Active Interaction Mapping Reveals the Hierarchical Organization of Autophagy

Highlights
- Diverse -omics data integrated to assemble a unified hierarchical model of autophagy
- Post hoc analysis prioritizes the most informative future data types
- Consequently, 156,364 genetic interactions measured in autophagy-activating conditions
- Multiple new functions involve Gyp1, Atg24, Atg26, Ssd1, Did4, Stp22, and others

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In Brief
Kramer et al. present a general procedure that guides molecular interaction mapping to assemble a hierarchical model of any biological system. Application to autophagy reveals the hierarchical organization of this process, including many new biological components and functions. This work provides an archetype for future studies in systems biology.

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Active Interaction Mapping Reveals the Hierarchical Organization of Autophagy

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SUMMARY
We have developed a general progressive procedure, Active Interaction Mapping, to guide assembly of the hierarchy of functions encoding any biological system. Using this process, we assemble an ontology of functions comprising autophagy, a central recycling process implicated in numerous diseases. A first-generation model, built from existing gene networks in Saccharomyces, captures most known autophagy components in broad relation to vesicle transport, cell cycle, and stress response. Systematic analysis identifies synthetic-lethal interactions as most informative for further experiments; consequently, we saturate the model with 156,364 such measurements across autophagy-activating conditions. These targeted interactions provide more information about autophagy than all previous datasets, producing a second-generation ontology of 220 functions. Approximately half are previously unknown; we confirm roles for Gyp1 at the phagophore-assembly site, Atg24 in cargo engulfment, Atg26 in cytoplasm-to-vacuole targeting, and Ssd1, Did4, and others in selective and non-selective autophagy. The procedure and autophagy hierarchy are at http://atgo.ucsd.edu/.

INTRODUCTION
A key promise of systems biology is to advance our understanding of biological systems by combining genome-scale data with model building in a virtuous cycle (Ideker et al., 2001a; Kitano, 2002). New genomics data improve the biological model and, in turn, analysis of the model informs collection of new datasets. Systems biology typically surveys the literature to assemble knowledge of specific molecules, interactions, and reactions involved in a biological system, then uses this knowledge to initialize a computational model (Covert et al., 2004; Davidson et al., 2002; Ideker et al., 2001b; Karr et al., 2012; King et al., 2004; Malleshaiah et al., 2010). Model predictions are systematically compared to laboratory measurements, with discrepancies used to improve the model and design further experiments.

Initially, formulating a model can be laborious, requires that much is already known about the system, and may be biased toward well-studied components. Alternatively, many studies have sought to construct models of biological systems directly from systematic datasets (Ergün et al., 2007; Ho et al., 2002). Genome-scale data, including profiles of mRNAs, proteins or metabolites, and gene and protein interactions, are analyzed to infer a network of genes describing the system (Behrends et al., 2010; Havugimana et al., 2012; Kim et al., 2011; Lefebvre et al., 2012; Picotti et al., 2013). Such network models have been increasingly used to describe key biological systems, including tissue specificity (Greene et al., 2015), cell differentiation (Cahan et al., 2014), and diseases such as cancer (Carro et al., 2010; Creixell et al., 2015; Leiserson et al., 2015).

At this juncture, we see two challenges. First, biological systems do not merely consist of flat networks of genes; they are exquisitely hierarchical in nature. Models should seek to capture this hierarchy, which extends over multiple scales from nucleotides (<1 nm scale) to proteins (1–10 nm), protein complexes (10–100 nm), cellular processes (100 nm), organelles (1 μm), cells (1–10 μm), tissues (100 μm–100 mm), and complex organisms (>1 m). Second, while the field has taken a foundational step by enabling the construction of models directly from data rather than only literature, a next step will be to determine how to iteratively improve these models with the most informative new data. The ability to rationally motivate and design new -omics datasets is sorely needed in genomics; far too many large-scale experiments are performed simply because one can, without justification or guidance for why those experiments might lead to an improved biological understanding.

Toward the first challenge, a hierarchical biological model already in widespread use is the Gene Ontology (GO) (Ashburner et al., 2000). Through extensive literature curation efforts, GO aims to factor the cell into a complete hierarchy of cellular components and processes, each represented by a GO “term.” Recently, we showed that an ontology of the cell very much like GO could be automatically constructed from diverse
Figure 1. Program for Active Interaction Mapping

(A) After initialization with public datasets (i), the approach proceeds by progressive iterations of data integration (ii), hierarchical model assembly (iii), and adaptive generation of new data (iv).

(legend continued on next page)
genome-scale datasets (Dutkowski et al., 2013; Kramer et al., 2014). Using this method, we built a data-driven ontology of yeast, the Network Extracted Ontology (NeXO), which recapitulated approximately 60% of known cellular components in GO. However, it was also clear that our hierarchical understanding of cell biology is far from complete, leaving open the second challenge: how to most efficiently improve cell function hierarchies with high-value experiments. Thus far, methods have been devised for selecting the optimal next experiment considering a particular kind of model (e.g., a gene network), data type (e.g., growth phenotyping), and a constrained repertoire of treatments (e.g., single or double gene knockouts) (Atlas et al., 2014; Barrett and Palsson, 2006; Ideker et al., 2000; King et al., 2009, 2004). However, such questions of experimental design have not been approached for building general hierarchical models and, given the plethora of -omics data types that can now be generated, guidance is needed on which type of data one should collect next to improve the models.

Here, we describe a general program for elucidating the hierarchy of functions underlying a cellular process, based on progressive cycles of mapping and modeling that we call Active Interaction Mapping, or AI-MAP (Figure 1A). We apply this approach to develop a hierarchical model of autophagy, the conserved process of “self-eating” by which cells respond to starvation and other stress by degrading macromolecules and recycling their constituent building blocks (Levine and Yuan, 2005). During autophagy, cellular contents are enclosed in a double-walled vesicle, the autophagosome, and delivered to the yeast vacuole or mammalian lysosome for degradation and recycling; such contents can include non-selective cytoplasmic contents (macroautophagy) or selective organelles, such as peroxisomes (pexophagy) or mitochondria (mitophagy). All non-selective and selective autophagy pathways share a core set of molecular machinery (Jin and Klionsky, 2013), which has wide-ranging effects on pathways such as tumor suppression (Liang et al., 1999), neurodegeneration (Rubinsztein et al., 2005), and aging (Rubinsteint al., 2011).

In what follows, we (1) construct a first-generation hierarchical model of autophagy from publicly available interaction networks for the budding yeast, Saccharomyces cerevisiae; (2) identify synthetic-lethal genetic interactions as the most useful type of data for systematically improving the model; (3) generate static and differential genetic networks involving growth measurements of over 156,000 yeast genotypes across autophagy-relevant conditions; and (4) integrate these new datasets with previous ones to arrive at a substantially improved understanding of autophagy. The active interaction mapping process creates a “living” ontology of cell function, which actively suggests new data types for self-improvement and highlights new functions discovered as data are added. AI-MAP has general utility for any biological process.

RESULTS

Integrating Diverse -omics Data Constructs a First-Generation Model of Autophagy

To initialize the AI-MAP process, we sought to construct a “first-generation” hierarchical model of autophagy, called the Autophagy Ontology (AtgO), based on publicly available molecular interaction networks in S. cerevisiae. Although not all datasets have been specifically designed to study autophagy, non-selective or selective autophagy pathways have some activity in nearly all conditions (Reggiori et al., 2012). In total, we obtained a compendium of 78 networks of nine different data types, including protein interactions, genetic interactions, gene co-expression, and gene-gene similarity based on shared protein sequence and structural information, as previously curated for YeastNet (Kim et al., 2014) (Figure 1Aii).

To build an ontology from these heterogeneous datasets, we first integrated all data into a single pairwise gene similarity network (Figure 1Aii) and then analyzed the hierarchical structure of this network to infer a gene ontology (Figure 1Aiii). Pairwise gene-gene similarities in the similarity network were determined using a statistical regression procedure that combines available interaction datasets into a single quantitative similarity score. To learn how the different data types should be combined, the regression was trained to model a “standard reference” gene similarity network derived from GO (gene-gene similarity scores based on relatedness in GO of the two genes; see STAR Methods). In this way, the data-derived similarity network captured known biological processes and components in GO when supported by data (Pearson r = 0.4 versus GO, p < 10−300, out-of-bag prediction); similar data combinations then identify new biological processes, components, and relationships not previously documented (Figure 1B).

For assembly of the ontology, we selected 492 candidate genes with potential relation to autophagy based on literature or data (Figure 1C). A broad net was intentionally cast to allow later steps of the AI-MAP process to dictate which of these 492 genes are most related to core autophagy functions. The Clique Extracted Ontologies algorithm (CiiXO; Kramer et al., 2014) was then applied to analyze the hierarchical structure of the data-derived gene similarity network among these 492 genes (Figure 1Aiii). By this method, nested communities of genes apparent in the pairwise similarity data were identified, resulting in a hierarchy of 218 terms and 310 term relations, which we call the Autophagy Ontology (AtgO 1.0; Figure 1D). To determine which terms represent known biological functions and which represent potential new biology, AtgO 1.0 was aligned with GO according to a previously described alignment procedure (Dutkowski et al., 2013). This process seeks a one-to-one mapping between terms in AtgO and terms in GO; aligned terms, term names, and descriptions are transferred from the GO reference.
Figure 2. First-Generation Autophagy Ontology
(A–C) AtgO 1.0 model of terms (rectangles), genes (ovals), and hierarchical interrelations among these (links). The rectangle size shows the number of genes annotated to that term; its color shows term alignment to GO: darker blue indicates higher similarity to GO; white terms/red outlines do not align. The oval color indicates the gene status. For compactness, the gene annotations are displayed only for terms AtgO:15 (B; no GO alignment) and AtgO:18 (C; aligned to GO: “macroautophagy”).
(D) Comparison to GO as curated from literature, showing GO terms and annotations for GO:0006914:autophagy and its descendants. The links of the same color.

Term AtgO:9, which aligned to GO:0006914 (autophagy), encapsulated 234 genes organized into 82 subterms (Figures 2A–2C), including 19 of the 20 core autophagy genes and 67 other genes annotated to GO: “autophagy” (Figure 2D). Also included were 148 genes newly associated with autophagy; within these, we observed a significant enrichment for functions in cell cycle (51 genes, p < 10⁻¹⁲), cellular response to stress (42 genes, p < 10⁻¹²), and vesicle transport (47 genes, p < 10⁻²⁴), placing these functions in broad relation to autophagy.

This AI-MAP protocol is available in a Jupyter Notebook at http://atgo.ucsd.edu; notebook execution constructs the AtgO model described herein; however, general ontology models can be constructed by running the notebook with new data supplied by the user.

Analysis of Model Reveals Genetic Interactions as Most Effective New Data
Next, we determined which new network data would be most capable of improving the AtgO model in further experiments (Figure 1Aiv). For this purpose, we removed all data of a given type and evaluated the resulting decrease in model performance, measured as the ability to capture the GO reference (Figure 3A). The largest contribution was from protein-protein interactions, closely followed by genetic interactions; that is, recovery of GO depends most strongly on data of these two types, making these more desirable. Beyond this absolute importance measure, we were also interested in the importance of data relative to the present time, i.e., the extent to which adding another dataset of a given type is expected to improve the model. Therefore, we
measured the decrease in model performance as individual studies of a particular type were sequentially removed (Figure 3B). The unit of one “study” was used to normalize for different temporal and financial costs of generating each type of data; it roughly estimates the amount of data one -omics lab can produce in one study on average (i.e., data per lab study). Removing single studies of genetic interactions caused the steepest instantaneous decrease in performance, suggesting that such studies may continue to provide the most information. A similar result was obtained when individual interactions rather than whole studies were progressively removed (Figure S1, available online). Interestingly, gene expression data provided very little information not captured by other data types, either when removed as a group (Figure 3A) or one study at a time (Figure 3B).

**Conditional Genetic Interaction Networks Incorporate New Autophagy Genes**

Guided by the above analysis, we designed a systematic screen for genetic interactions targeted at genes and conditions relevant to autophagy (Figures 3C and 3D). Synthetic genetic array technology (SGA) (Tong and Boone, 2006) was used to query 52 autophagy-related genes for genetic interactions against an array of 3,007 genes, covering approximately two-thirds of the non-essential yeast genome. SGA uses high-throughput robotic colony pinning on agar to create and score growth of many double gene deletion strains in parallel, here yielding $52 \times 3,007 = 156,364$ tests for gene-gene interaction (Table S1). These SGA networks were created in three conditions: rapamycin, which pharmacologically induces autophagy; amino-acid starvation, which metabolically induces autophagy; and an untreated control. An established computational workflow (Bean et al., 2014) was used to assign quantitative S-scores to all gene pairs, with positive S-scores indicating faster than expected growth (epistatic or suppressive interaction) and negative S-scores indicating slower than expected growth (synthetic-sick or lethal interaction). SGA networks were also computed in “differential” configurations (Bandyopadhyay et al., 2010), based on the differences in S-scores between (1) rapamycin and untreated, (2) nitrogen starvation and untreated, and (3) nitrogen starvation and rapamycin.

Several array genes displayed strong differential interactions with core autophagy genes, most specifically upon rapamycin treatment (Figure 3E). These included SSD1, encoding an mRNA-binding protein that represses translation (Jansen et al., 2009); DID4 and STP22, encoding subunits of the ESCRT complexes, which are required for autophagy in humans, but had not yet been examined in yeast (Rusten and Stenmark, 2009); GYP1, a GTPase-activating protein (GAP) (Du and Novick, 2001); IRA2, an inhibitor of RAS-cAMP (Rodkaer and Faergeman, 2014); PIB2, a phosphatidylinositol(3)-phosphate binding protein of unknown function (Shin et al., 2001); and YPL247C, an uncharacterized open reading frame. To investigate whether these genes play a direct role in macroautophagy, we scored their null mutants by the Pho8Δ60 assay, which provides a quantitative marker of autophagic flow into the vacuole (Noda et al., 1995). With the exception of YPL247C, autophagy showed a clear dependence on all of these genes (Figure 3F).

**New Targeted Interactions Markedly Improve the Hierarchical Model**

Next, we evaluated the extent to which the new interactions improved the AtgO ontology. When used as the sole source of data, networks from each of the static conditions showed some ability to reconstruct GO ($r = 0.13–0.18$; Figure 3G). The differential networks showed better performance ($r = 0.23–0.31$), consistent with previous findings on the utility of differential interaction mapping (Bandyopadhyay et al., 2010; Ideker and Krogan, 2012). Integrating all static and differential conditions into a single network yielded the best correspondence with GO ($r = 0.42$), suggesting that multiple conditions reveal different aspects of cell biology. Remarkably, this performance was better than all previous data combined ($r = 0.30$; Figure 3H). Integrating previous with new data performed best of all ($r = 0.48$). We designate the “second-generation” ontology of 220 terms resulting from these integrated data as AtgO 2.0 (Figure 4).

Beyond its improved ability to reconstruct GO, we found that the hierarchical structure of this model captured many potential new biological functions and relationships. In particular, analysis of AtgO 2.0 revealed that the majority of terms (56%) involved previously unknown biological findings, which we categorized into four broad types: (1) terms representing previously unknown subfunctions of autophagy (previously unknown groupings of primarily known autophagy genes), (2) terms representing previously unknown subfunctions related to autophagy (groupings of genes previously attributed to diverse functions), (3) terms representing superprocesses that integrate known processes, and (4) terms representing a known process, but expanded by adding genes (Figure 5A; Table S2). In what follows, we survey brief, but suggestive, experimental findings from several of these types. We have constructed a web portal to AtgO 2.0 (http://atgo.ucsd.edu/), which permits exploration of the autophagy hierarchy with visualization of the network data supporting each term.

**A Key Regulator of Vesicular Trafficking, Gyp1, Is Required for Autophagy-Related Pathways**

AtgO 2.0 suggested a broader involvement of vesicle trafficking, docking, and fusion pathways in autophagy (Figure 4C); for example, we noted AtgO:18 ("vesicle transport machinery of ER-EE-MVB-vacuole and autophagy"; 37 genes), which integrated core autophagy or pexophagy genes with many Rab GTPases and GAPs known primarily for their function in Golgi transport (GYP1, YPT1, YPT31-32, and SEC4) (Jean and Kiger, 2012) (Figure 4Ci). GYP1, the GAP of this pathway, displayed significant genetic interactions with core autophagy genes under starvation and rapamycin treatment, including positive interactions between Gyp1 and the Atg9-recycling system (Atg9 and Atg18), as well as with the two core Ubiquitin-like conjugation systems (Atg3, Atg5, and Atg7) (Figure 3E); these interactions were not observed in untreated conditions (Table S1). Gyp1 had been previously localized to the Golgi (Du and Novick, 2001); however, we observed that GFP-Gyp1 is also localized to the phagophore assembly site (PAS), the location of the autophagosome-generating machinery (Figure 6A). Functional experiments with a gyp1Δ strain revealed a requirement for Gyp1 in PAS formation, as deletion of GYP1 caused mislocalization.
Figure 3. Active Analysis of Data Types Leads to Conditional Genetic Interaction Maps
(A) Performance decrease in AtgO 1.0 when all data of one type are excluded from the model. The performance is measured as the Pearson correlation between pairwise similarity scores derived from integrated data versus GO, focusing on gene pairs within the 492 autophagy-related genes (see main text).
(B) Performance degradation as single studies of the indicated type (color) is cumulatively excluded. The mean (points) and SD (error bars) are calculated over 50 random sets of removed studies. See also Figure S1.
(C and D) New genetic interaction maps between 52 autophagy query genes and 3,007 non-essential array genes in untreated conditions (rich media; C) as well as rapamycin and starvation conditions (D). The differential maps between each pair of conditions are also displayed. See also Table S1.
(E) Differential genetic interactions (rapamycin, untreated) between core autophagy genes and implicated array genes.
(legend continued on next page)
of GFP-Atg8 (Figure 6B) and an Atg8-processing defect (Figure 6C), reduced maturation of prApe1 into mApe1 via selective autophagy (Figure 6D), and impaired macroautophagy under nitrogen starvation as measured by the Pho8Δ60 assay (Figure 3F). These assays confirm a general requirement of Gyp1 in autophagy-related pathways.

The Rab GTPase Ypt1 has also been implicated in autophagy (Wang et al., 2015); Gyp1 may act as the heretofore unknown GAP on Ypt1 at the PAS. To test this possibility, we used a Ypt1 overexpression system, which leads to increased PAS formation and rescues the autophagy defect of a Ypt1 GEF mutant (Trs85, upstream of Ypt1), but not of a Ypt1 effector mutant (Atg11, downstream of Ypt1) (Lipatova et al., 2012).

When Ypt1 was overexpressed in a gyp1Δ strain, the gyp1Δ defect in PAS formation was not rescued (Figure 6E). This indicates that Gyp1 acts downstream of Ypt1 during autophagy, similar to its function as the GAP for Ypt1 in ER-to-Golgi protein trafficking (Figure 6F).

Finally, some of the same autophagy-deficient phenotypes were observed for a vps1Δ strain (Figures 6C and 6D). VPS1

(F) Pho8Δ60 enzymatic activity measurement of gene deletions in (E). The samples were collected from growing cells (0 hr; mid-log phase in YPD) and after nitrogen starvation (4 hr SD-N). All activity measurements were normalized to Pho8Δ60 activity in the wild-type 4 hr sample (100%). The error bars indicate SD of three replicates.

(G and H) Performance of reconstructing GO, using new data only (G) or integrating new with prior data (H). The performance is measured over gene pairs within the set of 52 autophagy query genes, in comparison to randomized data or GO (mean of 100 trials).
Engulfment of Selective Autophagy Cargos Involves Atg8 and Atg24

AtgO:247 was a new term in AtgO 2.0 that grouped ATG8, encoding a ubiquitin-like protein that localizes to isolation membranes and mature autophagosomes (Noda et al., 2010), with ATG24, a regulator of membrane fusion (Ano et al., 2005; Kanki and Klionsky, 2008), based on a combination of strong genetic interaction profile similarity and co-expression (Figures 2Ci and 5B). Working with the yeast Pichia pastoris, where the very large peroxisomes serve as a model cargo for imaging of autophagy (Oku and Sakai, 2008), we followed Atg24-GFP in both wild-type and atg8Δ cells. We noted a change in Atg24 localization to PE, although previous work in S. cerevisiae had found no involvement of Atg26 in standard autophagy assays (Cao and Klionsky, 2007), we observed that Atg26 co-localizes in distinct, overlapping puncta with Atg19, the known receptor for prApe1 aggregates (Figure 7C). We then monitored processing of prApe1 in wild-type and atg26Δ strains, as well as in atg1Δ cells, as a positive control for disruption of prApe1 processing (Cheong et al., 2008). Normal maturation of prApe1 was observed in wild-type, but not atg1Δ cells (Figure 7D). The atg26Δ mutant also showed processing defects, especially in cells overexpressing prApe1, which produce larger aggregates (50 μM CuSO4; Figure 7F). In contrast, many aggregates remained in the vacuole in prApe1-overexpressing cells overexpressing Atg26, in transport of prApe1 aggregates to the vacuole. In the yeast Pichia pastoris, the Atg26 ortholog participates in pexophagy, where it is required for the degradation of large peroxisomes; hence, its designation as an "Atg" gene (Nazarko et al., 2009; Oku et al., 2003).

AtgO:185 implied involvement of the remaining gene, Atg26, in transport of prApe1 aggregates to the vacuole. We have provisionally named AtgO:247 “engulfment of selective autophagy cargo.”

Figure 5. Characterization of AtgO 2.0
(A) Summary of AtgO terms by types of biological findings.
(B) Pairwise gene similarity matrix and resulting AtgO 2.0 hierarchy for a subset of core autophagy genes.
had reduced processing for each of these cargos (Figures 7H and 7I). These results support a role for Atg26 in the transport of several cargos that use Atg19 as their receptor, including large prApe1-containing aggregates, Ty1, and Ape4; hence, we gave AtgO:185 the name “transport of Atg19-receptor cargos.”

**AtgO as an Automated Literature-Curation Device**

By incorporating evidence from all available -omics studies, AtgO 2.0 recovered many functions already reported in literature, but which had not yet been curated by GO (Figure 5A; representing 23% of all terms). For example, AtgO:138 recovered the Ubp3p-Bre5p ubiquitin protease complex implicated in ribophagy (Figure 4Bii) (Kraft et al., 2008); AtgO:160 recovered the Pho85-Pho80 CDK-cyclin complex, which regulates autophagy (Figure 4Biii) (Yang et al., 2010); AtgO:106 recovered Skm1, Ste20, and Cla4, part of a complex that downregulates sterol uptake (Figure 4Biv) (Lin et al., 2009); and AtgO:140 recovered the Vma12-Vma22 assembly complex (Figure 4Bv) (Graham et al., 1998).

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**Figure 6. Analysis of Gyp1 and Atg8/Atg24**

(A) Co-localization (yellow arrows) of Gyp1 and the PAS marker Ape1 in wild-type cells under nominal conditions (SD) or after rapamycin (SD + rapamycin) treatment, using fluorescence microscopy.

(B) Atg8 localization in wild-type and gyp1Δ cells.

(C and D) Atg8 cleavage (C) and prApe1 maturation assay (D) in wild-type, atg6Δ (positive control), gyp1Δ, and vps1Δ strains.

(E) Model proposing the conversion of GDP-bound Ypt1 into the GTP-bound, active form, catalyzed by guanine nucleotide exchange factor TRAPPIII during autophagy. Ypt1-GTP is recognized by at least two autophagy effectors, Atg11 and the COG complex, and is converted back to the GDP-bound, inactive form by the GTPase-activating protein Gyp1.

(G) Localization of Atg24 in wild-type, atg7Δ, and atg8Δ strains of *P. pastoris* under pexophagy conditions. The scale bar represents 5 μm.
We submitted each of these terms to GO curators and, given the ample prior evidence, all were accepted for inclusion in the Gene Ontology (GO:1990861, GO:1990860, GO:1990872, and GO:1990871, respectively). Other terms missing from GO, but supported by literature, include AtgO:246, which captured an E1/E2 enzyme complex that activates Atg8 in a ubiquitin-like cascade (Figures 4Bvi and 5B), and AtgO:240, a second ubiquitin-like cascade activating Atg12 (Figures 4Bvi and 5B) (Kaiser et al., 2012). Numerous new AtgO gene annotations were also supported by prior studies: for instance, term AtgO:96 was assigned Vac8, Nvj1, and Swh1, which form the nucleus-vacuole junction (Figure 4Bviii) during piecemeal microautophagy of the nucleus (Dawaliby and Mayer, 2010; Kvam and Goldfarb, 2004). Two of these genes (NVJ1 and SWH1) were not annotated as such in GO and thus at our request were annotated to this term (GO:0034727). As another example, we found that eight proteins annotated to autophagy by AtgO, but not GO, already had literature support for autophagy phenotypes: Hog1 (Prick et al., 2006), Pep12 (Kanki et al., 2009), Snf1 (Wang et al., 2012), Tpk1 (Budovskaya et al., 2006), Vps4 (Nebauer et al., 2007), Vps51 (Reggiori et al., 2003), and Vps52 (Reggiori et al., 2003). Here too, these new gene annotations were accepted for inclusion in GO. Thus, AI-MAP can automatically mine, structure, and unify knowledge of a cellular process to create a central research resource that is complementary to literature-curated models.

**Application to Human Autophagy**

To test the feasibility of the Active Interaction Mapping approach in the study of human biology, we applied the same procedure described in yeast to a compendium of human data including...
gene expression profiling, protein-protein interactions, genetic interactions, co-localization, and sequence similarity. As in yeast, we began with a seed set of genes (33) designated as having core functions common to non-selective and selective autophagy (Jin and Klionsky, 2013). This process resulted in a human autophagy ontology (hAtgO 1.0) of 1,452 genes and 1,664 terms, 173 of which align significantly to existing terms in the human GO, and the rest of which represent potential new autophagy subprocesses and components (Figure S2A; Table S3). Compared to yeast, a smaller percentage of the ontology (10% versus 35%) aligned to existing GO terms, which may be due to better GO annotation of yeast than human, more complete data in yeast than human, or both. As in yeast, much of the core machinery of autophagy was grouped together (hAtgO:2962), which contains 14 core and 8 other genes (Figure S2A). Also as before, our confidence in the model is increased by the presence of recently discovered autophagy biology that is not yet captured by GO; for example, the Huntingtonin protein, Htt, is placed among core autophagy proteins, consistent with the recent discovery that this important disease gene acts physiologically as a scaffold for selective autophagy (Rui et al., 2015). Turning to the data most valuable for constructing hAtgO, we see a striking difference as compared to yeast, as the Active Interaction Mapping process relies primarily on gene expression profiling to construct the model of the human autophagy system (Figure S2B). This result suggests the generality of Active Interaction Mapping, as it can quickly be adapted to a new species (human) and can rely on the data most available and useful for that species and system.

DISCUSSION

This study provides a roadmap for how to progressively elucidate the ontology of cellular functions underlying any biological process. Studying a new process is straightforward, requiring starting knowledge of some of the genes involved and access to public -omics datasets. At all points of the study, virtually any genomic data can be analyzed since almost any data linked to public -omics datasets. At all points of the study, virtually any genomic data can be analyzed since almost any data linked to public -omics datasets. However, the value of new interaction mapping efforts can thus be rigorously evaluated rather than assumed, and the design of these experiments can be guided rationally, based on current knowledge and data.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and three tables and can be found with this article online at dx.doi.org/10.1016/j.molcel.2016.12.024.

AUTHOR CONTRIBUTIONS

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REFERENCES


### Key Resources Table

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CONTACT FOR REAGENT AND RESOURCE SHARING

As Lead Contact, Trey Ideker is responsible for all reagent and resource requests. Please contact Trey Ideker at tideker@ucsd.edu with requests and inquiries.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Yeast Strains
Saccharomyces cerevisiae and Pichia pastoris laboratory strains as described in Key Resources Table with growth conditions as appropriate for experiments as described in Method Details.

METHOD DETAILS

Public Data for S. cerevisiae
Data from YeastNet v3 (Kim et al., 2014) were obtained from http://www.inetbio.org/yeastnet/, consisting of the following numbers of datasets classified by type: 50 co-expression, 1 domain co-occurrence, 1 genomic neighbor, 10 genetic interaction, 12 high-throughput protein-protein interaction, 1 phylogenetic profile, 1 protein network tertiary structure, 1 low-throughput protein-protein interaction, and 1 co-citation.
**Data Integration**

Input datasets were provided as features to a random forest regression system for prediction of gene-gene pairwise similarity, trained using pairwise gene similarities in GO as a bronze standard. The network of predicted similarities we call the ‘data-derived gene similarity network.’ We aimed to use the Gene Ontology (GO) as a standard reference for learning to integrate data in a supervised fashion. Previously, we showed that a weighted network of gene-gene similarities derived from a gene ontology can be analyzed to reconstruct the full hierarchy of terms and term relations with near perfect precision and recall (Kramer et al., 2014). Because the network and the ontology can be interconverted, both contain the same information. Based on this equivalence, we guided our integration of data using a gene similarity network derived from GO instead of using GO directly. We derived this network by calculating the Resnik semantic similarity for each pair of genes (Resnik, 1995). Semantic similarities are calculated across the Biological Process and Cellular Component branches of GO, downloaded on 6/2/2015 from http://www.geneontology.org.

The 78 input datasets were integrated into a single network by using them as features in a supervised learning of the GO gene similarity network. Learning was performed using random forest regression (Breiman, 2001) from the Python scikit-learn package (Pedregosa et al., 2011). Predictions were made “out of bag,” i.e., the similarity of a gene pair was predicted based on information learned from other gene pairs. In effect, the random forest learns patterns in the networks which recapitulate information in GO. Hence, only relations in GO that can be systematically explained from data are included, any relations not justified by the data are excluded, and new relations not in GO are added when the network data support them (Figure 1B).

**Construction of Autophagy Ontology (AtgO) 1.0**

We analyzed the data-derived gene similarity network using the Clique Extracted Ontology (CliXO) algorithm, version 0.3 (http://mhk7.github.io/clixo_0.3/; Kramer et al., 2014), with parameters $\alpha = 0.1$ and $\beta = 0.5$. We identified significantly aligned terms between AtgO and GO using a previously described ontology alignment procedure with FDR threshold of 10% and minimum alignment score of 0.24 (http://mhk7.github.io/alignOntology/; Dutkowski et al., 2013). Alignment was performed first against the sub-branch of GO rooted at GO:“autophagy” and then the entire GO, prepared as in Dutkowski et al. (2013); significant alignments to the autophagy sub-branch were given first priority.

**Genetic Interaction Mapping**

Strain construction, plating of mutants, mutant selection, and scoring of genetic interactions in each condition were performed using a previously defined protocol (Collins et al., 2006; Schuldiner et al., 2006). Using a replica pinning robot, haploid double mutants were grown on agar plates that were either untreated, rapamycin treated or nitrogen deficient. Plates were photographed and colony sizes normalized, spatially corrected, and quantified using the Colony Analyzer Toolkit (Bean et al., 2014). For the three replicates per double mutant, the resulting experimental data were used to assign a quantitative S-score based on a modified t-test that compares the observed double mutant growth rate to that expected assuming no interaction exists, again using the Colony Analyzer Toolkit. Differential interactions were calculated by subtracting the S-scores for the same double mutant pair across conditions. Resulting S-scores available in Table S1.

**Construction of Autophagy Ontology (AtgO) 2.0**

The data-derived gene similarity network was recalculated incorporating both prior data and the new genetic interaction screen. The CliXO algorithm was applied with previous parameters. Term names in AtgO 2.0 were manually curated by our team, considering the names of aligned terms in GO, when available, as well as an extensive literature review. Reasoning and citations for term names are included in Table S2.

**Biochemical Studies**

*The prApe1 processing assay*

The prApe1 processing assays for Figure 6D: cells were grown for 16 hr in YPD medium (1% yeast extract, 2% peptone and 2% glucose) without exceeding exponential phase (OD$_{600}$ above 1), 1 OD$_{600}$ equivalents of cells were collected and Trichloroacetic acid (TCA) precipitated using a final concentration of 12.5% TCA and incubated at least for 30 min at –80°C. Next TCA-treated cells were pelleted by centrifugation (10 min at 21,000 g, room temperature), washed twice with ice-cold 80% acetone, and air-dried. After dissolving the pellets in 100 μL of 1% SDS/0.1 N sodium hydroxide, 20 μL of 6× SDS sample buffer was added. Samples were boiled for 5 min. For SDS-polyacrylamide gel electrophoresis, 10 μL of each sample was used per lane and blotted onto nitrocellulose membranes. Western blots were blocked with 5% dry skim milk in Tris-buffered saline with 0.1% tween-20 (TTBS) and probed with anti-Ape1 (1:5000; rabbit; a gift from Dr. Daniel Klionsky) and anti-β-actin antibodies (1:5000; mouse; C4, Santa Cruz Biototechnology) diluted in TTBS with 5% dry skim milk and incubated overnight at 4°C. Membranes were probed with anti-rabbit or anti-mouse IgG HRP (1:5000; BioRad). All blots were detected with HyGLO Quick Spray (Denville Scientific Inc.) on a Medical film processor SRX-101A (Konica).

The prApe1 processing assays for Figure 7D: cells harboring the plasmid overexpressing prApe1 (pCK782, a gift from Dr. Claudine Kraft; Papinski et al., 2014) were grown for 16 hr in SD-Cu medium (0.67% yeast nitrogen base [YNB] without amino acids and copper, 2% glucose plus any required amino acid, nucleotide, or vitamin supplement) without exceeding exponential phase (OD$_{600}$ above 1) and transferred to SD-Cu medium with 200 nM Rapamycin and the indicated CuSO$_4$ concentration at an OD$_{600}$ of 1. One ml of cells was collected at different times, TCA precipitated and analyzed as described above.
The Pho8Δ60 assay (WT and atg8 Δ strains were a gift from Dr. Maho Niwa) was performed as described previously (Manjithaya et al., 2010; Noda and Klionsky, 2008). For the alkaline phosphatase assay, five OD600 equivalents of yeast cells were harvested, washed once with cold water and once with wash buffer (0.85% NaCl and 1mM PMSF) and resuspended in 500 μl lysis buffer (20 mM Pipes, pH 7.0, 0.5% Triton X-100, 50 mM KCl, 100 mM potassium acetate, 10 mM MgSO4, 10 μM ZnSO4, and 1 mM PMSF). The cells were lysed by vortexing at full speed 10 times with 250 μl equivalents of glass beads for 1 min and incubated for 1 min on ice in between. The lysate was centrifuged at 14,000 g for 5 min at 4°C and the supernatant was recovered without disturbing the pellet. 100 μl of this supernatant was added to 400 μl reaction buffer (250 mM Tris-HCl, pH 8.5, 0.4% Triton X-100, 10 mM MgSO4, and 1.25 mM p-nitrophenyl phosphate [pNPP]), and samples were incubated for 10-15 min at 30°C before terminating the reaction by adding 500 μl of stop buffer (2 M glycine, pH 11). Production of p-nitrophenol was monitored by measuring the absorbance at 400 nm (A400) using a spectrophotometer (DU-730; Beckman Coulter), and the concentration in nmol of p-nitrophenol in the samples was calculated by graphing the adjusted A400 values relative to a standard curve of commercial p-nitrophenol (0 to 100 nmol). Protein concentration in the extracts was measured with the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific), and the specific activity was calculated as nmol p-nitrophenol/min/mg protein.

The GFP-Atg8, GFP-Ape4 and GFP-Ty1 Gag processing assays

For the GFP-Atg8, GFP-Ape4 and GFP-Ty1 Gag processing assays, yeast strains harboring the plasmids GFP-Atg8 (pRS315-GFP-Atg8, in house plasmid), GFP-Ape4 (pTS551, a gift from Dr. Takahiro Shintani; Yuga et al., 2011) and GFP-Ty1 Gag (pYEX-BX[GFP–Ty1 Gag], a gift from Dr. Yoshinori Ohsumi; Suzuki et al., 2011) were grown to mid log phase in SD medium (0.67% YNB without amino acids, 2% glucose plus any required amino acid, nucleotide, or vitamin supplement) lacking auxotrophic amino acids and then shifted to SD-N medium (0.17% YNB without amino acids and ammonium sulfate, and 2% glucose) at an OD600 of 1 for the indicated time. At each indicated time point, 1 mL of cell culture was removed and TCA precipitated as described above. The protein extracts were resolved by SDS-PAGE and proteins were revealed by western blotting with anti-GFP (1:2500; mouse; JL8, Clontech) and anti-β-actin (1:5000; mouse; C4, Santa Cruz Biotechnology) antibodies. Densitometry was performed using ImageJ software.

Fluorescence Microscopy Studies

Cell culture conditions for microscopy were performed as described for biochemical studies. Fluorescence microscopy images were acquired at indicated times using a motorized fluorescence microscope (Axioskop 2 MOT, Carl Zeiss MicroImaging, Thornwood, NY) coupled to a monochrome digital camera (AxioCam MRm, Carl Zeiss MicroImaging) and processed using the AxioVision 4.8.2 software. To quantify prApe1 aggregates images, the fluorescence microscopy parameters such as exposition time, gain, and binning were kept constant. Pexophagy condition used for Pichia pastoris in Figure 6G: cells were grown in methanol medium (0.67% YNB without amino acids and 1% methanol plus any required amino acid) starting an OD600 of 0.2 for 15-16 hr and shifted to SD for 1 hr, then images were acquired.

Other Methods

Cloning, gene deletion and yeast transformation were performed using standard methods.

Human Autophagy Ontology (hAtgO)

Datasets utilized are listed in Table S3. All quantitative interaction/co-expression data were linearly transformed into 8 bit integers prior to data integration. Ontology constructed using available Active Interaction Mapping Jupyter notebook downloadable at atgo.ucsd.edu/download.html.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical tests performed using R and python. Significance of pearson correlation calculated using pearsonr function in python’s scipy package. Statistical GO gene set enrichments in the main text calculated using the hypergeometric test in R. For Figures 7H and 7I, statistical comparison of processed GFP for GFP-Ape4 and GFP-GAG Ty processing assay were calculated by comparing 3 replicates of each strain to 3 replicates of wild-type using the one sided t test in R.

DATA AND SOFTWARE AVAILABILITY

Genetic interaction S-scores as measured for 52 autophagy-related query genes and 3007 array genes under 3 static and 3 different conditions: Table S1. Active Interaction Mapping (AIM) software and data necessary to perform AIM in S. cerevisiae and H. sapiens are available for download as a Jupyter notebook at atgo.ucsd.edu/download.html. The AtgO 2.0 model is also available for browsing and exploration at atgo.ucsd.edu.