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Role of Human WIPIs in Macroautophagy

Tassula Proikas-Cezanne and Daniela Bakula

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1. Introduction

Eukaryotic cellular homeostasis is critically secured by autophagy, a catabolic pathway for the degradation of cytoplasmic material in the lysosomal compartment. Macroautophagy, one of the major autophagic pathway, is initiated upon PtdIns(3)P generation by activated PtdIns3KC3 in complex with Beclin 1, p150 and Atg14L. Subsequently, specific PtdIns(3)P-effector proteins permit the formation of double-membrane vesicles, autophagosomes, that sequester the cytoplasmic material. Autophagosomes then communicate and fuse with the lysosomal compartment for final cargo degradation. Members of the human WIPI family function as essential PtdIns(3)P-binding proteins during the initiation of macroautophagy downstream of PtdIns3KC3, and become membrane proteins of generated autophagosomes. Here, we discuss the function of human WIPIs and describe the WIPI puncta-formation analysis for the quantitative assessment of macroautophagy.

Autophagy (auto phagia: *greek*, self eating) is an ancient cellular survival pathway specific to eukaryotic cells. By promoting a constant turn-over of the cytoplasm, the process of autophagy coevolved with the endomembrane system to secure the functionality of organelles. Primitive eukaryotic cells employed the autophagic pathway to survive periods of nutrient shortage and to degrade invading pathogens [1,2]. The survival function of autophagy has been experimentally proven by landmark studies such as the analysis of essential autophagic factors in *C. elegans*, demonstrating that autophagy defines the lifespan of eukaryotic organisms [3], and the characterization of mice deficient for essential autophagic factors, demonstrating that autophagy functions to compensate for nutrients and energy during the post-natal starvation period [4].

The survival function of autophagy is based on the three major autophagic pathways, macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) that coexist in eukaryotic cells [5]. In the process of microautophagy, proteins or organelles are non-

selectively engulfed by the lysosome through lysosomal membrane invagination and vesicle scission [6]. CMA specifically targets cytoplasmic proteins containing the KFERQ-like consensus motif for an Hsp70-assisted transport to the lysosomal compartment and an LAMP2-assisted import into the lysosomal lumen in higher eukaryotic cells [7]. The process of macroautophagy is hallmarked by the formation of autophagosomes, double-membrane vesicles that sequester the cytoplasmic cargo (membranes, proteins, organelles) and that communicate with the lysosomal compartment for final degradation. Constitutively active on a low basal level, macroautophagy stochastically clears the cytoplasm and promotes the recycling of its constituents. In addition, upon a variety of cellular insults that lead to organelle damage and protein aggregation, macroautophagy is specifically induced and engaged to counteract toxicity.

The cytoprotective function of the three major forms of autophagy critically prevent the development of a variety of age-related human diseases, including cancer and neurodegeneration. However, autophagic pathways also play a vital role in the manifestation of certain pathologies, thus it is of urgent interest to monitor and understand the differential contribution of autophagic pathways to both human health and disease [5].

2. The process of macroautophagy

Central to the process of macroautophagy is the formation of autophagosomes that sequester and carry the cytoplasmic cargo – membranes, proteins and organelles - to the lysosomal compartment for subsequent degradation and recycling (Figure 1). For decades, the membrane origin of autophagosomes was uncertain [8]. Recently, a variety of independent studies provided evidence that multiple membrane sources should in fact become employed for the formation of autophagosomes [9]. Upon a hierarchical recruitment of autophagy-related (Atg) proteins [10], membrane origins are thought to undergo membrane rearrangements, including the formation of ER-associated omegasome structures [11], from which autophagosomal precursor membranes (phagophores) emerge [12,13]. By communicating with the endosomal compartment, the phagophore membrane is proposed to elongate and close to form the autophagosome that robustly sequesters the cytoplasmic cargo within a double-membraned vesicular structure [13]. Next, autophagosomes mature through communication with the endosomal/lysosomal compartment and the degradation of the sequestered cargo occurs in autolysosomes, fused vesicles of autophagosome and lysosomes [13]. Interestingly, *kiss and run* between autophagosomes and lysosomes has also been demonstrated to contribute to cargo final degradation [14].

The level of autophagosome formation is crucially balanced by the activity of the mTOR complex 1 (mTORC1), which inhibits macroautophagy when positioned at peripheral lysosomes and which releases its inhibitory role when positioned at perinuclear lysosomes [15] (Figure 2). The inhibition of mTORC1 mediates the activation of phosphatidylinositol 3-kinase class III (PtdIns3KC3 or Vps34) that phosphorylates PtdIns to generate PtdIns(3)P, an essential phospholipid for the forming autophagosomal membrane [16].

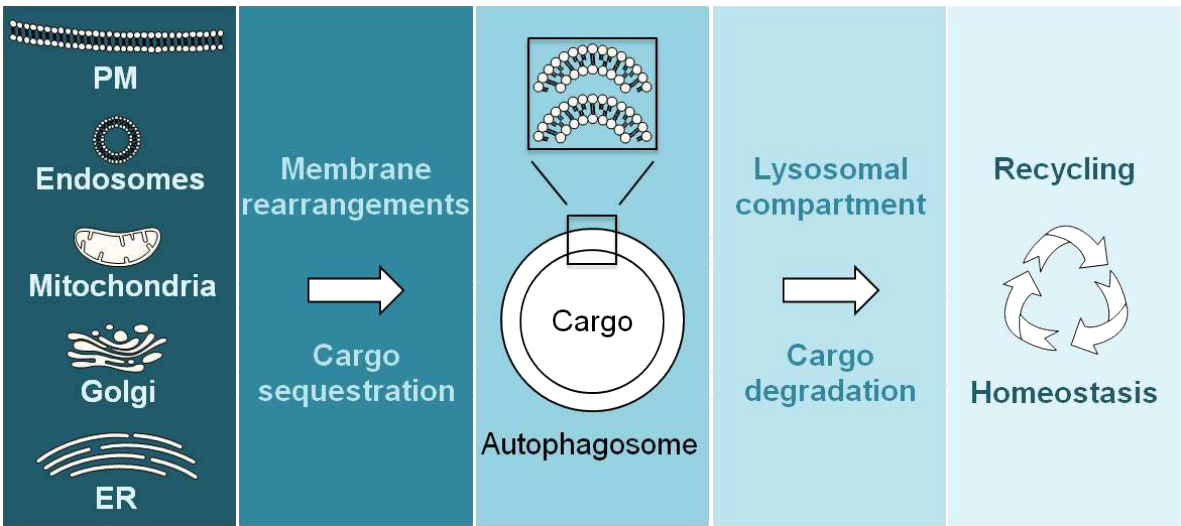


Figure 1. An overview of the process of macroautophagy.

PtdIns3KC3 functions in canonical macroautophagy in complex with Beclin 1 (Atg6 in yeast), p150 (or Vps15) and Atg14L [17], the latter recruiting PtdIns3KC3 to the ER [18] where membrane rearrangements are initialized by the PtdIns(3)P effector proteins DFCP1 [11] and WIPIs [19,20]. Macroautophagy can also be induced by non-canonical entries, e.g. independent of Beclin 1 [20,21,22].

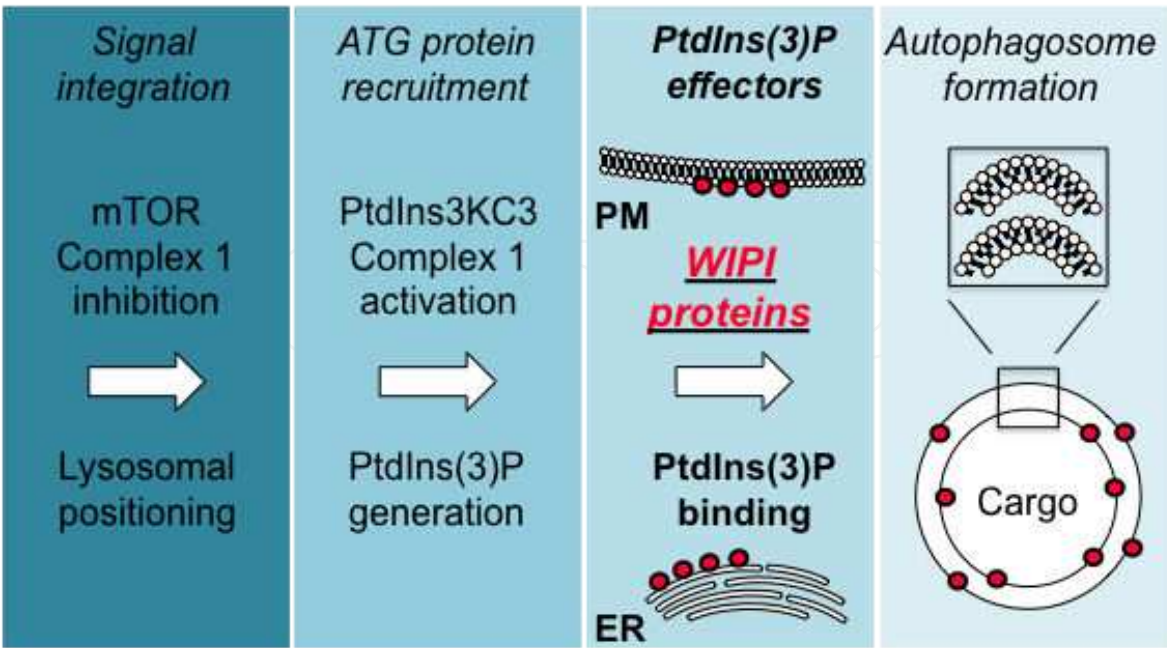


Figure 2. Evolutionarily conserved WIPIs function as essential PtdIns(3)P effectors to regulate macroautophagy.

3. Human WIPIs

By screening for novel p53 inhibitory factors, we identified a partial, uncharacterized cDNA fragment [23] and subsequently cloned the corresponding full-length cDNA from normal human liver and testis [19]. Using BLAST it became apparent that the isolated cDNA should be part of a novel human gene and protein family consisting of four members, which we subsequently cloned from normal human testis and placenta [19]. We proposed to term this novel human family WIPI (WD-repeat protein interacting with phosphoinositides) based on the following findings. First, the primary amino acid sequence suggested that the WIPIs contain seven WD40 repeats [19,24] that should fold into 7-bladed beta propeller proteins with an open Velcro configuration, as shown by structural homology modeling [19]. Second, WIPIs represent novel domains that specifically bind to PtdIns(3)P and PtdIns(3,5)P₂ [19,24,25,26]. Third, a comprehensive bioinformatic analysis demonstrated that the human WIPI family identified belongs to an ancient protein family of 7-bladed beta propellers with two paralogous groups, one group containing human WIPI-1 and WIPI-2, and the other group containing WIPI-3 and WIPI-4 [19,26]. Jeffries and coworkers found that WIPI-1 (WIPI49) should function in mannose-6-phosphate receptor trafficking [24], and our own studies demonstrated that WIPI-1 functions during macroautophagy in human tumor cells [19].

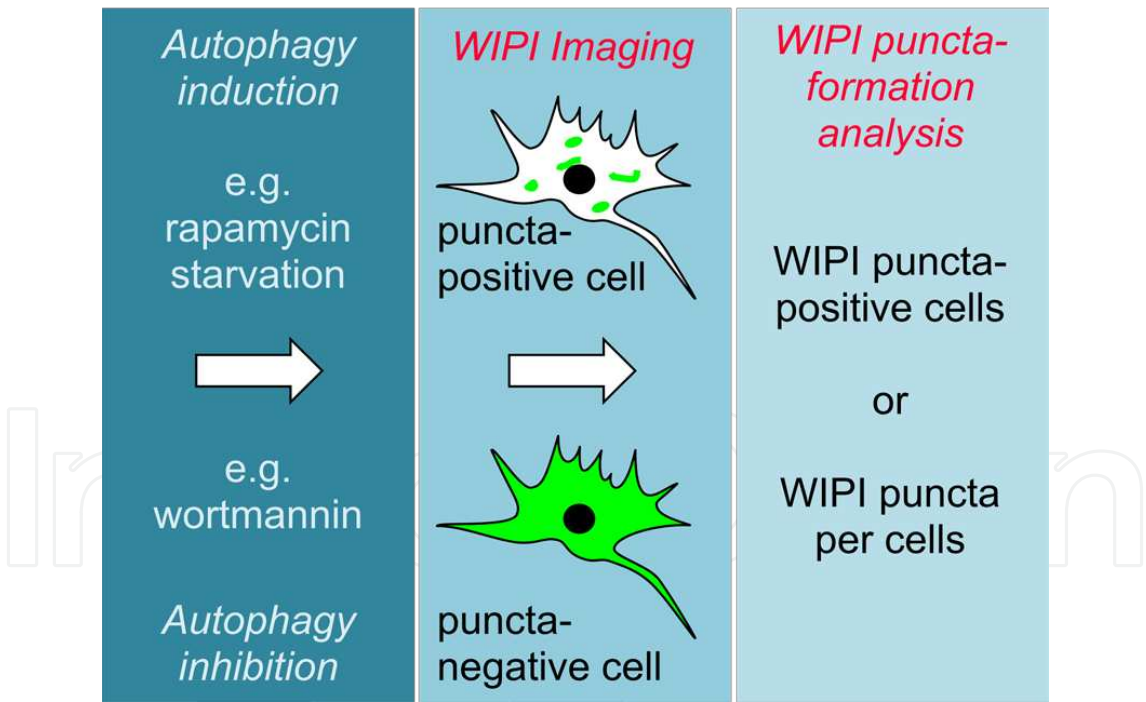


Figure 3. Assessing macroautophagy by WIPI puncta-formation analysis.

All human WIPI genes are ubiquitously expressed in normal human tissue, but show high levels in skeletal muscle and heart [19]. Moreover, in a variety of human tumor types the abundance of all WIPI genes was shown to be aberrant when compared to matched normal samples from the same patient; WIPI-1 and WIPI-3 seemed to be more abundant, and WIPI-2

and WIPI-4 less abundant in the tumor [19]. In human tumor cell lines the abundance of the four WIPIs also differs [19,26]. However, the contribution of WIPIs in tumor formation is as yet uncharacterized.

During the process of macroautophagy, essential PtdIns(3)P effector functions (Figure 2) have been assigned to members of the human WIPI family [10,19,22,26,27,28], according to the ancestral function of yeast Atg18 [25,29,30,31,32]. Moreover, human WIPIs were also shown to be involved in pathogen defense by promoting the degradation of internalized bacteria in the lysosomal compartment [33,34,35], and to further contribute to Parkin-mediated mitophagy [36].

Upon the initiation of autophagy (Figure 2) WIPI-1 and WIPI-2 specifically bind to generated PtdIns(3)P at phagophore membranes [10,19,26,37]. In addition, WIPI-1 and WIPI-2 also bind, although to a lesser extent, to PtdIns(3,5)P₂ [24,26,37], however with unknown functional consequences. Phospholipid binding is mediated by evolutionarily conserved amino acids positioned in blade 5 and 6 of the beta-propeller structure of human WIPI proteins [19] and yeast homologs [38,39,40]. Further, WIPI-1 and WIPI-2 act as PtdIns(3)P effectors upstream of both the Atg12 and LC3 ubiquitin-like conjugation systems, hence regulate LC3 lipidation [10,22,26,37] which is required for the elongation of the phagophore. Moreover, both WIPI-1 and WIPI-2 become membrane proteins of formed autophagosomes and probably also of autolysosomes [41].

From the further specific localization of WIPI-1 and WIPI-2 upon the induction of macroautophagy, conclusions about the membrane origin of WIPI-positive autophagosomes can be concluded (Figure 2): i) as WIPI-1 specifically accumulates at the ER and at the plasma membrane (PM) upon starvation-induced macroautophagy, both of these membrane systems might contribute to phagophore and autophagosome formation, ii) as WIPI-2 also accumulates at the plasma membrane upon starvation, and in addition to membranes close to the Golgi, a differential engagement of particular membrane systems for autophagosome formation might be mediated by the different WIPIs [41].

4. WIPI-1 puncta-formation analysis

The specific protein localization of WIPI-1 at both phagophores and autophagosomes has been employed for the quantitative assessment of macroautophagy in mammalian cells [37] and extended for usage of automated fluorescent image acquisition and analysis [22,34,42]. Upon the induction of macroautophagy, e.g. by rapamycin administration or starvation (Figure 3), WIPI-1 accumulates at autophagosomal membranes, termed puncta. Upon the inhibition of autophagy, e.g. by wortmannin treatment, WIPI-1 is distributed throughout the cytoplasm. Under nutrient-rich conditions few WIPI-1 puncta-positive cells are observed and this assessment reflects basal macroautophagy. To visualize endogenous WIPI-1, indirect immunofluorescence with specific anti-WIPI-1 antibodies is conducted. Alternatively, overexpressed WIPI-1 fusion proteins, e.g. tagged to GFP, can also be employed to quantify the status

of macroautophagy. Both, the number of cells displaying WIPI-1 puncta and the number of WIPI-1 puncta per cell can be used to assess macroautophagy [43,44].

5. Outlook

The notion that human WIPIs function as essential PtdIns(3)P effectors in macroautophagy needs to be addressed in more molecular detail as follows: i) analysing the individual contribution of the WIPIs to phagophore formation, ii) defining the function of WIPIs at autophagosomes and autolysosomes and iii) identification of WIPI interacting proteins and the signaling network regulating the PtdIns(3)P effector function of WIPIs. As WIPIs are aberrantly expressed in human tumors, the role of WIPIs during tumorigenesis, in particular the regulation of gene expression in normal and tumor cells is of further current interest. Moreover, the identification of compounds that permit a direct interference with the specific binding of WIPIs to PtdIns(3)P might become suitable in the future to specifically modulate macroautophagy in anti-tumor therapies.

Abbreviations

ATG, autophagy related; CMA, chaperone-mediated autophagy; PtdIns(3)P, phosphatidylinositol 3-phosphate; PtdIns(3,5)P₂, phosphatidylinositol 3,5-bisphosphate; PtdIns3KC3, phosphatidylinositol 3-kinase class III; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; WIPI, WD-repeat protein interacting with phosphoinositides.

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Author details

Tassula Proikas-Cezanne and Daniela Bakula

*Address all correspondence to: tassula.proikas-cezanne@uni-tuebingen.de

Autophagy Laboratory, Department of Molecular Biology, Interfaculty Institute for Cell Biology, Eberhard Karls University Tuebingen, Germany

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