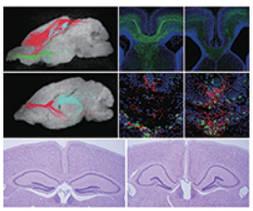
Autophagy



Autophagy

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AUTOPHAGIC PUNCTUM

Active Interaction Mapping as a tool to elucidate hierarchical functions of biological processes

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ABSTRACT

Increasingly, various 'omics data are contributing significantly to our understanding of novel biological processes, but it has not been possible to iteratively elucidate hierarchical functions in complex phenomena. We describe a general systems biology approach called Active Interaction Mapping (AI-MAP), which elucidates the hierarchy of functions for any biological process. Existing and new 'omics data sets can be iteratively added to create and improve hierarchical models which enhance our understanding of particular biological processes. The best datatypes to further improve an AI-MAP model are predicted computationally. We applied this approach to our understanding of general and selective autophagy, which are conserved in most eukaryotes, setting the stage for the broader application to other cellular processes of interest. In the particular application to autophagy-related processes, we uncovered and validated new autophagy and autophagy-related processes, expanded known autophagy processes with new components, integrated known non-autophagic processes with autophagy and predict other unexplored connections.

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Atg8; Atg24; Atg26; autophagy; Gyp1; hierarchical functions; OMICs; ontology

In a paper published in *Molecular Cell* by Kramer *et al.* (2017), we outlined a general systems biology approach, called Active Interaction Mapping (AI-MAP), which elucidates the hierarchy of functions for any biological process. Relevant 'omics data sets are used to create a hierarchical model. AI-MAP then evaluates the most suitable 'omics data for improved understanding of the particular biological process. The addition of new 'omics data creates an improved model. We applied the power of AI-MAP to bulk and selective autophagy in yeast, uncovering and validating new autophagy and autophagy-related processes, expanding known autophagy processes with new components, integrating known non-autophagic processes with autophagy and predicting other unexplored links.

Autophagy is a conserved, intracellular process whereby eukaryotic cells degrade and recycle macromolecules. This dynamic process responds to metabolic need, as well as extracellular and intracellular signals, and is orchestrated by a complex machinery of autophagy-related (Atg) proteins, many of which are conserved (core autophagy machinery). In addition to bulk autophagy, which functions via macroautophagy (hereafter called autophagy), many forms of selective autophagy target specific cargoes. These autophagy-related pathways play cytoprotective roles, resulting in cellular adaptation and survival, while also providing an essential defense mechanism against inflammation, infection, neurodegeneration and cancer. Deregulation of autophagy is implicated in the pathogenesis of human diseases. Importantly, because of evolutionary conservation of the core autophagy machinery, many insights gained from studies of yeast autophagy are generalizable to mammals.

A first-generation model, called Autophagy Ontology (AtgO) 1.0, was built from 78 published gene networks of 9 different types in *Saccharomyces cerevisiae*. This included protein and genetic interactions, gene co-expression, and gene-gene similarity based on shared protein sequence and structural information. AtgO includes a broad set of 492 candidate genes (including all autophagy-related genes) with potential links to autophagy based on literature or data. Once the AtgO 1.0 model was built, AI-MAP aligned it against the Gene Ontology to distinguish known components from novel ones, analogous to how a newly sequenced genome is aligned against a reference genome to transfer information about genes and genome structure. Atg0 1.0 restructures and unifies existing knowledge about autophagy and incorporates many new proteins and relationships.

Once AtgO 1.0 was constructed, we used AI-MAP to guide new data collection. First, we asked which existing network data sets were most informative to build AtgO 1.0 by removing all data of a given type and evaluating the resulting decrease in model performance. Protein-protein interactions followed by genetic interactions were the biggest contributors. Next, we asked which additional data would most improve our

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understanding of the hierarchical functions involved in autophagy. Systematic computational analysis with AI-MAP placed synthetic-lethal interactions at the top.

Armed with this insight, we designed a systematic screen for genetic interactions targeted at genes and conditions relevant to autophagy. Synthetic genetic array technology (SGA) was used to query 52 autophagy-related genes for genetic interactions against an array of 3,007 genes. These SGA networks were created in 3 conditions: rapamycin, which induces autophagy; amino-acid starvation, which metabolically induces autophagy; and an untreated control.

These 156,364 additional measurements across autophagy-activating conditions provided more information about autophagy than all previous data sets, producing a second-generation ontology (AtgO 2.0) of 220 functions, 56% of which were previously unknown.

In addition to improving AtgO 1.0, the SGA revealed several genes with strong condition-dependent interactions with core autophagy genes. These genes included *SSD1*, encoding an mRNA-binding protein that represses translation; *DID4* and *STP22*, encoding subunits of the ESCRT complexes (required for autophagy in humans but not yet examined in yeast); *GYP1*, encoding a GTPase-activating protein (GAP); *IRA2*, encoding an inhibitor of Ras-cAMP; *PIB2*, producing a phosphatidylinositol-3-phosphate binding protein of unknown function; and *YPL247C*, encoding an unknown protein. With the exception of *YPL247C*, autophagy assays showed a dependence on these genes.

The improved AtgO revealed new and expanded terms connected to autophagy, representing novel autophagy biology. For example, we validated a new autophagy process that links Atg8, a ubiquitin-like protein required for phagophore elongation, to Atg24, a phosphatidylinositol-3-phosphate-binding protein needed for selective autophagy. During peroxisome degradation by selective autophagy (pexophagy), Atg24 localizes to 2 dot-like structures in the vertex between the peroxisome cluster and the vacuole. However, in the absence of Atg8, but not in the absence Atg7 (the E1 activating enzyme required for proper Atg8 localization), Atg24 is not in these dot-like structures, but instead surrounds peroxisome clusters outside the interaction zone with the vacuole.

Similarly, while an autophagy phenotype was not previously reported for Atg26, its presence in AtgO 2.0 suggested an autophagy link. Atg26 was placed in the same term with Atg11, Atg19 and Atg27, implying an autophagy-related role. Consistent with this implication, *atg26*△ cells failed to efficiently process larger Ape1 complex cargo under autophagy-inducing conditions, showing that Atg26 indeed functions in autophagy.

Finally, AtgO integrated a known process involving a Rab-GAP cascade required for Golgi membrane traffic with autophagy. Core autophagy proteins, such as the Atg9-recycling system (Atg9 and Atg18), as well as the 2 core ubiquitin-like conjugation systems (Atg3, Atg5, and Atg7), were grouped with many Rab GTPases and GAPs (Gyp1, Ypt1, Ypt31, Ypt32, and Sec4) known to be involved in Golgi transport into a new term. Gyp1 is a Golgi-localized GAP that inactivates the Rab GTPase Ypt1 during a programmed series of Rab activation and inactivation steps, which changes the functional identity of membranes flowing along the exocytic pathway. We found that Gyp1 also localized to the phagophore assembly site (PAS), the location of the autophagosome-generating machinery, and acts downstream of Ypt1, suggesting a similar Rab conversion process acting during autophagy. These examples represent only a subset of new biology obtained from Atg0 2.0, leaving other new processes to be validated.

The AI-MAP protocol and AtgO 2.0 model are available at http://atgo.ucsd.edu. A downloadable Jupyter notebook constructs the AtgO model; new autophagy data can be added to the model by a user, and similar models can be constructed for other biological processes. We hope that the hierarchy proves of interest to the autophagy research community, and that the general method will enable many additional efforts to construct models of cell biological processes.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.