



# Autophagy in mitotic animal cells

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Mitosis is a highly dynamic cellular event that involves high energy consumption and active membrane remodeling. Autophagy is a process that cells digest their cellular contents to provide energy and nutrients to maintain homeostasis. Until now, whether autophagy persists during mitosis is still debated.

In most dividing animal cells, the whole cell cycle can be divided into about 20 h of interphase and 1–2 h of mitosis. Interphase consists of G1, S and G2 phases, where G stands for “gap” and S stands for “synthesis”. At the end of previous round of mitosis, two daughter cells are separated from each other before the new cell cycle starts with G1 phase. In this phase, cells synthesize proteins, carbohydrates and lipids necessary for cells growth. S phase is the DNA synthesis phase, where cells replicate their genomic contents. G2 phase is the last phase before mitosis, and the cells need to synthesize more proteins, such as microtubule-related proteins, and check all the factors to make sure they are ready for mitosis, a much shorter but highly dynamic process.

Mitosis is the process that a cell equally divides its chromosomes and cytoplasmic contents into two daughter cells. It includes prophase, prometaphase, metaphase, anaphase, telophase and cytokinesis. The cell starts to condense its chromatins into chromosomes and separate the centrosomes in prophase, breaks down its nuclear envelope and tries to connect all the kinetochores on the

chromosomes to microtubules in prometaphase, aligns all chromosomes in the middle in metaphase, splits chromosomes in anaphase, drags two sets of chromosomes close to the two spindle poles and reforms the nuclear envelope in telophase and finally divides the cellular contents into two separate daughter cells in cytokinesis. All these events have high energy demands and involve a large amount of cellular remodeling.

Autophagy is an evolutionarily conserved lysosome degradation pathway by which cells digest their cytoplasmic contents into amino acids and nucleotides to supply nutrition and degrade proteins and damaged organelles to maintain cellular homeostasis [1]. Autophagy includes macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy (hereafter autophagy) was studied most. Induced by autophagy stimuli, some membrane vesicles form preautophagosomal structure (PAS) where autophagic proteins are recruited and double-membrane structure is formed. They gradually elongate to enwrap the organelles and proteins to be degraded before finally forming closed double-membrane autophagosomes (Fig. 1). The outer membrane of autophagosome fuses with the lysosome and the inner membrane of autophagosome and inclusions were degraded in lysosome, providing amino acids, nucleotides and glucose to cytoplasm for cell growth and energy supply. Dysregulation in autophagy is related to multiple diseases, such as metabolic, neurodegenerative diseases and cancers. Therefore, it has been a hotspot in recent years. Current autophagy studies are mostly using unsynchronized cells, in which around 95 % of cells are in interphase. Therefore, the autophagy responses that researchers observed are likely to represent autophagy in interphase cells.

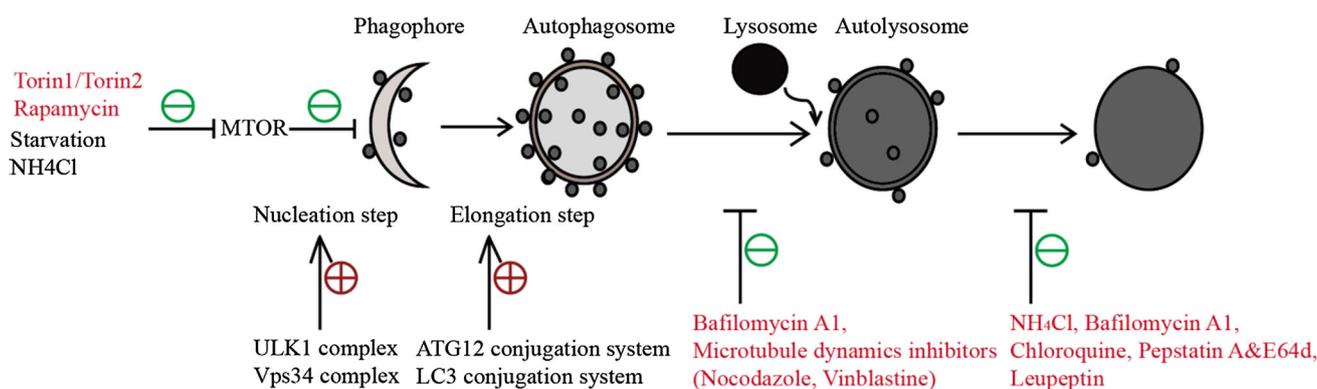
Multiple studies demonstrated the important role of autophagy in cytokinesis [2]. But whether autophagy

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**Fig. 1** (Color online) Autophagy is a highly dynamic and complex process. Autophagy process could be regulated in different steps, including nucleation step, elongation step and fusion step. Small molecules are usually used to induce or inhibit autophagy in early or late autophagic process

persists during the early phases of mitosis, such as prophase, prometaphase, metaphase, anaphase and telophase, remains a debate. Some studies indicate that autophagy is inhibited in mitosis. Mammalian cells undergo open mitosis, which means that their nuclear envelope is broken down during mitosis and mitotic spindles, dividing chromosomes, fragmented Golgi apparatus and mitochondria are all exposed. Theoretically, autophagy should be controlled temporally and spatially during mitosis to avoid harming these exposed organelles. Eskelinen et al. [3] first indicated that autophagy was inhibited in mitotic animal cells in 2002. They synchronized cells in mitosis using the microtubule-depolymerizing agent nocodazole and then starved the cells with Eagle's balanced salt solution (EBSS) to induce autophagy. They found that the fraction of autophagic vacuole in mitotic cell volume was much reduced compared to interphase and telophase (Fig. S1a). However, questions arise from these data. First of all, these results were all from morphological observations. Because both increased autophagic flux and inhibition at early steps were likely to decrease autophagosomes number (Fig. 1), it is not accurate to confirm the autophagy level only by morphological evidence. For example, the rapid autophagic flux may clear the autophagosomes faster and results in fewer autophagosomes detected [4]. Secondly, the author only determined that EBSS starvation-induced autophagy was inhibited in mitosis. In fact, most cells in mitosis may not experience starvation and the basal autophagy needs to be further investigated. Thirdly, they showed that EBSS could not induce autophagy in mitotic cells after removal of nocodazole. However, 45–90 min may not be insufficient for cells to fully recover from nocodazole treatment.

Another study supported the autophagy inhibition during mitosis was from Yuan's laboratory (Fig. S1b, d) [5]. The key finding is that Vps34 phosphorylation at Thr159 is increased in mitosis, which inhibits Vps34's function. This

is a very important finding that reveals the molecular regulation of Vps34 during mitosis (Fig. S1c, d). However, it was uncertain that phosphorylation at Vps34-Thr159 would be strong enough to inhibit all the function of Vps34 or to shut down autophagy during mitosis. Moreover, their results showed that phosphorylation of Vps34 inhibited starvation-induced autophagy rather than basal autophagy. Together with another study [6], these researches used reduced GFP-LC3 puncta in mitotic cells to demonstrate the decreased autophagy (Fig. S1b), which cannot rule out the possibility that fewer GFP-LC3 puncta observed were due to rapid clearance of autophagosomes [4] or the quenched GFP fluorescence in acidic autophagosomes [1].

There are also rationales behind the hypothesis that autophagy persists in mitosis, which was evidenced by increased endocytosis and decreased cell surface area in mitosis compared with that in interphase [7]. The increased vesicles coming from endocytosis might need more degradation pathways, such as autophagy. Meanwhile, autophagy could potentially provide the energy consumption required during mitosis. In addition, the damaged mitochondria caused by excessive energy metabolism during mitosis could also be removed by autophagy.

The first study indicating that autophagy persists during mitosis was published in 2009 [8]. It showed that after treated with NH<sub>4</sub>Cl or bafilomycin A1 for 20 h, cells in mitosis accumulated the same amount of LC3B as cells in interphase. But this work could not exclude the possibility that there was LC3B inherited from interphase due to the 20-h prolonged treatment. The authors also realized this problem and did another experiment by collecting mitotic cells using thymidine–nocodazole double synchronization. They then treated cells with 20 mmol/L NH<sub>4</sub>Cl for different time points and observed increased LC3BII within 30 min (Fig. S2a). However, as NH<sub>4</sub>Cl is also an autophagic flux inducer [9], the observed LC3BII increase may

be just a result of  $\text{NH}_4\text{Cl}$ -induced autophagy. Hence, it is necessary to confirm these results with the well-accepted autophagic flux inhibitors, such as bafilomycin A1 and chloroquine. A shorter interval between time points should also be applied. Furthermore, the results could be more convinced if interphase cells were set as control to show their response differences to autophagy inhibitors.

It was well known that LC3II directly correlates with the number of autophagosomes. Recently, flow cytometry was used to monitor the autophagy level in cell cycle by measuring LC3II level in different cell cycles [10]. This method accurately reflected all autophagosomes accumulation in specific timeframes and ruled out the possibility that GFP fluorescence could be quenched in acidic autophagosomes. Using different inducers and inhibitors, their results demonstrate that autophagy was at the same level in G2/M, S or G0/G1 phases (Fig. S2b). However, one caveat is that flow cytometry can only distinguish G0/G1, S and G2/M phases, but it cannot separate mitosis from G2 phase.

Recent studies also indicate roles of autophagy genes in mitosis. For example, Beclin-1 RNAi led to abnormal chromosome congression and outer kinetochore assembly (Fig. S2c, d) [11]. Further study implied that autophagy was indispensable for cell cycle progression [12]. Knock-down of ATG7 caused prolonged prophase, prometaphase and metaphase of mitosis as well as the overall duration of mitosis, which supports the idea that autophagy is functional and active in mitosis (Fig. S2e, f).

Interestingly, there has been a similar argument regarding whether endocytosis is inhibited [13] or persists [14] during mitosis. Kirchhausen's group analyzed the influence of experimental details on endocytosis, such as temperature and chemicals applied [15], and found that endocytosis persists in natural dividing cells. They showed that nocodazole-arrested pseudo-mitotic cells behave differently from those naturally occurring mitotic cells, which may due to the influence of microtubules in membrane trafficking. It suggests that chemicals like nocodazole should be used in caution in the study of cellular process, such as endocytosis study. Nocodazole-arrested pseudo-mitotic cells should also be avoided to study autophagy in mitosis. Instead, given that thymidine had been removed for a long time to allow cells to enter mitosis, using double

thymidine block may be more proper for cell synchronization in mitosis.

Both mitosis and autophagy are highly dynamic, multistage processes and require accurate temporal and spatial regulation. Further investigations to reveal the precise interplay between them will not only be critical for a better understanding of these basic cellular events, but also provide new insights into the related human diseases.

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