

Addendum

Neuronal autophagy

Going the distance to the axon

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Autophagy, a regulated cellular degradation process responsible for the turnover of long-lived proteins and organelles, has been increasingly implicated in neurological disorders. Although autophagy is mostly viewed as a stress-induced process, recent studies have indicated that it is constitutively active in central nervous system (CNS) neurons and is protective against neurodegeneration. Neurons are highly specialized, post-mitotic cells that are typically composed of a soma (cell body), a dendritic tree and an axon. The detailed process of autophagy in such a highly differentiated cell type remains to be characterized. To elucidate the physiological role of neuronal autophagy, we generated mutant mice containing a neural cell type-specific deletion of *Atg7*, an essential gene for autophagy. Establishment of these mutant mice allowed us to examine cell-autonomous events in cerebellar Purkinje cells deficient in autophagy. Our data reveal the indispensability of autophagy in the maintenance of axonal homeostasis and the prevention of axonal dystrophy and degeneration. Furthermore, our study implicates dysfunction of axonal autophagy as a potential mechanism underlying axonopathy, which is linked to neurodegeneration associated with numerous human neurological disorders. Finally, our study has raised a possibility that “constitutive autophagy” in neurons involves processes that are not typical of autophagy in other cell types, but rather is highly adapted to local physiological function in the axon, which is projected in a distance from one neuron to another for transducing neural signals.

Complex Roles of Constitutive Autophagy in the Neuron and the Axon: Protein Quality Control and Maintenance of Membrane Homeostasis

Historically, excessive autophagy has been shown in various neuropathological conditions and was thus suspected to be highly destructive.¹⁻⁵ While previous studies have not clarified a causal role for autophagy in neuropathogenesis, recent evidence has revealed a critical function of constitutive autophagy in the prevention of neurodegeneration. Genetic ablation of essential autophagy genes *Atg5* or *Atg7* in the brain causes the formation of ubiquitin-associated inclusions in neurons, highlighting the beneficial role of neuronal autophagy in protein quality control.^{6,7} Notably, the degree of vulnerability of neurons and the formation of intracellular inclusions vary significantly among different neuron types in the mutant mice deficient in autophagy, suggesting a cell-type specific cellular response to autophagy deficiency and a cell type-dependent mechanism contributing to the neurotoxicity in the mutant mice. For example, Purkinje cells deficient in *Atg5* or *Atg7* display very few ubiquitin-associated inclusions, whereas these cells are among the most vulnerable neuron types.^{6,7} In contrast, a large number of ubiquitin-associated inclusions were noted in the brain area where neuronal loss was hardly detected when autophagy was genetically inhibited (Waguri S, Komatsu M, unpublished data).

Our study and others have also revealed a prominent neuropathological feature associated with various neuron types in *Atg5* or *Atg7*-deficient brains—axonal dystrophy and degeneration.⁶⁻⁸ In particular, in the mutant Purkinje cells with *Atg7* deletion, axonal dystrophy and degeneration occur in a cell-autonomous manner and precede Purkinje cell death. This axonal dystrophy caused by autophagy failure is neither due to the action of neighboring glial cells, nor is secondary to the dying process of the mutant Purkinje cells. Rather, it is a primary event resulting directly from impaired autophagy and may contribute to the demise of the mutant Purkinje cells.⁸

Importantly, we found that dystrophic axon terminals of the mutant Purkinje cells tend to accumulate aberrant organelles or membrane structures which are rarely seen in wild-type controls.⁸ This result reveals a specific role for neuronal autophagy in the maintenance of local homeostasis at the axon terminals. We hypothesize

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that this highly specialized neuronal autophagy is required for keeping the balance of the membrane network, which normally involves cycling of membranous structures or vesicles, at the axon terminals to support synaptic activity. The role of this constitutive autophagy in maintaining membrane homeostasis is no surprise considering that the typical autophagic process involves dynamic membrane rearrangement and turnover;⁹ future study should solve the puzzle as to how this “self-eating” process participates in axonal membrane turnover and what membrane substrates axonal autophagy removes under physiological conditions. The answers to these questions are expected to advance our knowledge of cell biology in the neuron as well as of the disease processes that are associated with dysfunctional autophagy in the neuron and the axon.

Connection Between Axonal autophagy and Axonopathy

The axon is a highly specialized neuronal compartment that performs many functions independently from the soma. Axonal dystrophy, a hallmark of axonopathy, can be triggered by neuronal injuries, excitotoxicity and various neurodegenerative conditions.¹ Despite the prevalence of this pathology, the molecular mechanisms underlying axonopathy as well as the connection between axonopathy and neurodegeneration remain poorly understood.¹⁰

Autophagy has previously been suggested to be associated with axonal dystrophy or axonopathy. Following axotomy or excitotoxic stimuli, double-membrane vesicles resembling autophagosomes were originally observed to accumulate in the dilated axon terminals that resulted from the insults,^{11,12} a local phenomenon that was not observed in undisturbed axons. Autophagosome-like vesicles have also been shown to be present in the dysfunctional or degenerating axons associated with a range of chronic neurodegenerative conditions, including Alzheimer’s disease (AD),^{13,14} Parkinson’s disease (PD),¹⁵ Huntington’s disease (HD),¹⁶ and Creutzfeldt-Jakob disease¹⁷ and their animal models.^{1,18,19} Although it has been widely thought that these compartments are the sign of heightened autophagy, conclusive evidence for the autophagy-dependent formation of those structures is lacking.

We have previously shown that induction of autophagy in the mutant Purkinje cells suffering from excitotoxic insults (*Lurcher*) involves accumulation of LC3-positive double-membrane vesicles in the dystrophic axon,²⁰ in contrast to the lack of vesicles resembling autophagosomes in the dystrophic axon terminals of the mutant Purkinje cells with *Atg7* deletion.⁸ These *in vivo* studies strongly argue that the formation of a large number of vesicles in axonal dystrophic terminals of *Lurcher* Purkinje cells depends on autophagy, and that these vesicles are indeed autophagosomes. Importantly, these genetic studies in Purkinje cells have a broad implication in understanding the neuropathological process that is associated with axonal dystrophy in other types of neurons as aforementioned. We speculate that the observation of accumulated autophagosome-like vesicles in these injured axons/neurons as well as those in human neuropathological processes largely reflect the stimulated biosynthesis of autophagosomes that is needed to remodel neuronal structures during the crisis of neurological disorders. Furthermore, the axonal dystrophy that lacks autophagosomes in the *Atg7*-deficient Purkinje cells suggests that deficiency in axonal autophagy can be an alternative mechanism that potentially underlies the axonopathy that is associated with many neurological disorders. Thus, these two types

of mutant mice (*Lurcher* and *Atg7*-deficient Purkinje cells) under two distinct genetic settings provide excellent models for monitoring and further analyzing the process of how aberrant autophagic activity contributes to axonopathy and neurodegeneration. Future studies should look into the molecular details of how autophagy is affected (either hyperactive or impaired) in axonopathy associated with neurological disorders and the consequences of the altered autophagy in neuropathology.

Canonical Autophagy Versus Adapted Autophagy in Mammalian Neurons

Canonical autophagy is characterized by a dynamic process involving sequestering a portion of cytoplasm into double-membrane vesicles (autophagosomes) and delivering these autophagosomes to lysosomes for degradation. The biogenic part (vesicle formation) of autophagy is controlled by highly conserved autophagic machinery comprised of two ubiquitin-like conjugation systems.⁹ While our view of the autophagic process is largely limited to a response to nutrient limitation in lower eukaryotes, emerging evidence has implicated the autophagic process in divergent physiological functions in mammals. Recent studies using mice expressing an autophagy reporter, green fluorescent protein-tagged LC3 (GFP-LC3), have suggested that autophagy is distinctly regulated in different tissues.²¹ For example, food limitation triggers a rapid up-regulation of autophagy in mouse liver and heart as indicated by the formation of a large number of GFP-LC3 puncta (i.e., autophagosomes), whereas it fails to induce GFP-LC3 puncta in the mouse CNS despite high expression levels of GFP-LC3 in many types of neurons. Additionally, mouse liver and heart have many GFP-LC3 puncta even when food is not limited, suggesting constitutively active autophagy in these tissues. Furthermore, whereas the CNS neurons seem to be prohibited from autophagy induction in response to starvation,² some types of neurons develop ubiquitin-associated inclusions and suffer from axonal dystrophy and degeneration when autophagy is genetically blocked,⁶⁻⁸ demonstrating the presence of constitutive autophagy in the CNS neurons. Thus, these results not only show the tissue- and cell type-specific regulation of the autophagy process, but also suggest that the regulation of autophagy is highly specific even in the different compartments within a neuron.

We have previously proposed a model to explain local autophagic activity in the axon under physiological conditions: autophagosomes can be synthesized in the axons and transported in retrograde back to the soma, where lysosomes reside, for degradation.²² This model is based on the current knowledge of canonical autophagy and assumes that such an autophagic process is subjected to specific regulation in the axon. Here, we present an alternative explanation for the constitutive autophagy seen in the axon, which is not based on canonical autophagy. We hypothesize that, although all components of the autophagic machinery are ubiquitously expressed across tissues, cell types, and cell compartments, they are adapted to the highly specialized physiology of certain tissues (e.g., brain), cells (e.g., neuron) and cell compartments (e.g., axon) and thus confer different functions that are distinct from canonical autophagy. Consistent with this notion, we show the indispensability of *Atg7* for the formation of the double-membrane vesicular structures normally present within wild-type Purkinje cell axons.⁸ Notably, the majority of these structures are likely derived from invagination of neighboring

oligodendrocytes,^{23,24} rather than from the typical process of autophagosome biogenesis as in canonical autophagy. Therefore, an important question arising from these results concerns the specific role of Atg7 in the formation of these distinct double-membrane vesicles, whose nature and function are currently unknown. It is conceivable that Atg7 and other components of the autophagic machinery may have become highly adapted to the local environment and involved in tasks related to, but distinguished from, canonical autophagy that typically requires the formation of double-membrane autophagosomes. One well-known example for such a constitutive autophagy which is not canonical autophagy is the yeast Cvt (cytoplasm-to-vacuole targeting) pathway, which shares most protein components with autophagy but is biosynthetic and functions to specifically deliver certain enzymes to vacuoles.²⁵ We speculate that in mammalian cells there may exist many different adapted autophagy processes, which have evolved from the ancient role of autophagy for primarily supplying nutrients, to achieve sophisticated tissue-, cell type-, and cell compartment-specific novel functions.

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