

Crosstalk between apoptosis and autophagy within the Beclin 1 interactome

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Although the essential genes for autophagy (Atg) have been identified, the molecular mechanisms through which Atg proteins control ‘self eating’ in mammalian cells remain elusive. Beclin 1 (Bec1), the mammalian orthologue of yeast Atg6, is part of the class III phosphatidylinositol 3-kinase (PI3K) complex that induces autophagy. The first among an increasing number of Bec1-interacting proteins that has been identified is the anti-apoptotic protein Bcl-2. The dissociation of Bec1 from Bcl-2 is essential for its autophagic activity, and Bcl-2 only inhibits autophagy when it is present in the endoplasmic reticulum (ER). A paper in this issue of the *EMBO Journal* has identified a novel protein, NAF-1 (nutrient-deprivation autophagy factor-1), that binds Bcl-2 at the ER. NAF-1 is a component of the inositol-1,4,5 trisphosphate (IP₃) receptor complex, which contributes to the interaction of Bcl-2 with Bec1 and is required for Bcl-2 to functionally antagonize Bec1-mediated autophagy. This work provides mechanistic insights into how autophagy- and apoptosis-regulatory molecules crosstalk at the ER.

Autophagy is a catabolic pathway characterized by the formation of double-membrane vesicles (autophagosomes) that engulf cytoplasmic organelles and proteins, and then fuse with lysosomes, which degrade their luminal content. Autophagy acts as a cytoprotective mechanism, favouring stress adaptation that avoids cell death. In specific conditions, it may constitute an alternative pathway to cellular

demise (autophagic cell death). Despite major efforts, the crosstalk between ‘self-eating’ (autophagy) and ‘self-killing’ (apoptosis) remains unclear, even though the involvement of a functional and physical Bec1/Bcl-2 interaction has been suggested (Maiuri *et al*, 2007). Shore *et al* (Chang *et al*, 2009) provide new insights into the Bec1/Bcl-2 interaction at the ER. Bec1, a coiled-coil protein, has been identified as a haploinsufficient tumour suppressor gene and Bcl-2 interactor. Bec1 has a central role in incipient autophagy because of its interaction with PI3K, hVps34 and hVps15. Recently, numerous proteins interacting with Bec1 have been identified. UVRAG (UV radiation resistance-associated gene) or Atg14L interacts with Bec1 and promotes PI3K activity, activating autophagosome formation and maturation. Bif-1/endophilin B1 interacts with Bec1 via UVRAG acting as a regulator of the PI3K complex (Sinha and Levine, 2008). Barkor (Bec1-associated autophagy-related key regulator) competes with UVRAG for interaction with Bec1, and it has been suggested that these proteins interact with Bec1 in a stepwise manner, sequentially sustaining its function in early autophagosome formation and late autophagosome/lysosome fusion (Sun *et al*, 2008). Rubicon (RUN domain protein as Bec1 interacting and cysteine-rich containing) is a negative regulator of autophagy (Zhong *et al*, 2009). Ambra 1 (activating molecule in Bec1-regulated autophagy) binds to Bec1 and favours autophagosome formation in the central nervous system (Sinha and Levine, 2008). These data suggest

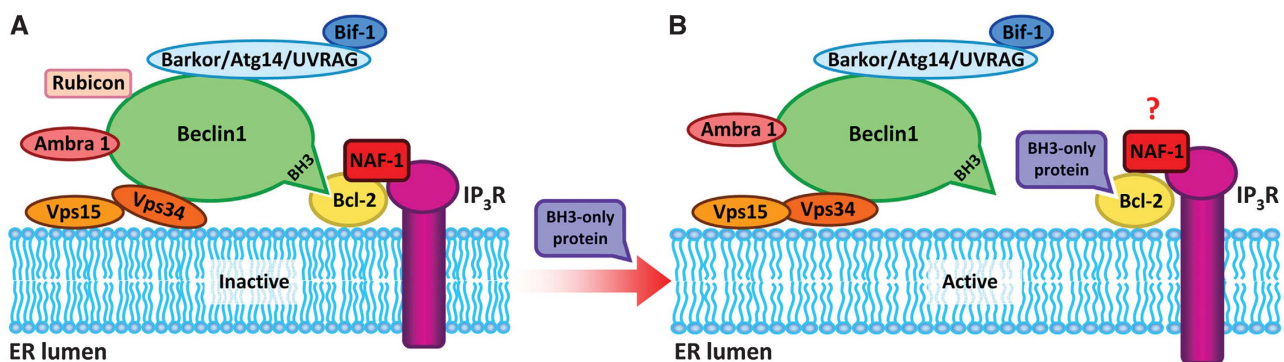


Figure 1 Model of the Bec1/Bcl-2/NAF-1 interaction and autophagy regulation at the ER. A simplified vision of the complex is shown before (A) and after autophagy induction (B). The exact stoichiometry of the Bec1 complex as well as possible intra-complex competitions (that would preclude the simultaneous interaction of all proteins) are unknown.

the existence of different Bec1 protein complexes that regulate autophagy in the particular context of tissue-specific cell types and the changing subcellular localization of the PI3K complex. Bec1 contains a Bcl-2 homology-3 (BH3) domain (Maiuri *et al*, 2007) that is necessary and sufficient for binding to the anti-apoptotic proteins of the Bcl-2 family (such as Bcl-2 and Bcl-X_L), which possess BH3-binding grooves. Despite the fact that Bec1 is a BH3-only protein, it cannot neutralize the anti-apoptotic function of Bcl-2, which is exerted at the mitochondrial membranes. In contrast, Bcl-2 or Bcl-X_L reduces the pro-autophagy activity of Bec1. Only ER-targeted Bcl-2 (or Bcl-X_L), and not mitochondrial-targeted Bcl-2 (or Bcl-X_L), can inhibit starvation-induced autophagy (Maiuri *et al*, 2007).

Shore *et al* add another piece to the Bec1/Bcl-2 crosstalk puzzle, showing that NAF-1 (CISD2: CDGSH iron sulphur domain 2; synonyms: ZCD2, Noxp70 and Miner1) contributes to Bec1/Bcl-2 interaction at the ER. NAF-1 binds Bcl-2 and this interaction is independent of a BH3 domain (absent in NAF-1) but depends at least in part on its CDGSH iron/sulphur-binding domain. The authors elegantly demonstrate that autophagy inhibition by Bcl-2 requires NAF-1. Knockdown of NAF-1 triggers autophagy and significantly reduces the Bec1/Bcl-2 interaction. Although NAF-1 does not contain a BH3 domain, the authors found that the ER-restricted pro-apoptotic BH3-only protein, Bik, was able to displace NAF-1 from Bcl-2. How can this be explained? The Bec1/Bcl-2 complex can be competitively disrupted by BH3-only proteins (Maiuri *et al*, 2007a), suggesting a differential affinity of Bcl-2 towards its partners. In Shore *et al*'s article, we learn that NAF-1 stabilizes the Bec1/Bcl-2 interaction. Possibly, Bec1 preferentially binds a Bcl-2 protein that changes its conformation upon interaction with NAF-1. Specific autophagic stimuli can trigger the phosphorylation of Bcl-2 or Bec1 (Sinha and Levine, 2008) within or close to their interaction domains, supporting the hypothesis that posttranslational modifications of Bec1 and Bcl-2 (and perhaps NAF-1 or others) may affect the interaction among these proteins. Future investigation must resolve the question whether NAF-1 might also modulate the anti-apoptotic function of Bcl-2 in specific circumstances. As NAF-1 contains a redox-active 2Fe–2S cluster, which determines its interaction with Bcl-2, it might link redox metabolism to autophagy regulation. This possibility should be investigated in the future.

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One target of Bcl-2 at the ER is the IP₃ receptor (IP₃R), a Ca²⁺ efflux channel. The IP₃R/Bcl-2 interaction does not involve BH3 domains. IP₃R acts as an endogenous buffer of IP₃ levels and IP₃R antagonists trigger autophagy. However, IP₃R-mediated Ca²⁺ fluxes are irrelevant to the regulation of autophagy. Possibly, IP₃R acts as a molecular scaffold that permits the inhibition of Bec1 by Bcl-2 (Vicencio *et al*, 2009). Shore *et al*, found that NAF-1 binds IP₃R type 1, suggesting that the crosstalk between Bcl-2 and Bec1 involves complex interactions with both NAF-1 and IP₃R at the ER. Although much of the mystery of this molecular pool game remains to be deciphered, the present data identify a novel player, NAF-1 (Figure 1).

Intriguingly, NAF-1 is truncated in Wolfram syndrome 2 (WFS2), an autosomal recessive neurodegenerative disorder. Knockout of NAF-1 increases autophagy, mitochondrial defects and premature ageing in mice (Chang *et al*, 2009). As stimulation of autophagy often extends the lifespan (Morselli *et al*, 2009), it appears counterintuitive to attribute the pathological manifestations of WFS2 to enhanced autophagy. NAF-1 is expressed both on the ER and on mitochondria (Chang *et al*, 2009); precise studies on whether WFS2 is due to primary mitochondrial defects or due to a dysregulation of autophagic flux have not been performed and may require the re-introduction of mitochondrion- or ER-targeted NAF-1 into knockout mice.

Another enigma that requires further studies is regarding the conditions in which autophagy prevents apoptosis or favours cellular demise. The identification of NAF-1 as a novel regulator of Bec1/Bcl-2 interaction has added another piece to this increasingly complex puzzle. Future studies will resolve the composition of the molecular complexes organized around Bec1 and Bcl-2 as they disassemble and reassemble during cellular stress resulting in either autophagy or apoptosis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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