Kinetic analysis of the phagosomal ROS production with an improved spatial resolution

Correlation with NOX2 assembly

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NOX2 and the phagosome chemistry

Recognition
Receptors
(Fc, Complement)

time (min)

Nordenfelt et al JLeukBiol 2011
Winterbourn et al JBC 2006
Nüße ScientificWorld J 2011
NOX2 and the phagosome chemistry

- Recognition
  - Receptors
    - (Fc, Complement)

- Phagosome
- Granules
- Phagocytosis

References:
- Nordenfelt et al. *J Leuk Biol* 2011
- Winterbourn et al. *JBC* 2006
- Nüße *ScientificWorld J* 2011
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Phagosome

Granules

Phagocytosis

Killing
ROS production
Proteases, peptides

time (min)

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Phagosome

Granules

Phagocytosis

Killing
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Proteases, peptides

Signalization steps

NOX2 and the phagosome chemistry

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NOX2 and the phagosome chemistry

- Recognition
  - Receptors (Fc, Complement)

- Phagocytosis
  - Granules

- Killing
  - ROS production
  - Proteases, peptides

Signalization steps:
- NOX2
- p67/p47/p40
- Rac
- p22/gp91

Time (min):
- Few mM/s
- 5 x 10^6 molecules/s

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NOX2 and the phagosome chemistry

- Recognition Receptors (Fc, Complement)
- Phagocytosis
- Killing ROS production Proteases, peptides

**Signalization steps**
- NOX2
- p67/p47/p40
- Rac
- p22/gp91

**Phagosome**
- MPO & Cl⁻
- H₂O₂
- HOCl
- Fe³⁺
- H₂O₂
- O₂⁻

**Granules**
- H₂O
- O₂
- HO²⁻

**Cell**
- few mM / s
- 5 10⁶ molecules /s

** MMO a few sec. after the phagosome closure**

**References**
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- Nüße ScientificWorld J 2011
Aims and tools

Activation / inactivation of NOX2 complex

Which subunit? Where? How Long?

pH, proteases, ROS

Who? Timing? How much?
Aims and tools

Activation / inactivation of NOX2 complex
Which subunit? Where? How Long?

pH, proteases, ROS
Who? Timing? How much?

Baker Yeast

4-5µm
Opsonisation:

IgG anti-yeast
Complement

Recognition
Receptors
(Fc, Complement)

Activation of NOX2 complex
ROS

Tlili et al FRBM 2011
Aims and tools

Opsonisation:

Recognition Receptors (Fc, Complement)

IgG anti-yeast

Complement

Activation of NOX2 complex ↔ ROS

Dye: 2',7'-dichlorodihydrofluorescein diacetate, succinimidyl ester / DCFH₂

Non fluorescent BEFORE oxidation

Fluorescent AFTER oxidation

 широкий диапазон использований

Нет специфичности

"накопление" и "непрерывное"

Устойчив к фотоокислению

Существует в виде сукининимидил эсера => Объект маркирования

Tlili et al FRBM 2011
Dye oxidation in vitro

$\text{DCFH}_2 \rightarrow \text{DCF} \ (\text{H}_2\text{O}_2/\text{HRP} \text{ or HO}^\circ)$

$\text{DCFH}_2 \rightarrow \text{Xfluo} \ (\text{HOCl})$

(Mass spectrometry)

Wardman FRBM 2007
Tili et al FRBM 2011
Dye oxidation in vitro

\[
\text{DCFH}_2 \rightarrow \text{DCF} \quad (\text{H}_2\text{O}_2/\text{HRP or HO}^\circ) \\
\text{DCFH}_2 \rightarrow \text{Xfluo} \quad (\text{HOCl})
\]

\[
\text{DCFH}_2\text{-yeast} + \text{H}_2\text{O}_2/\text{HRP} \rightarrow \text{“DCF-yeast”}
\]

\[
\text{DCFH}_2\text{-yeast} + \text{HOCl} \rightarrow \text{“Xfluo-yeast”}
\]

(Wardman FRBM 2007)

(Tlili et al FRBM 2011)
Yeast oxidation in cellulo
Fluorescence increase correlates with NOX2 activity
Limited photo-oxidation

- Yeasts oxidation in cellulo

Tilli et al. FRBM 2011
Fluorescence increase correlates with NOX2 activity

Limited photo-oxidation

The inhibition of ROS production limits the fluorescence increase

Tilli et al FRBM 2011
Correlation between ROS production and NOX2 assembly

p67-Citrine is present at the phagosome membrane
The time of presence of p67-Citrine depends on the opsonisation conditions

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The time of presence of p67-Citrine depends on the opsonisation conditions

The time course of phagosomal ROS production depends on the opsonisation.

Citrine is present at the phagosome membrane
The time of presence of p67-Citrine depends on the opsonisation conditions

Correlation between ROS production and NOX2 assembly

The time course of phagosomal ROS production depends on the opsonisation conditions

Good correlation between
- duration of ROS production
- presence of p67-Citrine at the phagosome

**What next?**

Proof of concept -> tool to measure the overall ROS production at the single phagosome level

1) Take advantage of the different spectral properties of Xfluo and DCF to improve their detection
2) Specific dye for each ROS (of different colors)
3) Simultaneous detection of two parameters (pH+ROS, ROS+ [protease], ratiometric detection)

From dead yeast to living pathogen using as sensors genetically encoded dyes as fluorescent proteins