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Mini-review

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Energy deprivation-induced autophagy and aggrephagy: insights from yeast and mammals

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Abstract: Autophagy plays a crucial role in maintaining cellular homeostasis in response to various stimuli. Compared to research on nutrient deprivation-induced autophagy, the understanding of the molecular mechanisms and physiological/pathological significance of autophagy triggered by energy deprivation remains limited. A primary focus of our lab is to elucidate how cells sense energy deprivation and initiate autophagy. Using the model organisms *Saccharomyces cerevisiae* and mammalian cells, we found that cellular reactive oxygen species (ROS), DNA damage sensor Mec1, and mitochondrial aerobic respiration play essential roles in the autophagy induced by energy deprivation. This review aims to provide a concise overview of these research findings.

Key words: Autophagy; Glucose starvation; Solid aggrephagy; Chaperonin-containing TCP-1, subunit 2 (CCT2)

Autophagy is a highly conserved material degradation pathway in eukaryotic cells that relies on vacuoles/lysosomes (Ohsumi, 2014). When cells confront external stress stimuli (such as amino acid starvation, nitrogen starvation, or glucose starvation), a double-layered membrane structure emerges within the cell. This structure encloses various redundant substances including proteins that need to be degraded, protein aggregates, pathogenic bacteria or viruses, damaged organelles, and lipid droplets. This double-layer membrane structure gradually grows, extends, and seals, eventually forming an autophagosome. Subsequently, the autophagosome fuses with a lysosome or vacuole, resulting in the formation of an autolysosome. The acidic hydrolytic enzymes housed within the lysosome degrade the substances enclosed in the autophagosome, thereby achieving the goal of clearing harmful substances within the cell and recycling them. This entire process is termed autophagy and is essential for maintaining cellular homeostasis (Ohsumi, 2014). Emerging evidence demonstrates a close association between autophagy dysfunction and several significant human diseases, such as neurodegenerative diseases, metabolic disorders, and cancer, among others (Mizushima and Levine, 2020). Consequently, exploring the molecular regulatory mechanisms and physiological-pathological functions of autophagy will offer new targets and perspectives for preventing and treating these diseases.

Like many cellular phenomena, autophagy is subject to regulation by cellular nutrition and energy status (He, 2022). As far back as 1992, the research team led by Nobel laureate in Medicine or Physiology in 2006, Yoshinori OHSUMI, observed that yeast cells generate numerous autophagosomes in response to nitrogen or carbon starvation (Suzuki and Ohsumi, 2007). Over the past three decades, various research groups, including Ohsumi's lab, have extensively investigated the molecular mechanisms and physiological functions of autophagy (Kametaka et al., 1998; Yurube et al., 2020). However, these groups have primarily concentrated on autophagy triggered by nutrient deprivation, such as nitrogen starvation, leaving research on autophagy induced by energy deprivation relatively unaddressed. So far, two primary signaling pathways have been identified for inducing autophagy: nutrient deprivation-induced autophagy, which is chiefly mediated through the mammalian target of rapamycin (mTOR)

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signaling pathway (Kamada et al., 2010), and energy deprivation-induced autophagy, which is primarily regulated through the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) signaling pathway (He, 2022).

Through a fortuitous experiment, we unveiled a unique molecular mechanism behind energy deprivation-induced autophagy. When subjecting yeast cells separately to nitrogen starvation and glucose starvation within enclosed tubes, we found that, while nitrogen source starvation-induced autophagy remained unaffected by low oxygen conditions, glucose starvation-induced autophagy was significantly blocked under similar low oxygen conditions (Yi et al., 2017). Given that cellular oxygen utilization primarily occurs in mitochondria through aerobic respiration, we proceeded to inhibit mitochondrial aerobic respiration (Vercellino and Sazanov, 2022). Subsequently, we observed a complete absence of autophagy induced by glucose starvation, while autophagy triggered by nitrogen starvation remained unaffected. This pivotal discovery led us to identify a key protein, mitosis entry checkpoint protein 1 (Mec1), which orchestrates this process. Mec1 functions as a DNA damage sensor that is essential for initiating DNA damage repair (Weinert et al., 1994; Maréchal and Zou, 2013). We found that the indole-3-acetic acid (IAA)-induced degradation of Mec1 specifically blocks glucose starvation-induced autophagy. Concurrently, the regulation of glucose starvation-induced autophagy by Mec1 is evolutionarily conserved, as knocking down its homolog, ataxia telangiectasia and Rad3-related protein (ATR), in mammals similarly inhibited glucose starvation-induced autophagy (Yi et al., 2017). Subsequent molecular mechanism studies revealed that, under glucose starvation conditions, Mec1 was specifically recruited to mitochondria. Conversely, under nitrogen starvation or rapamycin treatment, Mec1 remained localized in the nucleus. Furthermore, at the surface of mitochondria, the cellular energy sensor, sucrose non-fermentating protein 1 (Snf1, the homolog of AMPK in yeast), phosphorylated Mec1 (Weinert et al., 1994; Maréchal and Zou, 2013). This phosphorylated form of Mec1 facilitated the recruitment of the autophagy-related protein 1 (Atg1) complex to mitochondria, thereby initiating energy deprivation-induced autophagy. Hence, our findings underscore the significant and specific role that mitochondria play in the mechanism governing energy deprivation-induced autophagy (Yi et al., 2017).

After the revelation that mitochondria serve as the hub for initiating energy deprivation-induced autophagy, three critical questions arise: (1) Considering mitochondria as highly dynamic organelles undergoing continual fission and fusion, does their morphology influence energy deprivation-induced autophagy? (2) What mechanism underlies Mec1's recruitment to mitochondria, and is this recruitment essential for energy deprivation-induced autophagy? (3) How does Mec1 recruit the Atg1 complex to initiate energy deprivation-induced autophagy? Centered around these questions, we conducted a series of experiments. Primarily, we aimed to ascertain the potential role of mitochondrial morphology in energy deprivation-induced autophagy. Our observations under glucose starvation conditions indicated that the absence of mitochondrial fission had no impact on energy deprivation-induced autophagy. However, when mitochondrial fusion was impaired, energy deprivation-induced autophagy was completely blocked. Further investigations into the molecular mechanisms elucidated that the deficiency in mitochondrial fusion impeded mitochondrial aerobic respiration, subsequently blocking Snf1-mediated Mec1 phosphorylation (Wu et al., 2020). Consequently, we concluded that the mitochondrial fusion machinery promotes Snf1-mediated Mec1 phosphorylation by maintaining mitochondrial aerobic respiration, thereby initiating energy deprivation-induced autophagy.

Subsequently, we delved into identifying the signaling pathway governing Mec1 recruitment to mitochondria and assessing the necessity of Mec1's recruitment to mitochondria for energy deprivation-induced autophagy. Given that mitochondria serve as a primary source of reactive oxygen species (ROS) (Balaban et al., 2005), we measured ROS levels in yeast cells under different culture conditions. Utilizing flow cytometry analysis, we observed a substantial rise in cellular ROS levels solely under glucose starvation conditions, while there was no increase in ROS levels under nitrogen starvation or rapamycin treatment (Wu et al., 2021). This observation suggests a potentially significant role of ROS in glucose starvation-induced autophagy. To clarify the pivotal role of ROS in glucose starvation-induced autophagy, we employed the ROS scavenger butyl hydroxyanisole (BHA) to eliminate ROS generated during glucose starvation. We found that, under glucose starvation conditions, the removal of ROS prevented Mec1 recruitment to

mitochondria. By analyzing the number of cells with green fluorescent protein (GFP)-Atg8 entering vacuoles and the efficiency of GFP-Atg8 cleavage, we noted that the addition of the ROS scavenger BHA completely inhibited glucose starvation-induced autophagy, while nitrogen starvation-induced autophagy remained unaffected. Concurrently, we observed that the addition of the ROS scavenger BHA impaired the phosphorylation of Mec1 by Snf1 (Wu et al., 2021). Therefore, our findings indicate that glucose starvation prompts an elevation in cellular ROS levels, subsequently facilitating the recruitment of Mec1 to mitochondria, followed by Snf1-mediated Mec1 phosphorylation, thereby initiating energy deprivation-induced autophagy.

To address the third question, we initially conducted yeast two-hybrid (Y2H) assays to determine how Mec1 recruits the Atg1 complex to initiate energy deprivation-induced autophagy by identifying the specific protein within the Atg1 complex that directly interacts with Mec1. The Y2H assays revealed a direct interaction between Mec1 and Atg13. Subsequently, employing Y2H, in vitro pulldown assays, and immunoprecipitation experiments, we delineated their critical interaction region. The subsequent experiments we performed highlighted the indispensability of the interaction between Mec1 and Atg13 in recruiting the Atg1 complex and initiating energy deprivation-induced autophagy. Additionally, considering that DNA damage can also trigger autophagy and recognizing Mec1's crucial involvement in this process, we explored the necessity of their interaction for DNA damage-induced autophagy. Our investigations revealed that the interaction between Mec1 and Atg13 is equally indispensable for DNA damage-induced autophagy. Based on these results, we conclude that Mec1, through its interaction with the mitochondrial protein mitochondrial GTP/GDP carrier protein 1 (Ggc1) and the autophagic protein Atg13, facilitates the connection between mitochondria and autophagosomes under glucose starvation conditions. This connection led to the recruitment of the Atg1 complex onto the mitochondria, thereby initiating energy deprivation-induced autophagy (Yao et al., 2023b). Furthermore, we conducted a comparative analysis of the impacts of all identified yeast autophagy-related genes on autophagy induction under glucose starvation conditions. Remarkably, our findings highlighted the necessity of *ATG11*—a gene crucial for selective autophagy but dispensable for nitrogen starvation-induced autophagy—for the initiation of autophagy triggered by glucose starvation. Further investigation into the underlying mechanisms revealed that Atg11 is specifically required for activating the autophagy-related protein kinase Atg1 under glucose starvation conditions, whereas it is not necessary for autophagy induced by nitrogen starvation (Yao et al., 2023a). Conclusively, through our comprehensive series of experiments, we have elucidated significant distinctions between autophagy induced by energy deprivation and that induced by nutrient starvation in terms of their occurrence sites, signaling pathways, and molecular machinery (Fig. 1a).

On the other hand, our extensive research has focused on selective autophagy, particularly solid aggrephagy. Increasing studies have revealed a strong association between the accumulation of solid aggregates and the onset and progression of various human neurodegenerative diseases (Kuma et al., 2004; Mathieu et al., 2020). However, the mechanism of clearing solid aggregates through autophagy remains ambiguous (Lamark and Johansen, 2012). In collaboration with Prof. Liang GE's research group at Tsinghua University (Beijing, China), we utilized techniques such as the in vitro reconstruction of solid aggrephagy, autophagosome isolation using fluorescence-activated cell sorting (FACS), and mass spectrometry analysis. Through these methods, we successfully identified chaperonin-containing TCP-1, subunit 2 (CCT2) as the receptor responsible for solid aggrephagy, confirming its pivotal role in this process. Notably, our findings highlighted a high level of species conservation in the CCT2 receptor for solid aggrephagy. This revelation hints at a promising pathway for preventing and treating various human neurodegenerative diseases by targeting CCT2 to clear solid aggregates (Fig. 2) (Ma et al., 2022). Additionally, our research revealed that, during the process of selective autophagy, the autophagy-related protein kinase Atg1 enhances the binding of Atg11 to selective autophagy receptors through phosphorylating Atg11, thereby initiating the occurrence of selective autophagy (Fig. 1b) (Yao et al., 2023b). Collectively, these findings significantly enhance our understanding of selective autophagy, particularly in the context of solid aggrephagy.

In summary, we have outlined the progress of our lab's research on energy deprivation-induced

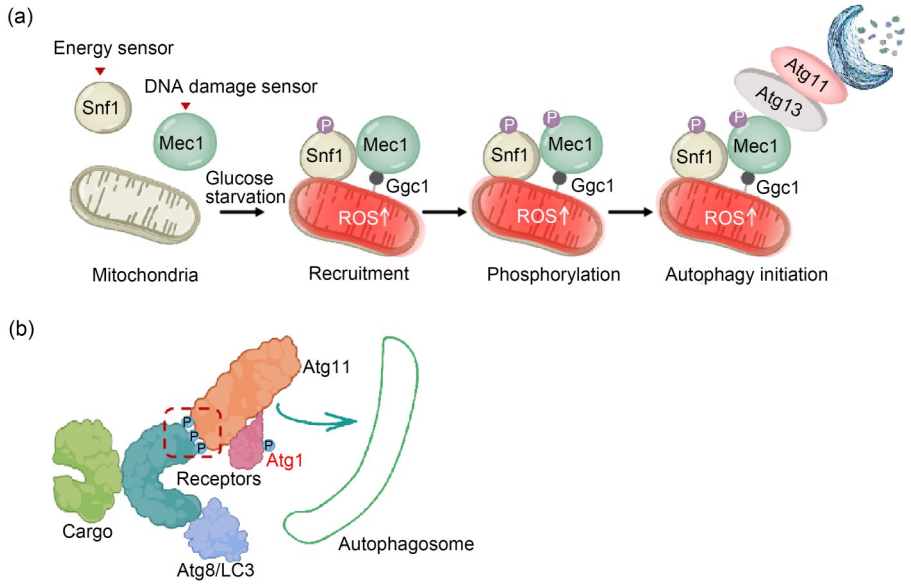

Fig. 1 Schematic diagram of the initiation of energy deprivation-induced autophagy and Atg1-mediated Atg11 phosphorylation regulating selective autophagy. (a) When cells face glucose starvation, there is a notable increase in cellular ROS, which induces the recruitment of Mec1 from the nucleus to the mitochondria. At the mitochondria, the energy sensor (Snf1) phosphorylates Mec1. Phosphorylated Mec1 then facilitates the recruitment of the Atg1 complex to the mitochondria by directly interacting with the autophagic protein Atg13, thereby initiating autophagy induced by energy deprivation. (b) When cells face autophagy-inducing stress, the activated Atg1 phosphorylates the selective autophagy marker protein Atg11, facilitating the binding of Atg11 to selective autophagy receptors, thereby initiating selective autophagy. Atg: autophagy-related protein; ROS: reactive oxygen species; Mec1: mitosis entry checkpoint protein 1; Snf1: sucrose nonfermentating protein 1; Ggc1: mitochondrial GTP/GDP carrier protein 1; P: phosphorylation; LC3: light chain 3.

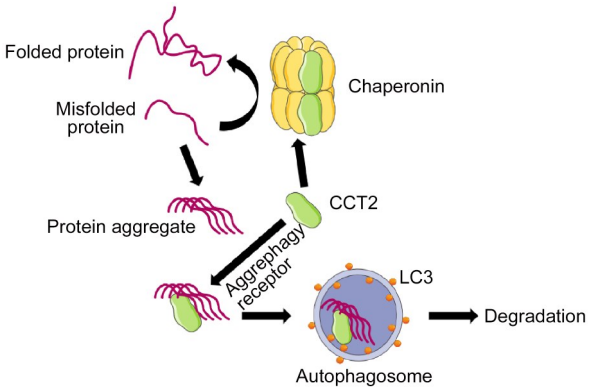

Fig. 2 Schematic diagram of the receptor CCT2 regulating solid aggrephagy. When solid aggregates accumulate within the cell, LC3 recruits the autophagic machinery to the protein aggregates by binding to the LIR motif of the receptor CCT2, thereby initiating solid aggrephagy. CCT2: chaperonin-containing TCP-1, subunit 2; LC3: light chain 3; LIR: LC3-interacting region.

autophagy and solid aggrephagy. The decrease in blood glucose levels following human starvation or exercise to some extent provides the physiological conditions for studying the physiological function of energy deprivation-induced autophagy. Meanwhile, neurodegenerative diseases serve as disease models for our investigation into the pathological function of solid aggrephagy in the onset and progression of these diseases. Despite strides in energy deprivation-induced autophagy and solid aggrephagy, comprehensively understanding their molecular mechanisms and physiological/pathological functions remains a formidable task. Building upon the current discoveries, we will utilize multiple biological tools such as genetics, cell biology, and chemical biology to uncover new targets and strategies associated with autophagy-related human diseases.

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Author contributions

Siyu FAN: conceptualization, formal analysis, data curation, writing original draft, and visualization. Yingcong CHEN: conceptualization. Weijing YAO: formal analysis. Cong YI: funding acquisition and writing – review & editing. All authors have read and approved the final version.

Compliance with ethics guidelines

Siyu FAN, Yingcong CHEN, Weijing YAO, and Cong YI declare that they do not have any conflicts of interest.

This review does not involve any studies conducted on human or animal subjects by any of the authors.

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