Autophagy is the major degradative process in eukaryotic cells that involves degradation of unnecessary or dysfunctional cellular components and even whole cell organelles. The autophagosomal mechanism is crucial for the cells since the inability to degrade defective organelles, clear microbes or remove harmful protein aggregates will result in the onset of diseases. Autophagy begins with the formation of the phagophore, a flat membrane cistern that enwraps portion of cell cytoplasm with/without cell organelles. When phagophore membrane achieves complete closure around the cargo, a double membrane vesicle, termed the autophagosome, is formed. The autophagosomes are then further maturated by fusing with lysosomes forming autolysosomes. The autolysosome degrades the sequestered cargo by the lysosomal hydrolases and degradation products are then recycled back to the cytoplasm. A consensus is emerging that the phagophore is interconnected with the endoplasmic reticulum (ER) and nucleates from a subdomain of the ER termed the omegasome [1]. However, several other organelles such as the mitochondria, Golgi complex, plasma membrane and lysosomes have also been linked with phagophore formation.

Here we will report on the findings that serial block face scanning electron microscopy (SB-EM) and electron tomography (ET) are offering in the field of phagophore biogenesis. SB-EM allows the generation of a three dimensional ultrastructural overview of cells revealing the occurrence and distribution of autophagosomes in large number of cells, as well as the organelles in close proximity to the autophagosomes (Figure 1). The actual membrane contact sites are below the resolution limit of SB-EM method but they can be revealed by utilizing ET (Figure 2) [2]. As SB-EM allows analysis of proximity in large volumes and ET specific contact sites of membrane bound organelles, these two methods nicely complement each other. As a result, this investigation has identified membrane contact sites between the phagophore and membranes originating from the mitochondria, ER exit site, Golgi complex and late endosomes/lysosomes. Identification of direct membrane contact between the phagophore membrane and adjoining organelles has potential to direct future research in membrane flux experiments to help determine whether membrane contacts also signify lipid translocation between the phagophore and the aforementioned organelles.


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Fig. 1: SB-EM indicates the presence of ER in close vicinity of the forming autophagosomes. NRK-52E cells were starved for 1 h and prepared for EM using chemical fixation. The block face was imaged at nominal magnification of 3500x with a voxel size of 11 x 11 x 40 nm$^3$.

Fig. 2: The phagophore and ER contact sites as revealed by ET. Tilt series were acquired at 14,500x or 19,000x nominal magnification and the membrane contact sites were primarily found between the forming phagophore membrane and the ER membrane located inside of forming autophagosome.