La dégradation sélective des mitochondries par autophagie:
de la levure aux cellules de mammifères

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7e colloque du réseau MeetOchondrie
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Autophagy is a degradative pathway of cytosolic constituents and organelles. Autophagy is induced by nutrient deprivation or by different stresses. Autophagy is conserved in mammals, plants, insects and yeasts.

This process is not selective
Selective autophagy

- Cargo
- Receptor scaffold
- autophagosome membrane
- Atg8
- Cargo
- Ligand
- Scaffold
Selective autophagy

Autophagosome membrane

Atg8

Cargo

Atg8

Receptor

LIR domain

WXXL-like

Atg8/LC3

Cargo

Peroxisomes

Mitochondria

Mitochondria

Mitochondria

Mitochondria

Proteins, Aggregates, Bacteria, Organelles...

ligand

scaffold

Atg30

Atg32

Nix

FUNDC1

BNIP3

P62

NBR1

Ub
Mitophagy: the beginning

In 2004

First evidence of the selective degradation of mitochondria by autophagy in yeast


Growth on lactate

Control
Starvation 24h

v m
Mitophagy: the beginning

**In 2004**

First evidence of the selective degradation of mitochondria by autophagy in yeast

Kissova et al. (2004) JBC

**In 2007**

Nutrient deprivation

Some mitochondria are sequestrated in autophagosomes

Kim et al. (2007) ABB 462, 245-253

Hepatocytes from transgenic mice expressing GFP-LC3 (autophagosomes and autophagolysosomes labeling)

TMRM labeling allows to follow the mitochondrial membrane potential

Irradiation of some mitochondria with a laser (488 nm)

Damaged mitochondria are sequestrated in autophagosomes

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Publications

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Molecular mechanism

Constitutive actors: Atg32, Atg11, Atg8 + other Atgs

Regulators: Uth1, Aup1 (Ptc6), Atg33
Molecular mechanism

**Mitophagy in yeast**

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In some conditions, glutathione level regulates mitophagy

Phosphorylation of S114 of the protein Atg32 is required for the interaction between Atg32 and Atg11 and for the mitophagy process

- Aoki et al. (2011) Mol Biol Cell
- Deffieu et al. (2009) JBC

**Signals Regulation**

- Hog1 pathway (Osmotic stress response)
  - Hog1
  - Bck1
  - Slt2

- Protein kinase c pathway (cell wall integrity)

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Molecular mechanism

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Constitutive actors: Atg32, Atg11, Atg8 + other Atgs
Regulators: Uth1, Aup1 (Ptc6), Atg33

Role of the mitophagy

Elimination of non damaged mitochondria
- Starvation on respiratory carbon sources
  - Induction of mitophagy
  - Induction of autophagy

Elimination of damaged mitochondria
- Stationary phase
  - Induction of mitophagy
  - Induction of autophagy

Mitochondrial degradations
- No induction of mitophagy
  - But induction of autophagy

Signals
Regulation

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Protein kinase c pathway
(cell wall integrity)
Bck1
Slt2

Hog1 pathway
(Osmotic stress response)
Hog1

Mitophagy in mammalian cells

Receptors identified with a LIR domain:
NIX
BNIP3
FUNDC1
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Ub as a ligand
Mitophagy in mammalian cells

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Role of mitophagy

- Elimination of non damaged mitochondria
- Elimination of damaged mitochondria
Elimination of non damaged mitochondria

Mitochondria are eliminated during development of some tissues (for ex. épithélium du cristallin) or maturation of some cells (for ex. erythroïd cells)

Model: Culture « in vitro » of mice réticulocytes Nix+/+ et Nix−/−

Role of NIX in Erythroid cells maturation

Schweers et al. (2007) PNAS 104, 19500-19505
Elimination of non damaged mitochondria

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Model: Culture « in vitro » of mice réticulocytes Nix+/− et Nix−−

Role of NIX in Erythroid cells maturation

Elimination of paternal mitochondria in fertilized Oocytes: different pathways involved

Mitophagy PINK/PARKIN dependant

Beginning of the PINK/PARKIN couple: Parkin and PINK1 are two of the genes involved in familial forms of Parkinson's disease.

- Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. 2006, Park et al. Nature 441, 1157
- Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. 2006, Clark et al., Nature, 441, 1162
- Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin 2006, Yang et al., PNAS 103, 10793
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Then the link between PINK/PARKIN and mitophagy

Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. 2008, Narendra et al., J Cell Biol., 183, 795

Models: Mammalian cells (KEK293), HeLa, MEF... Induction of mitophagy with CCCP, FCCP
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Recruitment of Parkin on mitochondria and induction of mitophagy

HeLa cells, 1h CCCP 10µM

HEK293 cells, 1h CCCP 10µM
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Recruitment of Parkin on mitochondria and induction of mitophagy

To better understand the mechanism, treatment with CCCP for 24h and surexpression of proteins: no physiological situation
Parkin and PINK1 are two of the genes involved in familial forms of Parkinson’s disease. PINK1p: mitochondrially targeted serine/threonine kinase. Parkin: cytosolic E3 ubiquitin-protein ligase.

Pink1 is addressed to inner mitochondrial membrane and is quickly degraded.

Parkin resides in the cytosol.
Parkin and PINK1 are two of the genes involved in familial forms of Parkinson’s disease.

**Pink1p:** mitochondrially targeted serine/threonine kinase

**Parkin:** cytosolic E3 ubiquitin-protein ligase

*Pink1p* is addressed to the inner mitochondrial membrane and is quickly degraded.

**Parkin** resides in the cytosol.

After mitochondria dysfunctioning, **Parkin** is recruited to mitochondria.

**Role of fission**
Transfer of cardiolipids from inner mitochondrial membrane to outer mitochondrial membrane
Model: Primary cortical neurons and SH-SY5Y cells, Induction of mitophagy with rotenone (1µM)
Cardiolipid synthase and scramblase (PLS3) are required
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Loss of iron triggers PINK/PARKIN independent mitophagy
Model U2OS and SH-SY5Y cells, Induction of mitophagy with iron chelator deferiprone (DFP)
Allen et al. (2013) Embo Rep., 14, 1127
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Hypoxia: Induction of mitophagy
Role of FUNDC1, BNIP3 and NIX
**Mitophagy in plants**

**AUTOPHAGY-RELATED 11** plays a critical role in general autophagy and senescence-induced mitophagy in Arabidopsis. *Li and al. (2014) The Plant Cell, 26, 788*

Plants possess the autophagy machinery

Atg11 ortholog of the yeast Atg11 protein

Dark-induced senescence induces mitochondrion turnover via an autophagic process. Mitochondria are associated with autophagic bodies.
<table>
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<tr>
<th>Take home message</th>
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<td>• Mitophagy has been evidenced in yeast, insect and mammalian cells.</td>
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<tr>
<th>Mitophagy receptors</th>
<th>Yeast</th>
<th>Insect and mammals cells</th>
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<tr>
<td>Atg32p</td>
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<td>NIX, BNIP3, FUNDC1, P62 with Ub</td>
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<td>PINK/PARKIN pathway</td>
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<tr>
<th>Induction</th>
<th>Starvation</th>
<th>Growth stationary phase</th>
<th>No effect of CCCP</th>
<th>No induction by mitochondrial alterations</th>
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Mitophagie

Questions?

Quelle est la définition d'une mitochondrie endommagée ?

Quel est le niveau requis d'altérations pour que les mitochondries soient dégradées par mitophagyie ?

Comment ces mitochondries altérées sont-elles reconnues ?

Quelle est la pertinence des modèles utilisés pour étudier ce processus par rapport à la physiologie des cellules ?

Quel est le niveau d'expression endogène des différents acteurs dans les différentes cellules ou tissus ?

Est-ce qu'il y a un lien entre l'induction de la mitophagie et le métabolisme des cellules (glycolysis versus OXPHOS) ?

…
Merci de votre attention
Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. 2008, Yang et al., PNAS, 105, 7070

The PINK1/Parkin pathway regulates mitochondrial morphology. 2008, Poole AC, PNAS, 105, 1638

Loss of parkin or PINK1 function increases Drp1-dependent mitochondrial fragmentation. 2009, Lutz et al., J Bio.Cell., 284, 22938

Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. 2008, Twig et al., EMBO J., 27, 433

Mitochondrial fission facilitates mitophagy in Saccharomyces cerevisiae. 2013, Mao and Klionsky, Autophagy, 9, 1900

Participation of mitochondrial fission during mitophagy. 2013, Mao and Klionsky, Cell cycle, 12, 3131

Role of mitochondrial fission
Does mitochondrial fusion/fission balance have a role in controlling the quality and the turnover of mitochondria?

InS1 cells (derived of rat insulinome (β cells of pancreatic tumor))
Tools: Photoactivable mitochondrial GFP
TMRE: mitochondrial membrane potential
Irradiation of 10% of the cell

Mitochondrial fusion is a selective process.
A subpopulation of depolarized mitochondria does not refuse.

Twig et al. (2008) Embo J. 27, 433-446
The amount of fusion protein Opa1 is reduced in depolarized mitochondria

Over-expression of Opa1 decrease the mitochondrial degradation

Inhibition of fission decreases autophagy of mitochondria

Depolarized mitochondria are sequestred in autophagosomes
Model proposed to describe life of mitochondria

Twig et al. (2008) Embo J. 27, 433-446
Mitochondria are eliminated during development of some tissues (for ex. épithélium du cristallin) or maturation of some cells (for ex. erythroïd cells)

NIX (BNIP3L): protein with a BH3 domain; expression increased during the last step of erythrocytes differentiation.

Model: Culture « in vitro » of mice réticulocytes Nix+/+ et Nix−/−

In NIX−/− cells, mitochondria are not eliminated

NIX−/− cells are able to form autophagosomes.

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**NIX (BNIP3L):** protein with a BH3 domain; expression increased during the last step of erythrocytes differentiation.

NIX-/− cells are able to form autophagosomes. Mitochondria are not sequestred by these autophagosomes.

Treatment with a decouplant allows the mitochondrial sequestration in autophagosomes in NIX-/− cells

Schweers et al. (2007) PNAS 104, 19500-19505*