Detection of mitophagy with Keima-Red

MBL
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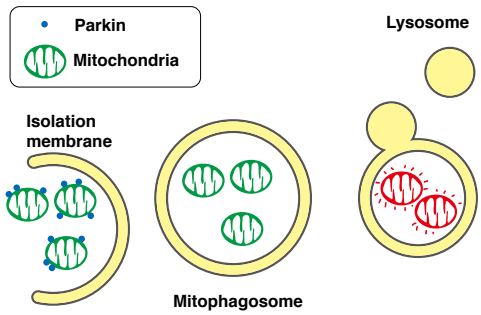
Mitophagy is the selective degradation of old or depolarized mitochondria by autophagy and contributes maintaining a healthy population of mitochondria. Since damaged mitochondria lead to collapse cell homeostasis, mitophagy is believed to protect against diseases related to mitochondrial dysfunction such as neurodegenerative disorders.



Parkin, an ubiquitin ligase known as the gene responsible for Parkinson's disease, plays an important role in autophagic elimination of mitophagy. When mitochondria are depolarized and dysfunctional, PTEN-induced putative kinase protein 1 (PINK1) accumulates on the outer membrane, and recruits Parkin on the damaged mitochondria. The outer membrane on the mitochondria is then ubiquitinated through the ubiquitin ligase activity of Parkin. Finally, the poly-ubiquitinated mitochondria are selectively recognized and executed by autophagic process.

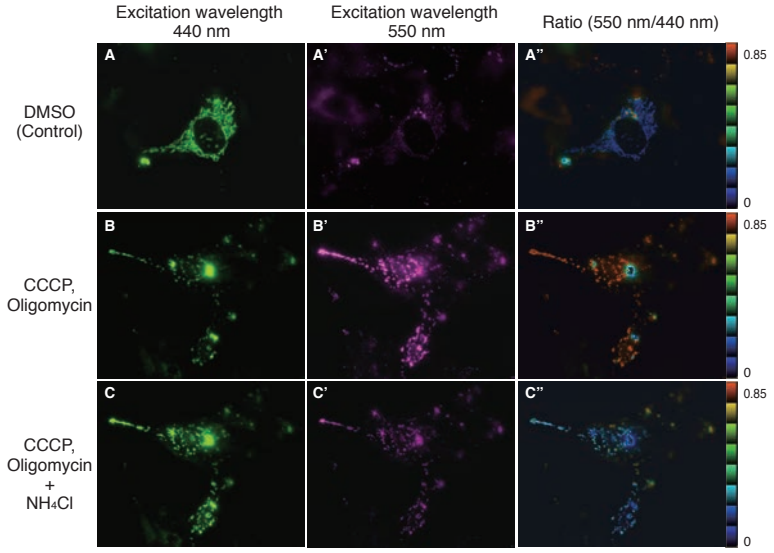
The fluorescent protein Keima has an excitation spectrum that changes according to pH. A short wavelength (440 nm) is predominant for excitation in a neutral environment, whereas a long wavelength (586 nm) is predominant in an acidic environment. When observing a ratio (586 nm/440 nm) image obtained from image data by using these two excitation wavelengths, Keima in a neutral environment has a low ratio value and is displayed in blue. However, in an acidic environment, Keima has a high ratio value and is displayed in red.

When MT-mKeima-Red, which is the Keima tagged with a mitochondria targeting signal peptide sequence, and Parkin is expressed in target cells, mitophagy is detected and visualized through the difference in fluorescent wavelengths observed before and after drug treatment.

Mitophagy is induced by administration of CCCP and oligomycin, drugs that affect mitochondrial membrane potential, and is observed with 550 nm/440 nm ratio images (A", B", C").

Through mitophagy induction, the mitochondria-localized MT-mKeima-Red is displayed in red in ratio images, showing its localization in an acidic environment (B-B"). The neutralizer NH_4Cl is then administered, which forces the entire cell into a neutral environment, and the image returns to blue (C-C").

This result matches findings in which the progression of mitophagy causes mitochondria to be engulfed in lysosomes when in an acidic environment.

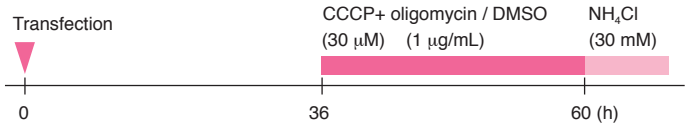


Outline of experiment

Plasmid, transfection

Plasmid1: pMT-mKeima-Red (AM-V0251)
Plasmid2: Mouse Parkin
↓
Co-transfected to mouse MEF cells
Plasmid1 : Plasmid2 = 2:1

Assay timeline



Filter set

440 nm (Ex : 440AF21, Em : 610ALP, DM : 590DRLP)
550 nm (Ex : 550DF30, Em : 610ALP, DM : 590DRLP)

Citations

- Choubey V *et al.* BECN1 is involved in the initiation of mitophagy: It facilitates PARK2 translocation to mitochondria. Autophagy 10, 1105–19 (2014).
- Safiulina D & Kaasik A. Energetic and Dynamic: How Mitochondria Meet Neuronal Energy Demands. PLoS Biol 11, e1001755 (2013).
- Togashi K *et al.* Na^+/H^+ Exchangers Induce Autophagy in Neurons and Inhibit Polyglutamine-Induced Aggregate Formation. PLoS ONE 8, e81313 (2013).
- Narendra DP *et al.* PINK1 rendered temperature sensitive by disease-associated and engineered mutations. Hum Mol Genet. 22, 2572-89 (2013).
- Bingol B *et al.* The mitochondrial deubiquitinase USP30 opposes parkin- mediated mitophagy. Nature 510, 370–5 (2014).

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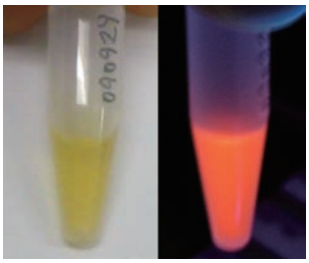
Keima-Red

CoralHue® dimeric Keima-Red (dKeima-Red) and CoralHue® monomeric Keima-Red (mKeima-Red) are red fluorescent proteins with extremely large Stokes shift. They absorb light maximally at 440 nm and emit red light at 616 nm and 620 nm, respectively. There are no other fluorescent proteins with this unique fluorescence. Because of this characteristic, they are excited by a very short wavelength but emit a long wavelength. The large Stokes shift property of Keima-Red allows effective applications such as for single wavelength excitation simultaneous multi-color imaging and single laser line FCCS.

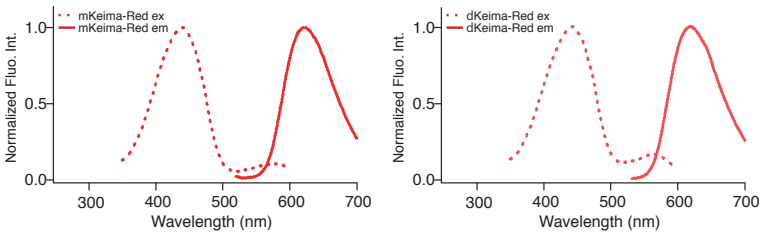
Keima is named after a shogi (Japanese chess) piece that can move in the hopping manner, similar to the knight in the game of chess.



Recombinant Keima-Red
Visible light (left), UV (right)

CHARACTERISTIC	dKeima-Red	mKeima-Red
Oligomerization	Dimer	Monomer
Number of amino acid	222	222
Excit./Emiss. Maxima (nm)	440/616	440/620
Molar Extinction Coefficient ($M^{-1}cm^{-1}$)	24,600 (440 nm)	14,400 (440 nm)
Fluorescence Quantum Yield	0.31	0.24
Brightness *1	7.6	3.5
pH sensitivity	p*Ka*=6.5	p*Ka*=6.5
Cytotoxicity *2	No	No

*1 Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000
*2 Toxicity when expressed in HeLa cells

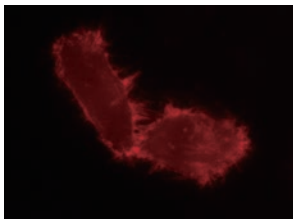


Excitation and emission spectra

Organelle targeting vectors (Keima-Red)

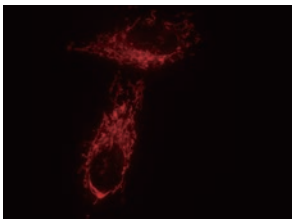
The vectors encode fusions of Keima-Red and localization signals, and allow visualization of subcellular structures in living cells.

Plasma membrane



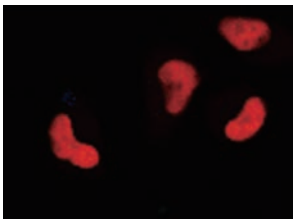
CoralHue® pPM-mKeima-Red

Mitochondria



CoralHue® pMT-mKeima-Red

Nucleoplasm



CoralHue® pNP-hdKeima-Red

CoralHue® Keima-Red vectors

CoralHue® fluorescent proteins were co-developed with the Laboratory for Cell Function and Dynamics, the Advanced Technology Development Center, the Brain Science Institute, RIKEN, and MBL possesses the license.

	Abbreviations	Oligomer	Excit. Maxima (nm)	Emiss. Maxima (nm)	Vectors			
					S1	MN1	MCLinker	MNLinker
Keima-Red	dKeima570	Dimer	440	570	AM-V0121M			
	hdKeima570	Dimer	440	570	AM-V0124M		AM-V0129M	AM-V0120M
	dKeima-Red	Dimer	440	616	AM-V0101M			
	mKeima-Red	Monomer	440	620	AM-V0091M	AM-V0093M		
	hdKeima-Red	Dimer	440	616	AM-V0104M		AM-V0109M	AM-V0100M
	hmKeima-Red	Monomer	440	620	AM-V0094M		AM-V0099M Recommended	AM-V0090M Recommended

CoralHue® Keima-Red vectors（Organelle targeting vectors）

	Abbreviations	Oligomer	Excit. Maxima (nm)	Emiss. Maxima (nm)	Mitochondria	Endoplasmic reticulum	Plasma membrane	Nucleoplasm
Keima-Red	mKeima-Red	Monomer	440	620	AM-V0251M Popular		AM-V0253M	
	hdKeima-Red	Dimer	440	616				AM-V0274M
	hdKeima570	Dimer	440	570				AM-V0324M

S1: subcloning vectors　MN1: expression vectors　m: monomers　d: dimers
h: expression vectors with fluorescent proteins optimized for mamalian codons
Linker: expression vectors with flexible linkers inserted between fluorescent proteins and MCS

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