Macroautophagy and endocytosis are two evolutionarily conserved catabolic processes that comprise vesicle trafficking events for the clearance of the sequestered intracellular and extracellular cargo, respectively. Both start differently: formation of a new organelle, the autophagosome, that engulfs cytoplasmic substrates (macroautophagy) and internalization of extracellular material and plasma membrane components within endocytic vesicles (endocytosis). However, they end in the same compartment, the lysosome.

In a proteomic analysis of purified lysosomal membranes from mouse fibroblasts, three Ca^{2+}-dependent phospholipid-binding proteins were found to increase their levels on these membranes under amino acid starvation, a condition that activates macroautophagy. Prompted by this initial finding and given that Ca^{2+} is an important second messenger, we sought to gain insights into the involvement of Ca^{2+} in the regulation of macroautophagy by amino acid starvation and the role of Ca^{2+}-binding proteins in macroautophagy.

We describe a Ca^{2+}-dependent signalling pathway that triggers the formation of autophagosomes. Thus, withdrawal of essential amino acids leads to an increase in cytosolic Ca^{2+}, arising from both extracellular medium and intracellular stores, which induces, *via* Ca^{2+}/calmodulin-dependent kinase kinase-β, the activation of adenosine monophosphate-activated protein kinase and the inhibition of the mammalian target of rapamycin complex 1. In the final step of this pathway, UNC-51-like kinase, a mammalian autophagy-initiating kinase, is activated and this leads to the formation of autophagosomes.

Annexin A1, annexin A5 and copine 1 are the three Ca^{2+}-dependent phospholipid binding proteins whose levels on lysosomal membranes increased under amino acid starvation in the proteomic analysis. Biochemical and immunofluorescence methods show that starvation causes the Ca^{2+}-dependent translocation of annexin A5 from Golgi complex to

lysosomal membranes and that it also induces the accumulation of annexin A1 and copine 1 in this localization. Using overexpression and silencing experiments, we found that all three proteins induce autophagosome fusion with lysosomes and that copine 1, and to a lesser extent annexin A1, enhance the effect of annexin A5 in this process. Moreover, annexin A5 inhibits endocytosis whereas copine 1 induces it. Thus, annexin A1, annexin A5, and copine 1 emerge as regulators of autophagosome maturation and the last two have opposite roles in endocytic trafficking.

Overall, our results highlight that the activation of autophagosome formation by the starvation of amino acids follows in part a Ca^{2+}-dependent signalling pathway and that this condition also activates the maturation of autophagosomes to autolysosomes through Ca^{2+}-binding proteins.