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Autophagy mediates phase transitions from cell death to life

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Abstract

Autophagy is a lysosomal degradation pathway, which is critical for maintaining normal cellular functions. Despite considerable advances in defining the specific molecular mechanism governing the autophagy pathway during the last decades, we are still far from understanding the underlying principle of the autophagy machinery and its complex role in human disease. As an alternative attempt to reinvigorate the search for the principle of the autophagy pathway, we in this study make use of the computer-aided analysis, complementing current molecular-level studies of autophagy. Specifically, we propose a hypothesis that autophagy mediates cellular phase transitions and demonstrate that the autophagic phase transitions are essential to the maintenance of normal cellular functions and critical in the fate of a cell, i.e., cell death or survival. This study should provide valuable insight into how interactions of sub-cellular components such as genes and protein modules/complexes regulate autophagy and then impact on the dynamic behaviors of living cells as a whole, bridging the microscopic molecular-level studies and the macroscopic cellular-level and physiological approaches.

Keywords: Autophagy, Mathematical model, Simulations, Phase transitions

1. Introduction

Macroautophagy (hereafter autophagy), an evolutionary conserved lysosomal degradation pathway, was discovered early in the 1960's. However, it is only lately that scientists can address various molecular facets of autophagy with confidence. It is regulated by complex positive-negative feedback mechanisms, wherein the output of the autophagic process such as recycled amino acids or ATP manipulates each of the consecutive steps of the process, i.e., autophagosome formation, autolysosome formation, and intralysosomal hydrolysis steps, in a concentration dependent manner, via mammalian target of rapamycin (mTOR) (for amino acids) and/or AMP-activated protein kinase (AMPK) pathways (for ATP) [1] [2] [3] [4] [5] [6] [7]. Such a carefully orchestrated autophagic process regulates cellular homeostasis and protein/organelle quality, and further it mediates cell death or survival depending on the context and degree of activation [8]. Ultimately, autophagy controls the onset and progression of human diseases such as cancers, metabolic disorders, and neurodegenerative diseases.

Despite that remarkable efforts have been made to unveil the cellular and molecular mechanisms involved in the autophagy machinery, we are still far from understanding the underlying principle of the autophagic process and function. Hence, in practice, a number of challenges still remain in assessing and interpreting the autophagy activity: Assessment of the autophagic flux, i.e., the rate of flow along the autophagy pathway [9] [10] [11], via conventional assays relying on a single specific marker, rather than systemic analyses, often leads to incorrect estimations of the autophagy status and misinterpretations of the cause-and-effect relationship between the autophagic flux and the concomitant functional changes at both cellular and sub-cellular levels [11] [12]. Furthermore, there exist difficulties in interpreting newly elucidating molecular mechanisms of autophagy; sometimes they contradict the existing hypotheses. From a medical viewpoint, there is a bottleneck in developing drugs and therapeutic strategies for targeting autophagy due to unsolved ambiguities as to the dual role of autophagy in the development and progression of various human diseases.

To overcome such limitations, as an alternative, we recently proposed the 'minimal autophagy model' [10] [11] [13] to integrate key individual molecular and cellular data sets on the autophagy pathway into a unified framework. Based on the model and extensive computer-aided analysis of the biological data on the pathway, in this study, we reveal the specific and quantitative information of the system at appropriate time resolution and propose a hypothesis which claims that autophagy mediates cellular phase transitions, which are critical for maintaining normal cellular functions and ultimately

determining cell death and survival in response to intra- and extra-cellular perturbations; such a new perspective on the autophagy may allow us to take a step toward the goal of understanding the principles of autophagy.

There are four sections in this article: In the second section, we propose the hypothesis of autophagy as a cellular phase transition and briefly introduce the autophagy model used for describing the target autophagy system of the present study. In support of our hypothesis, in the following section, we carry out the model-based computer simulations, using the realistic parameter values from *in vivo* and *in vitro* experiments. Finally, we discuss the biological/medical implications of the autophagic phase transitions and the importance of such an integrated theoretical-experimental approach in the autophagy research.

2. Materials and methods

2.1. Theoretical background

Phase transitions are long-established and familiar phenomena throughout the domains of physics and chemistry [14] [15] [16]. The most well-known examples include liquid-gas and liquid-solid phase transitions of water in which subtle changes in temperature or pressure induce an abrupt transition from liquid water (liquid phase) to water vapor (gaseous phase) or ice (solid phase) [17] [18]. On the other hand, the idea that such phenomena can potentially play a vital role in living systems such as cells was proposed just recently [19] [20] [21] [22] [23] [24] [25]. The phase transition occurring in (mammalian) cells (referred to hereafter as the cellular phase transition) can be defined as the transformation of a cell from one phase (phenotype) to another, with accompanying structural and/or functional changes at both cellular and sub-cellular levels.

With the molecular biology revolution, it is no longer a rare event to observe the cellular phase transition on laboratory benches [26] [27] [28] [29]. For example, during the cell cycle, a cell shifts from the G1 phase to other phases, e.g., S, G2, and M phases; in the cancer onset and progression, the phenotype of a cancer cell is transformed in response to distinct consecutive steps such as mutation, promotion, and invasion. More fundamentally, many essential processes in living cells, including development, differentiation, attainment of intrinsic properties, regulation of cellular functions, and execution of cell death, are likely to be attributable to the cellular phase transitions.

In addition to those well-known examples of the cellular phase transition, the operation mechanism of autophagy and the life phenomena associated with autophagy pathway could be interpreted in terms of the cellular phase transition. Specifically, we propose a hypothesis which claims that autophagy impacts on the cell death or survival, by mediating the cellular phase transitions.

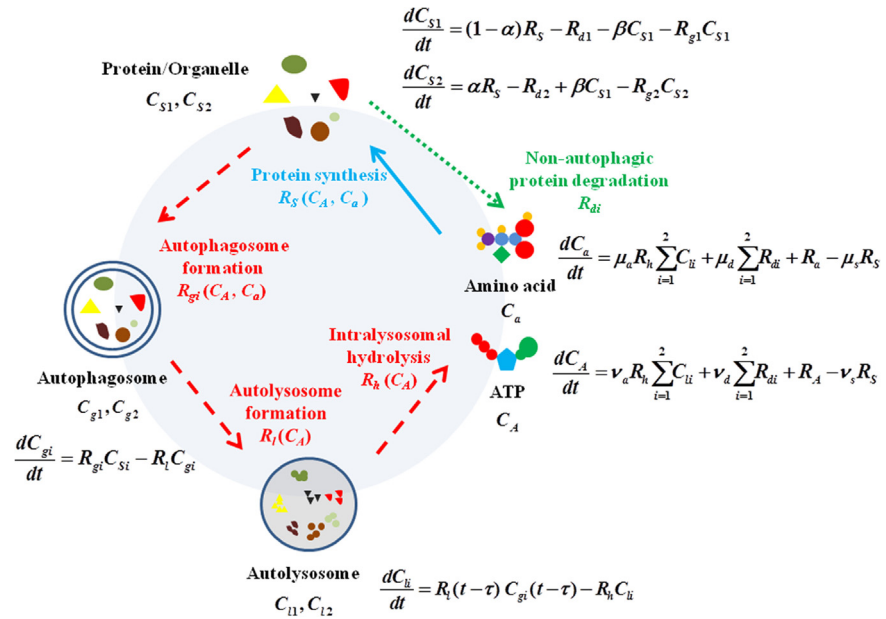


Fig. 1. Summary of the minimal autophagy model. The solid (blue) arrow describes the (total) protein/organelle synthesis R_S (from DNA). While resident protein/organelle S_1 is entirely synthesized from DNA, abnormal one S_2 is produced either directly from DNA or indirectly via the deterioration of S_1 : The production rates R_{S1} and R_{S2} of S_1 and S_2 thus read $R_{S1} = (1 - \alpha)R_S$ and $R_{S2} = \alpha R_S + \beta C_{S1}$, where α and β denote the fraction of S_2 in the (total) protein/organelle synthesis rate R_S (from DNA) and the (specific) deterioration rate of S_1 , respectively. The dotted (green) and three dashed (red) arrows depict, respectively, non-autophagic degradation of rates R_{di} and autophagic degradation steps including autophagosome formation of rates R_{gi} , autolysosome formation of rate R_l , and intralysosomal hydrolysis of rate R_h , where the subscript i labels resident ($i = 1$) and abnormal ($i = 2$) protein/organelle, respectively. The differential equations describe time evolutions of the corresponding intracellular concentrations $C_{gi}, C_{li}, C_{Si}, C_a$, and C_A of autophagosomes, autolysosomes, protein/organelles, amino acids, and ATP, respectively. All concentrations have time arguments t unless specified otherwise, e.g., $C_{g1} \equiv C_{g1}(t)$ and so on; the rate of changes of the autolysosome concentration C_{li} at time t depends on R_l, C_{g1} and C_{g2} at time $t - \tau$, earlier by the delay time τ , which is taken to be 8 min ($\tau = 480$ s).

In the last decades, various cellular and molecular mechanisms of autophagy have been elucidated, and many facets of them appear to resemble the cellular phase transition: In the autophagy machinery, alterations of nonlinear interactions among sub-cellular components such as genes and protein modules/complexes accumulate, without being unobserved until a threshold is reached. On approaching the critical point, however, it seems that the autophagy system becomes extremely sensitive to intra- and extra-cellular perturbations and the correlation length extends over the whole-cell system. These may result in qualitative changes in the global behavior of the cellular system as well as in the autophagy pathway; these are mediated by discontinuous jumps and continuous changes in the autophagic fluxes and the autophagosome/

autolysosome concentrations, which keep parallel with the first-order and continuous phase transition, respectively, in physical systems [15] [16].

This intriguing property would also indicate that once the whole-cell system is driven into certain (stable) phases, the corresponding sub-cellular systems have already been substantially modified. In this regard, the phase transition could provide information as to the sub-cellular dynamics as well as the global behavior of the whole-cell system. Therefore, to investigate the autophagy-mediated cellular phase transition should be valuable for the comprehensive understanding and further predicting the fate of a cell, i.e., (autophagy-mediated) cell death or survival. Such a view of autophagy as a phase transition is thus expected to provide valuable insight into the underlying principles of autophagy and its roles in the maintenance of normal cellular function and cell death/survival.

2.2. Mathematical model

The main idea and characteristic of the minimal autophagy model applied to analysis of the target system, the mammalian hepatocyte, is briefly outlined here (see Fig. 1): The model assumes a three-compartment description of the process, i.e., autophagosome, autolysosome, and protein/organelle compartments, and the rates at which the concentrations of autophagosomes, autolysosomes, and protein/organelles vary with time are expressed mathematically on the basis of the biological experiments [5] [6] [7] [30]. Specifically, the distinct dynamical characteristics between the autophagosome/autolysosome from resident protein/organelle S_1 and those from abnormal protein/organelle S_2 are considered.

We consider the autophagosome formation specific rates R_{g1} (from resident protein/organelle S_1) and R_{g2} (from abnormal protein/organelle S_2) as functions of the intracellular concentrations C_A of ATP and C_a of amino acids in the form:

$$R_{g1}(C_a, C_A) = r_g \frac{C_A^4}{C_A^4 + k_g^4} \frac{p_g^{12}}{C_A^{12} + p_g^{12}} \frac{a_g^8}{C_a^8 + a_g^8} \left(1 + \gamma_g e^{-\xi_g C_a}\right) \quad (1)$$

$$R_{g2}(C_a, C_A) = r_g \frac{C_A^4}{C_A^4 + k_g^4} \frac{p_g^{12}}{C_A^{12} + p_g^{12}} \left(1 + \gamma_g e^{-\xi_g C_a}\right) \quad (2)$$

where r_g is the rate constant for autophagosome formation, with the appropriate constants k_g , p_g (for ATP), a_g , γ_g , and ξ_g (for amino acids). In our simulations, the (basal) level of autophagic (autophagosome) flux [9] [10] [11] is suppressed or promoted by adjusting the value of r_g . Specifically, the flux is proportional to the rate constant r_g : When the rate constant r_g is set to be twice the normal value $r_g^{(0)}$ (i.e., $r_g = 2$ in units of $r_g^{(0)}$), the autophagic flux becomes double the normal flux.

We then describe the intracellular ATP dependence of the autolysosome formation step, and the specific rate R_l takes the form:

$$R_l(C_A) = r_l \frac{C_A^4}{C_A^4 + k_l^4} \frac{p_l^{12}}{C_A^{12} + p_l^{12}} \tag{3}$$

where r_l denotes the rate constant for autolysosome formation, with the appropriate constants k_l and p_l for ATP.

Next, the ATP dependent intralysosomal hydrolysis specific rate R_h is taken as a function of the intracellular ATP concentration:

$$R_h(C_A) = r_h \frac{C_A^{\delta_h}}{C_A^{\delta_h} + k_h^{\delta_h}} \tag{4}$$

with the appropriate exponent δ_h and constant k_h for ATP, where r_h is the rate constant for intralysosomal hydrolysis.

In addition to the autophagic process, we incorporate the (total) protein/organelle synthesis rate R_s , depending on the amino acids concentration C_a . We write the protein/organelle synthesis rate in the form:

$$R_s(C_a, C_A) = \begin{cases} r_s \frac{C_a}{C_a + k_s} \frac{\exp[C_A] - 1}{\exp[C_A^{(m)}] - 1} & \text{for } C_A < C_A^{(m)} \\ r_s \frac{C_a}{C_a + k_s} & \text{for } C_A \geq C_A^{(m)} \end{cases} \tag{5}$$

with the appropriate constant k_s for amino acids, where $C_A^{(m)}$ is the ATP concentration corresponding to the maximal protein/organelle synthesis rate and r_s denotes the rate constant for the protein/organelle synthesis.

Taking the rate of non-autophagic degradation to be 25% of autophagic degradation, we write the rate of non-autophagic degradation ($i = 1, 2$):

$$R_{di} = \frac{1}{4} R_h C_{li} \tag{6}$$

where C_{li} denotes the concentration of autolysosomes from S_i .

Finally, variations of the corresponding intracellular concentrations C_{gi} , C_{li} , C_{Si} , C_a , and C_A of autophagosomes, autolysosomes, protein/organelle, amino acids, and ATP are described by the coupled differential equations (see Fig. 1), which are solved via the 5th order Runge-Kutta method for very high precision. In simulations, we use the realistic parameter values, which are extracted from carefully selected biological experiments [5] [6] [7] [30] of the target autophagy system of the present study. The resulting parameter values are displayed in Table 1. Further details of the model can be found in literature [10] [11] [13].

Table 1. Parameters in computer simulations.

Parameter	Value	Unit	Description
$r_g^{(0)}$	$1.12 \times 10^{-5*}$	s^{-1}	Rate constant for autophagosome formation (normal value)
$r_g^{(1)}$	3.58×10^{-7}	s^{-1}	Rate constant for autophagosome formation (1st-order phase transition under $\beta^{(0)}$)
$r_g^{(2)}$	3.53×10^{-6}	s^{-1}	Rate constant for autophagosome formation (continuous phase transition under $\beta^{(0)}$)
$r_g^{(3)}$	3.36×10^{-7}	s^{-1}	Rate constant for autophagosome formation (1st-order phase transition under $\beta = 0.2\%$ /h)
$r_g^{(4)}$	1.80×10^{-6}	s^{-1}	Rate constant for autophagosome formation (continuous phase transition under $\beta = 0.2\%$ /h)
$\beta^{(0)}$	1.50×10^{-3}	h^{-1}	Rate constant for deterioration of S_1 (normal value)
α	1.00×10^{-2}	(unitless)	Constant for the protein/organelle synthesis (fraction of S_2 in protein/organelle synthesis rate R_s)
k_g	4.01^*	mM	Constant for autophagosome formation (ATP dependency)
p_g	3.00^*	mM	Constant for autophagosome formation (ATP dependency)
a_g	4.50	mM	Constant for autophagosome formation (amino acids dependency)
γ_g	1.22^*	(unitless)	Constant for autophagosome formation (amino acids dependency)
ξ_g	$7.49 \times 10^{-2*}$	mM^{-1}	Constant for autophagosome formation (amino acids dependency)
r_l	$2.47 \times 10^{-5*}$	s^{-1}	Rate constant for autolysosome formation
k_l	4.01^*	mM	Constant for autolysosome formation (ATP dependency)
p_l	3.00^*	mM	Constant for autolysosome formation (ATP dependency)
r_h	$1.39 \times 10^{-5*}$	s^{-1}	Rate constant for intralysosomal hydrolysis
δ_h	$7.24 \times 10^{-1*}$	(unitless)	Exponent for intralysosomal hydrolysis (ATP dependency)
k_h	2.99^*	mM	Constant for intralysosomal hydrolysis (ATP dependency)
r_s	$1.48 \times 10^{-5*}$	$mM \cdot s^{-1}$	Rate constant for protein/organelle synthesis
k_s	$1.77 \times 10^1^*$	mM	Constant for protein/organelle synthesis (amino acids dependency)
$C_A^{(m)}$	3.00	mM	ATP concentration corresponding to maximal protein/organelle synthesis rate

* Parameters with asterisks are fixed from the target biological experiments in Refs. [5] [6] [7] [30]. Those without asterisks are determined from computer simulations or adjustable depending on simulation setups.

3. Results

3.1. Autophagy-mediated cellular phase transitions

In this section, we analyze the target autophagy system, the mammalian hepatocyte [5] [6] [7] [30], via model-based computer simulations, with emphasis on the dynamic behavior of the system depending on the autophagic flux. In particular, implications of the behavior on cellular phase transitions are examined. In the simulations, we consider the intracellular concentrations of ATP, an essential energy source, and of amino acids, a metabolite precursor molecule, as the key biochemical parameters, which may represent the energy and metabolic state of the cellular system and further indicate the fate of the system, i.e., cell death or survival.

Fig. 2 shows how the concentrations C_a and C_A of amino acids and of ATP vary with the autophagosome formation rate constant r_g in Eqs. (1) and (2). When the rate constant r_g is lower than $r_g^{(1)}$ (≈ 0.032 in units of the normal value $r_g^{(0)}$ whereby the autophagic flux is far below (0.032 times) the normal flux), C_a and C_A remain at the extremely low-levels of about 5 and 0 mM, respectively, which may induce dysfunctions of the cells. Particularly, under such severe conditions, normal cellular functions could hardly be recovered, eventually resulting in cell death. Once, however, the rate constant r_g reaches $r_g^{(1)}$, the concentrations exhibit discontinuous jumps, dramatically restoring to the normal levels. Such discontinuous jumps are associated with a first-order phase transition in a physical system [16]. After undergoing this transition, the values of C_a and C_A gradually raise with the rate constant r_g . Further, they appear to undergo a continuous phase transition at $r_g = r_g^{(2)} \approx 0.315$ (in units of $r_g^{(0)}$); beyond it the concentrations display oscillatory behavior. As the rate constant r_g increases further, the amplitudes of the oscillations continuously grow. Whereas the minimum values remain constant, the maximum values in the oscillations gradually increase. It is thus tempting to conjecture that the system undergoes two phase transitions, one first-order and one continuous, depending on the autophagic flux. These intriguing properties appear to be deeply connected to the fate of the cellular system, cell death or survival.

From the biological viewpoint, the optimal maintenance level of ATP is vital for cell survival. Once intracellular ATP is exhausted, almost all the intracellular signaling/metabolic pathways may not work properly due to the failure of energy supply on the pathways. Then malfunction of various active membrane transporters, such as Na^+/K^+ -ATPase, plasma membrane Ca^{2+} ATPase, and proton pumps, may trigger dissipation of electrochemical gradients, eventually leading to necrosis. Further, depletion of the intracellular ATP level tends to switch apoptosis to necrosis [31] [32] [33] [34]: relatively high ATP levels

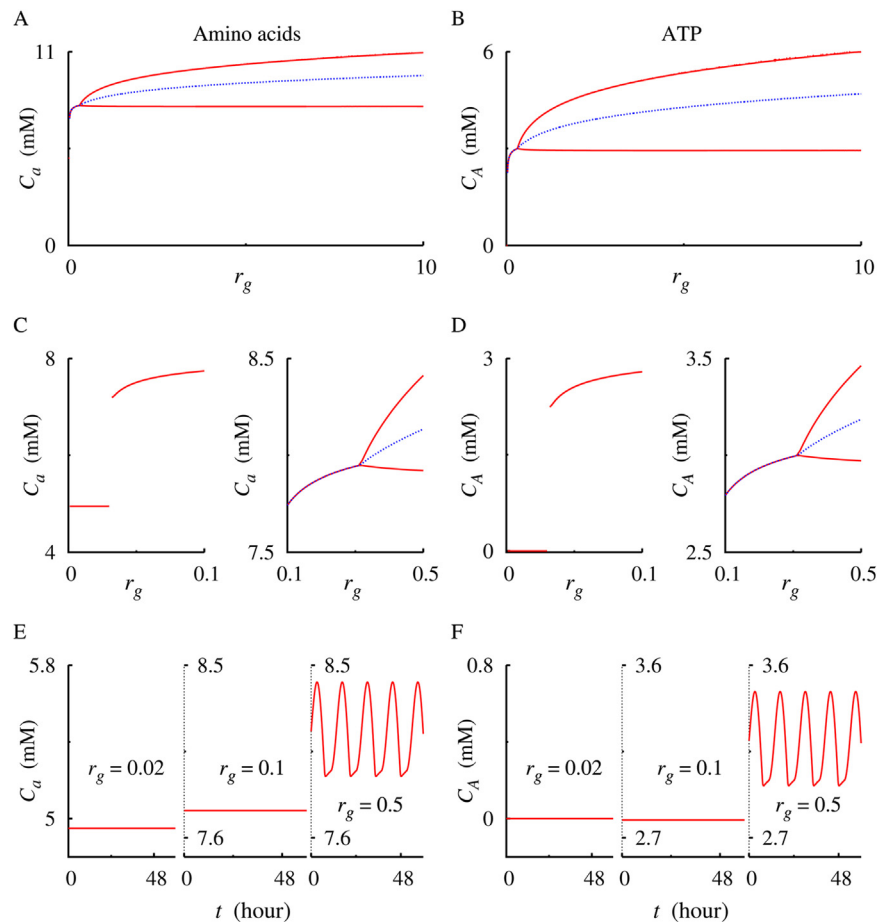


Fig. 2. Dynamics of amino acid and ATP concentrations depending on autophagic flux. **A** and **B** show how the concentrations C_a of amino acids and C_A of ATP change with the autophagic flux. The level of autophagic flux is suppressed or promoted by adjusting the value of r_g . Details of the corresponding behaviors for small values of r_g are shown in **C** and **D**. The concentrations (solid red lines) exhibit discontinuous jumps at $r_g = r_g^{(1)} \approx 0.032$ (in units of the normal value $r_g^{(0)}$) and continuous transitions at $r_g = r_g^{(2)} \approx 0.315$, beyond which oscillations develop. Splits in the lines manifest emergence of oscillations, with the upper and lower lines plotting the maximum and minimum values of the oscillations, respectively, and the dotted blue lines representing the average values. **E** and **F** exhibit the time evolutions of C_a and C_A for three values of r_g ($= 0.02, 0.1, \text{ and } 0.5$).

usually enable a cell to undergo energy-requiring apoptotic cell death whereas low ATP concentrations favor necrosis. Accordingly, autophagy functions as a key switch between apoptosis and necrosis by regulating the intracellular ATP level [35]. In addition, serious problems in the cellular protein homeostasis may arise if the intracellular amino acid levels fall down below certain thresholds, since amino acids not only provide primary building blocks of proteins but also control protein degradation via autophagy. Moreover, optimal levels for amino acids are essential for regulation of the transcription of DNA, mRNA stability, and other steps in the gene expression.

According to the theory of complex systems, global behaviors of the system may emerge from nonlinear interactions among a variety of sub-cellular components such as genes and protein modules/complexes. Therefore, oscillations of such whole-cell biochemical parameters as C_a and C_A are reflective of the related underlying dynamics of the sub-cellular system and should contain comprehensive information as to the conditions of the cellular system. Collectively, variations of C_a and C_A and/or the destruction/preservation of their intrinsic oscillatory behaviors can be used as key criteria for determining the cellular conditions and eventually the fate of the cellular system.

In this regard, restoration of the physiological levels and emergence of the oscillations of C_a and C_A via the autophagy-mediated cellular phase transition may indicate that autophagic flux (more generally, autophagy activity) is critical for the maintenance of the normal cellular functions and cell survival. In view of the accompanying abnormal behaviors of autophagosomes/ autolysosomes (see Refs. [4] and [5]), resident protein as well as amino acids and ATP (see Fig. 2), the cellular system is considered to remain in the “death state” for $r_g < r_g^{(1)}$, which is discussed in the following section. Then, for r_g larger than $r_g^{(1)}$, the system becomes restored drastically from the “death state” to the “intermediate state” via the first-order transition. As r_g is increased further, the system undergoes the continuous phase transition at $r_g^{(2)}$, and reaches the “survival state”. In particular, for $r_g^{(2)} < r_g$, there arise oscillatory behaviors of C_a and C_A , implying that the system is rescued fully from the “death state”. Altogether, these results certainly support our hypothesis that autophagy-mediated phase transitions impact on the cellular life and death.

3.2. Roles of autophagic phase transitions in cellular protein quality control

Autophagy plays an essential role in the cellular protein/organelle quality control, which is certainly crucial for the maintenance of the normal cellular functions and cell survival. Specifically, several researchers have reported that the substrate selectivity of autophagy, which claims that abnormal protein tends to be more easily sequestered within the autophagosome/autolysosome than resident protein, may contribute to the protein/organelle quality control [10] [11] [36] [37] [38] [39] [40] [41]. Extending those ideas and strategies, we have carried out computer simulations to analyze the roles of autophagy in the cellular protein/organelle quality control and discuss their simulation characteristics and biological implications in this section.

Fig. 3 shows the relationship between the average concentrations \bar{C}_{S1} and \bar{C}_{S2} of resident protein/organelle S_1 and abnormal one S_2 , i.e., average values of C_{S1}

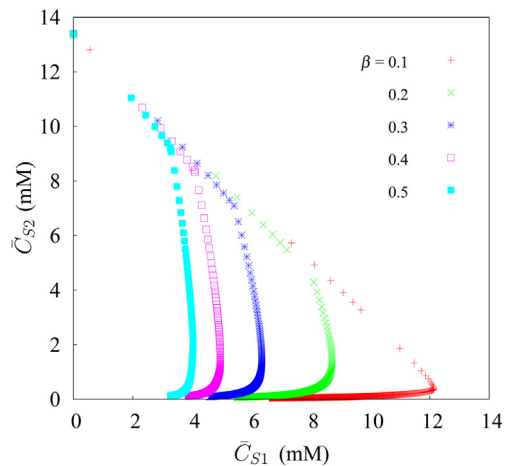


Fig. 3. Relationship between the average concentrations \bar{C}_{S1} and \bar{C}_{S2} of resident and abnormal protein/organelles, depending on autophagic flux, at various values of the cellular deterioration rate β . Data points are plotted from the upper left corner as the autophagosome formation rate constant r_g is increased from $r_g = 0$ to $r_g = 0.1$ (in units of $r_g^{(0)}$) at the increment of 0.01 and from $r_g = 0.1$ to $r_g = 100$ at the increment of 0.1.

and C_{S2} , respectively, depending on autophagic flux, at various values of the cellular deterioration rate β . The resident protein/organelle S_1 deteriorates and turns into the abnormal protein/organelle S_2 at given rates of deterioration, $\beta = 0.1, 0.2, 0.3, 0.4,$ and 0.5 %/h (for details see Fig. 1).

In case that autophagic flux is totally suppressed in the system ($r_g = 0$), the abnormal protein/organelle concentration stays at a very high level ($\bar{C}_{S2} = 13.4$ mM) whereas the resident protein/organelle concentration almost vanishes ($\bar{C}_{S1} \approx 0$) for all values of β . In other words, most of the protein/organelles are damaged. However, as shown in the case of $\beta = 0.2$ %/h, as r_g is enhanced from zero, \bar{C}_{S2} reduces drastically while \bar{C}_{S1} grows rapidly to the normal level via the discontinuous jump (first-order transition) at $r_g = r_g^{(3)}$ (≈ 0.03 in units of $r_g^{(0)}$). With r_g increased further, the system undergoes the continuous transition at $r_g = r_g^{(4)} \approx 0.16$ (in units of $r_g^{(0)}$), at which the slope of the curve \bar{C}_{S1} versus \bar{C}_{S2} changes abruptly; this indicates that \bar{C}_{S2} decreases more rapidly in response to the promotion of the autophagosome formation rate beyond $r_g^{(4)}$. Again, the oscillatory behaviors of C_{S1} and C_{S2} begin to appear after the continuous phase transition. As the rate constant r_g is increased further, the abnormal protein/organelle concentration C_{S2} reduces monotonically from the normal level, with the amplitudes of oscillations diminishing continuously. In particular, C_{S2} almost vanishes to 0 mM above ten-time promotion (i.e., for $r_g \gtrsim 10$ in units of $r_g^{(0)}$), whereas C_{S1} remains more or less within the normal range.

For a more quantitative analysis, the fractional abnormal protein/organelle concentration f_c can be defined as a simple surrogate index of the cellular

protein/organelle quality:

$$f_c \equiv \frac{\bar{C}_{S2}}{\bar{C}_{S1} + \bar{C}_{S2}}$$

which takes values between 0 and 1: While the value $f_c \approx 0$ addresses that most of the protein/organelles in the system are normal, $f_c \approx 1$ indicates that the majority of the protein/organelles are damaged.

Performing simulations, we have obtained the behavior of f_c in response to varying the rate constant r_g , which is displayed in Fig. 4. Specifically, we consider a stressful condition of the extremely elevated value of the cellular deterioration rate of S_1 , $\beta = 0.2$ %/h. At such a high level of the cellular deterioration rate, the production rate $R_{S2} = \alpha R_S + \beta C_{S1}$ of abnormal protein/organelles is approximately equal, on average, to the production rate $R_{S1} = (1 - \alpha)R_S$ of resident ones, although the fraction of S_2 in the (total) protein/organelle synthesis rate R_S (from DNA) is set one hundred times smaller than the fraction of S_1 , i.e., $\alpha = 0.01$.

In such severe suppression that the rate constant r_g is below $r_g^{(3)} \approx 0.03$ (in units of $r_g^{(0)}$), f_c remains at abnormally high levels (bottom of Fig. 4). It suggests that

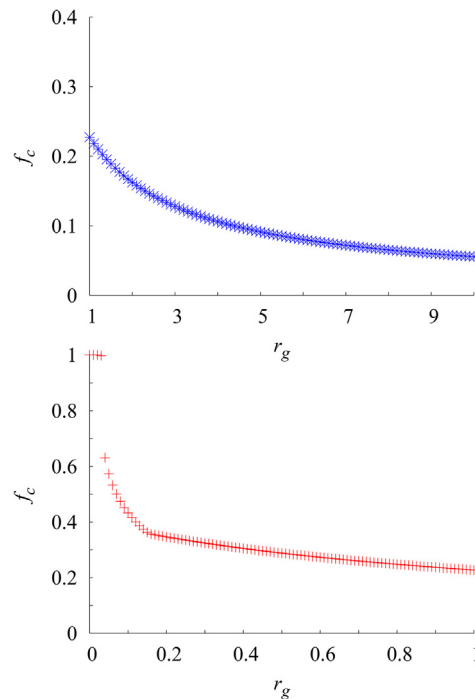


Fig. 4. Fractional abnormal protein/organelle concentration f_c versus the autophagosome formation rate constant r_g . The upper and lower panels display the dependence on the promotion and suppression, respectively, of the rate constant r_g (in units of $r_g^{(0)}$). Data have been obtained at the specific deterioration rate $\beta = 0.2$ %/h, higher than the normal value $\beta = 0.15$ %/h $\equiv \beta^{(0)}$, where the resident protein/organelle synthesis rate is approximately equal to the abnormal one.

almost all the protein/organelles in the system are abnormal: C_{S1} approaches 0 mM while C_{S2} remains at an abnormally high level at $r_g < r_g^{(3)}$. Once it undergoes the first-order transition at $r_g = r_g^{(1)}$, however, f_c becomes restored suddenly to the normal value as C_{S1} and C_{S2} jump to the normal (high and low, respectively) levels. Moreover, it is of interest that the system exhibits a continuous transition at $r_g = r_g^{(4)} \approx 0.16$ (in units of $r_g^{(0)}$): The slope of the curve f_c decreases abruptly, indicating that f_c becomes less sensitive to the suppression or promotion of the autophagosome formation rate r_g . In other words, the system appears more stable for larger values of r_g beyond $r_g^{(4)}$. As r_g is increased further, f_c gradually reduces and remains at the normal level.

It is thus likely that the autophagy-mediated cellular phase transitions are beneficial for the cellular protein/organelle quality control, which is essential for the normal functions of cells and their survival against cellular perturbations. The obtained results unambiguously support the validity of the hypothesis.

4. Discussion

With the molecular biology revolution, the mainstream of autophagy research has changed from the macroscopic physiological- and cellular-level studies to the microscopic description of individual genes and proteins: Since the early 1990's, several genes essential for the autophagy pathway and their functions have been identified, which has in turn brought forth a number of molecular-level studies of autophagy. Currently, based on the studies of the 'molecular autophagy', there is research being carried out on the prevention of the onset and progression of autophagy-related human diseases such as cancer, metabolic disorders, and neurodegenerative diseases [42] [43] [44].

Despite that contemporary biology can address various molecular facets of autophagy with confidence, scientists of this era are still far from the systemic and comprehensive understanding of the underlying principles of the autophagy machinery. This is highly challenging but indispensable for the realization of new treatment methods or drugs that can potentially regulate or control autophagy. So far, there has been far less concern about the comprehensive understanding of the dynamic behavior of the system, bridging the microscopic molecular-level studies and the macroscopic cellular- and physiological-level approaches, compared with the molecular mechanism in a specific cellular phase. To unveil the underlying principles of autophagy, in other words, we need to understand how the variations in gene expression patterns and the interactions among genes and protein modules/complexes affect the dynamic behavior of the whole-cell system during the change of the cellular phase due to intra- and extra-cellular perturbations.

Note here that living cells should also observe physical laws unless one adheres to vitalism. Therefore, although most existing investigations of autophagy were made from the biological viewpoint, it should be revealing to probe the principles of cellular phenomena by means of theoretical methods of physics. A complete view of the autophagic cell death/life phenomena could be obtained via the cooperation of theoretical and experimental approaches.

Given this context, we expect that the search for the principles of autophagy can be reinvigorated via modeling and computer-aided analysis of the target experimental systems. Modeling and computer simulations should provide a powerful tool to analyze the autophagy pathway, and allow one to test various experimental and theoretical hypothesis, which may be difficult or impossible to test under *in vitro* or *in vivo* experimental setups. In the model-based computer simulations, differently from biological experiments, it is possible to promote or suppress an individual step without interfering with other steps; this method is thus advantageous for identifying the effects of individual steps. Besides, with simple adjustments of parameters in the governing equations, various combination effects of promotion and/or suppression of interrelated pathways, i.e., three consecutive pathways of the autophagosome formation, autolysosome formation, and intralysosomal hydrolysis steps, could be efficiently probed.

In general a model should be simple enough to analyze and is thus far simpler than the real system. One may then raise a question as to the validity of the results from such simplification. Fortunately, it is well established that the characteristic collective behaviors emerging from co-operativity between constituents are rather insensitive to the details of the system [16]. Such a concept of *universality* justifies the use of a simple model for a system in probing the phase transitions in the system. Namely, thanks to universality, we are allowed to consider only relevant features of the system, disregarding other complicated details, to build a manageable “minimal model”. Indeed, with the help of the minimal autophagy model and the model-based computer simulations, we are able to discover the underlying dynamics of the target autophagy system and the corresponding cellular effects by reducing ambiguity as to causes and effects in the complex autophagy systems. Specifically, we have found that autophagy mediates cellular phase transitions and these are essential to the maintenance of normal cellular functions and further critical for determining the fate of a cell, i.e., death or survival.

As discussed, the simulation-based analysis of the target experimental system has revealed that the system, depending on autophagic flux, exhibits three different phases (phenotypes), which can be mapped to distinct states of a cell: death, intermediate, and survival. It has been observed that as the autophagosome formation rate constant r_g is raised, the biochemical parameters

C_A of ATP and C_a of amino acid concentrations first change abruptly to display discontinuous jumps and then change continuously to display oscillatory behaviors, which are reminiscent of a first-order and a continuous phase transition, respectively, in physical systems. The system remains in the “death state” for $r_g < r_g^{(1)}$; once r_g is increased to $r_g^{(1)}$, however, the system becomes quickly restored to the “intermediate state” via the first-order transition. As r_g is raised further, the system undergoes the continuous transition to the “survival state” at $r_g^{(2)}$, beyond which the biochemical parameters are remarkably well maintained within the normal range against cellular perturbations. In support of our hypothesis, after the continuous phase transition, biological oscillations emerge, which indicates that the system has been completely rescued from the “death state” (see Fig. 2).

Furthermore, as a testable application of the hypothesis, the roles of autophagic cellular phase transitions have been discussed with regard to the cellular protein/organelle quality control. We have carried out computer simulations designed to analyze quantitatively variations of resident and abnormal protein/organelle during the autophagy-mediated transitions and demonstrated that such cellular phase transitions are connected deeply to the cellular protein/organelle quality, which is critical for the maintenance of the normal cellular functions and cell survival. Indeed, it has been shown that a cell under severely deteriorated conditions is effectively restored by the autophagy-mediated phase transitions (see Fig. 3 and Fig. 4).

In this respect, this study provides a theoretical framework to understand the role of autophagy in human disease associated with decreased levels of autophagic flux. Examples include Huntington's disease, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, where decreased autophagic fluxes lead to the accumulation of (pathogenic) abnormal proteins/organelles while the protective effects of autophagy lacking [45]. With the development of more realistic mathematical models based on the minimal autophagy model [10] [11] [13], such an integrated theoretical-experimental approach should guide future therapeutic strategies to control and regulate the physio-pathological state in biological, pharmacological, and computational contexts.

Consequently, in view of both the behaviors of the representative whole-cell biochemical parameters such as ATP and amino acid concentration and the dynamics of the cellular protein/organelle quality in the process, the autophagy-mediated cellular phase transitions appear essential to maintaining normal cellular functions and further critical for determining the fate of a cell, i.e., life or death. It is thus disclosed how variations of autophagy affect the system as a whole, bridging the microscopic mechanism of the individual genes and proteins

and the macroscopic physiological- and cellular-level studies. Such an integrative approach is expected to provide new insight into the role of autophagy in the protein/organelle quality control and further in various human diseases, including cancer, metabolic disorders, and neurodegenerative diseases. Finally, in the medical perspective, the present hypothesis may shed light on the development of autophagy-targeted intervention strategies or drugs.

Declarations

Author contribution statement

MooYoung Choi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Jinwoong Kim: Conceived and designed the experiments.

Kyungreem Han: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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