Sensing Membrane Curvature in Macroautophagy

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Abstract

In response to intracellular stress events ranging from starvation to pathogen invasion, the cell activates one or more forms of macroautophagy. The key event in these related pathways is the de novo formation of a new organelle called the autophagosome, which either surrounds and sequesters random portions of the cytoplasm or selectively targets individual intracellular challenges. Thus, the autophagosome is a flexible membrane platform with dimensions that ultimately depend upon the target cargo. The intermediate membrane, termed the phagophore or isolation membrane, is a cup-like structure with a clear concave face and a highly curved rim. The phagophore is largely devoid of integral membrane proteins; thus, its shape and size are governed by peripherally associated membrane proteins and possibly by the lipid composition of the membrane itself. Growth along the phagophore rim marks the progress of both organelle expansion and ultimately organelle closure around a particular cargo. These two properties, a reliance on peripheral membrane proteins and a structurally distinct membrane architecture, suggest that the ability to target or manipulate membrane curvature might be an essential activity of proteins functioning in this pathway. In this review, we discuss the extent to which membranes are naturally curved at each of the cellular sites believed to engage in autophagosome formation, review basic mechanisms used to sense this curvature, and then summarize the existing literature concerning which autophagy proteins are capable of curvature recognition.

The Phagophore Is an Autophagic Intermediate with a Unique Membrane Architecture

The mechanistic details of autophagosome formation are only beginning to emerge, but the unique morphologies of the organelle and its intermediates have long been apparent by electron microscopy (EM). Mature autophagosomes were readily observed as early as the 1960s and defined as double-membrane vesicles filled with cytoplasmic material (e.g., Ref. [1]). Conditions that lead to the enrichment of these structures, such as starvation, also lead to the accumulation of other objects that were inferred to be autophagosome intermediates. The most striking structural intermediate is a cup-shaped double membrane called the phagophore or isolation membrane (IM; Fig. 1). In EM sections, the phagophore appears as a semicircular cisterna from 0.3 to 3 μm in diameter. The wall of the structure is composed of two membrane bilayers in very close apposition; the continuity of the convex- and concave-facing phagophore membranes generates a subregion that we call the phagophore rim (Fig. 2).

Topologically, this arrangement is reminiscent of multivesicular bodies during luminal vesicle budding events; however, the very close apposition of the two autophagosome bilayers suggests that the curvature at the phagophore rim is particularly dramatic. We have conducted a survey of the autophagosome literature in which EM was used to image these structures arising from a wide range of different model systems in an attempt to estimate typical dimensions of the phagophore (Table 1). EM from many groups using varying fixation and preservation strategies suggests that the separation between membranes in the luminal space of the phagophore is less than 30 nm (the limit of measure we can easily make in reproduced images from publications) and, in many cases, is essentially

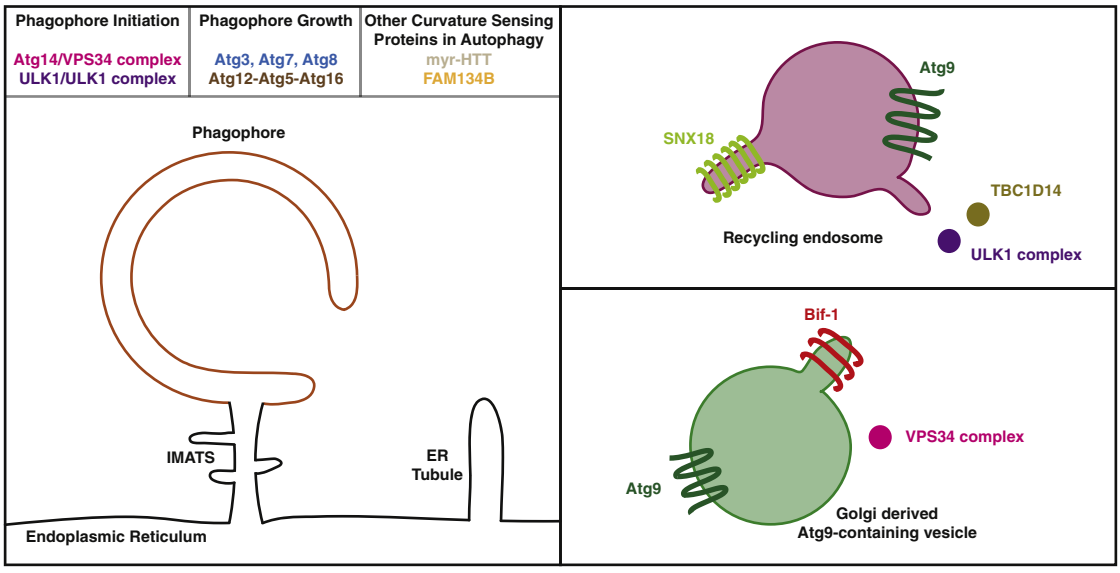

Fig. 1. Sources of membrane curvature associated with macroautophagy in the mammalian cell. Autophagosome biogenesis involves many structures that present strident positive curvature to the cytoplasm including very small vesicles, ER tubules, tubular protrusions from very small recycling endosome and Golgi vesicles, and the rim of the expanding phagophore (see Table 1). Proteins associated with each step in the organelle's maturation have been shown to possess membrane curvature sensing *in vitro* and rely on these motifs for proper function *in vivo*.

not detectable (e.g., Ref. [2]). Thus, the radius of curvature at the rim in these same structures may also be less than 30 nm (Fig. 2) and often appears to be at or below the limiting radius of curvature for a protein-free bilayer (Table 1) [3].

More recent studies using electron tomography have unambiguously confirmed the phagophore's bowl-like structure. In addition to corroborating previous observations, these studies also identify short, narrow tubular connections between the endoplasmic reticulum (ER) and the phagophore, termed “IM-associated tubular/vesicular structures” (IMATs shown in Fig. 1) [4]. Like the rim of the phagophore, these membrane structures are also highly curved (~30 nm diameter). Their direct

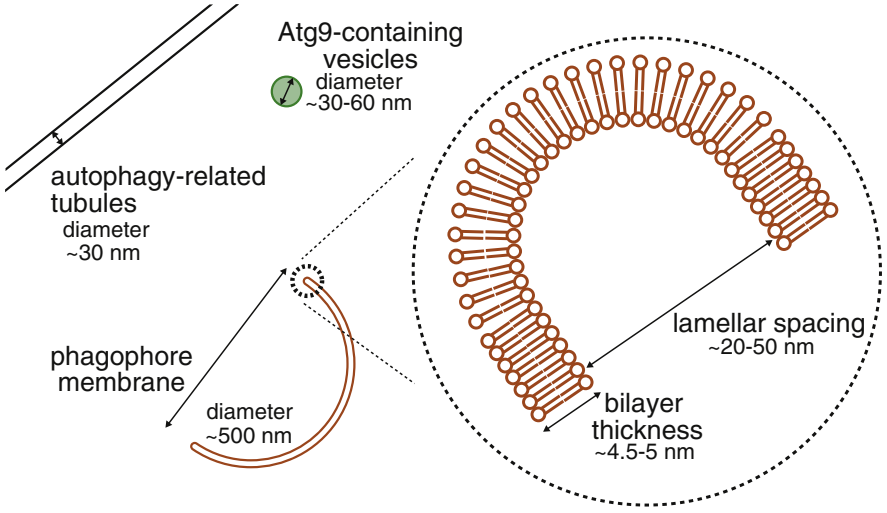

Fig. 2. Phagophore dimensions. The phagophore or isolation membrane is a cup-shaped intermediate in autophagosome development. It includes a concave inner and convex outer surface and a highly curved rim running along the open edge of the cup. Labeled are the diameters of several autophagy-related membrane structures exhibiting high curvature. In addition, we note the typical distances between the bilayers in the growing isolation membrane or the mature autophagosome (lamellar spacing). Although this distance does not directly represent a curvature, it sets a kind of lower bound for the radius of curvature connecting the two bilayers at the rim. In practice, actual measures of rim curvature are mostly not available. We have surveyed a representative set of articles in the literature to generate the range of measures in Table 1.