Computational model for autophagic vesicle dynamics in single cells

Dipak Barua[1*], Katie R. Martin[2*], Audra L. Kauffman[2], Laura M. Westrate[2,3], Richard G. Posner[4],
William S. Hlavacek[1,4], and Jeffrey P. MacKeigan[2]

Short Abstract —**We present a computational model describing autophagic vesicle dynamics in a mammalian system. We used time-resolved live-cell microscopy to measure the synthesis and turnover of autophagic vesicles in single cells. The stochastically simulated model was developed on the basis of data acquired during conditions of both basal and chemically-induced autophagy. The model was tested by genetic and chemical modulation of the autophagic machinery and found to accurately predict vesicle dynamics observed experimentally. Furthermore, the model generated an unforeseen prediction about vesicle size that is consistent with both published findings and our experimental observations.**

Key words — Macroautophagy, Single-cell imaging, Stochastic modeling and simulation.

I. PURPOSE

Macroautophagy (autophagy) is a cellular recycling process essential for homeostasis and survival during cytotoxic stress (1). This process is executed in four stages through the coordinated action of over thirty proteins. An effective strategy for studying complicated cellular processes, such as autophagy, involves the construction and analysis of computational models. When developed and refined on the basis of experimental knowledge, computational models can be used to interrogate signaling pathways, formulate novel hypotheses about systems, and make predictions about cellular responses to specific interventions.

Here, we present the development and analysis of a stochastic model, which we formulated to characterize the single-cell dynamics of autophagic vesicle synthesis and degradation under treatments with a variety of compounds and their combinations. Predictions of the model were tested experimentally.

II. MODEL

The model accounts for key signaling proteins and lipids that mediate autophagy downstream of mammalian target of rapamycin (mTOR), a serine/threonine kinase. An input representing an mTOR inhibitor in the model induces autophagy signaling via activation of Vps34, a Class III PI3K. Vps34 catalyzes conversion of membrane lipid PtdIns into PtdIns3P, which recruits and activates other signaling proteins, leading to nucleation of isolation membranes. An isolation membrane grows in size as specific protein and lipid components are incorporated. The model assumes that the rate of maturation of an isolation membrane into a free autophagic vesicle is proportional to the accumulation of a protein called LC3-II. LC3-II is produced from LC3-I, a protein added at a constant rate to an isolation membrane after nucleation. A mature autophagic vesicle is subject to degradation via fusion with a lysosome or endosome destined for lysosome fusion.

The model is simulated using a modified form of Gillespie's method (2). The model incorporates a dynamic list of reactions occurring both outside and inside of autophagic membranes. Each autophagic membrane represents a separate reaction compartment. Thus, the reaction list is determined by the instantaneous number of membranes and their states of maturation.

III. RESULTS

We carried out experiments to measure autophagic vesicle abundance in single cells in a basal steady state and under treatments with various compounds that modulate the rate of autophagy initiation or autophagic vesicle turnover via inhibition of mTOR or lysosome activity. We parameterized the model by fitting the model to experimentally measured time series of vesicle counts. The model accurately captured the observed population averaged dynamics and stochastic dynamics at the single-cell level. Using the model we predicted the effects of knockdown of Atg9, a lipid-carrier implicated in initiation of isolation membrane synthesis, and inhibition of Vps34. We tested these predictions through siRNA knockdown of Atg9 and wortmannin-inhibition of Vps34. We also tested an unexpected prediction that vesicle size should positively correlate with LC3-I abundance. The experimental tests confirmed the model predictions. Thus, our model provides a reliable foundation for future, more detailed modeling of autophagy regulation and dynamics.

References

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[1]Theoretical Biology and Biophysics Group and Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, NM 87545.
[2]Laboratory of Systems Biology, Van Andel Research Institute and [3]Van Andel Institute Graduate School, Grand Rapids, MI 49503.
[4]Clinical Translational Research Division, Translational Genomics Research Institute, Scottsdale, AZ 85259.
*These authors contributed equally.