2012 Senri Life Science International Symposium on "Cutting-edge of Autophagy Study"

Proceedings

Date : January 20th (Friday), 2012 9:30-17:10

Venue : Senri Life Science Center Building 5th floor
"Life Hall" & "Science Hall"

Sponsored by Senri Life Science Foundation

The front and rear cover:

“The Tower of the Sun”

<The symbol of the Expo ’70 Commemorative Park >

The famous Expo’70 Commemorative Park is located in the vicinity of the venue of our symposium, the Senri Life Science Center Building. Built on the former grounds of the Japan World Exposition held in 1970, the park encompasses 260 hectares of land full of trees and flowers, and now accommodates many facilities appropriate for various cultural and sports activities. One of the most popular facilities is the Japanese garden, which was created in various styles from different eras in the Japanese history using supreme landscaping skills and techniques from all over Japan. Another notable place to visit is the National Museum of Ethnology, which houses a rich collection of ethnological materials from around the world. The two fundamental concepts of this park are “Co-existence of Humans and Nature” and “Interaction among People.”

The monument featured on the front of this brochure is called the Tower of the Sun. It was designed by late Taro Okamoto, a Japanese prominent artist (1911-1996), as a symbol of Expo ‘70. The tower has three different faces; the “Golden Mask” on the top of the tower indicates the future, the “Face of the Sun” at the front shows the present, and the “Black Sun” at the back represents the past. Last year is the centennial anniversary of the birth of Taro Okamoto. Among the existing works of art that have been created during the last fifty years, the Tower of the Sun is certainly one of those that are most known and loved by the Japanese people.

Courtesy of Commemorative Organization for the Japan World Exposition ‘70

2012 Senri Life Science International Symposium

"Cutting-edge of Autophagy Study"

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Keiji Tanaka (Tokyo Metropolitan Institute of Medical Science)

Opening Address

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Honors & Awards:

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1983 Osaka Science Prize

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1991 Foreign Associate, The US National Academy of Science

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Introduction to “Autophagy World”

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Honors & Awards:

Fujiwara Award, 2005; The Japan Academy Prize, 2006;

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Abstract

Autophagy is a bulk degradation system of cellular constituents which is well conserved in eukaryotes. Since identification of genes autophagy has attracted a good deal of attention for biology and medical researchers and now become one of the most attractive fields of biology. Protein turnover via autophagy is so fundamental function of eukaryotic cells, now almost every day important findings appear, showing autophagy is relevant to many physiological events and diseases. However, there are many questions remained to answer to understand induction, membrane dynamics and physiological roles at a molecular level. This symposium aims to introduce and discuss about recent progresses in autophagy by leading scientists of this field from Japan and abroad.

“New approaches toward the elucidation of molecular mechanisms of autophagy”

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2009-2011	Assistant Professor, Tokyo Institute of Technology
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Honors & Awards:

2003	Inoue Research Award for Young Scientists

Abstract

Autophagy involves the formation of double membrane-bound vesicles called autophagosomes that selectively or non-selectively engulf cytoplasmic constituents including organelles to deliver them into lytic compartments such as lysosomes or vacuoles for degradation. In this process, small flattened vesicles are formed in response to autophagy-inducing signals, and they grow while curving and finally close to become autophagosomes. We have studied molecular mechanisms underlying this unique and dynamic membrane biogenesis using the budding yeast *Saccharomyces cerevisiae* as a model organism (1).

Autophagosome formation requires a specific set of proteins called Atg (autophagy-related). Previous studies revealed that these proteins organize an assembly called the pre-autophagosomal structure in the vicinity of the vacuole and cooperatively act to form the autophagosome. However, how these proteins mediate membrane formation is still enigmatic. In addition, while the whole process of autophagosome formation is roughly illustrated as described above, questions about more detailed processes, including how early small vesicles are generated, how they grow, and what is the source(s) of the membrane, remain unanswered. Moreover, recent studies have revealed that some proteins that function in other cellular activities are also involved in autophagy, implying that we do not yet have a complete set of cellular systems to execute autophagy. To address these critical issues, we have taken different approaches. First, we have employed in vitro reconstitution systems with purified components, which provided us with important information on two ubiquitin-like conjugation systems composed of Atg proteins, including the mechanisms of the conjugation reactions and the functions of the ubiquitin-like protein conjugates (2). Secondly, our recent biochemical approaches identified a novel membrane structure thought to be a precursor of the autophagosomal membrane. Further morphological and biochemical analyses of this structure will dissect an early stage of autophagosome biogenesis. Finally, whereas previous studies identified *ATG* genes among genes not essential for yeast growth, we have conducted a new screening and identified a number of essential genes suggested to be involved in autophagy. In this symposium, I will present results of these approaches and would also like to discuss issues remained to be addressed.

References

(1) Nakatogawa, H. et al. (2009) Nat. Rev. Mol. Cell Biol., 10, 458-467.

(2) Nakatogawa, H. et al. (2007) Cell, 130, 165-178.

“Insights into Membrane Dynamics in Selective and Non-Selective Autophagy”

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2006-2010	Full Professor in Department of Cellular Regulation, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
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Abstract

We identified Atg14L as a component of Class III phosphatidylinositol 3-kinase (PI3-kinase) complex and demonstrated that it positively regulates autophagosome formation. Furthermore, we found that Atg14L localizes to the endoplasmic reticulum (ER) as well as autophagosome. A point mutation in Atg14L resulting in defective ER localization was also defective in autophagosome formation. The addition of the ER-targeting motif of another ER protein to this mutant fully complemented the autophagic defect. Based on these results, we suggest that Atg14L recruits a subset of class III PI3-kinase to the ER and the Atg14L-dependent appearance of PI3P there makes part of the organelle into the platform for autophagosome formation. On the other hands, electron tomography revealed that a subdomain of the ER forms a cradle encircling an immature autophagosome and the membranes were interconnected. Our data provides a clue for the longstanding question where and how autophagosome membrane generates. I will also discuss about the relationship between the ER and mitochondria, which is another candidate of the autophagosome generator.

In addition, we have investigated formation of autophagosome against invading *Salmonella*, which is quite selective in contrast to non-selectivity in starvation-induced canonical autophagy. The membrane formation requires Atg9L1. Atg9L1 and FIP200 are important for autophagy-specific recruitment of the PI3-kinase complex. In the absence of Atg9L1, FIP200, and PI3-kinase activity, LC3 is still recruited near Salmonella. We propose a novel model in which the mechanism of LC3 recruitment is separate from isolation membrane generation. Finally, I will show some data supporting our current hypothesis about how cells selectively sequester the targets by autophagosomes.

References

1. Matsunaga K, et al. Two Beclin-1 binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat Cell Biol. 11, 385-96 (2009)
2. Matsunaga K, et al. Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. J Cell Biol. 190, 511-21. (2010)
3. Hayashi-Nishino M, et al. A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. Nat Cell Biol. 11, 1433-7 (2009)
4. Kageyama S, et al. The LC3 recruitment mechanism is separate from Atg9L1-dependent membrane formation in the autophagic response against *Salmonella*. Mol Biol Cell. 22, 2290-2300. (2011)

Memo:

“Selective autophagy mediated by autophagic adapter proteins”

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1990-1991: Associate Professor, Department of Biotechnology, Institute of Medical Biology, University of Tromsø.

1991-: Professor, Biochemistry Department, Institute of Medical Biology, University of Tromsø

Honors & Awards:

1985-1987: Pre-Doctoral Fellow grant from the Norwegian Research Council.

1987-1988: Graduate student scholarship University of Tromsø.

1988-1990: Post-Doctoral Fellowship grant from the Norwegian Research Council.

2000-2007: Investigator of the Top Research Programme, Norwegian Research Council

2010: Innovation prize from TTO Nord, University of Tromsø

Abstract

The process of macroautophagy (hereafter autophagy), where cytosolic components including organelles, intracellular viruses and bacteria are degraded in the lysosome following encapsulation by a double membrane to form an autophagosome, has been studied since the early sixties. Autophagy was long considered a bulk degradation process with little or no selectivity. However, observation of selective removal of damaged mitochondria and peroxisomes by autophagy suggested that there should be selective mechanisms involved too. The Cytoplasm-to-Vacuole targeting pathway in yeast also hinted strongly to such a selectivity. Studying the signalling adaptor p62/SQSTM1 we found that this protein was involved in forming cytosolic punctate structures we called p62 bodies and that these bodies became degraded by autophagy. Subsequently, we could show that a short sequence motif we named LIR (LC3-Interacting Region) mediated a direct interaction between p62 and the LC3B protein associated with the autophagosomal membrane. This was confirmed by structural studied performed by Masaaki Komatsu and co-workers. We found that the p62 bodies was identical to the so-called ALIS (Aggresome-Like Inducible Structures) first identified in dendritic cells and called DALIS (D=dendritic ALIS). These structures contain ubiquitin and misfolded, aggregated proteins and it was reasonable to assume that p62 would be recruited to such structures because p62 contains a ubiquitin-binding UBA domain. In cell lines and animal models, a clear correlation is seen between inhibition of autophagy and accumulation of ubiquitinated proteins in p62-containing inclusion bodies. p62 has been found in ubiquitinated protein inclusions in all major neurodegenerative diseases and in liver diseases such as Mallory bodies of alcoholic and nonalcoholic steatohepatitis. The presence of p62 in all these types of protein inclusions suggest that p62 could contribute to their clearance by autophagy.We therefore proposed that p62 could act as a cargo receptor for the degradation of ubiquitinated substrates/targets by autophagy. It is also possible that p62 may bind directly to another protein and cause its degradation by autophagy without the presence of any Ub modification. Confirming our hypothesis of p62 as a cargo receptor for autophagic degradation of ubiquitinated targes, p62 has recently been shown to be involved in selective autophagy of protein aggregates, soluble proteins, midbody rings, damaged mitochondria, peroxisomes, intracellular bacteria, phagocytic membrane remnants, bacteriocidal precursor- and viral capsid proteins.

In a collaborative effort, involving several other groups, we found that NBR1, a protein with a similar domain architecture as p62, was also a selective autophagy substrate that could act as cargo receptor for degradation of ubiquitinated protein aggregates by autophagy. Looking into the evolution of p62 and NBR1 we found that homologs of NBR1 are distributed throughout the eukaryotic kingdom, while p62 is confined to the metazoans. Plant NBR1 is more similar to mammalian NBR1 than to p62 in domain architecture and amino acid sequence but has hybrid properties of mammalian NBR1 and p62. Likely, NBR1 is involved in targeting for selective autophagy many of the same substrates as p62. Based on our studies of p62 we have developed several tools to study autophagic degradation of p62 and LC3 including a tandem tag strategy employing acid-stable mCherry and acid-labile EGFP.

p62 is not only a cytosolic proteins but undergoes a fast nucleo-cytoplasmic shuttling. We have mapped a nuclear localization signal (NLS) and a nuclear export signal (NES) using mutagenesis and live cell imaging. p62 accumulates in the nucleus in response to aggregation of proteins as

exemplified using a nuclear mutant of Ataxin-1. We suggest this may be linked to a role of p62 in mediating degradation of misfolded proteins (as soluble forms) via the proteasome connected to PML bodies.

Many groups have contributed to show that p62 is a major stress response protein. With Japanese researchers in the forefront, several groups, including ours, showed that p62 binds to KEAP1 to regulate the oxidative response regulating transcription factor Nrf2. We could additionally show that p62 is involved in a positive feedback loop here because Nrf2 is regulating the expression of p62 by binding to its upstream region inducing its transcription.

Following our discovery of the LIR motifs in p62 and NBR1, we and other research groups have identified several other human proteins that bind to ATG8 family proteins using a LIR motif. One of these proteins is FYCO1 which we discovered is involved in microtubule +-end directed transport of autophagosomes, late endosomes and lysosomes via its interaction with LC3, PI3P and Rab7. Currently, we have found that members of the Ulk1 kinase complex, regulating autophagy, interact with ATG8 family proteins via LIR motifs. A summary of our groups published findings as well as recent data on LIR-interactions in the Ulk1 complex will be presented.

References

1. **Bjørkøy, G., Lamark, T., Brech, A., Outzen, H., Øvervatn, A., Stenmark, H. and Johansen, T.** 2005. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. ***Journal of Cell Biology,* 171,** 603-614.
2. **Pankiv, S., Høyvarde Clausen, T., Lamark, T., Brech, A., Bruun, J-A., Outzen, H., Øvervatn, A., Bjørkøy, G. and Johansen, T.** 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. ***Journal of Biological Chemistry,* 282,** 24131-24145.
3. **Bjørkøy, G., Lamark, T., Pankiv, S., Brech, A. and Johansen, T.** 2009. Monitoring autophagic degradation of p62/SQSTM1. ***Methods of Enzymol.* 452,** 181-197.
4. **Kirkin, V., Lamark, T., Sou, Y.S., Bjørkøy, G., Nunn, J.L., Bruun J-A., Shvets, E., McEwan, D.G., Clausen, T.H., Wild, P., Bilusic, I., Theurillat, J.P., Øvervatn, A., Ishii, T., Elazar, Z., Komatsu, M., Dikic, I. and Johansen, T.** 2009. A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. ***Mol. Cell* 33,** 505-516.
5. **Zheng, Y.T., Shahnazari, S., Brech, A., Lamark, T., Johansen, T. and John H. Brumell.** 2009. The adaptor protein p62/SQSTM1 targets invading bacteria to the autophagy pathway. ***Journal of Immunology,* 183,** 5909-5916.
6. **Pankiv, S., Lamark, T, Bruun, J.-A., Øvervatn, A., Bjørkøy, G. and Johansen, T.** 2010. Nucleocytoplasmic shuttling of p62/SQSTM1 and its role in recruitment of nuclear polyubiquitinated proteins to PML bodies. ***Journal of Biological Chemistry,* 285,** 5941-5953.
7. **Pankiv, S., Alemu, E. A., Brech, A., Bruun, J.-A., Lamark, T, Øvervatn, A., Bjørkøy, G. and Johansen, T.** 2010. FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus

end-directed vesicle transport. ***Journal of Cell Biology,* 188,** 253-269.

8. **Høyvarde Clausen, T., Lamark, T., Isakson, P., Finley, K., Bowitz Larsen, K., Brech, A., Øvervatn, A., Stenmark, H., Bjørkøy, G., Simonsen, A. and Johansen, T.** 2010. p62/SQSTM1 and ALFY interact to facilitate the formation of p62 bodies/ALIS and their degradation by autophagy. ***Autophagy,* 6,** 330-344.
9. **Jain, A., Lamark, T., Sjøttem, E., Bowitz Larsen, K., Awuh, J.A., Øvervatn, A., McMahon, M., Hayes, J.D. and Johansen, T.** 2010. *p62/SQSTM1* is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. ***Journal of Biological Chemistry,* 285,** 22576–22591
10. **Johansen, T. and Lamark, T.** 2011. Selective autophagy mediated by autophagic adapter proteins. ***Autophagy*, 7,** 279-296.
11. **Svenning, S., Lamark, T., Krause, K. and Johansen, T.** 2011. Plant NBR1 is a selective autophagy substrate and a functional hybrid of the mammalian autophagic adapters NBR1 and p62/SQSTM1. ***Autophagy*, 7,** 993-1010

“The mechanism of secretory autophagosomes mediated unconventional protein secretion”

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Awards and Honors

1982-1985	Pirie-Reid Scholar, Oxford University.
1985-1987	Damon-Runyon Walter-Winchell Post-doctoral Fellow.
1988-1990	American Cancer Society Senior Post-doctoral Fellow.
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1995-2000	Established Investigator of the American Heart Association.
2005	Elected Fellow AAAS.
2009	Elected Member EMBO.
2011-2016	ERC advanced grant.

Abstract

The unconventional secretion of the Acyl-CoA binding protein of *S. cerevisiae*, Acb1, requires proteins involved in the formation of autophagosomes, in the fusion of membranes with the endosomes, proteins of the multivesicular body pathway, the cell surface t-SNARE Sso1 and the GRASP ortholog Grh1 (Kinseth et al, 2007, Duran et al, 2010). Our new findings reveal that upon nutrient starvation, Grh1 concentrates in a Phosphatidylinositol 3 phosphate (PI3P)-kinase dependent manner to unique membranes near the Sec13 containing ER exit sites. Furthermore, these membranes are enriched in PI3P and contain the ESCRT protein Vps23, and the autophagy-related gene products Atg8 and Atg9 thereby offering commonality between the different proteins required for secretion of Acb1. Electron microscopy revealed that these membranes are CUP shaped and we have therefore dubbed them CUPS for *C*ompartment for *U*nconventional *P*rotein *S*ecretion. The biogenesis of CUPS is starvation specific but independent of the Rapamycin induced autophagy. A number of genes are involved in the biogenesis of CUPs including Grh1, Bug1, Vps34, and the ESCRT-II and III proteins Vsp25, 36, 20, and 2. Based on our findings we believe that CUPS serve as a station for the biogenesis of autophagosomes that contain Acb1. These secretory autophagosomes do not fuse with the vacuole and instead, in an Sso1 dependent reaction, fuse with the cell surface to release Acb1 into the extracellular space. We suggest that secretion of the cytokine IL-1ß and many other signal sequence lacking proteins is likely mediated by a similar process involving secretory autophagosomes. I will present our new findings on the biogenesis of CUPS and downstream events involved in Acb1 secretion.

References

1. Kinseth, M.A., Anjard, C., Fuller, D., Guizzunti, G., Loomis, W.F., and **Malhotra, V**. The Golgi-associated protein GRASP is required for unconventional protein secretion during development. **Cell** 130: 524-534 (2007).
2. Bossard, C., Bresson, D., Polishchuk, R.S., and **Malhotra, V**. Dimeric PKD regulates membrane fission to form transport carriers at the TGN. **J. Cell Biol**. 179: 1123-1131 (2007).
3. Tsang, W., Bossard, C., **Malhotra, V** and Dynlacht, B. CP110 suppresses primary cilia formation through its interaction with CEP290, a protein deficient in human ciliary disease. **Dev. Cell**. 16: 187-197 (2008).
4. Saito, K., Chen, M., Bard, F., Chen, S., Zhou, H., Woodley, D., Polischuk, R., Schekman, R., and **Malhotra. V**. TANGO1 facilitates cargo loading at Endoplasmic reticulum exit sites. **Cell** 136: 891-902 (2009).

5. Von Blume, J., Duran, J.M., Forlanelli, E., Alleaume, A.M., Egorov, M., Polischuk, R., Molina, H and **Malhotra, V**. Actin remodeling by ADF/Cofilin is required for cargo sorting at the Trans Golgi Network. **J. Cell Biol**. 187:1055-1069 (2009).
6. Duran, J.M., Anjard, C., Stefan, C, Loomis, W.F., and **Malhotra, V**. Unconventional secretion of Acb1 is mediated by autophagosome. **J. Cell Biol**. 188: 527-536 (2010).
7. Pusapati, G.V., Krndija. D., Armacki, M., vonWickert. G., vonBlume, J., **Malhotra, V**., Adler, G., and Seufferlein, T. Role of the second cysteine-rich domain and Pro 275 in PKD2 interaction with ARF1, TGN recruitment and protein transport. **Mol. Biol. Cell**.21: 1011-1022 (2010).
8. Cabral, M., Anjard, C., **Malhotra, V**., Loomis, W., Kuspa, A. Unconventional Secretion of AcbA in *Dictyostelium discoideum* through a vesicular intermediate. **Euk. Cell**. 9.1009-1017 (2010).
9. Von Blume, J., Alleaume, A.M., Recasens, G.C., Valverde, M.A, and **Malhotra, V**. ADF/Cofilin regulates secretory cargo sorting at the TGN via the Ca2+ ATPase SPCA1. **Dev. Cell**. 20: 652-662. 2011.
10. Saito, k., Yamashiro, k., Ichikawa, Y., Erlmann, P., Kontani, K., **Malhotra, V** and Katada. T. cTAGE mediates collagen secretion through interaction with TANGO1 at Endoplasmic reticulum exit sites. **Mol. Biol. Cell** 22: 2301-2308. (2011).
11. **Malhotra, V** and Erlmann, P. Protein export at the ER: TANGO1 helps COPII carriers grow in size. **EMBO. J**. in press (2011).

Memo:

“Parkinson’s disease (PD) and autophagy: two familial PD gene products, PINK1 and Parkin, cooperate to identify, label and clear damaged mitochondria”

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Awards and Honors

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2009 Best Poster Presentation Award, Annual Internal Meeting of Tokyo Metropolitan Institute of Medical Science

Abstract

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder, and thus the understanding of its pathogenic mechanism(s) is important. The majority of PD cases are sporadic; however, the discovery of genes linked to rare familial forms of the disease has provided crucial insight into the molecular mechanisms of disease pathogenesis. In 2000, our laboratory found that Parkin, whose dysfunction causes autosomal recessive juvenile Parkinsonism (AR-JP), is an E3 ubiquitin-ligase (1). Since then, many papers have been published, but the pathogenic mechanisms of AR-JP caused by loss-of-function of Parkin have largely remained obscure.

PINK1 (*PARK6*) is another causal gene of early-onset Parkinson's disease. PINK1 is a mitochondrial kinase that keeps the integrity of the mitochondria (2). Interestingly, in 2006, three groups of investigators reported the tight relationship between PNK1 and Parkin (3-5). By the genetic study using *Drosophila* system, they found that PINK1 and Parkin function in the same pathway, with PINK1 functioning upstream of Parkin. However, the exact mechanism involved in regulation of Parkin by PINK1 as the upstream factor remained elusive. Furthermore, the functional interplay between Parkin-catalyzed ubiquitylation and PINK1-regulated mitochondrial quality control was completely missing.

In 2008, Youle and colleagues in NIH reported that Parkin is selectively recruited to damaged mitochondria with low membrane potential ($\Delta\Psi m$), and leads it to degradation by selective autophagy (alias mitophagy) (6). We soon confirmed the reproducibility of their results. Moreover we revealed that PINK1 is the Parkin recruitment factor that retrieves Parkin from the cytoplasm to the mitochondria with low $\Delta\Psi m$. PINK1 itself is localized on mitochondria upon decrease of $\Delta\Psi m$ because PINK1 is rapidly and constitutively degraded under steady-state conditions in a mitochondrial $\Delta\Psi m$-dependent manner and that a loss in mitochondrial $\Delta\Psi m$ stabilizes PINK1 mitochondrial accumulation. Interestingly, the ubiquitin-ligase activity of Parkin is usually repressed in the cytoplasm; however, PINK1-dependent mitochondrial localization liberates the latent enzymatic activity of Parkin. Some pathogenic mutations of PINK1 and Parkin interfere with this phenomenon, demonstrating the etiological importance (7). Our conclusion is depicted in Fig. 1, and several groups of investigators independently reached the same conclusion (8-10).

The essence of the aforementioned model is that PINK1 monitors mitochondrial quality via its escape from $\Delta\Psi m$-dependent degradation. However, homeostatic controlling mechanisms of PINK1 in cell remain obscure. Recently we realized that PINK1 is autophosphorylated upon decrease of $\Delta\Psi m$ and most disease-relevant mutations hinder this event. Importantly, the mitochondrial localization of

PINK1 is necessary for the discrimination of damaged mitochondria but not sufficient to fulfill downstream events such as Parkin recruitment onto mitochondria. Indeed, low-accumulation of PINK1 on the outer membrane cannot retrieve cytosolic Parkin, and PINK1 activation coincided with its autophosphorylation is required for optimized mitochondrial localization of Parkin. We propose that the integrity of mitochondria is sensed and transduced by two distinct mechanisms: one is the escape from ΔΨm-dependent degradation of PINK1, and the other is the functional activation of PINK1 kinase (11).

References

(1) Shimura *et al*., (2000) Nat. Genet 25, 302.

(2) Valente *et al.*, (2004) Science 304, 1158

(3) Park *et al*., (2006) Nature 441, 1157.

(4) Clark *et al*., (2006) Nature 441, 1162.

(5) Yang *et al.*, (2006) Proc Natl Acad Sci USA 103, 10793.

(6) Narendra *et al*., (2008) J. Cell Biol. 183, 795.

(7) Matsuda *et al.*, (2010) J. Cell Biol. 189, 211.

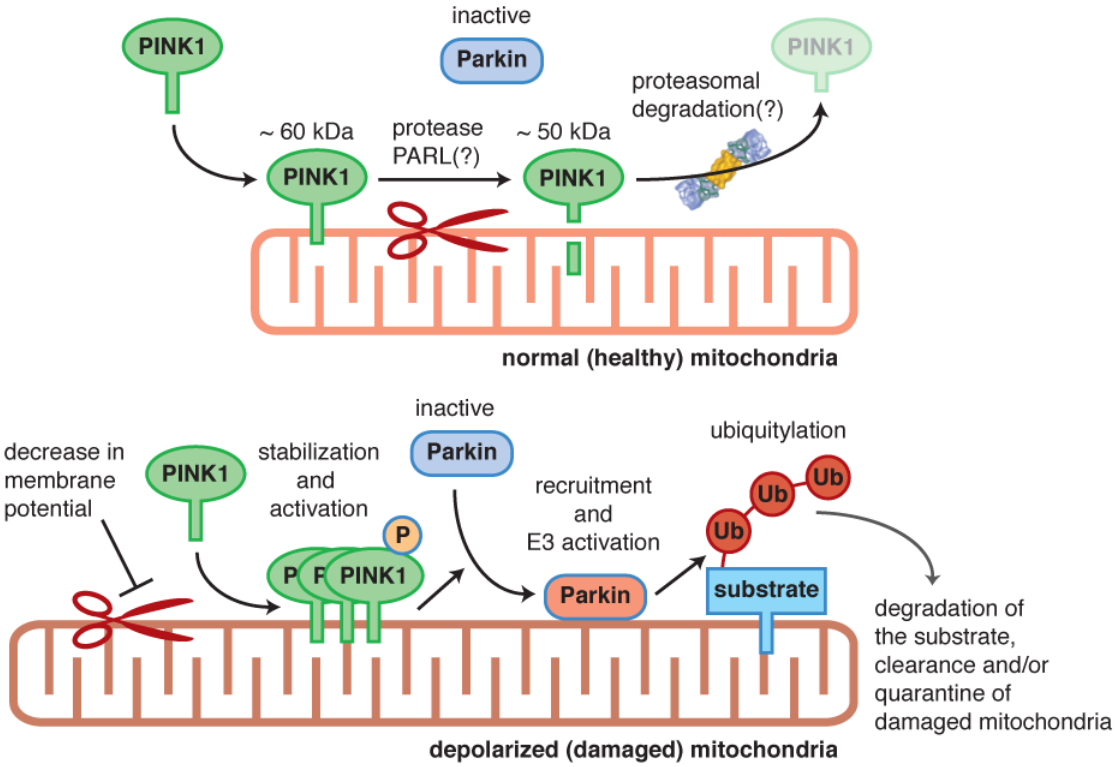
(8) Vives-Bauza *et al.*, (2010) Proc Natl Acad Sci USA 107, 378

(9) Geisler *et al.*, (2010) Nat Cell Biol. 12, 119

(10) Narendra *et al*., (2010) PLoS Biol. 26, e1000298

(11) Okatsu *et al.*, (2011) manuscript in preparation

Matsuda Figure 1, Model of mitochondrial quality control mediated by PINK1 and Parkin



“Neuronal autophagy and its therapeutic potential for neurodegenerative disease”

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Educational History:

1982–1986	B.S.	Wheaton College, Wheaton, IL
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Research and professional experience

1987–1991	Research Assistant, Yale University, New Haven, CT
1989	Teaching Assistant, Hopkins Marine Station, Stanford University, Stanford, CA
1991–1992	Intern, Department of Internal Medicine, University of California, San Francisco, CA
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1995–1998	Research Fellow and Clinical Instructor, Department of Neurology, Children’s Hospital, Harvard Medical School, Boston, MA
1998	Instructor, Department of Molecular Neurobiology, Woods Hole Marine Biology Laboratory, Woods Hole, MA
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1999–2005	Assistant Professor, Departments of Neurology and Physiology, UCSF, San Francisco, CA
1999–2005	Assistant Investigator, Gladstone Institute of Neurological Disease, San Francisco, CA
2005–2008	Associate Professor, Departments of Neurology and Physiology, UCSF, San Francisco, CA
2005–2008	Associate Investigator, Gladstone Institute of Neurological Disease, San Francisco, CA
2006–	Associate Director, Gladstone Institute of Neurological Disease, San Francisco, CA
2009–	Professor, Departments of Neurology and Physiology, Graduate Program in Biological Sciences, Graduate Program in Neuroscience, Graduate Program in Biomedical Sciences, Medical Scientist Training Program, University of California, San Francisco, CA
2009–	Senior Investigator, Gladstone Institute of Neurological Disease, San Francisco, CA

2009–	Director, Taube-Koret Center for Huntington's Disease Research, San Francisco, CA
2011–	Director, Hellman Family Foundation Program in Alzheimer's Disease Research, San Francisco, CA
2011–	Associate Editor, Autophagy
2011–	Editorial board, Journal of Huntington's Disease.

Awards and Honors

Medical Scientist Training Program Fellowship, Yale University (1988); AΩA Honor Medical Society, Yale University (1991); The William U. Gardner Prize for an outstanding doctoral thesis, Yale University (1991); The Medical Scientist Training Program Prize, Yale University (1991); Medical Licensure, California (1991); Medical Licensure, Massachusetts (1995); NINDS Clinical Investigator Development Award (1995); NICHD MRRC New Investigator Award (1996); Children's Hospital Research Scholars Award (1997); Harcourt General Medical Foundation, New Investigator Award (1998); Member, American Society for Cell Biology (1989–); Member, Society for Neuroscience (1990–); Member, Biophysical Society (1990–); Charles E. Culpeper Medical Scholar Award (1999); W. M. Keck Distinguished Medical Scholar Award (semi-finalist) (2001); Klingenstein Award in Neurosciences (2001); NIH Study Section Member for MDCN 2/NBDG (2001–08); Huntington's Disease Society of America Therapeutics Initiative Award (2003); Lieberman Award for work on Huntington's Disease (2005); Elected Member, American Neurological Association (2005–); Member, Hereditary Disease Foundation Board (2005–); Taube Award for Outstanding work on Huntington's disease (2006); Thompson "Hot Paper" (http://www.in-cites.com/hotpapers/2006/november06-neu.html) (2006); Editorial Board Member, Molecular Brain (2007); UCSF Award for Outstanding Faculty Mentorship (2008); Huntington's Disease Society of America Distinguished Leadership Award (2008); Huntington's Disease Society of America Coalition for the Cure Team Member (2010); Grant Reviewer for the ALS Association (2011); Award for Outstanding Research Achievement, *Nature Biotechnology* (2011); *Autophagy,* Associate Editor (2011).

Abstract

Work from our laboratory has led to the discovery that deficits in protein homeostasis may be a common thread in a variety of neurodegenerative diseases. Using automated microscopy and longitudinal single cell analysis, data from neuron models of Parkinson's disease, Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) have been used to construct dynamic predictive models of neurodegeneration and they indicate that stimulating endogenous protein clearance pathways, such as autophagy, could be a helpful therapeutic strategy. To pursue this strategy, we conducted a screen that led to the discovery of small molecules that stimulate autophagy in primary neurons from rodents and in human neurons differentiated from induced pluripotent stem cells. These inducers were subsequently shown to have beneficial effects in primary neuron models of HD and ALS including the promotion of cell survival, the reduction in levels of the disease-causing protein, and a reduction in abnormal intracellular protein deposits called inclusions. These findings further suggest that induction of autophagy could be a therapeutic strategy for neurodegenerative diseases.

Selected Peer-reviewed Publications (From over 60)

1. Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. (1990) Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science* 247:470–473.
2. Saudou F*, Finkbeiner SM*, Devys D, Greenberg ME. (1998) Huntingtin acts in the nucleus to induce apoptosis, but death does not correlate with the formation of intranuclear inclusions. *Cell* 95:55–66. (*Authors contributed equally to this work.)
3. †Arrasate M, Mitra S, Schweitzer E, Segal M, Finkbeiner S. (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431:805–810.
4. Arrasate M, Finkbeiner S. (2005) Automated microscope system for determining factors that predict neuronal fate. *Proc. Natl. Acad. Sci. U.S.A.*102:3840–3845.
5. Mitra S, Tsvetkov A, Finkbeiner S. (2009) Single neuron ubiquitin-proteasome dynamics accompanying inclusion body formation in Huntington's disease. *J. Biol. Chem.* 284:4398–4403. PMCID: PMC2640959
6. Tsvetkov AS, Mitra S, Finkbeiner S. (2009) Protein turnover differences between neurons and other cells. *Autophagy* 5:1–3. PMCID: PMC2892253
7. Daub A, Sharma P, Finkbeiner S. (2009) High-content screening of primary neurons: Ready for prime time. *Curr. Opin. Neurobiol.* 19:537–543. PMCID: PMC2787795
8. Thompson LM, Aiken CT, Agrawal N, Kaltenbach LS, Illes K, Khoshnan A, Martinez-Vincente M,

Arrasate M, O'Rourke JG, Lukacsovich T, Zhu Y-Z, Lau AL, Massey A, Hayden MR, Zeitlin SO, Finkbeiner S, Huang L, Lo DC, Patterson PH, Cuervo AM, Marsh JL, and Steffan JS. (2009) IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. *J. Cell Bio.* 187:1083–1099. PMCID: PMC2806289

9. Barmada SJ, Skibinski G, Korb E, Rao E, Wu JY, Finkbeiner SM. (2010) Cytoplasmic mislocalization of TDP-43 is toxic to neurons and enhanced by a mutation associated with familial amyotrophic lateral sclerosis. *J. Neurosci.* 13:639–649. PMCID: PMC2821110
10. Vossel KA, Zhang K, Brodbeck J, Daub AC, Sharma P, Finkbeiner S, Cui B, Mucke L. (2010) Tau reduction ameliorates Ab-induced impairments in axonal transport. Science 330:198.
11. Finkbeiner S. Bridging the Valley of Death of therapeutics for neurodegeneration. (2010) Nat. Med. 16:1227–1232.
12. Tsvetkov AS, Miller J, Arrasate M, Wong JS, Pleiss MA, Finkbeiner S. (2010) A small-molecule scaffold induces autophagy in primary neurons and protects against toxicity in a Huntington's disease model. *Proc. Natl. Acad. Sci. U.S.A.* 107:16982–16987. PMCID: PMC2947884
13. Miller J, Arrasate M, Mitra S, Masliah E, Finkbeiner S. (2010) Quantitative relationships between huntingtin levels, polyglutamine length, inclusion body formation, and neuronal death provide novel insight into Huntington's disease molecular pathogenesis. *J. Neurosci.* 30:10541–10550.
14. Skibinski G, Finkbeiner, S. Drug discovery in Parkinson's disease—Updates and developments in the use of cellular models. Int. J. of High Throughput Screening, 2:15–25, 2011.
15. Wray S, Self M, Lewis PA, Taanman J-W, Ryan N, Mahoney C, Devine MJ, Sheerin UM, Houlden H, Morris H, Healy D, Marti-Masso J-F, Sutherland M, Shapira AJ, Uitti RJ, Guttman M, Opala G, Jasinska-Myga B, Puschmann A, Nilsson C, Guttmann L, Boeve B, Boylan K, Stoessl J, Gerpen JV, Gerstenhaber M, Gwinn K, Dawson T, Isacson O, Marder K, Przedborski S, Finkbeiner S, Rothstein JD, Wszolek Z, Rossor M, Hardy J. (2011) Creation of an open-access mutation-defined fibroblast resource for neurological disease research. PLoS One, *in the press*.
16. Klionsky DJ, Baehrecke EH, Brumell JH, Chu CT, Codogno P, Cuervo AM, Debnath J, Deretic V, Elazar Z, Eskelinen L, Finkbeiner S, Fueyo-Margareto J, Gewirtz D, Jäättelä M, Kroemer G, Levine B, Melia TA, Mizushima N, Rubinsztein DC, Simonsen A, Thorburn A, Thumm M, Tooze SA. (2011) A comprehensive glossary of autophagy-related molecules and processes. Autophagy, *in the press*.
17. Miller J*, Arrasate M*, Brooks E, Peters-Libeu C, Newhouse Y, Krishnan P, Widjaja, K, Cheung K, Tran T, Curtis J, Hatters D, Legleiter J, Lotz G, Saudou F, Muchowski P, Weisgraber K, Finkbeiner S. (2011) Identifying polyglutamine protein species *in situ* that best predict neurodegeneration. Nat. Chem. Biol., *in press*.
18. Klionsky DJ et al., (2011) Guidelines for the Use and Interpretation of Assays for Monitoring Autophagy in Higher Eukaryotes, *in the press*.

[†]The most cited paper in neuroscience and behavior for the decade in which it was published according to Thompson Scientific. Collectively, work from the Finkbeiner lab has garnered over 6,000 citations.

Memo:

“Autophagy regulation in cells and whole organisms”

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Educational History:

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Research and professional experience

1996-1998	Research fellow (PD) of the Japan Society of the Promotion of Science
1998-1999	Post Doctoral Fellow of National Institute for Basic Biology
1999-2002	PRESTO, Japan Science and Technology Corporation (JST)
2002-2004	Assistant Professor, Department of Cell Biology, National Institute for Basic Biology, Okazaki, Japan
2004-2006	Laboratory Head, Department of Bioregulation and Metabolism, The Tokyo Metropolitan Institute of Medical Science
2006-present	Professor, Department of Physiology and Cell Biology, Tokyo Medical and Dental University

Awards and Honors

2001	Young investigator award of the Japanese Biochemical Society
2005	Mitsubishi Chemical Award of the Molecular Biology Society of Japan
2006	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, The Young Scientists’ Prize
2007	FEBS Letters Young Scientist Award
2008	JSPS (Japan Society for the Promotion of Science) Prize
2008	Brain Science Foundation, Tsukahara Award
2009	Inoue Prize for Science, Inoue Foundation for Science
2010	Japanese Biochemical Society, Kakiuchi Saburo Memorial Award
2011	The Takeda Prize for Medical Science

Abstract

Autophagy is the primary means for the degradation of cytoplasmic constituents in the lysosome. A portion of cytoplasm is sequestered by an autophagosome, and then delivered to the lysosome. Autophagy is important for various physiological processes such as adaptive response to starvation, quality control of intracellular proteins and organelles, tumor suppression, preimplantation development, and immune responses.

Yeast genetic studies have identified autophagy-related genes, many of which are conserved in mammals. We have shown that the ULK1 protein complex is the most upstream unit, which is directly regulated by mTORC1. Downstream of the ULK1 complex, the Atg14/PI3-kinase complex, its effectors (DFCP1 and WIPI-1), the Atg12—Atg5–Atg16L1 complex and LC3 function to form the autophagosome. The punctate structures containing upstream Atg proteins such as ULK1 and Atg14 associate with the ER, where VMP1 and Atg9A also transiently localize. These structures should represent the autophagosome formation site or preautophagosomal structure (PAS) in mammalian cells. Mammalian Atg2A and 2B are also recruited to this site and are essential for a rather late step of autophagosome formation. Interestingly, mammalian Atg2 proteins localize on both autophagic membrane and lipid droplets. Depletion of both Atg2A and Atg2B causes clustering of enlarged lipid droplets in an autophagy-independent manner. These data suggest that mammalian Atg2 proteins function both in autophagosome formation and regulation of lipid droplet morphology and dispersion.

We previously found that the autophagy selective substrate, p62 can localize to the ER-associated autophagosome formation site independently of most Atg proteins, suggesting that p62–LC3 binding is not the only mechanism explaining substrate selectivity. Similarly in Parkin-mediated mitophagy, although it has been proposed that p62–LC3 interaction may mediate the mitochondrial recognition by autophagosome, structures containing upstream Atg proteins including ULK1, Atg14, DFCP1, WIPI-1 and Atg16L1 can associate with depolarized mitochondria independently of LC3. The Atg9A structures are also recruited to these damaged mitochondria in a ULK1-independent manner. In Atg3 KO cells, small autophagosome-like structures, most of which are not completely sealed, are generated in the mitochondrial area, but they fail to enclose damaged mitochondria. These results suggest that (1) the isolation membrane is generated de novo on damaged mitochondria, (2) at least two distinct Atg components, Atg9A and the ULK1 complexes, are independently involved at initial stages of mitophagosome formation, and (3) LC3-mediated recognition is important for incorporation of damaged mitochondria inside autophagosomes.

We have also analyzed how autophagy is regulated in mice in vivo. Although autophagy is differently activated among tissues following food withdrawal, autophagy induction well correlates

with mTOR suppression in each tissue. Insulin seems to have a major role in mTOR activation and autophagy suppression in skeletal muscle of refed mice, but a relatively minor role in the liver. Finally, we found that autophagy is suppressed in denerved muscle through proteasome-dependent mTOR activation. These data suggest that mTOR is a major regulator of autophagy in vivo as well as in vitro.

References

1. Takamura, A., Komatsu, M., Hara, T., Sakamoto, A., Kishi, C., Waguri, S., Eishi, Y., Hino, O., Tanaka, K., Mizushima, N. Autophagy-deficient mice develop multiple liver tumors. ***Genes Dev.*** 25: 795-800 (2011)
2. Itakura, E., Mizushima, N. p62 targeting to the autophagosome formation site requires self-oligomerization but not LC3-binding. ***J. Cell Biol.*** 192: 17-27 (2011).
3. Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S., Natsume, T., Takehana, K., Yamada, N., Guan, J.L., Oshiro, N., Mizushima, N. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. ***Mol. Biol. Cell*** 20: 1981-1991 (2009)
4. Itakura, E., Kishi, C., Inoue, K., Mizushima, N. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. ***Mol. Biol. Cell*** 19: 5360-5372 (2008)
5. Tsukamoto, S., Kuma, A., Murakami, M., Kishi, C., Yamamoto, A., Mizushima, N. Autophagy is essential for preimplantation development of mouse embryos. ***Science*** 321: 117-120 (2008)
6. Hara, T., Takamura, A., Kishi, C., Iemura, S., Natsume, T., Guan, J.L., Mizushima, N. FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. ***J. Cell Biol.*** 181: 497-510 (2008)

Memo:

Closing Remarks

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Honors & Awards:

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