Live cell imaging reveals PI(3)P-enriched ER subdomains with a role in autophagosome biogenesis

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The term autophagy (literally meaning “to eat oneself”) is widely used to describe an important evolutionarily conserved intracellular process whereby cells sequester proteins and organelles in a double-membrane enclosed vesicle targeted to the lysosome for degradation (1).

Autophagy occurs at a basal level and is important for the recycling of long-lived proteins and organelles. However, under certain physiological conditions such as starvation, the rate at which autophagy occurs can dramatically increase and is an important response to enable cell survival. Deregulation of autophagy has been implicated in developmental defects, neurodegeneration and cancer (1).

Although the process of autophagy is well understood and major components of the pathway have been identified, there are still fundamental questions which remain to be answered. One such question is what is the source of the membrane used to form the double-membrane enclosure of the autophagocytic vesicle (2)?

We have used a variety of imaging techniques to try and address this question. We have generated a clonal HEK 293 cell line stably expressing a low level of GFP-tagged DFCP1. This protein contains twin FYVE domains and is know to bind PI(3)P. During amino acid starvation the cytosolic/ER localised GFP-DFCP1 became concentrated at discrete foci distributed throughout the cell. These foci grew into small rings which collapsed and disappeared. Total Internal Reflection Fluorescence microscopy revealed that the foci formed on the ER and that the rings grew out from the ER. Co-expression of the autophagocytic marker protein LC3 revealed an intimate and dynamic interaction between LC3 particles and the DFCP1-labelled compartment. This was confirmed by deconvolution of widefield images which revealed the LC3 to be enveloped by the growing and collapsing ring.

We conclude that DFCP1 labels a specific pool of PI(3)P present on the ER which is destined to become the autophagosomal double membrane.
