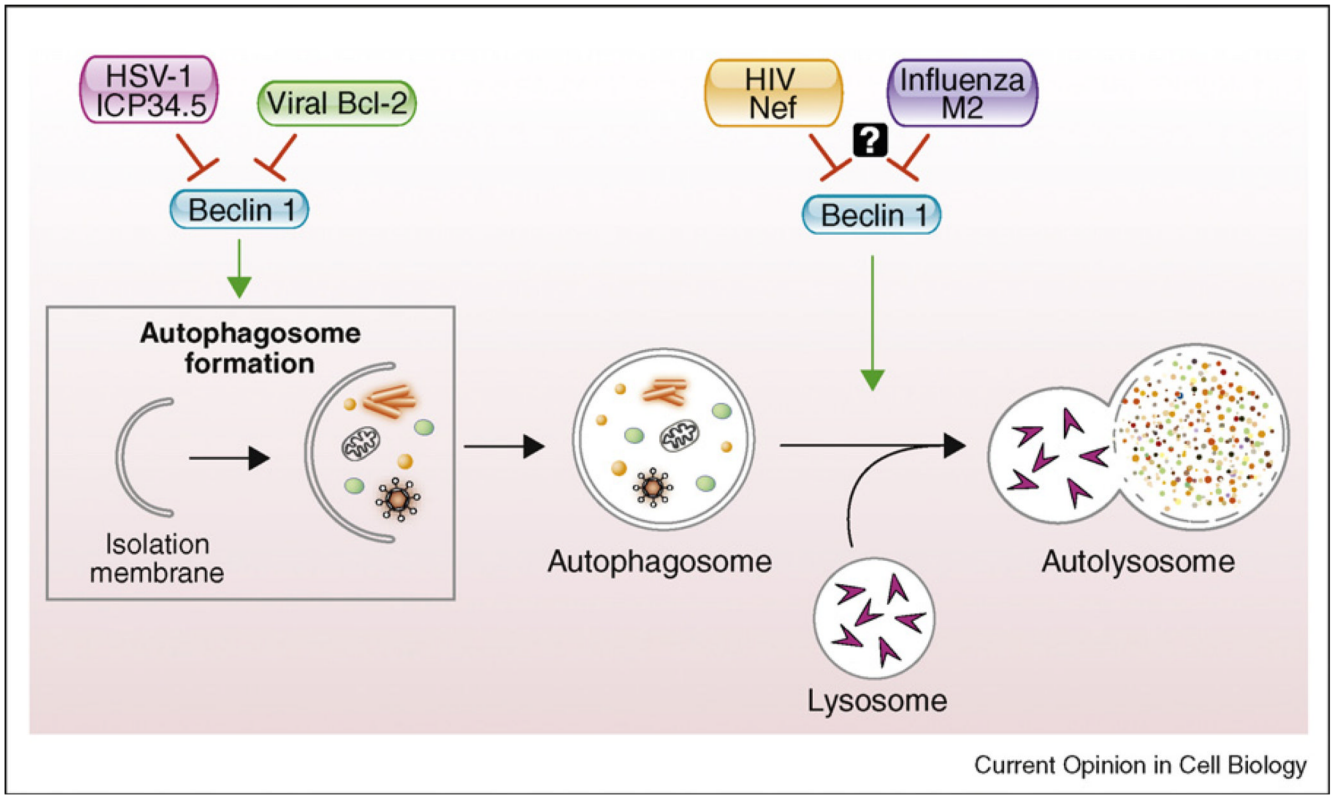

Figure 3.
The Beclin 1 viral protein interactome and their presumed sites of anti-autophagy action. HSV-1 ICP34.5 and γ-herpesvirus-encoded viral Bcl-2 molecules inhibit autophagosome formation through their interactions with Beclin 1, whereas HIV-1 Nef and influenza M2 block the maturation step of autophagy. The anti-autophagic maturation activity of HIV-1 Nef is thought to be mediated by interactions with Beclin 1 (see text), whereas it is not yet known whether the anti-autophagic maturation activity of influenza M2 is mediated by its interaction with Beclin 1.

Table 1

Summary of studies that have confirmed endogenous interactions in mammalian cells between Beclin 1 and the indicated Beclin 1-binding partners

Beclin 1-binding partner	References
A. Class III PI3K complex components	
Autophagy inducers:	
Vps34	[3••,9•,10•]
Vps15	[9•]
Ambra1	[12••]
Atg14	[9•,10•,11•]
UVRAG	[9•,10•,14]
Bif	[15]
Autophagy inhibitors:	
Rubicon	[9•]
B. Bcl-2 family members	
Bcl-2	[20••,23•,32•,33]
Bcl-X_L	[36]
C. Receptor and adaptor proteins	
Inositol 1,4,5-trisphosphate receptor (IP_3R)	[41]
Estrogen receptor (ERα)	[45]
MyD88 and TRIF	[42]
nPIST	[43]