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Bringing It All Together: The Vital Role of Motor Proteins in Autophagy

Introduction

Autophagy is a vital catabolic process in which damaged organelles and other cellular structures are targeted for degradation and recycling of their constituent components. It is important for quality control and cellular homeostasis but also has other recognized roles, such as in differentiation and development.[1,2] Broadly known as a "self-eating" phenomenon, autophagy is not a single pathway but actually encompasses three related mechanisms[3] under complex regulatory control: macroautophagy (the subject of this newsletter), chaperone-mediated autophagy (where specific proteins are directed to lysosomes for degradation), and microautophagy, in which cellular material is directly sequestered by membrane invaginations in late endosomes or lysosomes.[1]

Macroautophagy, hereafter referred to as autophagy and summarized in Figure 1, may operate as either a nonselective bulk process or by recognizing particular cargos through specific autophagy receptors (SARs);[4] for example, as seen in the elimination of damaged mitochondria by mitophagy.[5] Either way, the selected cargo is surrounded by a double-membrane vesicle known as an autophagosome that must then fuse with a lysosome to initiate enzymatic degradation and complete the autophagic process. As explained below, the fusion step requires coordinated vesicle trafficking that is mediated by the cytoskeleton and associated motor proteins through mechanisms that continue to be unraveled.[6]

The Critical Role of the Cytoskeleton

Early work in the field identified functional roles in autophagy for both the actin and tubulin components of the cytoskeleton.[7] In this newsletter, we will focus on the latter since microtubules are known to be essential for autophagosome–lysosome fusion in mammals.[8] Specifically, maturing autophagosomes are transported along microtubules to perinuclear microtubule organizing centers (MTOCs), where the cellular concentration of lysosomes and late endosomes is highest.[9] Fusion of autophagosomes or amphisomes with lysosomes then produces autolysosomes[10] to initiate degradation of the cargo (Figure 1).

During transport, autophagosomes are tethered to motor proteins through various adapters that regulate intracellular trafficking. The two key adapters are FYVE and coiled-coil domain-containing protein 1 (FYCO1), which links autophagosomes to kinesins to drive anterograde movement (i.e., toward the cell periphery), and Rab-interacting lysosomal protein (RILP), which recruits dynein-1 to promote retrograde transport (i.e., toward the nucleus).[8]

While full details of the relevant mechanisms remain elusive,[11] the small GTPase Rab7 is thought to act as a molecular switch controlling the direction of transport. Interaction of autophagosome-associated Rab7 with FYCO1 recruits kinesin-1, apparently under control of the centrosomal factor NINL/NLP.[12] Alternatively, the cholesterol sensor OSBPL1A/ORP1L can bind to Rab7 to promote complex formation with RILP,[13] tethering the autophagosome to dynein-1 for transport in the opposite direction.

Certain posttranslational modifications (PTMs) introduce an additional layer of control. For instance, the Hippo kinase STK4/MST1 can phosphorylate the autophagosome protein MAP1LC3B/LC3B (often referred to as simply LC3)—which also binds to FYCO1—to reduce the affinity of this interaction and promote preferential dynein-1 recruitment for retrograde transport.[14,15] Microtubule acetylation is also required, since knockdown of α tubulin acetyltransferase 1 (ATAT1) resulted in random autophagosome distribution in mouse embryonic fibroblasts.[16] Acetylation of long-lived microtubules is well established in neurons,[17,18] where autophagosome transport over long distances is necessary in axons.

The Unique Case of Neuronal Autophagy

Autophagy in neurons is notable for its distinct compartmentalization, with autophagosome biogenesis occurring in the distal axon and lysosomal degradation confined mainly to the soma.[19] Autophagosomes initially undergo bidirectional movement in the growth cone region because they can be linked with both dynein-1 and kinesin-1,[20] but a switch to retrograde transport occurs on acquiring endolysosomal markers such as Rab7 and LAMP1. This event is seemingly controlled by JNK-interacting protein 1 (MAPK8IP1/JIP1), as its binding to LC3 induces dephosphorylation and competitively inhibits activation of kinesin-1, thereby favoring dynein-mediated retrograde motion.[21,22] As noted above, STK4-mediated phosphorylation of LC3 may also play a role.[15,19]

As autophagosomes move toward the soma, they mature through endosomal or lysosomal fusion and undergo progressive acidification. In the mid-axon, retrograde trafficking relies on both

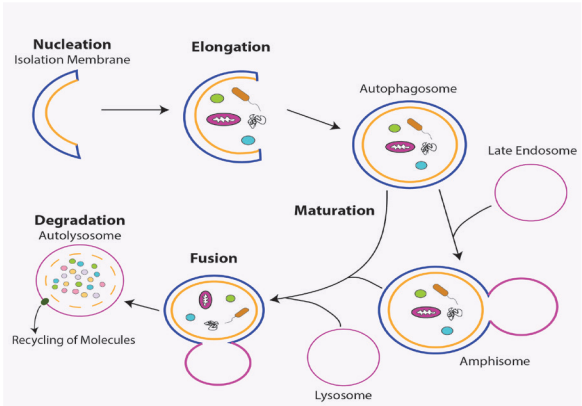

Figure 1. Schematic showing the general process of autophagy from nucleation to degradation. Adapted from Nakamura and Yoshimori, 2017 (Ref # 8).

Continued from Page 1

huntingtin and huntingtin-associated protein 1 (HAP1). Sufficiently acidic autophagic vesicles are then bound by another adapter, MAPK8IP3/JIP3,[20] to promote continued retrograde transport in the proximal axon. Finally, entry into the soma is a "one-way ticket" that is required for effective degradation of the cargo.[19,23]

Functional deficiencies in this complex trafficking process are associated with a range of neurological disorders. Dynein downregulation or loss-of-function mutations have been found in human motor neuropathies, neurodevelopmental disorders, and late-stage Parkinson's disease (PD).[24–26] The pathogenic LRRK2 G2019S mutation, one of the foremost causes of genetic PD,[27] results in enhanced kinase activity and hyperphosphorylation of Rab proteins. In neurons, this manifests as aberrant recruitment of the kinesin adapter SPAG9/JIP4 to autophagosomes, inducing a futile "tug of war" between opposing molecular motors and hindering retrograde transport and cargo degradation.[28]

Other findings suggest that neuronal protein aggregates are cleared by autophagy and that this process is dysregulated in protein aggregation diseases. In a landmark early study, inhibition of dynein-1 function was shown to impair autophagic clearance of aggregation-prone α-synuclein and huntingtin mutants.[29] Later work established that excessive accumulation of α-synuclein interferes with autophagosome transport by sequestering dynein-1, rationalizing its apparent reduced activity in PD.[24,30] Huntington's disease (HD) is associated with expansions in the polyglutamine (polyQ) tract of huntingtin that promote protein aggregation. While these aggregates can be cleared by autophagy,[31] huntingtin's role as an autophagy factor paradoxically impairs this process and may explain why larger expansions increase disease severity.[32,33]

Motor Proteins Also Position Lysosomes

For efficient autophagosome–lysosome fusion, the coordinated transport and positioning of both vesicles is essential.[8] As well as mediating retrograde trafficking of autophagosomes via Rab7, the dynein adapter RILP is involved in lysosomal positioning under the elaborate control of two other small GTPases, ARL8B and Rab34.[34,35] The lysosomal membrane protein ARL8B can, on the one hand, bind to the kinesin adapter PLEKHM2/SKIP to promote anterograde transport.[36] Alternatively, ARL8B may interact with DENND6A, a guanine nucleotide exchange factor, to activate Rab34, which then recruits RILP to tether the lysosome to dynein-1 and drive perinuclear clustering.[35] In support of this mechanism, DENND6A knockout led to altered lysosome distribution and decreased autophagic flux in HeLa cells.

Recent Developments and Outlook

Since its efficiency declines with age,[37] autophagy has received considerable recent attention in the context of aging and age-related diseases. The mechanistic details remain only partially resolved, but motor proteins are clearly involved.[6] One study found impaired autophagosome transport and lysosome positioning in old versus young mice, and respectively attributed these effects to reduced dynein-1 recruitment and downregulation of the kinesin superfamily member KIFC3.[38]

There is particular interest surrounding neurodegenerative diseases, where the therapeutic potential of inducing autophagy has been demonstrated in animal models.[39] Consistent with this, recent human data have suggested aberrant overexpression of ARL8B in Alzheimer's disease that hinders autophagy through altered lysosome distribution and pH.[40] Moving forward, it is hoped that new interventions targeting autophagy will offer therapeutic benefit in aging and associated pathogeneses as the molecular mechanisms continue to be elucidated.[39]

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KIF3C kinesin motor domain protein GST tagged: Homo sapiens recombinant	2 x 25µg	KF01-A
Kinesin ELIPA Kit	96 assays	BK060
Microtubules **(Taxol Stabilized and Lyophilized)**	4 x 500 µg 1 x 10 mg	MT002-A MT002-XL

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