Origins reticulum (er), mitochondria and the plasma membrane

Sociology, Identity

Origins of the autophagosome: biogenesis of the contributingmembranes. The formation of theautophagosome has long been a widely debated cellular process, with theidentity of contributing membranes and cellular components involved unclear. A recentmodel has proposed that autophagosomal membrane formation is initiated throughthe complex contribution of components localised to the membrane of twoorganelles, with localised regulation of phosphatidylinositol-3-phosphate(PI3P) playing a key role. Keywords: autophagosome, endoplasmic reticulum, plasma membrane, biogenesis, localised regulation, phosphatidyl-inositol-3-phosphate. Autophagy is a key cellular process involved in thedegradation of intracellular complexes, and is a critical for cellularhomeostasis (1).

The formation of the autophagosomal structure is an importantmechanistic step in macroautophagy, however the exact signalling networks andmembrane components involved is not wholly understood (2). The biogenesis ofthe autophagosomal membrane is complex and the membrane contribution of severalorganelles have been implicated in this process, however this is widely debatedamongst researchers. The initiation of autophagy involves two major proteincomplexes: the unc51-like autophagy activating kinase 1 (ULK1) protein kinasecomplex and the class III phosphatidylinositol-3-kinase complex I (PI3KC3-C1)lipid kinase complex (3). The components of these complexes are largely wellcharacterised and composed of a small number of catalytic subunits, with a considerableproportion of the complexes providing a structural role. However, in comparisonto the molecular components involved, there is

dispute over the membrane sourceof origin of the autophagosomal structure. The autophagosomal initiationpathways under different autophagy-inducing conditions is providing wide debate. Previous published evidence has linked the endoplasmic reticulum (ER), mitochondria and the plasma membrane (PM) as possible organelle membranesources for autophagosome formation (2).

Independent models for autophagosome formation have beenproposed by various researchers whom have provided evidence to support modelsfor single organellar membrane contribution as the origin of the autophagosome. In contrast to this, Hamasaki et al.(4) proposed a two organellar model, based on the presence of ATG14 (AutophagyRelated Gene 14; marker of pre-autophagosome/autophagosome structures) atER-mitochondria contact sites which lead to the subsequent formation of ATG5(Autophagy Related Gene 5; marker of autophagosome formation) puncta underconditions of cellular starvation. They suggested that this indicates that theER-mitochondria contact site provides the site of biogenesis for autophagosomeformation, illustrated in Figure 1A. During autophagy, phosphatidylinositol-3-phosphate(PI3P) is present in the omegasome region of the ER, and has been attributed tothe signalling network involved in autophagosomal biogenesis (5). Nascimbeni et al.(6) have identified a role for ER-PM contact sites in the formation ofautophagosomal structures, through the interplay with PI3P regulation anddirect interaction with the tethering properties of extended synaptotagmins(E-Syts), as shown in Figure 1B. This was partially identified usingsuper-resolution two-colour stimulated

emission depletion (STED) microscopy, which aided in the positive identification of early autophagic andautophagosomal structures within the vicinity of the ER-PM contact sites.

Additionally, the role of E-Syts in autophagosome biogenesis was further supported though thepresence of early autophagic markers at sites containing E-Syt proteins. Furtherto this, Nascimbeni et al. (6) showedthat over-expression of E-Syt2/3 resulted in induction of autophagosomeformation, whereas in E-Syt deficient cells, as generated through siRNAknockdown via transfection, reduced autophagosome formation was seen. This datafurther supported the proposed notion for the functional role of the E-Sytclass proteins in the formation of autophagosomal structures. Moreover, the results indicate a role for PI3P synthesisthrough E-Syt function, near to the ER-PM contact sites via recruitment of VacuoleMembrane Protein 1 (VMP1) (6). VMP1 has been shown to be involved in theformation of the PI3K/Beclin-1 complex, as well as the positive recruitmentregulator of the autophagosome initiator proteins ATG14L1 and LC3, hence itsidentified role as a positive inducer of autophagosomal membrane formation (7).

It has been shown through siRNA knockdown and confocal microscopy that in cellsdeficient in E-Syt proteins under autophagy inducing conditions, the amount ofPI3P structures is reduced (6). Additionally, through co-transfection, confocalmicroscopy, western blotting and co-immunoprecipitation, the authors were ableto establish a co-distribution between VMP1 and E-Syt2/3 during the formationof the autophagosomal

structure, as well as establishing an interaction atER-PM contact sites between E-Syt2/3 with VMP1 and Beclin-1 (6). It has been previously shown that ER-mitochondria contactsites are under regulation of basal autophagy (8), whereas the E-Syt tetheringin ER-PM contact sites appear to be important in response to stress-inducedautophagy (6). In addition to this, the precise domain of the E-Syt proteinsresponsible for ER-PM contact site tethering is undefined (9).

In theNascimbeni (6) study, the C2C domain was shown to be critical in this process, however the SMP domain of E-Syts have previously identified to be involved inthe transfer of lipids (10). Further exploration of the E-Syt domain functionmay reveal the exact mechanistic role of E-Syt proteins in autophagosomalmembrane formation. It has been proposed by Nascimbeni et al. (6) that the origin of the autophagosomal membrane will bedependent upon the autophagic-inducer, as this may dictate the localisation ofthe autophagy-dependent synthesis of PI3P pool within the cell, as well as thesubsequent formation of the initiator complex. This proposal may be the focusof the next experimental strategies in advancing understanding of the origin, regulation, and signalling networks involved in autophagosomal membraneformation, as well as the impact on cellular autophagy. Inconclusion, the data presented by Nascimbeni et al.

(6) provides evidence to suggest the functional role ofE-Syt proteins in the formation of ER-PM contact sites, with the controlledregulation of PI3P synthesis allowing for the formation of the autophagosomalstructures at these double membrane contact sites. This furthers understandingas to the

origin of the autophagosomal membrane, and eludes the necessity ofcontact sites between two membranes in order for the initiation ofautophagosome formation to occur. This may provide the fundamental knowledge toexplore this cellular mechanism further, and may be a future therapeutic targetfor a number of diseases, in which the dysregulation of autophagy is implicatedin the disease pathology.