# Autophagy and microtubules – new story, old players

**Rafah Mackeh[1], Daniel Perdiz[1], Séverine Lorin[1], Patrice Codogno[2,*] and Christian Poüs[1,3,*]**

[1]UPRES EA4530, Université Paris-Sud, Faculté de Pharmacie, 92296 Châtenay-Malabry, France
[2]INSERM U845, Université Paris Descartes, 75014 Paris, France
[3]Biochimie-Hormonologie, AP-HP, Hôpital Antoine Béclère, 92141 Clamart, France
*Authors for correspondence (patrice.codogno@u-psud.fr; christian.pous@u-psud.fr)

*Journal of Cell Science 126, 1071–1080*

*doi: 10.1242/jcs.115626*

## Summary

Both at a basal level and after induction (especially in response to nutrient starvation), the function of autophagy is to allow cells to degrade and recycle damaged organelles, proteins and other biological constituents. Here, we focus on the role microtubules have in autophagosome formation, autophagosome transport across the cytoplasm and in the formation of autolysosomes. Recent insights into the exact relationship between autophagy and microtubules now point to the importance of microtubule dynamics, tubulin post-translational modifications and microtubule motors in the autophagy process. Such factors regulate signaling pathways that converge to stimulate autophagosome formation. They also orchestrate the movements of pre-autophagosomal structures and autophagosomes or more globally organize and localize immature and mature autophagosomes and lysosomes. Most of the factors that now appear to link microtubules to autophagosome formation or to autophagosome dynamics and fate were identified initially without the notion that sequestration, recruitment and/or interaction with microtubules contribute to their function. Spatial and temporal coordination of many stages in the life of autophagosomes thus underlines the integrative role of microtubules and progressively reveals hidden parts of the autophagy machinery.

**Key words:** Microtubule, Macroautophagy, Phagocytosis, Phagophore, Cell signaling, Lysosome, Acetylation, Molecular motor

## Introduction

Autophagy is a catabolic process that leads to the degradation of cytoplasmic components by lysosomes. There are several types of autophagy, such as macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) (for a review, see Mizushima and Komatsu, 2011). Here, we will focus on macroautophagy (hereafter referred to as autophagy). The initial step of autophagy is the formation of a cup-shaped structure, named the phagophore (Fig. 1A). In most cell types, autophagy proceeds at a basal rate to ensure a level of quality control of the cytoplasm and to maintain cellular homeostasis, by eliminating long-lived or aggregated proteins and damaged organelles, including mitochondria (for a review, see Yang and Klionsky, 2010). However, under several stress conditions (e.g. metabolic stress, hypoxia, chemotherapy), autophagy is greatly induced to cope with the stress and to fulfil the energetic or biosynthetic demand, thereby allowing cell survival (for reviews, see Kroemer et al., 2010; Singh and Cuervo, 2011; Yang and Klionsky, 2010). Autophagy not only has an important physiological role in stress management but is also involved in development and immunity (reviewed by Deretic and Levine, 2009; Levine and Klionsky, 2004). Deregulation of autophagy leads to a wide range of disorders, such as cancers, neurodegenerative disorders, heart and liver diseases, myopathies and ageing (Ravikumar et al., 2010; Rubinsztein et al., 2011).

The autophagosome membrane elongates to surround the cytoplasmic material to be degraded (e.g. proteins, lipid droplets, organelles and pathogens), before it expands, curves and then closes to form a double-membrane vesicle. The sequestered material is then degraded by acid hydrolases that reside in the lysosome. The resulting degradation products (e.g. amino acids, fatty acids and nucleotides) are exported through membrane permeases to the cytoplasm, where they are recycled for the biosynthesis of new macromolecules or the production of ATP (Mehrpour et al., 2010; Yang and Klionsky, 2010).

Similar to most of the membrane-bound organelles and vesicles, various aspects of autophagosome dynamics rely in part on their interactions with the cytoskeleton and especially with microtubules (MTs) (Monastyrska et al., 2009). Although the involvement of MTs in many steps of autophagosome dynamics has been a matter of considerable debate over the past years, it appears that a consistent picture is now emerging in which MTs have essential roles in coordinating and organizing many crucial steps of autophagosome life. After providing a brief overview of autophagy and of microtubule function and dynamics, we will focus here on the roles that MTs and microtubule molecular motors have in autophagosome formation, their movements and their interaction with lysosomes. We will also discuss recent advances that highlight the importance of global organelle positioning and of MT acetylation in autophagy.

## Overview of the autophagic machinery and its regulation

More than 30 autophagy-related gene products (ATGs) are dedicated to the execution of autophagy (for reviews, see Mizushima et al., 2011; Yang and Klionsky, 2010). Most of them have been identified by genetic screens in yeast and are evolutionarily conserved in mammals. The core machinery of autophagy includes the following steps: (i) initiation of autophagy and vesicle nucleation; (ii) vesicle expansion and closure; (iii) autophagosome maturation and its fusion with the late endosome, and degradation (Fig. 1A). Initiation of

Journal of Cell Science

autophagy starts with the activation of the ULK1 complex (Atg1 complex in budding yeast) that contains the serine/threonine-protein kinase ULK1, ATG13, focal adhesion kinase family kinase-interacting protein of 200 kD (FIP200, also known as RBCC1) and ATG101 (reviewed by Mizushima, 2010). Upon induction of autophagy, this complex localizes to the site of phagophore formation to regulate the nucleation machinery. Phagophore nucleation is highly dependent on the production of phosphatidylinositol 3-phosphate [PtdIns(3)*P* (PI3P)] by a phosphoinositide 3-kinase (PI 3-kinase) complex that is recruited to the phagophore membrane during the initiation step. This complex comprises the class III PI 3-kinase Vps34

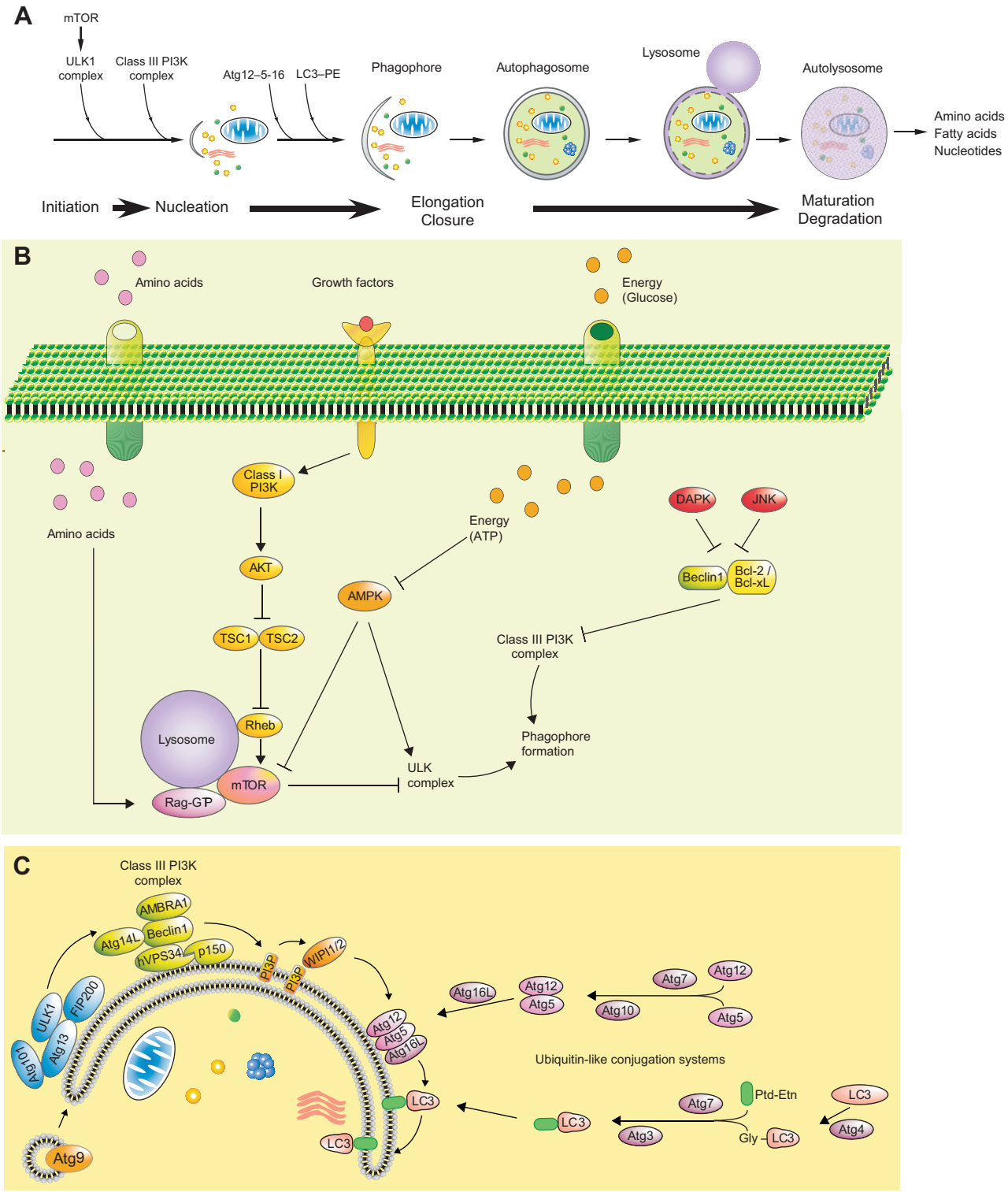


**Fig. 1.** See next page for legend.

(PK3C3), Beclin 1 (the mammalian ortholog of Atg6 in yeast), ATG14, Vps15 and activating molecule in BECN1-regulated autophagy protein 1 (AMBRA1) (Funderburk et al., 2010). The production of PtdIns(3)*P* in the phagophore membrane allows the recruitment of the WD repeat domain phosphoinositide-interacting proteins WIPI1 and WIPI2 (the mammalian orthologs of Atg18 in budding yeast) (Polson et al., 2010). Both contribute to the expansion and the closure of the vesicle in concert with two ubiquitin-like conjugation systems, resulting in the ATG12–ATG5-ATG16L complex and the phosphatidylethanolamine (PtdEtn) conjugate of microtubule-associated protein 1 light chain 3 [LC3 (MLP3); the mammalian ortholog of Atg8 in budding yeast] (Fujita et al., 2008; Hanada et al., 2007; for a review, see Xie and Klionsky, 2007). The transmembrane protein ATG9 also participates in the nucleation and the expansion of the phagophore membrane by cycling between different compartments and the phagophore (Orsi et al., 2012) (Fig. 1C). Autophagosome maturation and its fusion with the lysosome occur in the vicinity of the centrosome and depend on several lysosomal membrane proteins, such as the small GTPase Ras-related protein Rab7 and the transmembrane lysosome-associated membrane glycoprotein 2 (LAMP2) (Gutierrez et al., 2004; Jäger et al., 2004; Tanaka et al., 2000). Degradation of autophagosomal cargoes is then achieved by the acid hydrolases and the cathepsin proteases that are present in the lysosomal lumen.

Induction of autophagy is strictly regulated through upstream signaling pathways that are governed by growth factors, amino acids, glucose and the energy status (Fig. 1B). Most of these pathways control the two initiation complexes ULK1 and PI 3-kinase . Activity of the ULK1 complex is controlled by one of the main players in autophagy regulation, the mammalian target of rapamycin complex 1 (mTORC1), which comprises the mTOR serine/threonine-protein kinase and auxiliary proteins. As long as mTOR is activated, it inhibits autophagy by inhibitory phosphorylation of ULK1 (for a review, see Yang and Klionsky, 2010). Under diverse stresses including amino acid starvation, mTORC1 is inhibited, thereby promoting ULK1 complex activity and autophagy (Hosokawa et al., 2009; Jung et al., 2009). One of the important pathways that regulates mTORC1 is initiated by growth factors and involves the class I PI 3-kinase s and Akt serine/threonine-protein kinases (for a review, see Sengupta et al., 2010). Another pathway that controls autophagy in response to amino acids involves the Rag GTPase, which allows the recruitment of mTOR at the lysosomal membrane, where its direct activators reside (Sancak et al., 2010). Upon energy depletion, when the AMP:ATP ratio rises, AMP-activated protein kinase (AMPK) can also activate the ULK1 complex by directly phosphorylating ULK1 or, indirectly, by inactivating mTORC1 (Alers et al., 2012; Kim et al., 2011). Activity of the PI 3-kinase complex is also regulated, mainly through the interaction of Beclin 1 with the anti-apoptotic members of the Bcl-2 family (Kang et al., 2011). Interaction of Beclin 1 with Bcl-2 or Bcl-xL through its BH3 domain inhibits autophagy. Upon amino acid starvation, activation of c-Jun N-terminal kinase-1 [JNK1 (mitogen-activated protein kinase 8)] leads to Bcl-2 phosphorylation and the release of Beclin 1, which in turn induces autophagy (Wei et al., 2008). Interaction between Beclin 1 and apoptosis regulator Bcl-2 or Bcl-xL can also be disrupted following the phosphorylation of Beclin 1 by death-associated protein kinase (DAPK) (Zalckvar et al., 2009).

The origin of the autophagosomal membrane and the process of phagophore nucleation have long been an enigma. The phagophore appears to expand by membrane addition rather than by *de novo* lipid synthesis (for a review, see Yang and Klionsky, 2010). Several organelles could serve as membrane donors for autophagosome formation, such as the endoplasmic reticulum (ER), the Golgi complex, mitochondria, endosomes, the plasma membrane or the nuclear envelope (for a review, see Mizushima et al., 2011). Recent studies suggest that a specialized domain of the ER called the omegasome (Axe et al., 2008) is a privileged site for phagophore biogenesis. The autophagosomal membrane elongates inside a cradle that is formed by ER membranes, which acts as a template for the spherical shape of the autophagosome (Hayashi-Nishino et al., 2009; Ylä-Anttila et al., 2009). As we discuss below, many steps in the life cycle of autophagosomes involve MTs and their associated molecular motors.

**Fig. 1. The autophagic pathway and its regulation.** (**A**) Autophagy (specifically, macroautophagy) begins with the nucleation of an isolation membrane, termed the phagophore, which surrounds a fraction of cytoplasm to be degraded. After elongation and closure, the newly formed autophagosome receives input from the endocytic pathway and ultimately fuses with a lysosome, allowing the degradation of autophagic substrates. The resultant macromolecules are exported to the cytosol and recycled for ATP production and biosynthesis. (**B**) Autophagy is regulated by upstream signaling, which integrates stimuli mediated by growth factors as well as the sensing of available energy (ATP) and amino acids. These signaling pathways converge on two initiation complexes – ULK1 and class III phosphoinositide 3-kinase (PI3K). The kinase mTOR, a master regulator of autophagy, integrates multiple signals and inhibits ULK1 by phosphorylation. As two of its activators, Rag and Rheb, localize to the lysosomal membrane, mTOR is activated at the lysosomal surface. (**C**) Phagophore formation. Once activated, the ULK1 complex localizes at sites of phagophore formation together with the class III PI3K complex containing Beclin 1. Newly synthesized PtdIns(3)*P* then recruits the FYVE-domain-containing proteins WIPI1 and WIPI2. They contribute to the expansion and the closure of the autophagic vesicle in concert with ATG12–ATG5–ATG16L and LC3–PtdEtn. The formation of these two complexes involves a covalent linkage of ATG12 to ATG5 and of LC3 to PtdEtn by ubiquitin-like conjugation systems. The latter involve the E1-like enzyme ATG7, which activates ATG12 and LC3 (previously cleaved by ATG4), and two distinct E2-like enzymes, ATG10 and ATG3, which transfer, respectively, ATG12 to ATG5 and LC3 to PtdEtn. The ATG12–ATG5 conjugate then forms a complex with ATG16L and associates with the phagophore, which is necessary for insertion of LC3–PtdEtn in the membrane. ATG9-enriched vesicles might provide lipids to the phagophore membrane, allowing its expansion.

## Microtubule dynamics and functions

MTs are hollow cylinders of ~25 nm in diameter that are formed by polymerization of α–β tubulin dimers. In most mammalian cells, interphase MTs assemble at the centrosome and/or at membrane MT-organizing centers (MTOCs) such as the Golgi complex. γ-Tubulin-containing ring complexes nucleate MTs and interact with their minus-ends (for a review, see Kollman et al., 2011). MT plus-ends grow towards the cell periphery by incorporating GTP-bound tubulin subunits. Growing MTs generally hydrolyze GTP in the inner regions of the polymer, yielding a GTP–tubulin cap (David-Pfeuty et al., 1977; Dimitrov et al., 2008). Once this cap is lost at the cell periphery, MTs disassemble and release GDP–tubulin into the cytosol. The alternation between growth and shrinking phases of MTs that are

separated by transitions that are termed either catastrophes (from MT assembly or pausing to disassembly) and rescues (from phases of MT shrinking or pausing to their re-growth) is called dynamic instability (Mitchison and Kirschner, 1984). Such a continuous remodelling operates independently for each MT and allows MTs to interact temporarily with cellular components, to explore the intracellular space and to position organelles dynamically (for reviews, see Desai and Mitchison, 1997; Howard, 2006). For instance, MTs maintain the ER and mitochondria that are dispersed throughout the cytoplasm, while they at the same time also maintain the Golgi complex and the endosomes that are clustered in the vicinity of the nucleus (reviewed by Cole and Lippincott-Schwartz, 1995). MTs move and position organelles by functioning as tracks, on which plus-end- (kinesins) or minus-end-directed (cytoplasmic dynein) molecular motors carry membranes, by exerting pushing and pulling forces during their assembly and depolymerization or by sliding along each other in a way that is powered by molecular motors (for a review, see Tolić-Nørrelykke, 2008). MTs could also interact directly or indirectly with a wealth of proteins that might be sequestered, released, assembled into complexes and/or transported to organize and modulate signal transduction (for a review, see Etienne-Manneville, 2010; Gundersen and Cook, 1999).

At a given time, not all the MT network of a cell is subjected to remodelling by dynamic instability. Indeed, a subpopulation comprises long-lived polymers, which can persist even for an entire interphase (so-called stable MTs) (Webster et al., 1987). MT stabilization, which is enhanced by cell confluence, might occur at the cell periphery, where MT plus-ends are capped with proteins that prevent tubulin exchange (Bartolini et al., 2008; Infante et al., 2000; Palazzo et al., 2001). This stabilization is not stochastic and can result from specific cortical interactions (reviewed by Gundersen et al., 2004) or from interactions with organelles such as the Golgi complex (Chabin-Brion et al., 2001; Efimov et al., 2007; Miller et al., 2009). In addition, stable MTs exhibit numerous tubulin post-translational modifications, such as de-tyrosination, polyglutamylation or acetylation (for a review, see Janke and Bulinski, 2011). These modifications might prevent the binding of disassembling factors or modulate MT functions by fine-tuning the binding of proteins to the MT surface. Stable and dynamic MT subpopulations are functionally specialized in organizing signaling pathways or vesicle trafficking. For example, dynamic MTs are involved in basolateral-directed post-Golgi trafficking in epithelial cells, as well as in transcytosis (Hunziker et al., 1990; Lafont et al., 1994; Poüs et al., 1998) or in caveolae-mediated pathogen internalization (Guignot et al., 2001). Stable MTs participate in ER-to-Golgi traffic (Mizuno and Singer, 1994), the Golgi-to-plasma-membrane traffic (Cai et al., 2009) or in the recycling of endosomes to the plasma membrane (Lin et al., 2002). Stable MTs are also involved in alcohol-induced alterations in protein traffic in hepatocytes (Joseph et al., 2008). Vesicular carriers are thought to recognize the MT tracks they require through interaction with molecular motors, especially with kinesins (Cai et al., 2009). Indeed, the kinesin-1 KIF5C preferentially moves on stable and post-translationally modified MTs, whereas the kinesin-2 KIF17 and the kinesin-3 KIF1A are not selective (Cai et al., 2009).

Various pharmacological MT-targeting agents (anti-polymerization drugs, such as nocodazole or vinca alkaloids, and MT-stabilizing drugs such as Taxol) and biochemical procedures are used to identify the role the MT network has in biological processes, including the specific involvement of dynamic and stable MT subpopulations (see Box 1). An indirect relationship between macroautophagy and MTs was first proposed over 35 years ago (Amenta et al., 1977). This study reported that the vinca alkaloids vincristine or vinblastine inhibit the autophagic protein degradation occurring in response to serum deprivation. Such a global response includes: first, the formation of autophagosomes and, second, their fusion with lysosomes and subsequent protein degradation. The following sections address the roles of MTs and of their associated molecular motors in these two steps of autophagy.

## The role of microtubules and molecular motors in autophagosome formation

The role of MTs in autophagosome formation appears to be different between basal and stress-induced autophagy. Under basal conditions, several studies using nocodazole and Taxol suggest that MTs do not participate in autophagosome formation (Aplin et al., 1992; Köchl et al., 2006; Reunanen et al., 1988). Upon amino acid starvation, disassembling MTs with high doses of nocodazole prevents autophagosome formation, highlighting the role of MTs in this step (Geeraert et al., 2010; Köchl et al., 2006). In addition, MT stabilization by Taxol or by submicromolar nocodazole concentrations has the same effect, suggesting that MT dynamics are also important (Geeraert et al., 2010; Köchl et al., 2006). In the above studies, the functional importance of MTs in autophagosome formation essentially relies on the use of tubulin-acting drugs and thus might be subject to misinterpretations owing to possible side effects. This is especially the case with the experiments performed with vinca alkaloids, in which autophagosome formation is enhanced both under basal or amino acid starvation conditions (Köchl et al., 2006). This puzzling behaviour might result from the fact that vinca alkaloids cause tubulin precipitation into paracrystals. These structures, even if they do not resemble functional MTs, could perhaps act as molecular scaffolds that facilitate autophagosome formation.

### Box 1. Tools to study microtubule subpopulations

Tubulin-binding drugs are used to identify the biological role of the whole MT network or of dynamic and stable MT subsets. To depolymerize the whole MT network, long-term treatments (within hours) with high concentrations (micromolar) of anti-polymerizing agents such as nocodazole or vinca alkaloids are used. They trigger MT depolymerization as they prevent tubulin incorporation into MTs but do not affect MT catastrophes and disassembly of pre-existing MTs. Note that, in contrast to nocodazole, vinca alkaloids cause tubulin precipitation into paracrystals. To analyze the role of dynamic MTs, they can be depolymerized using short-term treatment (usually $<5$ minutes) with high concentrations of the same drugs. Alternatively, dynamic MTs can be stabilized using low concentrations (submicromolar, within hours) of these agents. Indeed, a few molecules of vinca alkaloids bound to high-affinity sites at the MT plus-end block MT dynamics. Similarly, the incorporation of a few tubulin dimers bound to nocodazole in growing MTs also suppresses their dynamics (for a review, see Jordan and Wilson, 2004). Another possibility is to use Taxol (paclitaxel), which binds to polymerized tubulin to stabilize MTs.

The hypothesis of MT involvement in autophagosome formation has been reinforced and refined by complementary findings. First, both the non-lipidated and the PtdEtn-conjugated forms of LC3 are found in MT-containing subcellular fractions, suggesting that an interaction occurs between LC3 and MTs (Fass et al., 2006; Geeraert et al., 2010). LC3 has been shown to associate with MTs indirectly through an interaction with microtubule-associated proteins MAP1A and MAP1B (Mann and Hammarback, 1994) or its homolog MAP1S (Xie et al., 2011). LC3 might also interact directly with tubulin through its N-terminal domain (Kouno et al., 2005). Moreover, several other ATG proteins (ULK1, Beclin 1, WIPI1, ATG5, ATG12) that are involved in the early steps of autophagosome formation are enriched in the dynamic MT fraction (Table 1), suggesting that the dynamic subset of MTs supports the assembly of pre-autophagosomal structures. Unlike phagophores, which are immobile (Fass et al., 2006; Kimura et al., 2008), WIPI1-positive pre-autophagosomal structures move along MTs upon starvation of the cell (Geeraert et al., 2010). Interestingly, such movements are highly sensitive to nocodazole treatment under conditions that affect only highly dynamic MTs. This raises the possibility that motors associated with these MTs could drive other important steps of autophagosome formation such as the homotypic fusion of ATG16-positive membranes, which then maturate into phagophores (Moreau et al., 2011). Second, it has been shown that MTs and MT motors regulate two major complexes involved in the initiation of the autophagic response: mTORC1 and the class III PI 3-kinase complex. mTOR binds to the cytoplasmic face of lysosomes (Sancak et al., 2010), and its activity is controlled by lysosome localization. This localization is organized by MTs and involves two kinesins: the kinesin-2 KIF2A and the kinesin-3 KIF1B (Korolchuk et al., 2011). When nutrients are available, these kinesins localize to lysosomes at the cell periphery, and mTORC1 is kept active, thereby preventing autophagosome formation. When nutrients are in short supply, the cytoplasmic pH increases, these motors are released from lysosomes, facilitating their centripetal movement, mTORC1 inactivation and autophagosome formation (Fig. 2A) (Korolchuk et al., 2011; Poüs and Codogno, 2011). Beclin 1 is a major component of the class III PI 3-kinase complex. It is sequestered on MTs in two complexes containing the dynein light chain 1 (DLC1) and either AMBRA1 or Bim (Bcl2-like protein 11). Upon induction of autophagy, AMBRA1 phosphorylation by the kinase ULK1 releases Beclin 1 (Di Bartolomeo et al., 2010). In parallel, activated JNK1 phosphorylates Bim to release Beclin 1 (Luo et al., 2012), allowing it to be recruited to membranes such as omegasomes, from which autophagosomes originate (Fig. 2B). JNK1 also disrupts the ER-associated complex formed by Beclin 1 and Bcl-2 (Wei et al., 2008) (Fig. 2B). Interestingly, upon amino acid starvation, JNK1 activation is a kinesin-1-dependent process (Geeraert et al., 2010; Verhey et al., 2001), and it seems to require the hyperacetylation of the dynamic subset of MTs (Geeraert et al., 2010).

The mechanism by which tubulin acetylation (in the lumen of MTs) could modulate the recruitment and walking of molecular motors (on the outer face of MTs) is still a matter of debate and

**Table 1. Autophagosomal proteins and their association with microtubules**

| Protein | Role in autophagy[1] | Link with microtubules | References[2] |
|---|---|---|---|
| ATG proteins | | | |
| LC3 (ATG8) | Two post-translationally modified forms: cytosolic LC3-I and membrane-bound LC3-II (LC3–PtdEtn) (autophagosome) | Subunit of the neuronal microtubule-associated proteins MAPlA and MAPlB. | Mann and Hammarback, 1994 |
| | | Co-purifies with Taxol-assembled rat MTs. | |
| | Contributes to the closure of the autophagosome membrane | Binds to tubulin through ionic interactions. | |
| | | LC3-I binds to the labile MT fraction, LC3-II to the stable MT fraction. | Geeraert et al., 2010 |
| | | Participates in minus-end- and plus-end-directed autophagosome transport. | Kimura et al., 2008; Pankiv et al., 2010 |
| ATG5–ATG12 | Contribute to the expansion and the closure of the autophagosome membrane together with ATG16L | Co-purify with labile MTs in starved cells. | Geeraert et al., 2010 |
| Beclin 1 (ATG6) | Participates in a complex with the class III phosphoinositide 3-kinase VPS34, ATG14L, VPS15 and AMBRA1 | Binds to MTs through interactions with AMBRA1 and DLC1 with Bim and DLC1. | Di Bartolomeo et al., 2010; Luo et al., 2012 |
| | Contributes to the initiation of autophagosome formation | Sequestered on MTs at the basal level and relocalized to phagophores under starvation | Di Bartolomeo et al., 2010; Luo et al., 2012 |
| WIPI1 (ATG18) | Binds to PtdIns3*P* on phagophores | Binds to MTs through recruitment into mobile pre-autophagosomal structures; co-purifies with labile MTs in starved cells | Geeraert et al., 2010 |
| ULK1/UNC-51 (ATG1) | Phosphorylates raptor and AMBRA1 | Binds to a kinesin heavy chain adaptor in neurons | Toda et al., 2008 |
| | | Phosphorylates AMBRA1 to release AMBRA1 and Beclin 1 from their interaction with MTs | Di Bartolomeo et al., 2010 |
| Non-ATG proteins | | | |
| p62 | Autophagy substrate, interacts with ubiquitin | Co-purifies with both labile and stable microtubules in starved cells | Geeraert et al., 2010 |
| AMBRA1 | Participates in a complex with class III PI 3-kinase VPS34, ATG14L, VPS15 and Beclin 1 | Mediates binding of Beclin 1 to MTs through interaction with dynein light chain; released with Beclin 1 after phosphorylation by ULK1 upon induction of autophagy | Di Bartolomeo et al., 2010 |
| | Contributes to the initiation of autophagosome formation | | |
| FYCO1 | Interaction with LC3, RAB7 and PtdIns3*P* | Promotes plus-end-directed transport of autophagic vesicles | Pankiv et al., 2010 |

[1]For specific references, see the main text (for reviews, see Kroemer et al., 2010; Mizushima et al., 2011; Singh and Cuervo, 2011; Yang and Klionsky, 2010).
[2]Cited references only correspond to the interactions with microtubules (MTs).

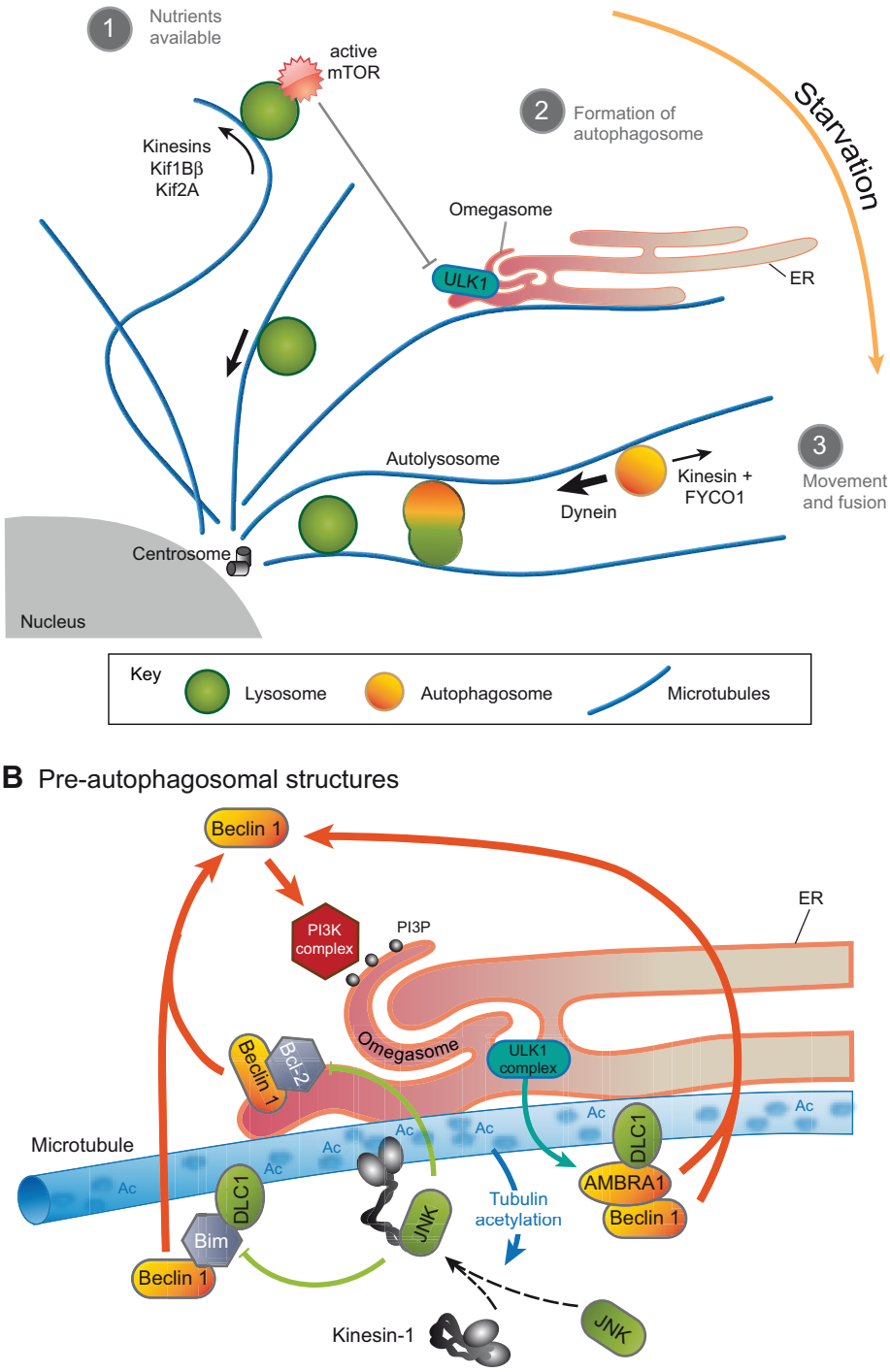


**Fig. 2. Microtubules as global and local integrators of the autophagic response.** (**A**) Microtubules (blue) organize the large-scale organelle positioning throughout the autophagy process. Under conditions in which there is a sufficient supply of nutrients (1), mTOR (through its lysosomal localization) is transported towards the cell periphery, where it is kept active. Microtubules and kinesins are essential for this localization. Upon autophagosome formation (2), lysosomes (green) undergo centripetal movement while, at the same time, mTOR inhibition of ULK1 is relieved (see Fig. 1). Newly formed autophagosomes (orange) move along microtubules in two directions as a result of the opposing activities of the minus-end-directed motor protein dynein and a plus-end-directed motor kinesin/FYCO1 (3). Thereafter, the autophagosomes cluster in a perinuclear region (close to the centrosome), where they can fuse with lysosomes (3). ER, endoplasmic reticulum. (**B**) At a more local level, microtubules participate in the formation of pre-autophagosomal structures. Prior to induction of autophagy, they contribute to sequestration of Beclin 1 through their association with dynein light chain 1 (DLC1), which is found in the two complexes that Beclin 1 forms – one containing AMBRA1, and the other containing Bim. Beclin 1 also associates with Bcl-2 at the cytoplasmic face of the endoplasmic reticulum, a membrane-bound organelle that is organized by the microtubule network. Upon stimulation of autophagy, activated ULK1 phosphorylates AMBRA1, thus releasing it from the Beclin 1 complex and allowing the recruitment of Beclin 1 into the class III PI3K complex. In parallel, hyperacetylation of tubulin stimulates recruitment of kinesin-1 to microtubules, which activates c-Jun N-terminal kinase-1 (JNK1) and allows the subsequent phosphorylation of Bcl-2 and Bim, thus releasing Beclin 1 and contributing to autophagosome formation.

will require further investigations to determine whether it is a key actor or a more indirect phenomenon in regulating motor activity. Acetylation has been proposed to trigger changes in the lateral interaction between tubulin subunits (Cueva et al., 2012; Topalidou et al., 2012). A consecutive change in MT conformation might therefore enhance motor recruitment to MTs (Dompierre et al., 2007; Hammond et al., 2009; Reed et al., 2006). Conversely, the role of tubulin acetylation upon molecular motor recruitment and function has been questioned in motility assays using tubulin acetylated *in vitro* (Soppina et al., 2012; Walter et al., 2012). These studies proposed that the recruitment of kinesin-1 to acetylated MTs and its velocity were not affected, but other tubulin modifications (de-tyrosination and polyglutamylation) could have masked the effect of acetylation. The level of tubulin acetylation depends on the histone deacetylase HDAC6 and the NAD-dependent protein deacetylase sirtuin-2 (SIRT2) (Hubbert et al., 2002; North et al., 2003; Zhang et al., 2003), which have also been shown to

regulate autophagy. SIRT2 inhibits this process (Zhao et al., 2010), whereas HDAC6 functions as a scaffold that binds to polyubiquitylated proteins to allow the formation of aggresomes and binds to damaged mitochondria that are cleared by autophagy (Kawaguchi et al., 2003; Lee et al., 2010; Pandey et al., 2007). The relationship between MTs and the function of SIRT2 in autophagy is not straightforward given its inhibitory activity, but HDAC6 sequestration in cytoplasmic regions engulfed by autophagosomes could perhaps contribute to its inactivation and thus to the induction of tubulin acetylation. Whatever the exact roles deacetylases have in autophagosome formation, the functional importance of tubulin acetylation will deserve further clarification.

Altogether, the above data support the idea that stress-induced autophagosome formation involves MTs, and many proteins involved in this process localize on the dynamic MT subset. Interestingly, the rate of autophagosome formation (~1/minute) (Fass et al., 2006) or the lifetime of omegasomes (<3 minutes) (Ktistakis et al., 2011) is consistent with the life span of dynamic MTs (a few minutes). By contrast, mature autophagosomes exist for ~30 minutes before they fuse with lysosomes (Fass et al., 2006; Ktistakis et al., 2011). This period fits well with the life span of stable MTs, along which they could get clustered near the nucleus and meet lysosomes, as discussed in the next section.

## The role of microtubules and molecular motors in autolysosome formation

The role of MTs in the fusion of autophagosomes with lysosomes has long been controversial, as most studies did not clearly distinguish transport from fusion. The involvement of MTs in autolysosome formation was initially proposed based on the observation that a complete disassembly of MTs inhibits the colocalization of autophagosomes and lysosomes and/or protein degradation by autophagy (Aplin et al., 1992; Köchl et al., 2006; Webb et al., 2004). The role of MTs in autolysosome formation is likely to depend on stable MTs. Indeed, Taxol-mediated MT stabilization does not affect autophagosome and lysosome fusion, suggesting that MT dynamics are not involved in the gathering of autophagosomes and lysosomes or in their fusion (Köchl et al., 2006). Also, under basal conditions, stable acetylated MTs seem to participate in autolysosome formation (Xie et al., 2010). Fass and colleagues precisely determined MT participation in autophagosome-to-lysosome fusion events in CHO cells starved of amino acids and confirmed that protein degradation is impaired by MT disassembly. They also found that the lifetime of autophagosomes does not change in the absence of MTs (Fass et al., 2006). The authors proposed that fusion would still occur in the absence of MTs, but that nocodazole impairs lysosomal degradation owing to a drop in protease transport to lysosomes, as shown in the early 1990s (Fass et al., 2006; Scheel et al., 1990). Taken together, these results suggest that MTs are dispensable for the fusion between autophagosomes and lysosomes. However, a trafficking of autophagosomes along MTs towards lysosomes is necessary to allow effective fusion, as has been shown by Kimura and colleagues (Kimura et al., 2008) using fluorescence recovery after photobleaching (FRAP) assays.

Once they have formed, autophagosomes move bi-directionally along MTs and finally concentrate around the centrosome in the perinuclear region. The centripetal movement of autophagosomes is mediated by the MT minus-end-directed motor dynein, as shown using an inhibitor of dynein ATPase activity (Jahreiss et al., 2008). Furthermore, dominant-negative mutations of the gene encoding dynein were shown to decrease the autophagy-mediated clearance of a mutant form of α-synuclein, which causes familial Parkinson disease (Ravikumar et al., 2005). In this study, the expression of dynein mutants also increased the number of autophagosomes and impaired their fusion with lysosomes. Dynein involvement was confirmed by a decrease in autophagosome movements in HeLa cells that expressed GFP–LC3 and p50 dynamitin, which disrupts the dynein–dynactin complex and impairs centripetal organelle movements (Ravikumar et al., 2005). A similar reduction was observed after blocking LC3 function by microinjecting a specific antibody during amino acid starvation (Kimura et al., 2008). LC3 might participate in the recruitment of dynein to autophagosomes. Indeed, LC3 can bind to Rab7 (Pankiv et al., 2010), which has been shown to be involved in the recruitment of the dynein-associated dynactin subunit 1 protein ($p150^{Glued}$) (Johansson et al., 2007) and in autophagosome maturation (Gutierrez et al., 2004; Jäger et al., 2004). Regarding the centrifugal movement of autophagosomes, kinesin-1 is involved under basal conditions (Cardoso et al., 2009; Geeraert et al., 2009), but the kinesin motor(s) involved in stress conditions are still unknown. A possible functional link between autophagosomes and kinesins in stressed cells might involve FYVE and coiled-coil domain-containing protein 1 (FYCO1), which contains a possible kinesin binding site and is recruited to autophagosomes by means of Rab7 and LC3 (Pankiv et al., 2010) (Fig. 2A).

Altogether, the importance of MTs in the formation of autolysosomes mainly relies on their role in the localization of autophagosomes and lysosomes in the juxta-nuclear region. This gathering most likely results from their ‘motorization’ by dynein after inhibition of centrifugal trafficking that is mediated by kinesins. The emerging picture thus shows that the spatial control of autophagosome location during their formation and movements depends both on their compartmentalization between dynamic and stable MTs and on their subcellular positioning through the balance between dynein and kinesin activities. These mechanisms might thus prevent the premature fusion of immature autophagosomes with lysosomes.

## Conclusions and perspectives

It is now clear that MTs are involved in different steps of autophagy – that is, in the formation and motility of autophagosomes, but not in their fusion with lysosomes. MTs might serve as local suppliers or as scaffolds to promote the interaction of proteins that are required during the early stages of autophagy, such as Beclin 1 or AMBRA1. In addition, numerous studies have demonstrated that MTs have a role in the motility of autophagosomes. Here, we have discussed how two characteristics of MTs are also important for autophagosome formation: the dynamics of MTs and the post-translational modification of tubulin, in particular its acetylation. This adds a novel layer to the growing importance of acetylation in the overall regulation of autophagy (Hamaï and Codogno, 2012). MT dynamics also provide cells with a way to compartmentalize and organize, first, the upstream signaling of autophagosome formation and, second, autophagosome biogenesis and trafficking. The relationship between MTs and autophagy thus expands the repertoire of MT function in terms of signaling and membrane trafficking.

Finally, the role of MTs in autophagy needs to be considered from a therapeutic point of view. MT-targeting agents are being

used in tumours as they inhibit mitosis. However, human tumour cells divide more slowly than cultured cell lines and tumour cells in animal models (for a review, see Komlodi-Pasztor et al., 2011). As MTs have a main role in intracellular trafficking, signaling and autophagy in non-mitotic cells, these processes might also constitute effective targets in cancer cells that are treated with MT-targeting agents. Indeed, at least part of the cytotoxic effect of the MT-stabilizer Taxol is dependent on its ability to block autophagosome maturation (Veldhoen et al., 2012). Conversely, other tubulin-binding drugs, such as vinblastine, naphtharazin or 2-methoxyestradiol, might instead stimulate autophagy, which could contribute to their anticancer activity (Acharya et al., 2011; Chen et al., 2008; Kamath et al., 2006; Köchl et al., 2006; Lorin et al., 2009). In the context of neurodegenerative diseases, the link between MT-acting drugs and autophagy could also prove to be important. For example, in hybrid cells harbouring mitochondria from Parkinson's patients, mitochondria dysfunction alters the MT network and causes an accumulation of autophagosomes and an impaired clearance of both p62 and α-synuclein (Arduíno et al., 2012). Interestingly, in these cells, Taxol improves autophagic flux, most likely because it restores MT integrity.

Regardless of the mechanisms by which MT dynamics might influence autophagy, these examples demonstrate the increasing evidence that autophagy is a 'drugable' process that can be exploited for the future development of human disease therapies (reviewed by Rubinsztein et al., 2012) and that MT-targeting agents should be considered as drugs that can target autophagy.

## Funding

R.M., S.L., D.P. and C.P. are supported by institutional funding from the Université Paris-Sud. P.C. is supported by institutional funding from the Institut National de la Santé et de la Recherche Médicale (INSERM) and Université Paris Descartes.

## References

**Acharya, B. R., Bhattacharyya, S., Choudhury, D. and Chakrabarti, G.** (2011). The microtubule depolymerizing agent naphthazarin induces both apoptosis and autophagy in A549 lung cancer cells. *Apoptosis* **16**, 924-939.

**Alers, S., Löffler, A. S., Wesselborg, S. and Stork, B.** (2012). Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: cross talk, shortcuts, and feedbacks. *Mol. Cell. Biol.* **32**, 2-11.

**Amenta, J. S., Sargus, M. J. and Baccino, F. M.** (1977). Effect of microtubular or translational inhibitors on general cell protein degradation. Evidence for a dual catabolic pathway. *Biochem. J.* **168**, 223-227.

**Aplin, A., Jasionowski, T., Tuttle, D. L., Lenk, S. E. and Dunn, W. A., Jr** (1992). Cytoskeletal elements are required for the formation and maturation of autophagic vacuoles. *J. Cell. Physiol.* **152**, 458-466.

**Arduíno, D. M., Esteves, A. R., Cortes, L., Silva, D. F., Patel, B., Grazina, M., Swerdlow, R. H., Oliveira, C. R. and Cardoso, S. M.** (2012). Mitochondrial metabolism in Parkinson's disease impairs quality control autophagy by hampering microtubule-dependent traffic. *Hum. Mol. Genet.* **21**, 4680-4702.

**Axe, E. L., Walker, S. A., Manifava, M., Chandra, P., Roderick, H. L., Habermann, A., Griffiths, G. and Ktistakis, N. T.** (2008). Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J. Cell Biol.* **182**, 685-701.

**Bartolini, F., Moseley, J. B., Schmoranzer, J., Cassimeris, L., Goode, B. L. and Gundersen, G. G.** (2008). The formin mDia2 stabilizes microtubules independently of its actin nucleation activity. *J. Cell Biol.* **181**, 523-536.

**Cai, D., McEwen, D. P., Martens, J. R., Meyhofer, E. and Verhey, K. J.** (2009). Single molecule imaging reveals differences in microtubule track selection between Kinesin motors. *PLoS Biol.* **7**, e1000216.

**Cardoso, C. M., Groth-Pedersen, L., Høyer-Hansen, M., Kirkegaard, T., Corcelle, E., Andersen, J. S., Jäättelä, M. and Nylandsted, J.** (2009). Depletion of kinesin 5B affects lysosomal distribution and stability and induces peri-nuclear accumulation of autophagosomes in cancer cells. *PLoS ONE* **4**, e4424.

**Chabin-Brion, K., Marceiller, J., Perez, F., Settegrana, C., Drechou, A., Durand, G. and Poüs, C.** (2001). The Golgi complex is a microtubule-organizing organelle. *Mol. Biol. Cell* **12**, 2047-2060.

**Chen, Y., McMillan-Ward, E., Kong, J., Israels, S. J. and Gibson, S. B.** (2008). Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells. *Cell Death Differ.* **15**, 171-182.

**Cole, N. B. and Lippincott-Schwartz, J.** (1995). Organization of organelles and membrane traffic by microtubules. *Curr. Opin. Cell Biol.* **7**, 55-64.

**Cueva, J. G., Hsin, J., Huang, K. C. and Goodman, M. B.** (2012). Posttranslational acetylation of α-tubulin constrains protofilament number in native microtubules. *Curr. Biol.* **22**, 1066-1074.

**David-Pfeuty, T., Erickson, H. P. and Pantaloni, D.** (1977). Guanosinetriphosphatase activity of tubulin associated with microtubule assembly. *Proc. Natl. Acad. Sci. USA* **74**, 5372-5376.

**Deretic, V. and Levine, B.** (2009). Autophagy, immunity, and microbial adaptations. *Cell Host Microbe* **5**, 527-549.

**Desai, A. and Mitchison, T. J.** (1997). Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* **13**, 83-117.

**Di Bartolomeo, S., Corazzari, M., Nazio, F., Oliverio, S., Lisi, G., Antonioli, M., Pagliarini, V., Matteoni, S., Fuoco, C., Giunta, L. et al.** (2010). The dynamic interaction of AMBRA1 with the dynein motor complex regulates mammalian autophagy. *J. Cell Biol.* **191**, 155-168.

**Dimitrov, A., Quesnoit, M., Moutel, S., Cantaloube, I., Poüs, C. and Perez, F.** (2008). Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues. *Science* **322**, 1353-1356.

**Dompierre, J. P., Godin, J. D., Charrin, B. C., Cordelières, F. P., King, S. J., Humbert, S. and Saudou, F.** (2007). Histone deacetylase 6 inhibition compensates for the transport deficit in Huntington's disease by increasing tubulin acetylation. *J. Neurosci.* **27**, 3571-3583.

**Efimov, A., Kharitonov, A., Efimova, N., Loncarek, J., Miller, P. M., Andreyeva, N., Gleeson, P., Galjart, N., Maia, A. R. R., McLeod, I. X. et al.** (2007). Asymmetric CLASP-dependent nucleation of noncentrosomal microtubules at the trans-Golgi network. *Dev. Cell* **12**, 917-930.

**Etienne-Manneville, S.** (2010). From signaling pathways to microtubule dynamics: the key players. *Curr. Opin. Cell Biol.* **22**, 104-111.

**Fass, E., Shvets, E., Degani, I., Hirschberg, K. and Elazar, Z.** (2006). Microtubules support production of starvation-induced autophagosomes but not their targeting and fusion with lysosomes. *J. Biol. Chem.* **281**, 36303-36316.

**Fujita, N., Itoh, T., Omori, H., Fukuda, M., Noda, T. and Yoshimori, T.** (2008). The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol. Biol. Cell* **19**, 2092-2100.

**Funderburk, S. F., Wang, Q. J. and Yue, Z.** (2010). The beclin 1-VPS34 complex – at the crossroads of autophagy and beyond. *Trends Cell Biol.* **20**, 355-362.

**Geeraert, C., Ratier, A., Pfisterer, S. G., Perdiz, D., Cantaloube, I., Rouault, A., Pattingre, S., Proikas-Cezanne, T., Codogno, P. and Poüs, C.** (2010). Starvation-induced hyperacetylation of tubulin is required for the stimulation of autophagy by nutrient deprivation. *J. Biol. Chem.* **285**, 24184-24194.

**Guignot, J., Bernet-Camard, M. F., Poüs, C., Plançon, L., Le Bouguenec, C. and Servin, A. L.** (2001). Polarized entry of uropathogenic Afa/Dr diffusely adhering Escherichia coli strain IH11128 into human epithelial cells: evidence for alpha5beta1 integrin recognition and subsequent internalization through a pathway involving caveolae and dynamic unstable microtubules. *Infect. Immun.* **69**, 1856-1868.

**Gundersen, G. G. and Cook, T. A.** (1999). Microtubules and signal transduction. *Curr. Opin. Cell Biol.* **11**, 81-94.

**Gundersen, G. G., Gomes, E. R. and Wen, Y.** (2004). Cortical control of microtubule stability and polarization. *Curr. Opin. Cell Biol.* **16**, 106-112.

**Gutierrez, M. G., Munafó, D. B., Berón, W. and Colombo, M. I.** (2004). Rab7 is required for the normal progression of the autophagic pathway in mammalian cells. *J. Cell Sci.* **117**, 2687-2697.

**Hamaï, A. and Codogno, P.** (2012). New targets for acetylation in autophagy. *Sci. Signal.* **5**, pe29.

**Hammond, J. W., Huang, C. F., Kaech, S., Jacobson, C., Banker, G. and Verhey, K. J.** (2010). Posttranslational modifications of tubulin and the polarized transport of kinesin-1 in neurons. *Mol. Biol. Cell.* **21**, 572-83

**Hanada, T., Noda, N. N., Satomi, Y., Ichimura, Y., Fujioka, Y., Takao, T., Inagaki, F. and Ohsumi, Y.** (2007). The Atg12-Atg5 conjugate has a novel E3-like activity for protein lipidation in autophagy. *J. Biol. Chem.* **282**, 37298-37302.

**Hayashi-Nishino, M., Fujita, N., Noda, T., Yamaguchi, A., Yoshimori, T. and Yamamoto, A.** (2009). A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat. Cell Biol.* **11**, 1433-1437.

**Hosokawa, N., Sasaki, T., Iemura, S., Natsume, T., Hara, T. and Mizushima, N.** (2009). Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy* **5**, 973-979.

**Howard, J.** (2006). Elastic and damping forces generated by confined arrays of dynamic microtubules. *Phys. Biol.* **3**, 54-66.

**Hubbert, C., Guardiola, A., Shao, R., Kawaguchi, Y., Ito, A., Nixon, A., Yoshida, M., Wang, X. F. and Yao, T. P.** (2002). HDAC6 is a microtubule-associated deacetylase. *Nature* **417**, 455-458.

**Hunziker, W., Mâle, P. and Mellman, I.** (1990). Differential microtubule requirements for transcytosis in MDCK cells. *EMBO J.* **9**, 3515-3525.

**Infante, A. S., Stein, M. S., Zhai, Y., Borisy, G. G. and Gundersen, G. G.** (2000). Detyrosinated (Glu) microtubules are stabilized by an ATP-sensitive plus-end cap. *J. Cell Sci.* **113**, 3907-3919.

**Jäger, S., Bucci, C., Tanida, I., Ueno, T., Kominami, E., Saftig, P. and Eskelinen, E.-L.** (2004). Role for Rab7 in maturation of late autophagic vacuoles. *J. Cell Sci.* **117**, 4837-4848.

**Jahreiss, L., Menzies, F. M. and Rubinsztein, D. C.** (2008). The itinerary of autophagosomes: from peripheral formation to kiss-and-run fusion with lysosomes. *Traffic* **9**, 574-587.

**Janke, C. and Bulinski, J. C.** (2011). Post-translational regulation of the microtubule cytoskeleton: mechanisms and functions. *Nat. Rev. Mol. Cell Biol.* **12**, 773-786.

**Johansson, M., Rocha, N., Zwart, W., Jordens, I., Janssen, L., Kuijl, C., Olkkonen, V. M. and Neefjes, J.** (2007). Activation of endosomal dynein motors by stepwise assembly of Rab7-RILP-p150Glued, ORP1L, and the receptor betalll spectrin. *J. Cell Biol.* **176**, 459-471.

**Jordan, M. A. and Wilson, L.** (2004). Microtubules as a target for anticancer drugs. *Nat. Rev. Cancer* **4**, 253-265.

**Joseph, R. A., Shepard, B. D., Kannarkat, G. T., Rutledge, T. M., Tuma, D. J. and Tuma, P. L.** (2008). Microtubule acetylation and stability may explain alcohol-induced alterations in hepatic protein trafficking. *Hepatology* **47**, 1745-1753.

**Jung, C. H., Jun, C. B., Ro, S. H., Kim, Y. M., Otto, N. M., Cao, J., Kundu, M. and Kim, D. H.** (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol. Biol. Cell* **20**, 1992-2003.

**Kamath, K., Okouneva, T., Larson, G., Panda, D., Wilson, L. and Jordan, M. A.** (2006). 2-Methoxyestradiol suppresses microtubule dynamics and arrests mitosis without depolymerizing microtubules. *Mol. Cancer Ther.* **5**, 2225-2233.

**Kang, R., Zeh, H. J., Lotze, M. T. and Tang, D.** (2011). The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ.* **18**, 571-580.

**Kawaguchi, Y., Kovacs, J. J., McLaurin, A., Vance, J. M., Ito, A. and Yao, T. P.** (2003). The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. *Cell* **115**, 727-738.

**Kim, J., Kundu, M., Viollet, B. and Guan, K. L.** (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132-141.

**Kimura, S., Noda, T. and Yoshimori, T.** (2008). Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes. *Cell Struct. Funct.* **33**, 109-122.

**Köchl, R., Hu, X. W., Chan, E. Y. and Tooze, S. A.** (2006). Microtubules facilitate autophagosome formation and fusion of autophagosomes with endosomes. *Traffic* **7**, 129-145.

**Kollman, J. M., Merdes, A., Mourey, L. and Agard, D. A.** (2011). Microtubule nucleation by γ-tubulin complexes. *Nat. Rev. Mol. Cell Biol.* **12**, 709-721.

**Komlodi-Pasztor, E., Sackett, D., Wilkerson, J. and Fojo, T.** (2011). Mitosis is not a key target of microtubule agents in patient tumors. *Nat. Rev. Clin. Oncol.* **8**, 244-250.

**Korolchuk, V. I., Saiki, S., Lichtenberg, M., Siddiqi, F. H., Roberts, E. A., Imarisio, S., Jahreiss, L., Sarkar, S., Futter, M., Menzies, F. M. et al.** (2011). Lysosomal positioning coordinates cellular nutrient responses. *Nat. Cell Biol.* **13**, 453-460.

**Kouno, T., Mizuguchi, M., Tanida, I., Ueno, T., Kanematsu, T., Mori, Y., Shinoda, H., Hirata, M., Kominami, E. and Kawano, K.** (2005). Solution structure of microtubule-associated protein light chain 3 and identification of its functional subdomains. *J. Biol. Chem.* **280**, 24610-24617.

**Kroemer, G., Mariño, G. and Levine, B.** (2010). Autophagy and the integrated stress response. *Mol. Cell* **40**, 280-293.

**Ktistakis, N. T., Andrews, S. and Long, J.** (2011). What is the advantage of a transient precursor in autophagosome biogenesis? *Autophagy* **7**, 118-122.

**Lafont, F., Burkhardt, J. K. and Simons, K.** (1994). Involvement of microtubule motors in basolateral and apical transport in kidney cells. *Nature* **372**, 801-803.

**Lee, J. Y., Nagano, Y., Taylor, J. P., Lim, K. L. and Yao, T. P.** (2010). Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. *J. Cell Biol.* **189**, 671-679.

**Levine, B. and Klionsky, D. J.** (2004). Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev. Cell* **6**, 463-477.

**Lin, S. X., Gundersen, G. G. and Maxfield, F. R.** (2002). Export from pericentriolar endocytic recycling compartment to cell surface depends on stable, detyrosinated (glu) microtubules and kinesin. *Mol. Biol. Cell* **13**, 96-109.

**Lorin, S., Borges, A., Ribeiro Dos Santos, L., Souquère, S., Pierron, G., Ryan, K. M., Codogno, P. and Djavaheri-Mergny, M.** (2009). c-Jun NH2-terminal kinase activation is essential for DRAM-dependent induction of autophagy and apoptosis in 2-methoxyestradiol-treated Ewing sarcoma cells. *Cancer Res.* **69**, 6924-6931.

**Luo, S., Garcia-Arencibia, M., Zhao, R., Puri, C., Toh, P. P. C., Sadiq, O. and Rubinsztein, D. C.** (2012). Bim inhibits autophagy by recruiting Beclin 1 to microtubules. *Mol. Cell* **47**, 359-370.

**Mann, S. S. and Hammarback, J. A.** (1994). Molecular characterization of light chain 3. A microtubule binding subunit of MAP1A and MAP1B. *J. Biol. Chem.* **269**, 11492-11497.

**Mehrpour, M., Esclatine, A., Beau, I. and Codogno, P.** (2010). Overview of macroautophagy regulation in mammalian cells. *Cell Res.* **20**, 748-762.

**Miller, P. M., Folkmann, A. W., Maia, A. R. R., Efimova, N., Efimov, A. and Kaverina, I.** (2009). Golgi-derived CLASP-dependent microtubules control Golgi organization and polarized trafficking in motile cells. *Nat. Cell Biol.* **11**, 1069-1080.

**Mitchison, T. and Kirschner, M.** (1984). Dynamic instability of microtubule growth. *Nature* **312**, 237-242.

**Mizuno, M. and Singer, S. J.** (1994). A possible role for stable microtubules in intracellular transport from the endoplasmic reticulum to the Golgi apparatus. *J. Cell Sci.* **107**, 1321-1331.

**Mizushima, N.** (2010). The role of the Atg1/ULK1 complex in autophagy regulation. *Curr. Opin. Cell Biol.* **22**, 132-139.

**Mizushima, N. and Komatsu, M.** (2011). Autophagy: renovation of cells and tissues. *Cell* **147**, 728-741.

**Mizushima, N., Yoshimori, T. and Ohsumi, Y.** (2011). The role of Atg proteins in autophagosome formation. *Annu. Rev. Cell Dev. Biol.* **27**, 107-132.

**Monastyrska, I., Rieter, E., Klionsky, D. J. and Reggiori, F.** (2009). Multiple roles of the cytoskeleton in autophagy. *Biol. Rev. Camb. Philos. Soc.* **84**, 431-448.

**Moreau, K., Ravikumar, B., Renna, M., Puri, C. and Rubinsztein, D. C.** (2011). Autophagosome precursor maturation requires homotypic fusion. *Cell* **146**, 303-317.

**North, B. J., Marshall, B. L., Borra, M. T., Denu, J. M. and Verdin, E.** (2003). The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. *Mol. Cell* **11**, 437-444.

**Orsi, A., Razi, M., Dooley, H. C., Robinson, D., Weston, A. E., Collinson, L. M. and Tooze, S. A.** (2012). Dynamic and transient interactions of Atg9 with autophagosomes, but not membrane integration, are required for autophagy. *Mol. Biol. Cell* **23**, 1860-1873.

**Palazzo, A. F., Cook, T. A., Alberts, A. S. and Gundersen, G. G.** (2001). mDia mediates Rho-regulated formation and orientation of stable microtubules. *Nat. Cell Biol.* **3**, 723-729.

**Pandey, U. B., Nie, Z., Batlevi, Y., McCray, B. A., Ritson, G. P., Nedelsky, N. B., Schwartz, S. L., DiProspero, N. A., Knight, M. A., Schuldiner, O. et al.** (2007). HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. *Nature* **447**, 859-863.

**Pankiv, S., Alemu, E. A., Brech, A., Bruun, J. A., Lamark, T., Overvatn, A., Bjørkøy, G. and Johansen, T.** (2010). FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport. *J. Cell Biol.* **188**, 253-269.

**Polson, H. E., de Lartigue, J., Rigden, D. J., Reedijk, M., Urbé, S., Clague, M. J. and Tooze, S. A.** (2010). Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. *Autophagy* **6**, 506-522.

**Poüs, C. and Codogno, P.** (2011). Lysosome positioning coordinates mTORC1 activity and autophagy. *Nat. Cell Biol.* **13**, 342-344.

**Poüs, C., Chabin, K., Drechou, A., Barbot, L., Phung-Koskas, T., Settegrana, C., Bourguet-Kondracki, M. L., Maurice, M., Cassio, D., Guyot, M. et al.** (1998). Functional specialization of stable and dynamic microtubules in protein traffic in WIF-B cells. *J. Cell Biol.* **142**, 153-165.

**Ravikumar, B., Acevedo-Arozena, A., Imarisio, S., Berger, Z., Vacher, C., O'Kane, C. J., Brown, S. D. and Rubinsztein, D. C.** (2005). Dynein mutations impair autophagic clearance of aggregate-prone proteins. *Nat. Genet.* **37**, 771-776.

**Ravikumar, B., Sarkar, S., Davies, J. E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z. W., Jimenez-Sanchez, M., Korolchuk, V. I., Lichtenberg, M., Luo, S. et al.** (2010). Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol. Rev.* **90**, 1383-1435.

**Reed, N. A., Cai, D., Blasius, T. L., Jih, G. T., Meyhofer, E., Gaertig, J. and Verhey, K. J.** (2006). Microtubule acetylation promotes kinesin-1 binding and transport. *Curr. Biol.* **16**, 2166-2172.

**Reunanen, H., Marttinen, M. and Hirsimäki, P.** (1988). Effects of griseofulvin and nocodazole on the accumulation of autophagic vacuoles in Ehrlich ascites tumor cells. *Exp. Mol. Pathol.* **48**, 97-102.

**Rubinsztein, D. C., Mariño, G. and Kroemer, G.** (2011). Autophagy and aging. *Cell* **146**, 682-695.

**Rubinsztein, D. C., Codogno, P. and Levine, B.** (2012). Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat. Rev. Drug Discov.* **11**, 709-730.

**Sancak, Y., Bar-Peled, L., Zoncu, R., Markhard, A. L., Nada, S. and Sabatini, D. M.** (2010). Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* **141**, 290-303.

**Scheel, J., Matteoni, R., Ludwig, T., Hoflack, B. and Kreis, T. E.** (1990). Microtubule depolymerization inhibits transport of cathepsin D from the Golgi apparatus to lysosomes. *J. Cell Sci.* **96**, 711-720.

**Sengupta, S., Peterson, T. R. and Sabatini, D. M.** (2010). Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol. Cell* **40**, 310-322.

**Singh, R. and Cuervo, A. M.** (2011). Autophagy in the cellular energetic balance. *Cell Metab.* **13**, 495-504.

**Soppina, V., Herbstman, J. F., Skiniotis, G. and Verhey, K. J.** (2012). Luminal localization of α-tubulin K40 acetylation by cryo-EM analysis of fab-labeled microtubules. *PLoS ONE* **7**, e48204.

**Tanaka, Y., Guhde, G., Suter, A., Eskelinen, E. L., Hartmann, D., Lüllmann-Rauch, R., Janssen, P. M., Blanz, J., von Figura, K. and Saftig, P.** (2000). Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* **406**, 902-906.

**Toda, H., Mochizuki, H., Flores, R., 3rd, Josowitz, R., Krasieva, T. B., Lamorte, V. J., Suzuki, E., Gindhart, J. G., Furukubo-Tokunaga, K. and Tomoda, T.** (2008). UNC-51/ATG1 kinase regulates axonal transport by mediating motor-cargo assembly. *Genes Dev.* **22**, 3292-3307.

**Tolić-Nørrelykke, I. M.** (2008). Push-me-pull-you: how microtubules organize the cell interior. *Eur. Biophys. J.* **37**, 1271-1278.

**Topalidou, I., Keller, C., Kalebic, N., Nguyen, K. C. Q., Somhegyi, H., Politi, K. A., Heppenstall, P., Hall, D. H. and Chalfie, M.** (2012). Genetically separable functions of the MEC-17 tubulin acetyltransferase affect microtubule organization. *Curr. Biol.* **22**, 1057-1065.

**Veldhoen, R. A., Banman, S. L., Hemmerling, D. R., Odsen, R., Simmen, T., Simmonds, A. J., Underhill, D. A. and Goping, I. S.** (2013). The chemotherapeutic agent paclitaxel inhibits autophagy through two distinct mechanisms that regulate apoptosis. *Oncogene* **32**, 736-746.

**Verhey, K. J., Meyer, D., Deehan, R., Blenis, J., Schnapp, B. J., Rapoport, T. A. and Margolis, B.** (2001). Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. *J. Cell Biol.* **152**, 959-970.

**Walter, W. J., Beránek, V., Fischermeier, E. and Diez, S.** (2012). Tubulin acetylation alone does not affect kinesin-1 velocity and run length in vitro. *PLoS ONE* **7**, e42218.

**Webb, J. L., Ravikumar, B. and Rubinsztein, D. C.** (2004). Microtubule disruption inhibits autophagosome-lysosome fusion: implications for studying the roles of aggresomes in polyglutamine diseases. *Int. J. Biochem. Cell Biol.* **36**, 2541-2550.

**Webster, D. R., Gundersen, G. G., Bulinski, J. C. and Borisy, G. G.** (1987). Differential turnover of tyrosinated and detyrosinated microtubules. *Proc. Natl. Acad. Sci. USA* **84**, 9040-9044.

**Wei, Y., Pattingre, S., Sinha, S., Bassik, M. and Levine, B.** (2008). JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol. Cell* **30**, 678-688.

**Xie, Z. and Klionsky, D. J.** (2007). Autophagosome formation: core machinery and adaptations. *Nat. Cell Biol.* **9**, 1102-1109.

**Xie, R., Nguyen, S., McKeehan, W. L. and Liu, L.** (2010). Acetylated microtubules are required for fusion of autophagosomes with lysosomes. *BMC Cell Biol.* **11**, 89.

**Xie, R., Nguyen, S., McKeehan, K., Wang, F., McKeehan, W. L. and Liu, L.** (2011). Microtubule-associated protein 1S (MAP1S) bridges autophagic components with microtubules and mitochondria to affect autophagosomal biogenesis and degradation. *J. Biol. Chem.* **286**, 10367-10377.

**Yang, Z. and Klionsky, D. J.** (2010). Mammalian autophagy: core molecular machinery and signaling regulation. *Curr. Opin. Cell Biol.* **22**, 124-131.

**Ylä-Anttila, P., Vihinen, H., Jokitalo, E. and Eskelinen, E. L.** (2009). 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* **5**, 1180-1185.

**Zalckvar, E., Berissi, H., Mizrachy, L., Idelchuk, Y., Koren, I., Eisenstein, M., Sabanay, H., Pinkas-Kramarski, R. and Kimchi, A.** (2009). DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. *EMBO Rep.* **10**, 285-292.

**Zhang, Y., Li, N., Caron, C., Matthias, G., Hess, D., Khochbin, S. and Matthias, P.** (2003). HDAC-6 interacts with and deacetylates tubulin and microtubules in vivo. *EMBO J.* **22**, 1168-1179.

**Zhao, Y., Yang, J., Liao, W., Liu, X., Zhang, H., Wang, S., Wang, D., Feng, J., Yu, L. and Zhu, W. G.** (2010). Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity. *Nat. Cell Biol.* **12**, 665-675.