OTKA K83509

Final report

During this project, we have established new cell culture, antibody production and immunoprecipitation facilities. Reagents and experiments produced with the help of OTKA funding were critical in obtaining key data that were central in the publication of 13 peer-reviewed papers. All these concern the evolutionarily conserved catabolic pathway called autophagy (self-eating). During the main pathway of autophagy, a phagophore membrane cistern forms in the cytosol. Material to be degraded is the engulfed into a double-membrane autophagosome, which fuses with a lysosome where biological material is degraded and recycled to fuel biosynthesis or energy production.

Main findings of the works published during the course of this project are the following:

1. In a theoretical paper, I argued that lysosomal inhibitors that are commonly used to block autophagic degradation (flux) may decrease the acitivity of TOR kinase, a central regulator of cell growth and autophagy. This way, inhibitor treatment can enhance autopagosome formation. Citation:

Interpretation of bafilomycin, pH neutralizing or protease inhibitor treatments in autophagic flux experiments: novel considerations.
Juhász G.
Autophagy. 2012 Dec;8(12):1875-6.
PMID: 22874642

2. We identified the autophagosomal SNARE Syntaxin 17 that forms a fusogenic SNARE complex with SNAP29 and lysosomal Vamp7. This finding has been confirmed in mammalian cells as well (please see Cell. 2012 Dec 7;151(6):1256-69. doi: 10.1016/j.cell.2012.11.001., and Nature. 2015 Apr 23;520(7548):563-6. doi: 10.1038/nature14147). Citation:

Autophagosomal Syntaxin17-dependent lysosomal degradation maintains neuronal function in Drosophila.
Takáts S, Nagy P, Varga Á, Pircs K, Kárpáti M, Varga K, Kovács AL, Hegedűs K, Juhász G.
J Cell Biol. 2013 May 13;201(4):531-9. doi: 10.1083/jcb.201211160.
PMID: 23671310

3. We wrote an invited, peer-reviewed review article that discussed recent Syntaxin 17 findings. Citation:

Evolutionarily conserved role and physiological relevance of a STX17/Syx17 (syntaxin 17)-containing SNARE complex in autophagosome fusion with endosomes and lysosomes.

Hegedűs K, Takáts S, Kovács AL, Juhász G.

Autophagy. 2013 Oct;9(10):1642-6. doi: 10.4161/auto.25684. Epub 2013 Jul 22. Review.

PMID: 24113031

4. We showed that Myc, the well-known oncogene is a major positive regulator of autophagy. This has been confirmed in mammalian cells after the acceptance of our paper (please see Hum Mol Genet. 2013 Dec 20;22(25):5237-48. doi: 10.1093/hmg/ddt381). Citation:

Myc-driven overgrowth requires unfolded protein response-mediated induction of autophagy and antioxidant responses in Drosophila melanogaster.

Nagy P, Varga A, Pircs K, Hegedűs K, Juhász G.

PLoS Genet. 2013;9(8):e1003664. doi: 10.1371/journal.pgen.1003664. Epub 2013 Aug 8.

PMID: 23950728

5. I contributed advice and antibodies to a study that identified acetyl-coenzyme A as a major regulator of autophagy and aging. Citation:

Nucleocytosolic depletion of the energy metabolite acetyl-coenzyme a stimulates autophagy and prolongs lifespan.

Eisenberg T, Schroeder S, Andryushkova A, Pendl T, Küttner V, Bhukel A, Mariño G, Pietrocola F, Harger A, Zimmermann A, Moustafa T, Sprenger A, Jany E, Büttner S, Carmona-Gutierrez D, Ruckenstuhl C, Ring J, Reichelt W, Schimmel K, Leeb T, Moser C, Schatz S, Kamolz LP, Magnes C, Sinner F, Sedej S, Fröhlich KU, Juhasz G, Pieber TR, Dengjel J, Sigrist SJ, Kroemer G, Madeo F.

Cell Metab. 2014 Mar 4;19(3):431-44. doi: 10.1016/j.cmet.2014.02.010.

PMID: 24606900

6. We used computer modeling to correctly predict the structure of Atg101 (according to 3 structural biochemistry papers that were published later), an autophagy protein in mammals that we showed to be also function as part of the Atg1 kinase complex during autophagy induction in Drosophila. Citation:

The putative HORMA domain protein Atg101 dimerizes and is required for starvation-induced and selective autophagy in Drosophila.

Hegedűs K, Nagy P, Gáspári Z, Juhász G.

Biomed Res Int. 2014;2014:470482. doi: 10.1155/2014/470482. Epub 2014 May 8.

PMID: 24895579

7. I acted as a Guest Editor for an Autophagy Special Issue, and I wrote a peer-reviewed review on autophagy in Drosophila together with another Editor (we are shared senior authors). Citation:

Autophagy in Drosophila: from historical studies to current knowledge.
Mulakkal NC, Nagy P, Takats S, Tusco R, Juhász G, Nezis IP.
Biomed Res Int. 2014;2014:273473. doi: 10.1155/2014/273473. Epub 2014 May 18. Review.
PMID: 24949430

8. We showed that Atg18 acts earlier than Atg2, as loss of the latter one allows the formation of phagophores. This is different from yeast cells, where these two proteins function together. Citation:

Different effects of Atg2 and Atg18 mutations on Atg8a and Atg9 trafficking during starvation in Drosophila.
Nagy P, Hegedűs K, Pircs K, Varga Á, Juhász G.
FEBS Lett. 2014 Jan 31;588(3):408-13. doi: 10.1016/j.febslet.2013.12.012. Epub 2013 Dec 24.
PMID: 24374083

9. We showed that Atg17/FIP200 activates Atg1 during autophagy induction in Drosophila, and mapped the interacting regions of proteins in this complex. Citation:

Atg17/FIP200 localizes to perilysosomal Ref(2)P aggregates and promotes autophagy by activation of Atg1 in Drosophila.
Nagy P, Kárpáti M, Varga A, Pircs K, Venkei Z, Takáts S, Varga K, Erdi B, Hegedűs K, Juhász G.
Autophagy. 2014 Mar;10(3):453-67. doi: 10.4161/auto.27442. Epub 2014 Jan 6.
PMID: 24419107

10. Our cell culture facilities and the help of my cell culture expert postdoc, Krisztina Hegedus (employed on this OTKA project) were critical to described the interaction of the endocytotic regulator Hook with Rab11, a newly identified promoter of autophagosome maturation. Citation:

Rab11 facilitates cross-talk between autophagy and endosomal pathway through regulation of Hook localization.
Szatmári Z, Kis V, Lippai M, Hegedus K, Faragó T, Lorincz P, Tanaka T, Juhász G, Sass M.

Mol Biol Cell. 2014 Feb;25(4):522-31. doi: 10.1091/mbc.E13-10-0574. Epub 2013 Dec 19.
PMID: 24356450

11. We showed that Syntaxin 17 cooperates with the HOPS tethering complex during autophagosome-lysosome fusion. This biochemical interaction is specific for autophagy, because Syntaxin 17 is dispensable for endosome maturation and for the delivery of lysosomal hydrolasas that also require HOPS. Citation:

Interaction of the HOPS complex with Syntaxin 17 mediates autophagosome clearance in Drosophila.
Takáts S, Pircs K, Nagy P, Varga Á, Kárpáti M, Hegedűs K, Kramer H, Kovács AL, Sass M, Juhász G.
Mol Biol Cell. 2014 Apr;25(8):1338-54. doi: 10.1091/mbc.E13-08-0449. Epub 2014 Feb 19.
PMID: 24554766

12. An Autophagy Special Issue was published by the journal Methods, with the leading experts of the field explaining how to study this process in various models. I was selected by the Editors to write the paper on Drosophila. Citation:

How and why to study autophagy in Drosophila: it's more than just a garbage chute.
Nagy P, Varga Á, Kovács AL, Takáts S, Juhász G.
Methods. 2015 Mar;75:151-61. doi: 10.1016/j.ymeth.2014.11.016. Epub 2014 Dec 3.
PMID: 25481477

13. We showed that the loss of HOPS leads to decreased TOR activity and increases autophagosome formation. Since HOPS is required for autophagoome clearance, this causes the accumulation of much more vesicles than in the case of Syntaxin 17 mutation. This paper agrees well with the first, theoretical paper that was published in this OTKA project (please see above). Citation:

Loss of Drosophila Vps16A enhances autophagosome formation through reduced Tor activity.
Takáts S, Varga Á, Pircs K, Juhász G.
Autophagy. 2015;11(8):1209-15. doi: 10.1080/15548627.2015.1059559.
PMID: 26061715

These results represent a major step forward in the understanding of the molecular mechanisms of autophagy. Its relevance is well illustrated by the role of autophagy (the breakdown and recycling of intracellular self-material via self-degradation) in various human diseases, including cancer, aging, infections and neurodegeneration.

Budapest, 1/30/2016.

Gabor Juhasz
Principal Investigator