SQST-1/p62 and SKN-1/Nrf2 promote phagocytosis under stress by promoting lysosomal trafficking

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Abstract

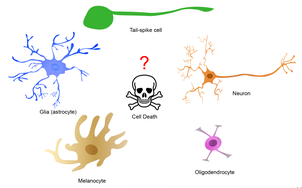
Programmed cell death and autophagy are among conserved quality control processes that regulate cell homeostasis and development. Autophagy and cell death have been linked, with autophagy shown to be involved in cell clearance. Our understanding of autophagy and programmed cell death in morphologically complex cells, which are polarized with long processes, is incomplete owing to unique features of such cells such as vastly different environments between different domains and differences in subcellular architecture.

We present a novel in vivo system to decipher the mechanisms of autophagy and programmed cell death and the link between them in complex cells. In this "tri-partite" killing program, Compartmentalized Cell Elimination, or CCE, three segments of the C. elegans tail-spike cell and the sex-specific CEM neurons die in different ways-the soma rounds as in apoptotic death, the proximal segment of the process beads and fragments and the distal process undergoes a bidirectional retraction.

We asked whether there is a link between CCE and autophagy. We subjected wild-type worms to a heat-shock stress and found CCE of the tail-spike cell to take place normally. We found that while there was no defect under normal conditions, following heat shock stress, mutants for the genes sqst-1 and uba-1 show significantly hampered CCE. The gene sqst-1 encodes for the C. elegans ortholog of SQSTM1/Sequestosome-1/p62. SQSTM1/p62 is a scaffold protein that targets and anchors ubiquitinated proteins to the autophagosome membrane, promoting their degradation by selective autophagy. Additionally, SQSTM1/p62 acts as a signaling hub for many pathways linked to neurodegeneration. The gene uba-1 encodes for the C. elegans ortholog the E1 ubiquitin-ligase enzyme UBA1. Together these data implicate selective autophagy in aiding CCE under stress.

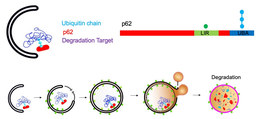
One degradation target of SQSTM1/p62 is KEAP1, a negative regulator of the stress response transcription factor NRF2. SQSTM1/p62 activates NRF2 by inactivating KEAP1. NRF2 is best known as a regulator of antioxidant and xenobiotic defense and is recently implicated in additional functions that include proteostasis and metabolic regulation. In worms, SKN-1 is the ortholog of NRF2. SKN-1 has been showed to promote DNA damage-induced germline apoptosis in worms and the NRF2-KEAP1 pathway has been linked to in neuronal remodeling in flies. We find that skn-1 mutants also have a CCE defect under stress conditions and that SKN-1/NRF2 translocates to the phagocyte nucleus following heat stress. One transcriptional target of SKN-1/NRF-2 is the lysosomal trafficking gene lyst-1. We find that lyst-1 mutants also have a similar CCE defect, as do mutants for lmp-1/Lamp-1, an important lysosomal gene. LMP-1/LAMP-1 also localizes discretely in the phagocyte following heat stress. Together, our data suggests autophagy and cellular stress resistance mechanisms bolster a developmental death program of complex cells under stress conditions by enhancing lysosomal trafficking and subsequent corpse digestion.

Cell elimination in complex cells is poorly understood



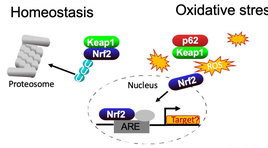
Morphologically complex cells (MCCs) are commonplace. Due to their long projections, MCCs have distinguishable cellular compartments.
How do MCCs die as a whole? How does localized elimination occur?

SQSTM-1/p62 is a ubiquitin- and LC3-dependent autophagic receptor



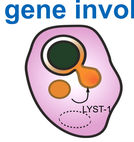
- SQSTM-1/p62 is an autophagic scaffolding protein.
- LC3/LGG-1 (LIR domain) is used as a marker for autophagosomes.
- UBA-1 is an E1 ubiquitin-activating enzyme.

SKN-1/Nrf2 is a transcription factor involved in antioxidant resistance



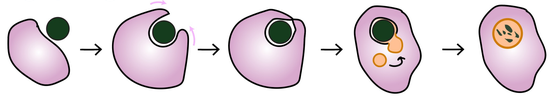
- Keap1 is a target of p62
- Under normal conditions, Keap1 tags Nrf2 for degradation in the proteosome.
- In times of stress, p62 is upregulated, and inhibits Keap1, freeing Nrf2 and allowing it to translocate to the nucleus and transcribe its target proteins.

lyst-1 is a gene involved in lysosomal trafficking



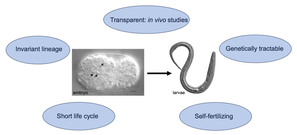
- Encoded by the LYST (Lysosomal Trafficking Regulator) protein
- Plays an important role in regulating intracellular protein trafficking and/or fission of endosomal vesicles such as lysosomes

Cell-eating: phagocytosis removes dead cells

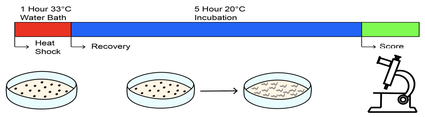


1. Corpse recognition
2. Pseudopod extension
3. Phagosome sealing
4. Lysosomal trafficking and fusion
5. Corpse digestion

C. elegans is an ideal tool to study cell elimination



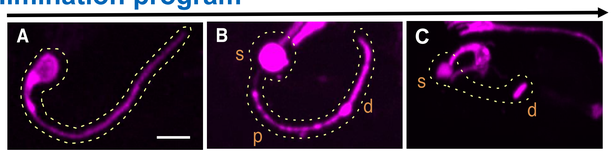
Assay: Heat shock and recovery



Objective

To understand the link between complex cell elimination during normal development and stress.

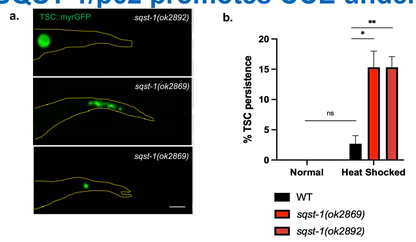
Compartmentalized Cell Elimination (CCE) is a novel tripartite cell elimination program



a. – *c.* TSC undergoing CCE.

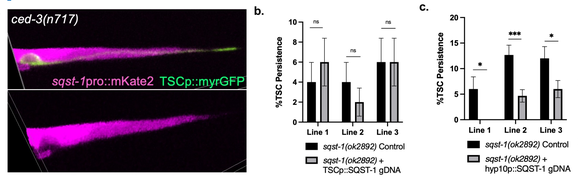
Results

SQST-1/p62 promotes CCE under stress



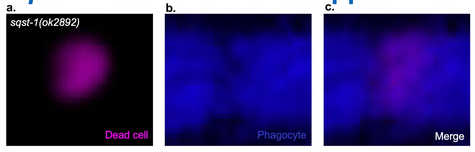
a. Range of phenotypes of persisting TSC fragments in *sqst-1* mutants
b. Graph showing percentage of *sqst-1* mutants with persisting TSC fragments

sqst-1 does does not function cell autonomously



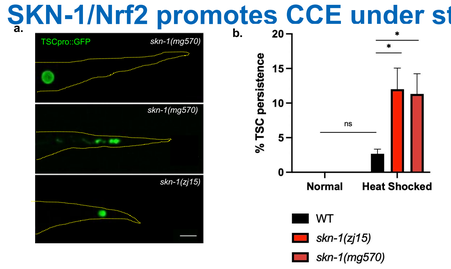
a. Expression of *sqst-1* in phagocyte
b. Cell specific rescue of *sqst-1* mutants
c. Phagocyte specific rescue of *sqst-1* mutants

sqst-1 mutant remnants appear internalized



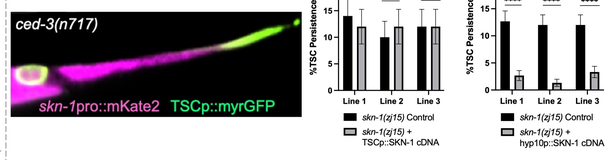
a. Persisting cell corpse labeled in mCherry
b. Phagocyte labeled in iBlueberry
c. Merge

SKN-1/Nrf2 promotes CCE under stress



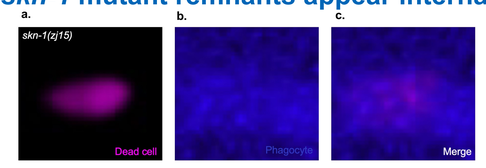
a. Range of phenotypes of persisting TSC fragments in *skn-1* mutants
b. Graph showing percentage of *skn-1* mutants with persisting TSC fragments

skn-1 does not function cell autonomously



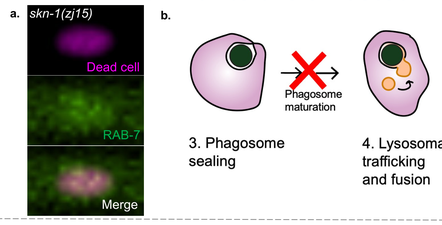
a. Expression of *skn-1* in TSC and phagocyte
b. Cell specific rescue of *skn-1* mutants
c. Phagocyte specific rescue of *skn-1* mutants

skn-1 mutant remnants appear internalized



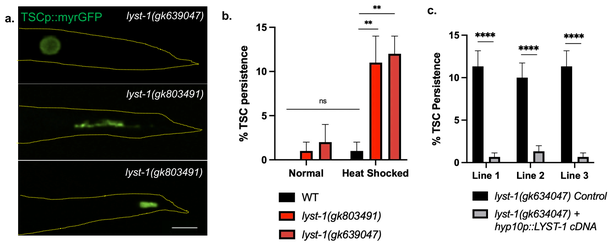
a. Persisting cell corpse labeled in mCherry
b. Phagocyte labeled in iBlueberry
c. Merge

skn-1 mutant remnants are arrested in the late phagosome



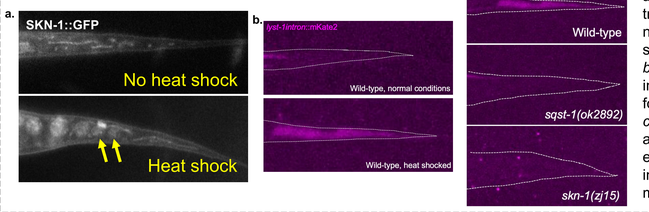
a. RAB-7::GFP localizes around *skn-1* mutant remnant
b. Schematic showing arrestation of remnants is occurring prior to lysosome fusion during phagosome maturation steps

LYST-1 promotes CCE under stress



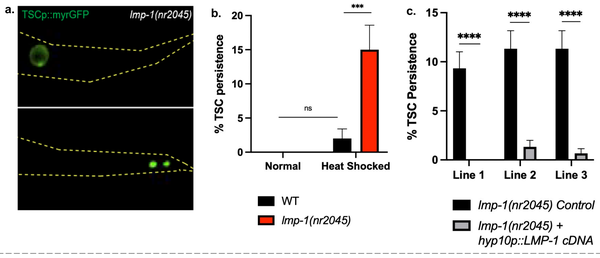
a. Range of phenotypes of persisting TSC fragments in *lyst-1* mutants
b. Graph showing percentage of *lyst-1* mutants with persisting TSC fragments
c. Phagocyte specific rescue of *lyst-1* mutants

SKN-1/Nrf2 translocates to the nucleus and *lyst-1* is expressed in the phagocyte following heat stress



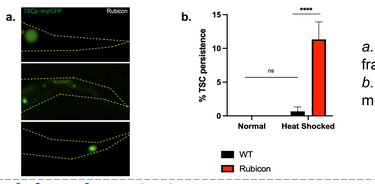
a. SKN-1::GFP translocating to the nucleus following heat shock
b. Expression of *lyst-1* in the phagocyte following heat shock
c. Percentage of animals with *lyst-1* expression decreases in *sqst-1* and *skn-1* mutants

LMP-1/LAMP-1 promotes CCE under stress



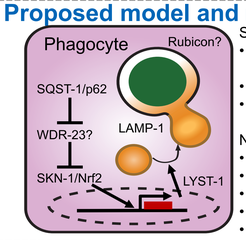
a. Range of phenotypes of persisting TSC fragments in *lmp-1* mutants
b. Graph showing percentage of *lmp-1* mutants with persisting TSC fragments
c. Phagocyte specific rescue of *lmp-1* mutants

LC3-Associated Phagocytosis may assist in CCE corpse clearance



a. Range of phenotypes of persisting TSC fragments in Rubicon mutants
b. Graph showing percentage of Rubicon mutants with persisting TSC fragments

Proposed model and next steps



Significance:
- CCE as a model for studying complex cell elimination in normal development and under stress
- New role of SKN-1/Nrf2 not previously shown
- New context of LAP in *C. elegans*

Next steps:
- WDR-23 overexpression
- Rubicon cell-specific rescue and expression
- LMP-1/LAMP-1 localization in WT and all mutants
- SKN-1 binding site CRISPR in *lyst-1* expression strains
- Internalization and phagosome maturation markers on all other mutants

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