Compartmentalized regulation of autophagy regulators: fine-tuning AMBRA1 by Bcl-2

Sharon A Tooze^{1,*} and Patrice Codogno²

¹London Research Institute, Cancer Research UK, London, UK and ²INSERM U984, Faculté de Pharmacie, Université Paris-Sud, Châtenay-Malabry, France

*Correspondence to: sharon.tooze@cancer.org.uk

The EMBO Journal (2011) 30, 1185–1186. doi:10.1038/emboj.2011.75

Members of the anti-apoptotic Bcl-2 family and AMBRA1 (activating molecule in beclin 1-regulated autophagy) interact with Beclin 1 and control autophagy. Bcl-2 is a negative regulator of autophagy whereas AMBRA1 stimulates autophagy. A paper in this issue of *The EMBO Journal* reveals an interaction between AMBRA1 and Bcl-2 at the mitochondrial outer membrane. In response to nutrient withdrawal, this interaction is dissociated, and AMBRA1 associates with Beclin 1 at the endoplasmic reticulum (ER) and mitochondria to stimulate autophagy. Bcl-2 can thus regulate the initiation of autophagy at the ER as well as at mitochondrial membranes. In addition, binding between AMBRA1 and mito-BCL-2 is reduced during apoptosis and this may enhance the anti-apoptotic functions of Bcl-2. This work provides important novel insight into the importance of subcellular location on the activity of autophagy proteins and the crosstalk between autophagy and apoptosis.

Autophagy is a conserved process of self-digestion, ‘self-eating’, which is utilized by cells in all multi-cellular organisms to survive a variety of stressful conditions, including nutrient deprivation, environmental changes, and infection. In addition, autophagy has a key role during development, in prevention of disease, and aging, although paradoxically autophagy may not always be beneficial. Given the complex and wide-ranging role of autophagy, it is perhaps not surprising that it is a highly regulated process. Currently,

there are over 34 proteins that are required for autophagy. They were first systematically identified in yeast and called Atg (autophagy related) proteins (Yang and Klionsky, 2010). The function of these 34 Atg proteins is almost entirely dedicated to making autophagosomes with the correct composition and content. A subset of these Atg proteins function at the site of autophagosome formation that remains somewhat enigmatic: recently the ER, the Golgi, mitochondria, and plasma membrane have all been implicated in autophagosome formation. Under nutrient deprivation, amino-acid starvation in particular, the ER appears to be the site where most autophagosome formation occurs (Tooze and Yoshimori, 2010).

One key Atg-containing complex is the autophagy-specific class III phosphatidylinositol 3-kinase (PI3P-kinase), which produces PI3P at the site of autophagosome formation (Simonsen and Tooze, 2009). In metazoans, this lipid kinase complex is composed of a kinase, hVps34, its regulatory partner p150, and Beclin 1 (Atg6 in yeast). Beclin 1 exists in several complexes involved in autophagosome formation and maturation. Two proteins that are essential for the function of the complex in autophagosome formation are Atg14L and AMBRA1. Atg14L is one of the first proteins that is translocated to the ER site of autophagosome formation (Matsunaga *et al*, 2010). In response to autophagic stimuli, AMBRA1 associates with Beclin 1 and becomes part of the

Figure 1 Bcl-2, Beclin 1, and AMBRA1 form distinct complexes under normal conditions (left) and upon nutrient deprivation and induction of autophagy (right) on the mitochondrial membrane and endoplasmic reticulum (ER). Under normal conditions, Bcl-2 interacts with Beclin 1 and AMBRA1 on the mitochondria and the ER and appears to function as an important negative regulator of autophagy.

core autophagic PI3K complex (called the Beclin 1 complex). In resting cells, AMBRA1 is found in a complex with the Dynein motor complex. Upon induction of autophagy, it is released from the motor complex and translocates to the ER site of autophagosome formation (Di Bartolomeo *et al*, 2010). AMBRA1 release from dynein light chains requires phosphorylation by ULK1 (Atg1 in yeast), which is itself regulated by the TOR kinase.

While the Atg proteins are essential for autophagy, it is becoming increasingly apparent that the regulation of autophagy is a complex process using pathways and molecules that are essential for other cellular processes. In particular, Beclin 1 appears to be regulated by the anti-apoptotic protein Bcl-2 (He and Levine, 2010). Bcl-2 and other members of this family have an anti-autophagic activity via their interaction with Beclin 1, and dissociation of the Beclin 1:Bcl-2 complex stimulates autophagy. This dissociation can occur through phosphorylation of Beclin 1 or Bcl-2, by competition with BH3 domain-containing proteins that disrupt the interaction of Bcl-2 family proteins with the BH3 motif of Beclin 1, or by phosphorylation of the BH3 domain through DAP kinase (He and Levine, 2010).

A key aspect of these regulatory functions may be cellular location. Bcl-2 inhibits Beclin 1 when localized to the ER, but not at the mitochondria (He and Levine, 2010). In this issue of *The EMBO Journal*, Strappazzon *et al* (2011) provide a novel link between Bcl-2 and the regulation of autophagy (Figure 1). Bcl-2 localized to the mitochondria (mito-Bcl-2) can bind AMBRA1, but ER-localized Bcl-2 (ER-Bcl-2) does not. Importantly, the interaction between mito-Bcl-2 and AMBRA1 is downregulated by nutrient starvation, and interestingly also appears to be decreased during apoptosis. Mechanistically, Bcl-2 can thus regulate autophagy both directly, through regulation of Beclin 1 by direct association, and indirectly by sequestering the positive regulator, AMBRA1. Bcl-2 interaction with AMBRA1 may prevent AMBRA1 from associating with the Beclin 1 complex on the mitochondria, or prevent it from translocating to the Beclin 1 complex at the ER site of autophagosome formation. Either way, Bcl-2 would be exerting a regulatory role highlighting its importance as a negative regulator of autophagy, and the interaction between Bcl-2 and AMBRA1 represents a novel mechanism to regulate autophagy.

This very interesting paper raises additional questions about the role of Bcl-2 and AMBRA1 interactions during autophagy and apoptosis, and further studies are required to complete our understanding. The cellular location of the interactions should be further explored, in particular given the recent identification of the mitochondria as a site of

autophagosome formation. Furthermore, it will be exciting to learn more about how and why Bcl-2 binds AMBRA1, and if this provides an additional means for Bcl-2 to regulate autophagy. As an aside, it will be particularly interesting to explore how the Bcl-2 bound pool of AMBRA1 is related to that bound by the Dynein motor complex.

A major unanswered question is what controls the dissociation of the AMBRA1:Bcl-2 complex. Phosphorylation of Bcl-2 by JNK that dissociates the Bcl-2:Beclin 1 complex under starvation is not responsible for the dissociation of the AMBRA1:Bcl-2 complex. Whether ULK1, which phosphorylates AMBRA1 to dissociate it from microtubules is responsible for its dissociation from Bcl-2 is an intriguing possibility to be investigated. Moreover, it is possible that the dissociation of the AMBRA1:Bcl-2 complex not only triggers autophagy at the ER via the association of Beclin 1 with AMBRA1 but also at the mitochondrial membrane where AMBRA1 is also able to interact with Beclin 1 (Strappazzon *et al*, 2011). However, this assumption would require the identification of a complex containing AMBRA1 and Beclin 1 with Atg14L, hVps34, and p150. At present, it is not known if such a complex is recruited to the outer membrane of mitochondria, which have also been proposed to be a source for autophagosomal membrane (Tooze and Yoshimori, 2010).

Another important point emerging from the study of Strappazzon *et al* (2011) is that AMBRA1 is able to regulate autophagy and modulate apoptosis via its interaction with Bcl-2 at the mitochondrial level whereas Beclin 1 regulates neither autophagy nor apoptosis at the mitochondria (He and Levine, 2010). In addition, after autophagy induction AMBRA1 is able to displace Bcl-2 from Beclin 1 (Strappazzon *et al*, 2011). The molecular details supporting this displacement remain to be investigated because Bcl-2 and Beclin 1 interact with different domains of AMBRA1.

In conclusion, this study highlights that the subcellular location of autophagy regulators has an important role in balancing autophagy and apoptosis. This special parameter needs to be added to the emerging wiring diagrams of signalling pathways linking autophagy and apoptosis (Bialik *et al*, 2010).

Acknowledgements

SAT is supported by Cancer Research UK and PC is supported by INSERM, ANR, and INCa.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Bialik S, Zalckvar E, Ber Y, Rubinstein AD, Kimchi A (2010) Systems biology analysis of programmed cell death. *Trends Biochem Sci* **35**: 556–564
- Di Bartolomeo S, Corazzari M, Nazio F, Oliverio S, Lisi G, Antonioli M, Pagliarini V, Matteoni S, Fuoco C, Giunta L, D'Amelio M, Nardacci R, Romagnoli A, Piacentini M, Cecconi F, Fimia GM (2010) The dynamic interaction of AMBRA1 with the dynein motor complex regulates mammalian autophagy. *J Cell Biol* **191**: 155–168
- He C, Levine B (2010) The Beclin 1 interactome. *Curr Opin Cell Biol* **22**: 140–149
- Matsunaga K, Morita E, Saitoh T, Akira S, Ktistakis NT, Izumi T, Noda T, Yoshimori T (2010) Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. *J Cell Biol* **190**: 511–521
- Simonsen A, Tooze SA (2009) Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. *J Cell Biol* **186**: 773–782
- Strappazzon F, Vietri-Rudan M, Campello S, Nazio F, Florenzano F, Fimia GM, Piacentini M, Levine B, Cecconi F (2011) Mitochondrial BCL-2 inhibits AMBRA1-induced autophagy. *EMBO J* **30**: 1195–1208
- Tooze SA, Yoshimori T (2010) The origin of the autophagosomal membrane. *Nat Cell Biol* **12**: 831–835
- Yang Z, Klionsky DJ (2010) Eaten alive: a history of macroautophagy. *Nat Cell Biol* **12**: 814–822