New Ca²⁺-dependent regulators of autophagosome maturation

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Autophagy is a membrane trafficking pathway responsible for the breakdown of unwanted intracellular materials and crucial for the cell healthiness and survival. In the autophagic flux, various dynamic membrane rearrangements occurs starting with the elongation of the phagophore and its closure to build an autophagosome and ending with its fusion with late endosomes and lysosomes to form an autolysosome. Although Ca²⁺ is a well established regulator of membrane fusion events, little is known about its role in these processes during autophagy. Recent studies, based on proteomic analyses of lysosomal membranes, have provided new insights into this field of study. Thus, the levels on lysosomal membranes of annexin A1, annexin A5 and copine 1, three proteins that bind to phospholipid membranes in a Ca²⁺-dependent manner, increased under nutrient deprivation, a condition that promotes autophagic degradation. In addition, two different studies showed that annexin A5 and annexin A1 are involved in autophagosome maturation. Here, we discuss the molecular mechanisms by which the fusion of autophagosomes with endosomes and lysosomes could be regulated by these three proteins and Ca²⁺.

Background

The clearance of cell components, in particular those that are damaged, is essential for cell welfare and survival. This is mainly, but not exclusively, performed by two different degradation pathways involving proteasomes or lysosomes.[1] The delivery of intracellular material to lysosomes for breakdown is principally mediated by double membrane vesicles called autophagosomes. In the last years, the origin of the autophagosomal membrane and the molecular mechanisms of autophagosome formation have been extensively analyzed and discussed. However, much less attention has been paid to later steps in the autophagic process, when autophagosomes deliver their content to acidic compartments for degradation, which are nonetheless the final destiny of the sequestered material. At this stage, autophagosomes mature by fusing with different endocytic and lysosomal vesicles, which add complexity to these fusion events.

Autophagosomal Fusion Machinery

Although the available information on this machinery is still fragmentary, several cell components have been described to be involved in the fusion of autophagosomes with endo-lysosomal compartments. For example, microtubules are thought to direct the traffic of autophagosomes toward lysosomes and endosomes.[2,3] Also SNAREs (Soluble N-ethylmaleimide-sensitive factor attachment protein receptors), proteins with a well known role in tethering/docking of vesicles in the presence of Ca²⁺,[4–6] induce, in association with the Rab7 GTPase and the HOPS (Homotypic fusion and protein sorting) complex, the fusion of autophagosomes with lysosomes.[7-10] In addition, it has been reported that the three ESCRT (Endosomal sorting complex required for transport) complexes, I–III, which were originally associated with the sorting of ubiquitinated membrane proteins into multivesicular bodies,[11-13] participate in the fusion of autophagosomes with lysosomes by mechanisms that are still unknown.[14]

Involvement of Annexins in Autophagy

Annexins are a family of ubiquitous and Ca²⁺-dependent membrane-binding proteins whose functions depend on their ability to attach to specific lipid microdomains. Using a proteomic approach, we recently identified annexin A5 as a regulator of autophagosome maturation, especially in the starvation response, where it localizes on lysosomal membranes in a Ca²⁺-dependent way.[15] Under starvation conditions, annexin A5 translocates from the Golgi complex to lysosomes and, to a lesser extent, to late endosomes. Interestingly, this protein was found to inhibit fluid phase and cholera toxin endocytosis. Since annexin A5 localizes in late endosomes considerably more than in early endosomes, it is likely that the observed inhibition of endocytosis occurs at the late steps of this process. Moreover, annexin A5 induces autophagosome fusion with lysosomes, but inhibits the formation of amphisomes, hybrid organelles produced by the fusion of autophagosomes with late endosomes. Although the molecular basis of these two opposite roles of annexin A5, activation of

autophagy and inhibition of endocytosis, remains to be elucidated, it is possible that lysosomal and late endosomal membranes have different molecular characteristics in terms of their respective mechanisms of fusion with autophagosomes. In accordance with this concept, at least one protein, Rab7, is required in autophagosome-lysosome fusions,[16] but is dispensable in fusions of autophagosomes with late endosomes.[17]

Experimental findings also support the involvement in autophagy of another protein of the same family, annexin A1. In fact, in the same proteomic analysis we found that, like annexin A5, the levels of annexin A1 increased on lysosomal membranes upon starvation.[15] Also, a different group showed that annexin A1 promotes autophagy and suggested that this protein plays a role in the formation of amphisomes.[18] Somewhat complementary to these results, a small dimeric Ca^{2+}-binding protein that can form a complex with annexin A1, S100A11, was identified as another component of the lysosomal membrane.[19]

Mounting evidences support that, in spite of their lack of transmembrane domains, various members of the annexin family can induce membrane fusions.[20-22] Thus, several studies (reviewed in Monastyrskaya et al, see ref. 23) described a Ca^{2+}-dependent role of some of these proteins in the formation and traffic of specific endo-lysosomal compartments including annexin A1 (early endosomes, multivesicular bodies and lysosomes) and annexin A5 (late endosomes and lysosomes). Since Ca^{2+} promotes the fusion of autophagosomes with lysosomes under in vitro conditions,[24] these two annexins may be involved in Ca^{2+}-regulated interactions of the autophagosomal and lysosomal membranes that finally lead to their fusion and to the delivery of the autophagosomal content to the lysosome.

Another interesting association of annexin A1 with autophagy was revealed in preliminary maps of interaction networks in autophagy, which are based on a shotgun proteomic analysis.[25] These maps identified annexin A1 as a putative interactor of Atg4B. This protease has a crucial role in the formation and maturation of autophagosomes,[26,27] because it participates in the conjugation/deconjugation of phosphoethanolamine to LC3, the mammalian ortholog of yeast Atg8. In fact, the balance between lipidation and delipidation of LC3 controls the tethering and hemifusion during closure of the autophagosomal membranes,[28] and this is an important requirement for the fusion of autophagosomes with endosomes and lysosomes that occurs later.[29] Therefore, it is tempting to speculate that annexin A1 stimulates the fusogenic potential of autophagosomes by regulating the activity of Atg4B.

Possible Involvement in Autophagy of Copine 1 in Relationship with Annexins

Copine 1 is another protein whose levels increased on lysosomal membranes under high proteolysis conditions.[15] It shares with the annexin family of proteins the property of binding to phospholipid membranes in a Ca^{2+}-dependent manner.[30] In addition, its presence in autophagosomal, phagosomal and lysosomal delimiting membranes has been previously reported.[31,32] Interestingly, an in vitro study showed that annexin A1 creates membrane domains enriched in phosphatidyl serine (PS) that assemble copine 1 aggregates. This provides a possible scaffold to cluster signaling proteins in the presence of Ca^{2+}.[33]

Also, annexin A5 is known to bind with high specificity to PS, using Ca^{2+} as a bridge between the negatively charged convex side of the C-terminal domain of the protein and the anionic phospholipid.[34] PS is known to be distributed in all cellular membranes, but it only confers a negative charge (ideal for Ca^{2+} binding) at the cytosolic face of endosomes and lysosomes. This is probably because in other organelles, such as mitochondria, Golgi and endoplasmic reticulum, PS is localized in the luminal leaflets of their membranes.[35] Although annexins A1 and annexin A5 lack a coiled-coil domain, which according to a yeast two-hybrid screening study facilitates copine 1 binding,[36] it is possible that annexin A5, like annexin A1, also forms suitable domains for copine 1 recruitment, after its translocation under high proteolysis conditions from cytosol to lysosomal membranes, to facilitate the fusion of lysosomes with autophagosomes,

Conclusions and Future Work

Annexin A1, annexin A5, and probably also copine 1, emerge as possible regulators of autophagosome maturation by mechanisms that require Ca^{2+}. Both annexins could interact on the lysosomal surface with copine 1, by mechanisms regulated by phospholipid rearrangements and Ca^{2+} (see **Fig. 1**), to promote the fusion of autophagosomes with lysosomes.

To determine which of the proposed molecular mechanism are involved in this process, it will be necessary to identify other proteins and, perhaps more important, the specific lipids that interact with annexins A1 and annexin A5 and also with copine 1 at the cytosolic surface of the endo-lysosomal membranes. In particular, it would be interesting to find differences in the interacting proteins and lipid domains between lysosomes and late endosomes and also to verify whether these interactions produce phospholipid rearrangements at specific lipid domains.
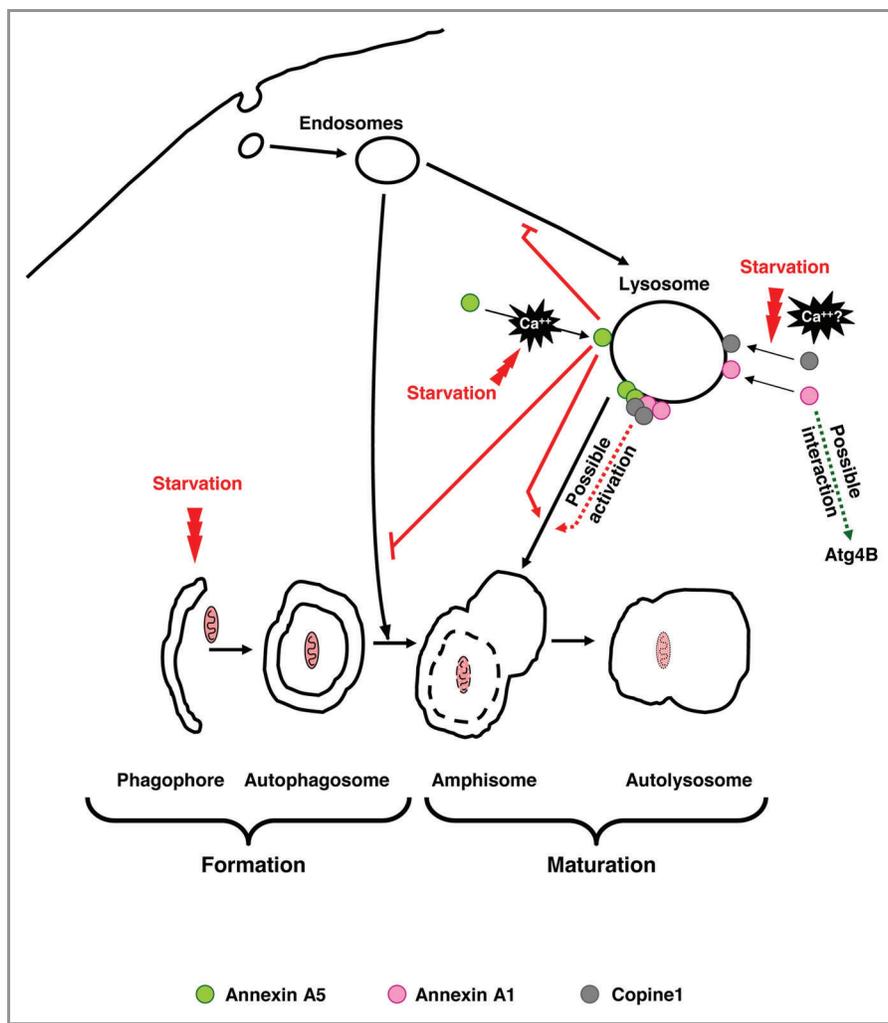
Figure 1. Possible mechanisms for the involvement of annexin A5, annexin A1 and copine 1 in autophagosome maturation: The sequence of the different steps in autophagy is shown below. Starvation induces annexin A5 translocation to lysosomal membranes in a Ca^{2+}-dependent way. This protein inhibits (indicated by the blunted red lines) endocytosis and amphisome formation and induces (red arrow) autophagosome fusion with lysosomes. Likewise, two other Ca^{2+}-dependent phospholipid binding proteins, annexin A1 and copine 1, are localized on lysosomal membranes under starvation. It is likely that annexin A5 and annexin A1 aggregate on lysosomal membranes in a Ca^{2+}-dependent way in order to form domains for the subsequent binding of copine 1, and that interactions between these and perhaps other proteins promote the fusion of autophagosomes with lysosomes. An additional possibility, at least for annexin A1, is that its interaction with Atg4B regulates the activity of this protease in the lipidation/delipidation of LC3 and thus contributes to increase the ability of autophagosomal membranes to fuse with lysosomes.

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