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LOUVAIN DRUG RESEARCH INSTITUTE

Intracellular mechanisms of apoptosis induced by aminoglycoside antibiotics

Sophie Denamur

Promoter : Prof. Marie-Paule Mingeot-Leclercq

Co-promoter : Prof. Paul M. Tulkens

Introduction

What do we expect from an antibiotic ?

Aminoglycoside antibiotics : potent and useful…

- Treatment of severe infections caused by Gram negative bacteria (Drusano et al., 2007; Lopez-Novoa et al, 2011)
- Treatment of genetic diseases ? (Malik et al., 2010)
- Antiviral ? (Houghton et al., 2010)

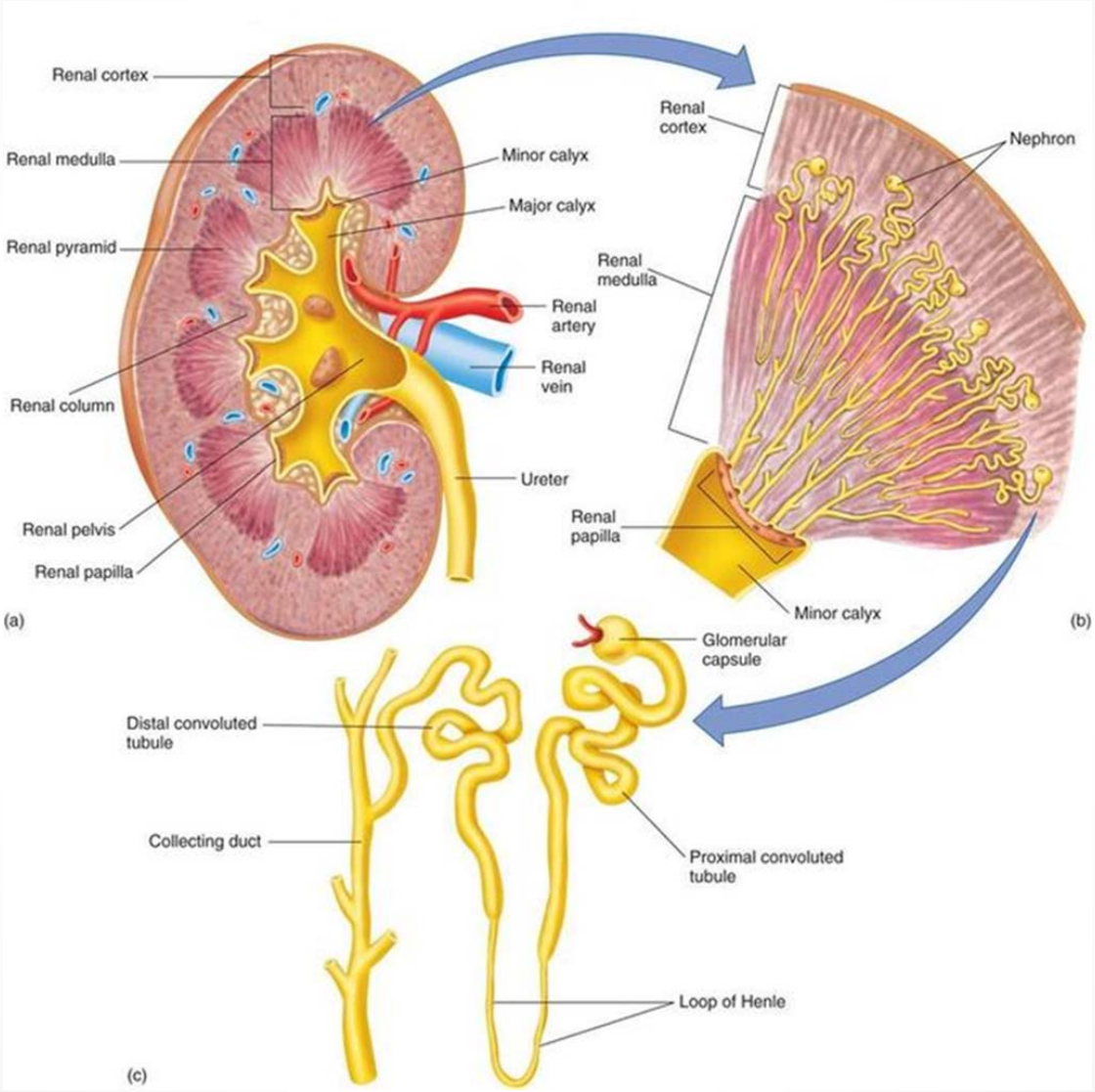
BUT… toxic !

- Cochlear and vestibular toxicity
- Nephrotoxicity : 10-25% of treatments
  - o <u>Clinical features</u> :
    non-oliguric renal failure
    Slow rise in creatinine
  - o <u>Risk factors</u> : duration of the treatment, older age, reduced renal function, hepatic dysfunction, interactions with other drugs

Nephrotoxicity of aminoglycosides

De Broe et al., 1984

Kidney structure



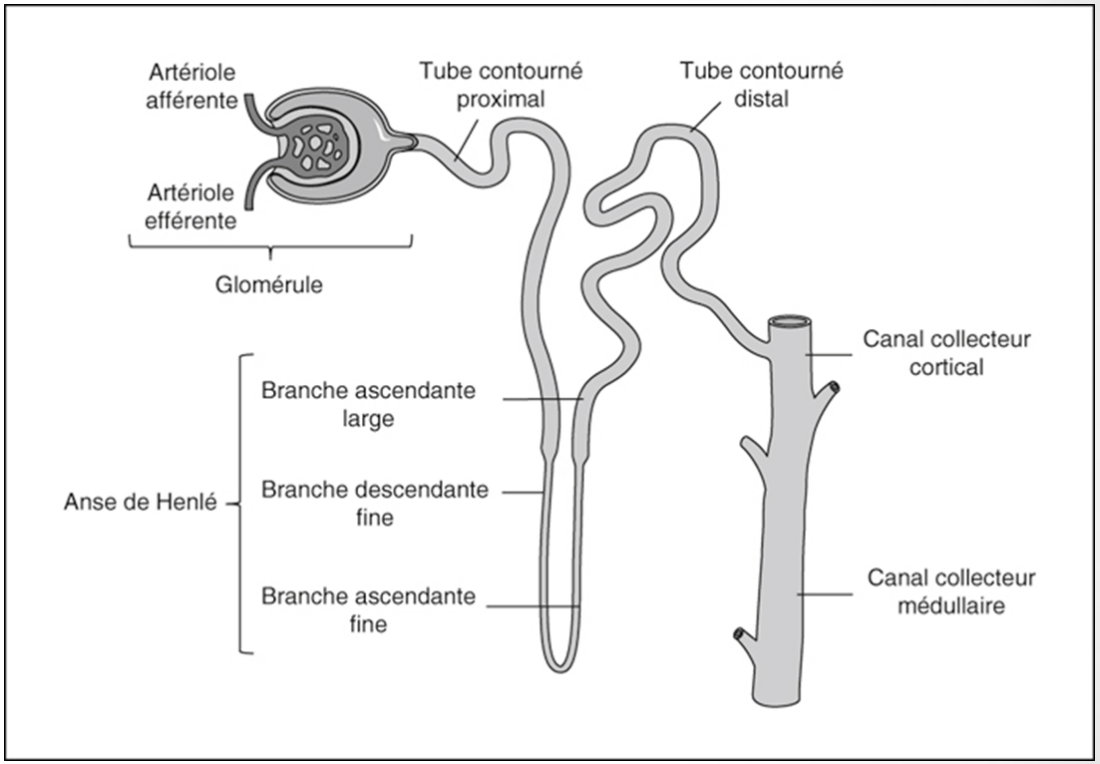
Nephrotoxicity of aminoglycosides

De Broe et al., 1984

Giurgia-Marion et al., 1986
El Mouedden et al., 2000a

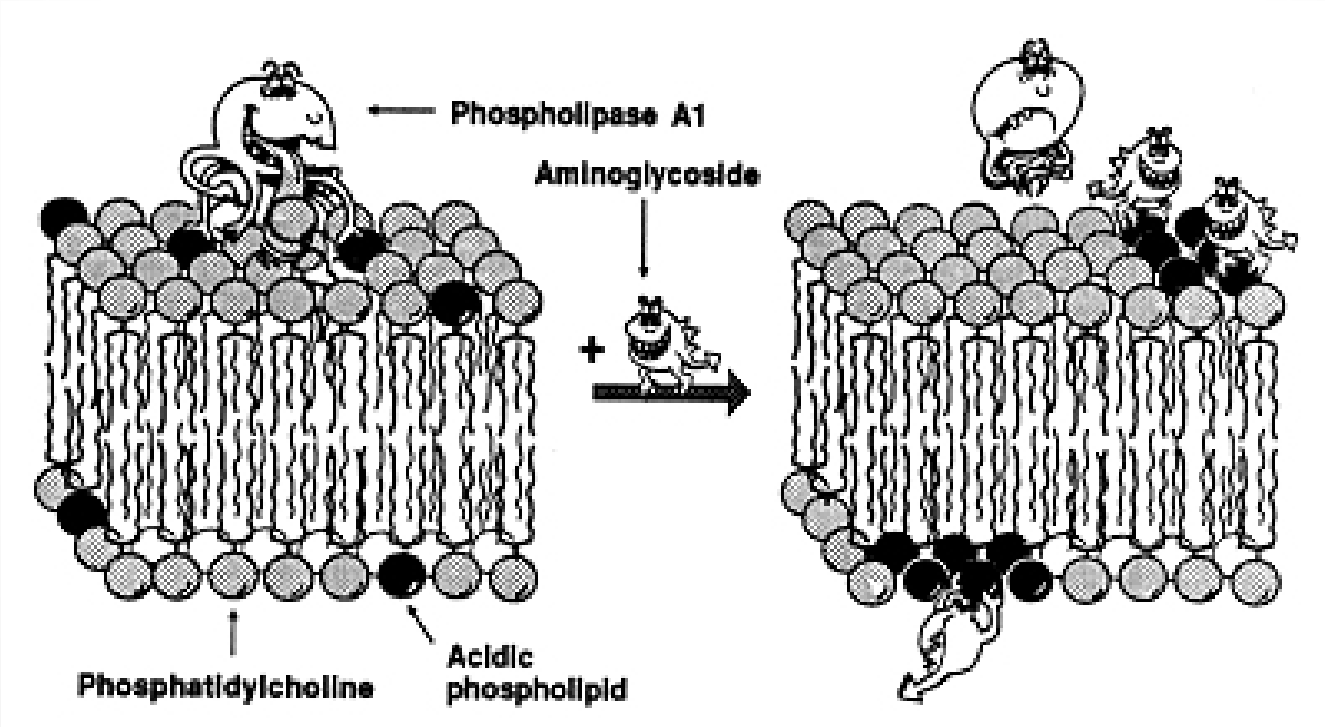
Tubular toxicity

- Release of brush border and lysosomal enzymes
- Decreased reabsorption of filtered proteins
- Wasting K^+, Mg^{2+},Ca^{2+} and glucose
- Phospholipiduria



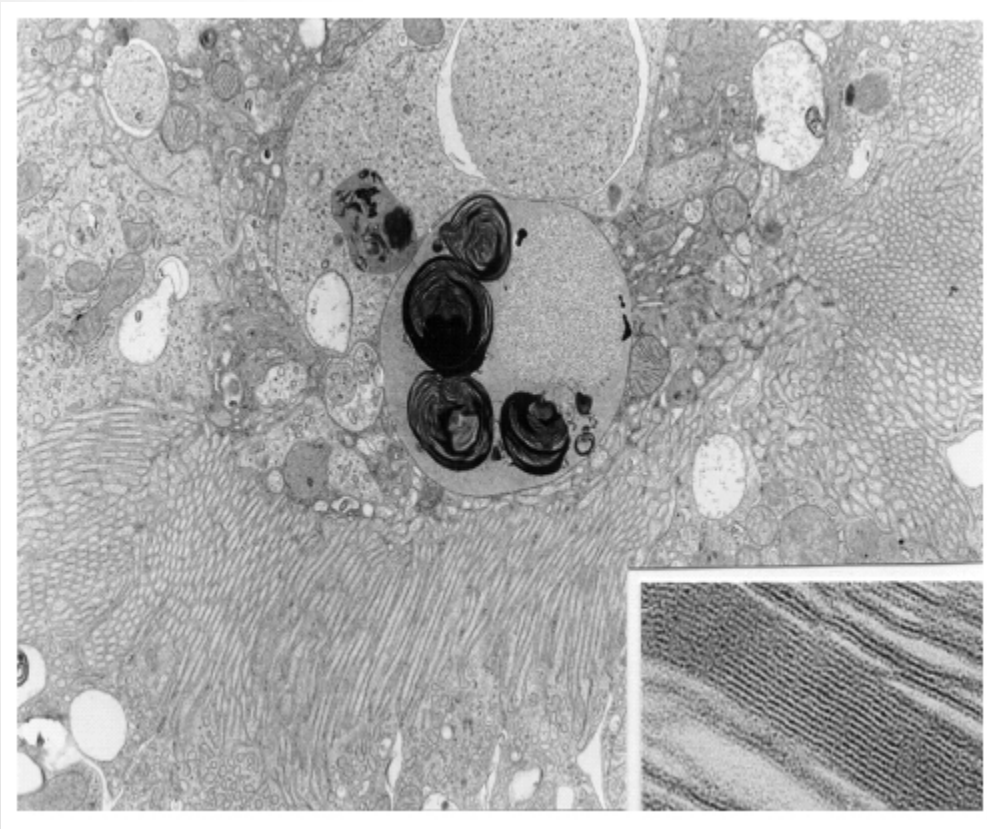
Lysosomal alterations induced by aminoglycoside antibiotics

Inhibition of lysosomal phospholipases



From Mingeot-Leclercq, 1999

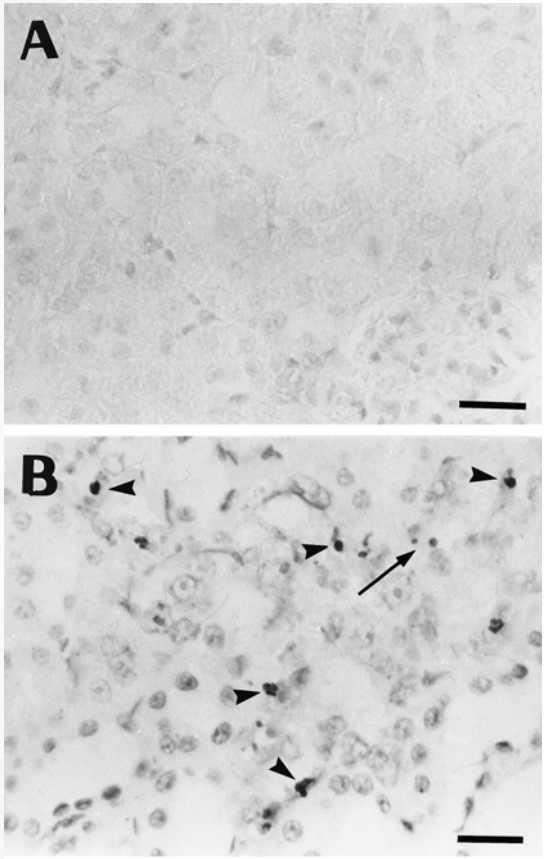
Lysosomal alterations induced by aminoglycoside antibiotics



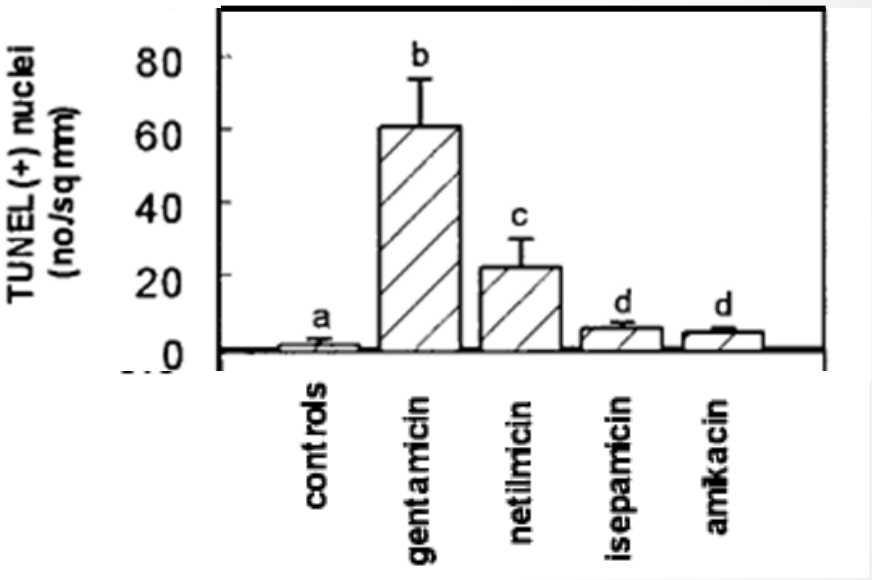
Phospholipidosis induced in renal proximal tubular cells of rats treated with low, therapeutically-relevant doses.

From Tulkens, 1986

Induction of apoptosis

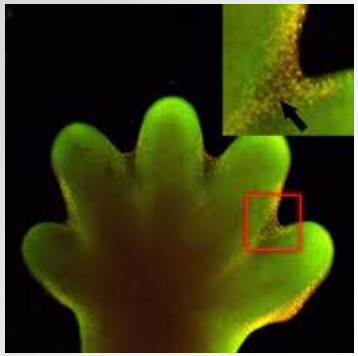


Renal cortex of control rats (A) or animals treated for 10 days with 10 mg/kg gentamicin



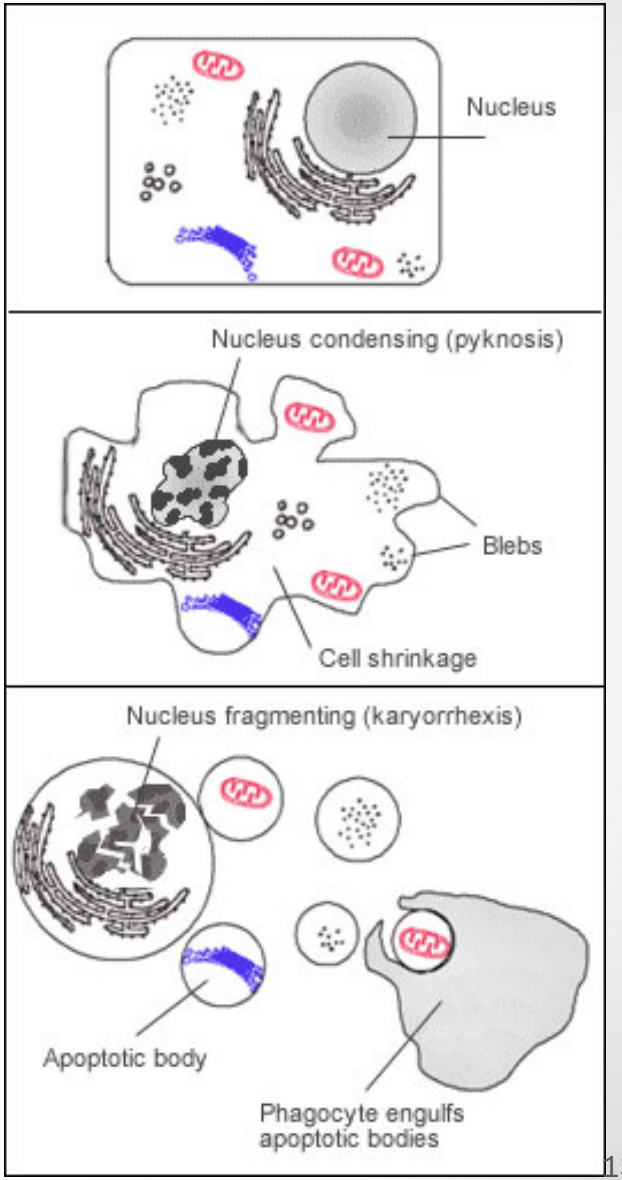
Rats treated for 10 days with saline (control), 10 mg/kg of gentamicin and netilmicin, or 40 mg/kg of isepamicin and amikacin

El Mouedden et al., 2000a



Apoptosis ?

- Active form of cell death
- Embryogenesis
- Maintenance of homeostasis
- Implicated in cancer, neurodegenerative diseases, …
- Can be induced by exposure of the cell to a toxic substance or drug



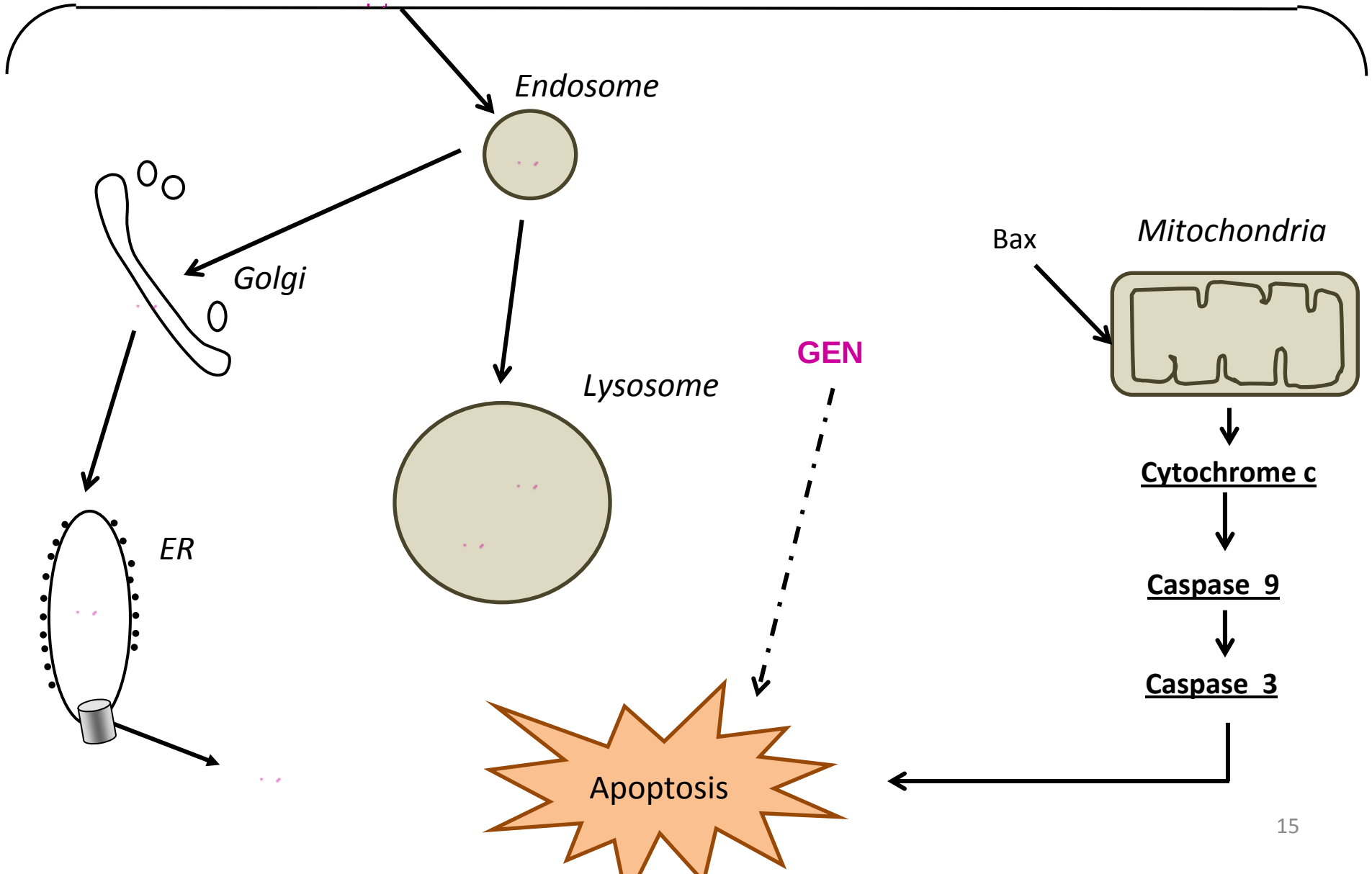
Nephrotoxicity of aminoglycosides

De Broe et al., 1984

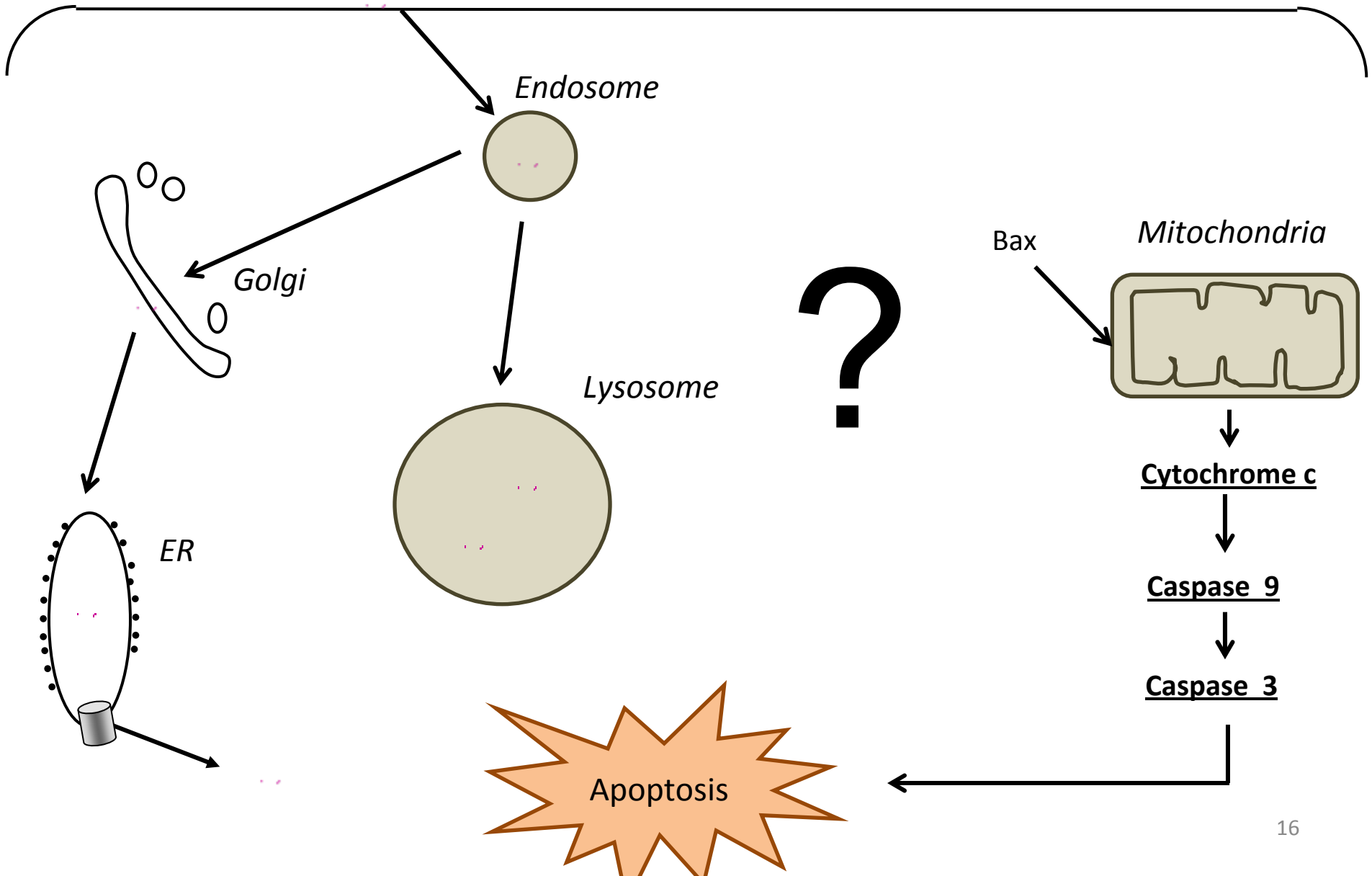
Giurgia-Marion et al., 1986
El Mouedden et al., 2000a

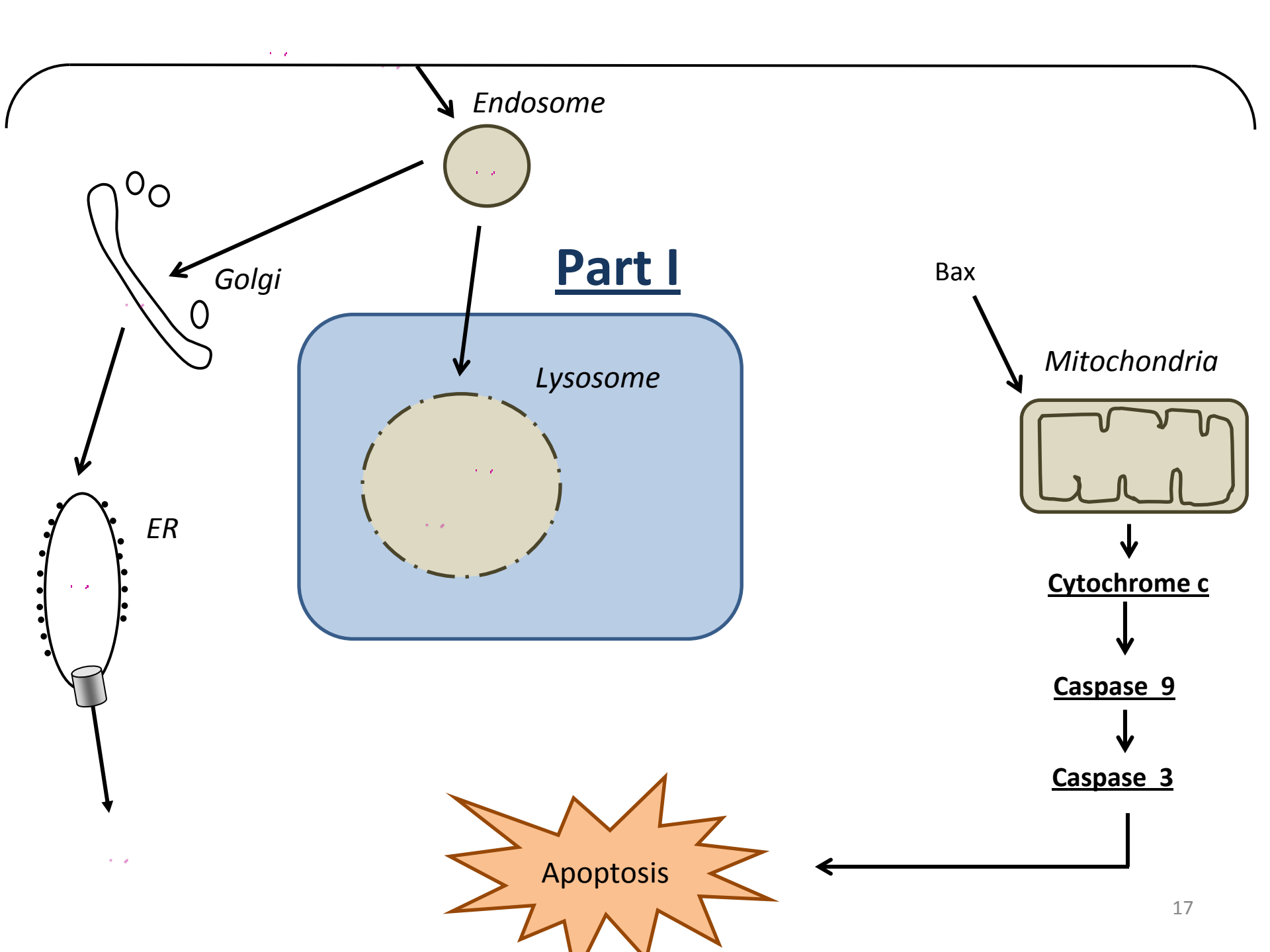
El Mouedden et al., 2000b
Servais et al., 2005
Servais et al., 2006

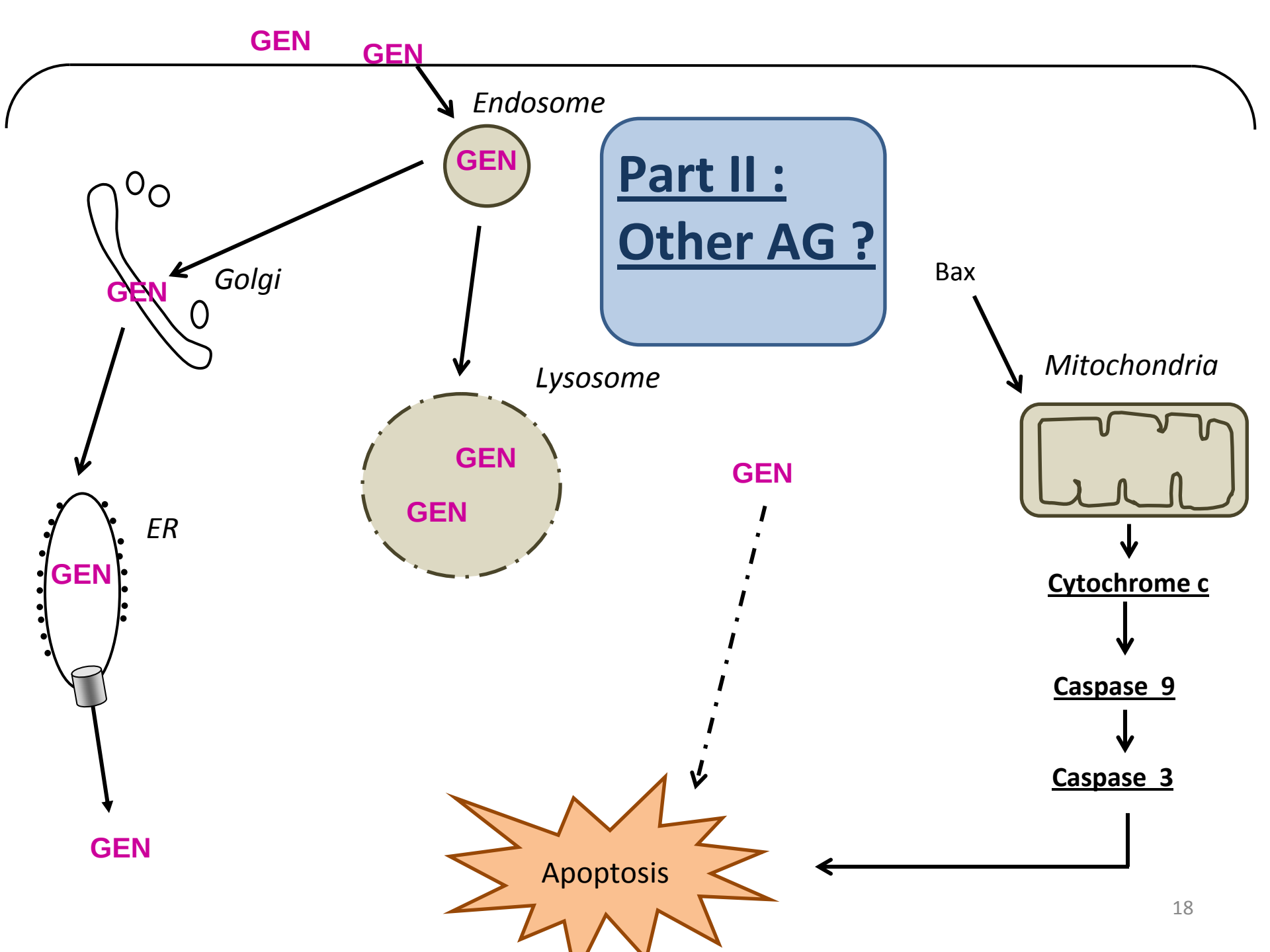
Apoptosis induced by gentamicin

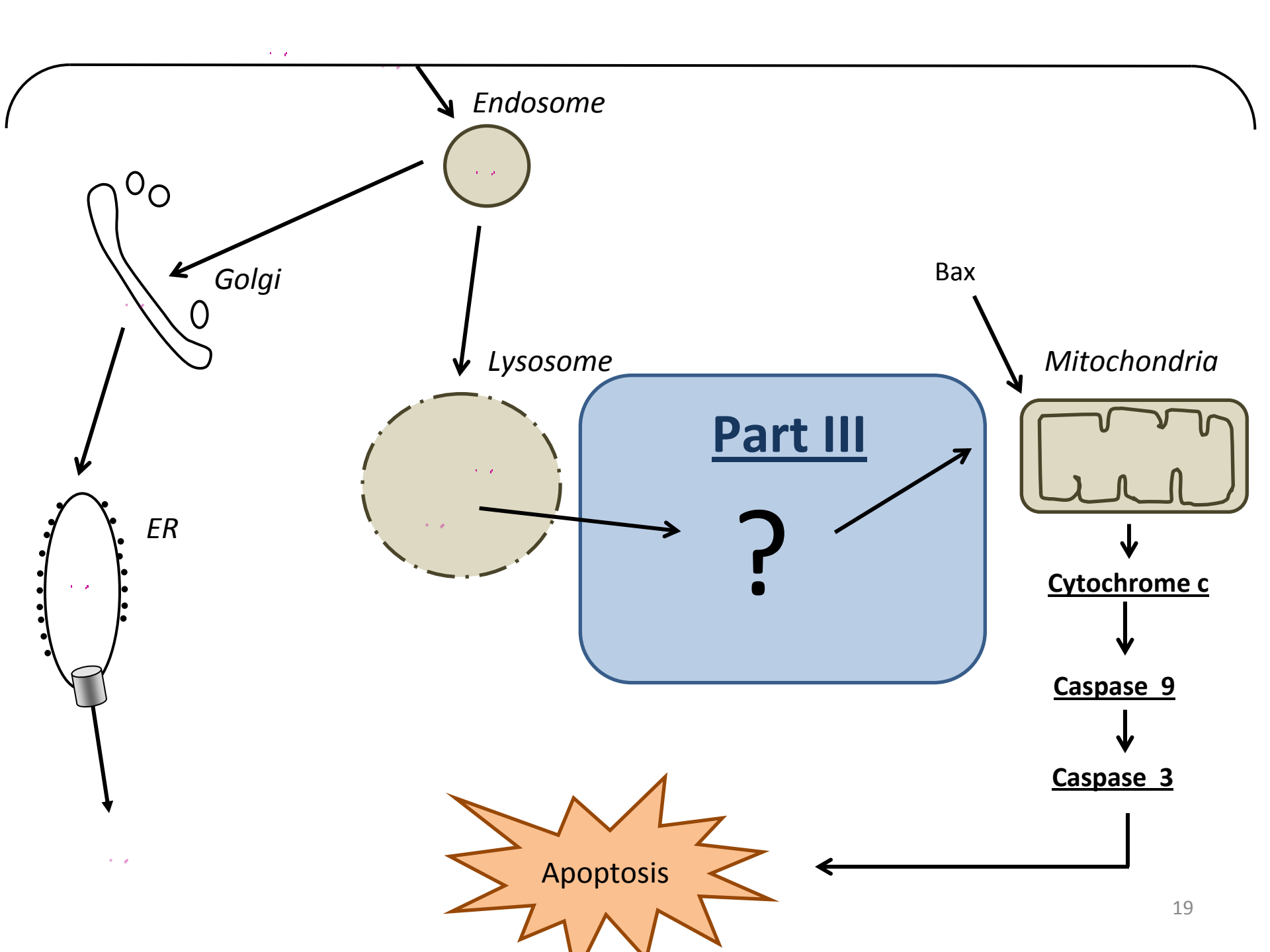


Aim of the study : How gentamicin induces apoptosis ?

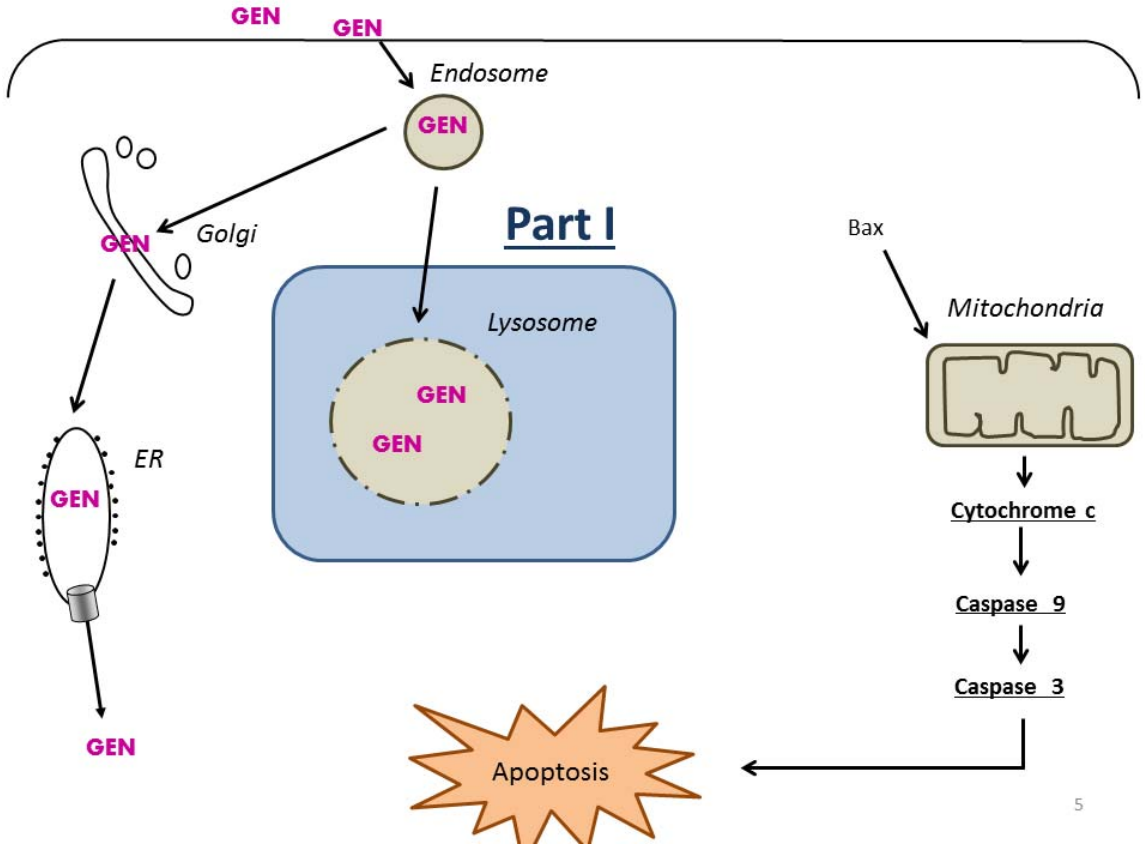



Endosome
Golgi
Part I
Bax
Lysosome
Mitochondria
ER
Cytochrome c
Caspase 9
Caspase 3
Apoptosis


GEN
GEN
Endosome
GEN
Part II :
Other AG ?
Golgi
GEN
Bax
Mitochondria
Lysosome
GEN
GEN
GEN
ER
GEN
Cytochrome c
Caspase 9
Caspase 3
GEN
Apoptosis


Endosome
Golgi
ER
Lysosome
Part III
?
Bax
Mitochondria
Cytochrome c
Caspase 9
Caspase 3
Apoptosis

Results



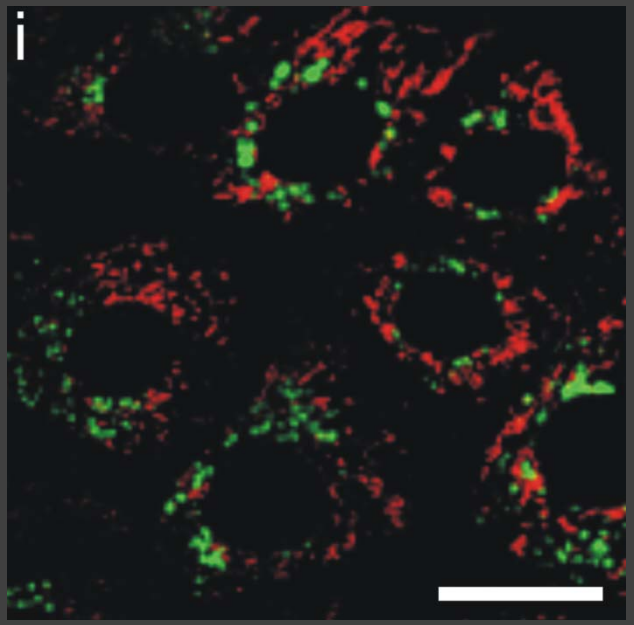
Part I

GENTAMICIN-INDUCED LYSOSOMAL MEMBRANE PERMEABILIZATION AND MECHANISM INVOLVED

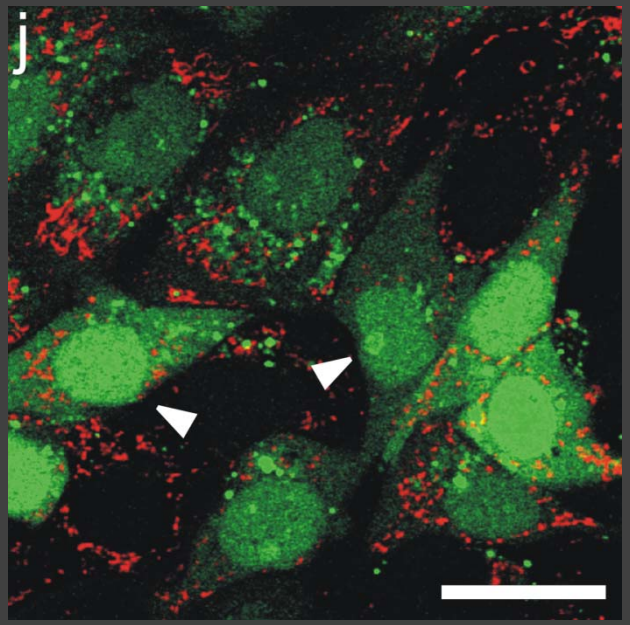
Does gentamicin induce lysosomal membrane permeabilization?

Follow of LMP by vital imaging with Lucifer Yellow

- pH insensitive, membrane bilayer-impermeant
- Increase of sensitivity of the method by inhibiting the organic anion transporter with probenecid
- MitoTracker red

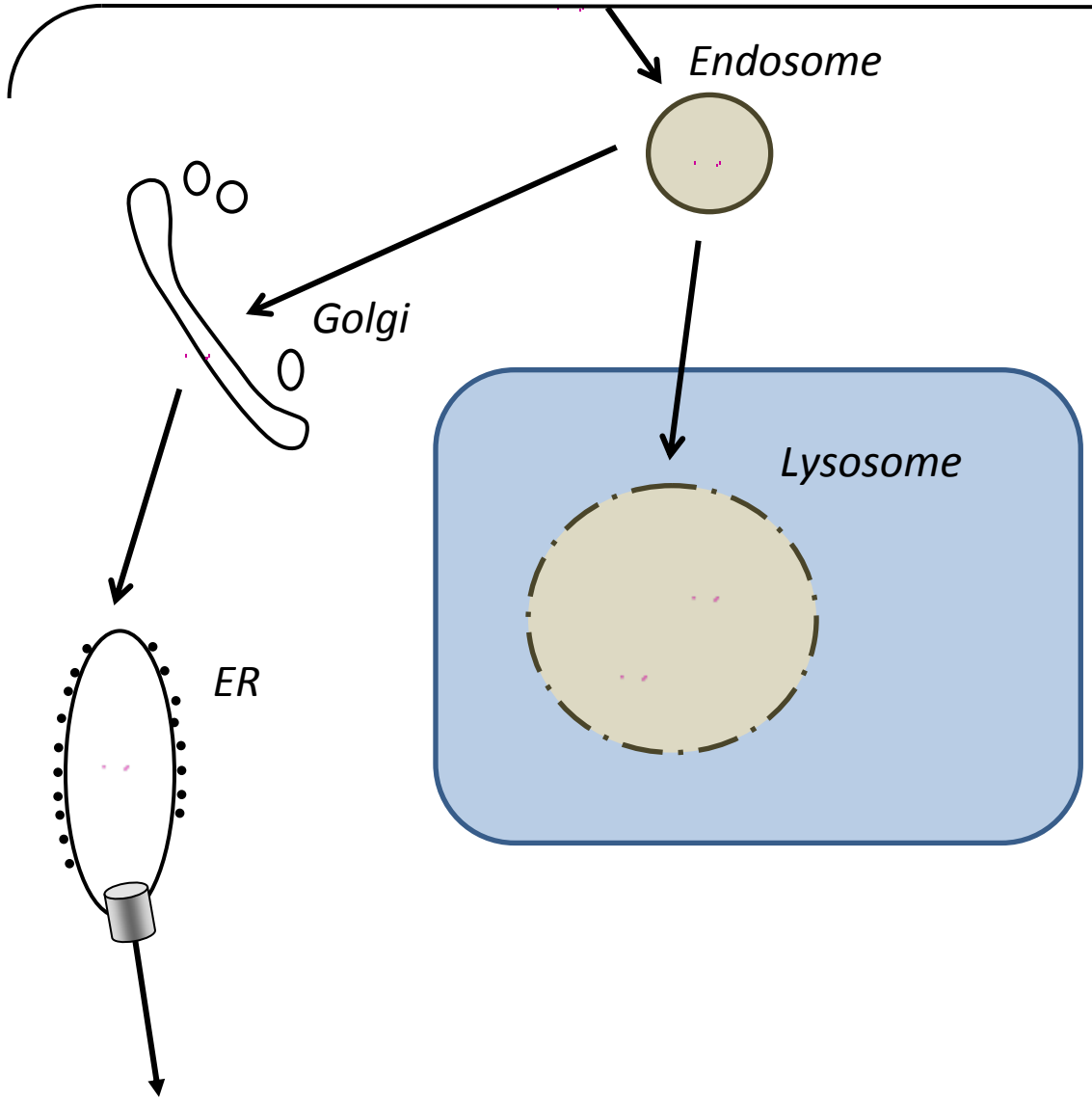


Control



Gentamicin 3 mM – 6h

Scale bars = 20 μM



Gentamicin induces lysosomal membrane permeabilization

What is the underlying mechanism?

Implication of Reactive Oxygen Species ??

DNA

$O_2^{\bullet -}$

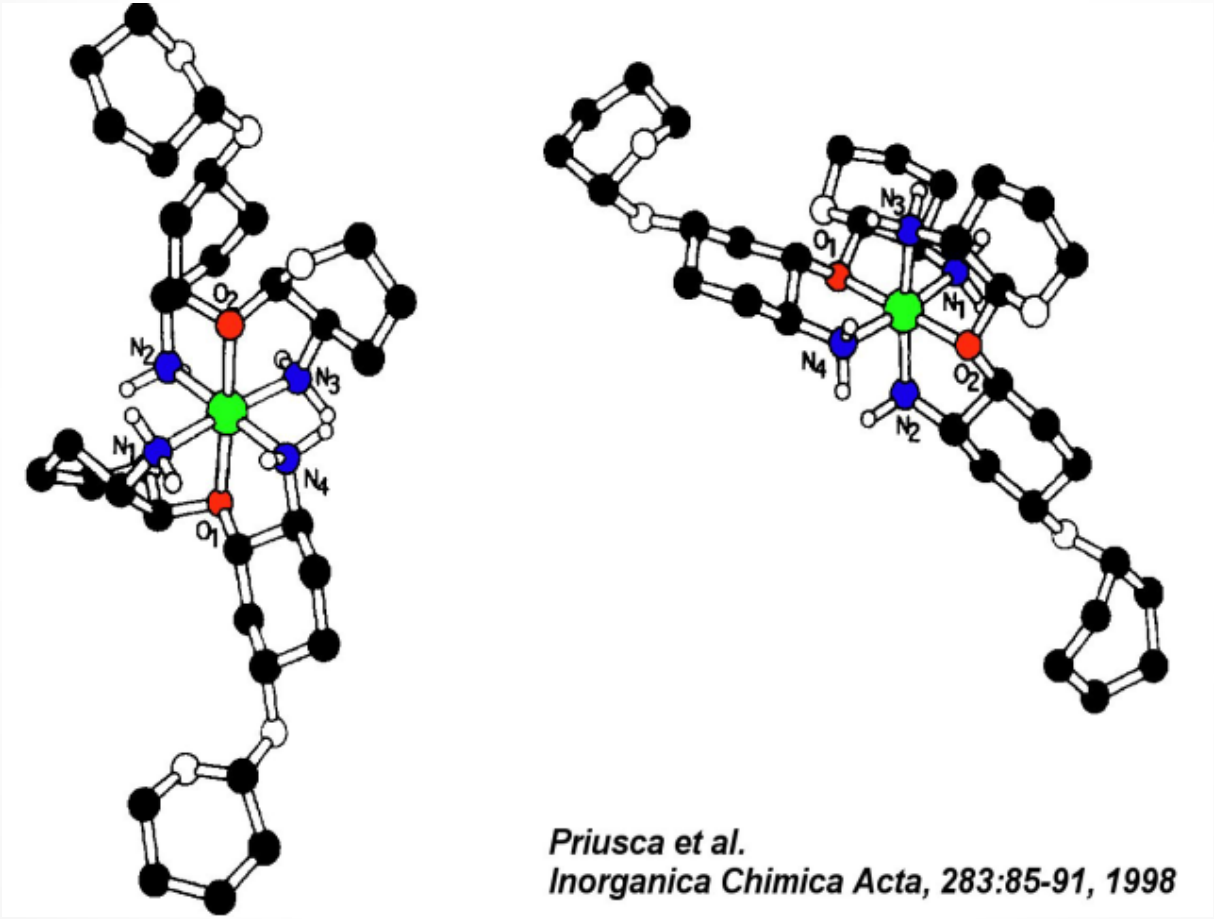
H_2O_2

$^{\bullet}OH$

Proteins

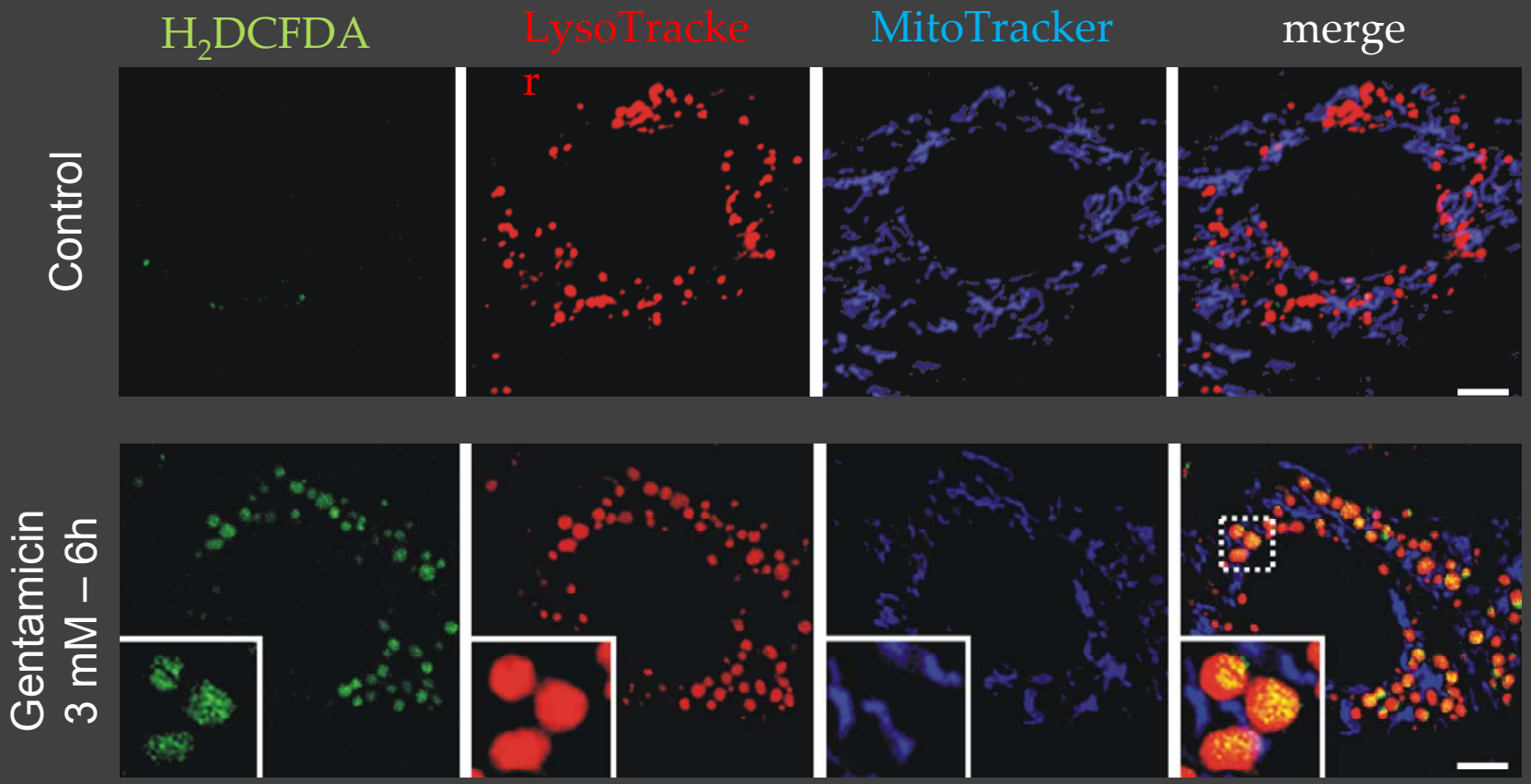
Lipids

Role of ROS in GEN-induced lysosomal permeabilization



Priusca et al.
Inorganica Chimica Acta, 283:85-91, 1998

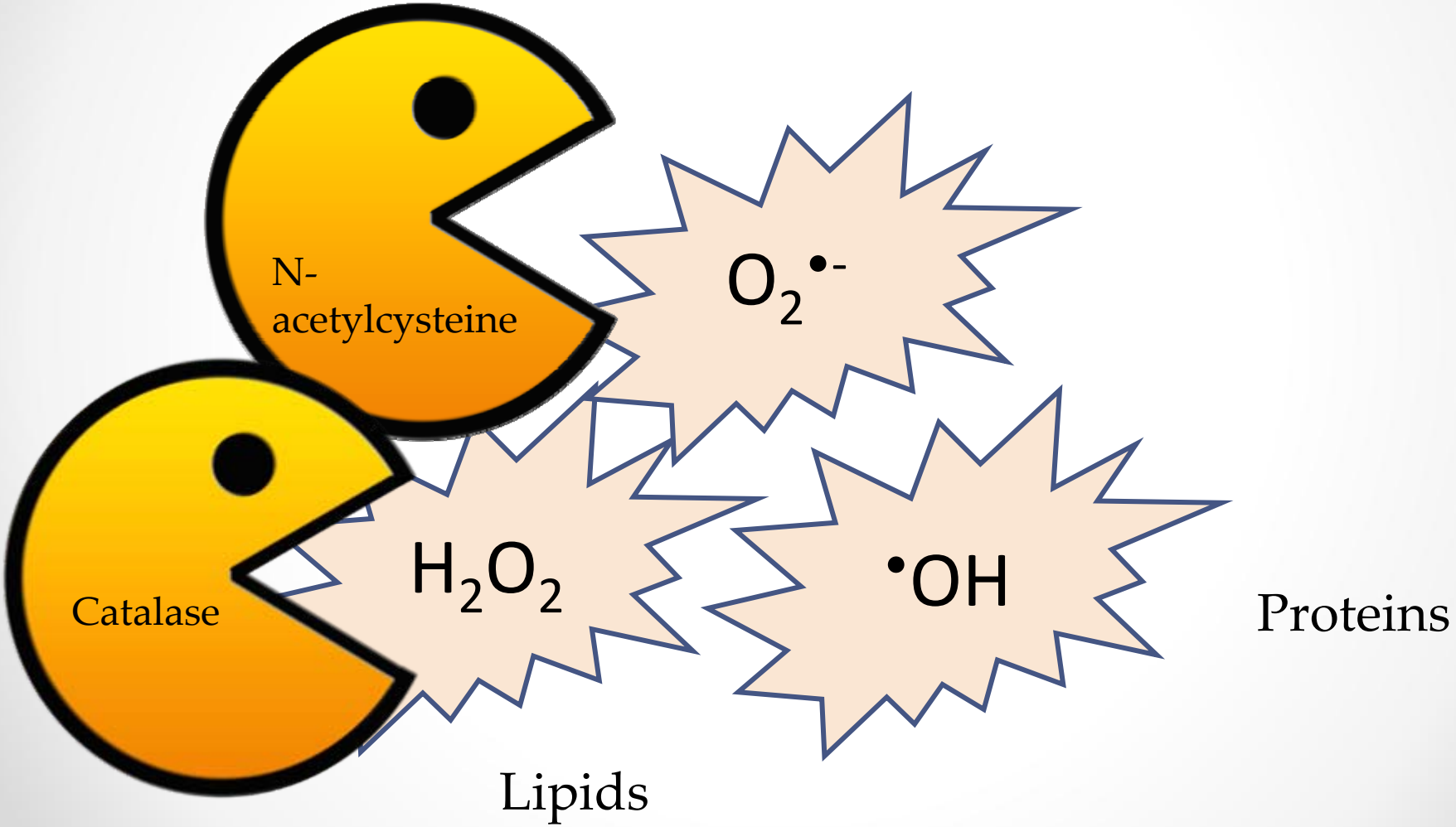
Intracellular localisation of GEN-induced ROS production



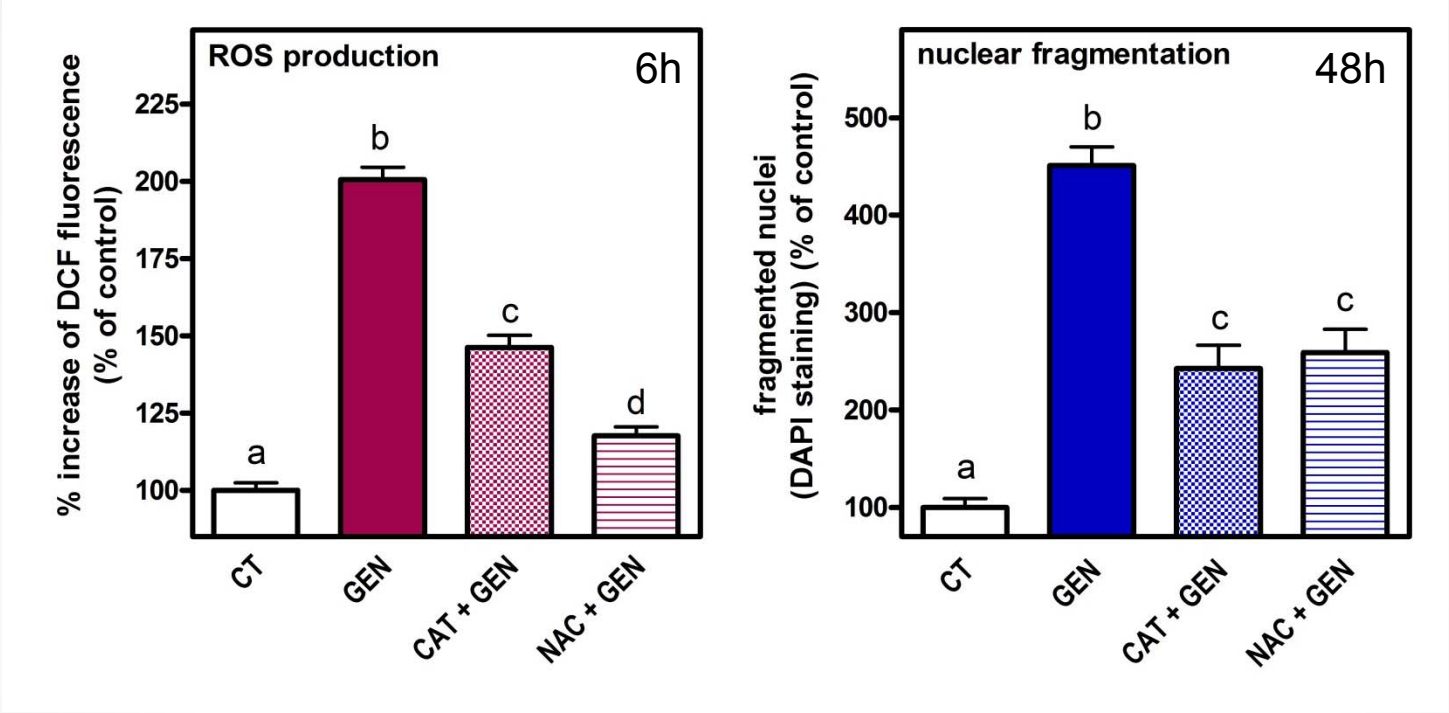
GEN-induced ROS production occurs in lysosomes

Scale bars = 5 µM

Implication of Reactive Oxygen Species ??

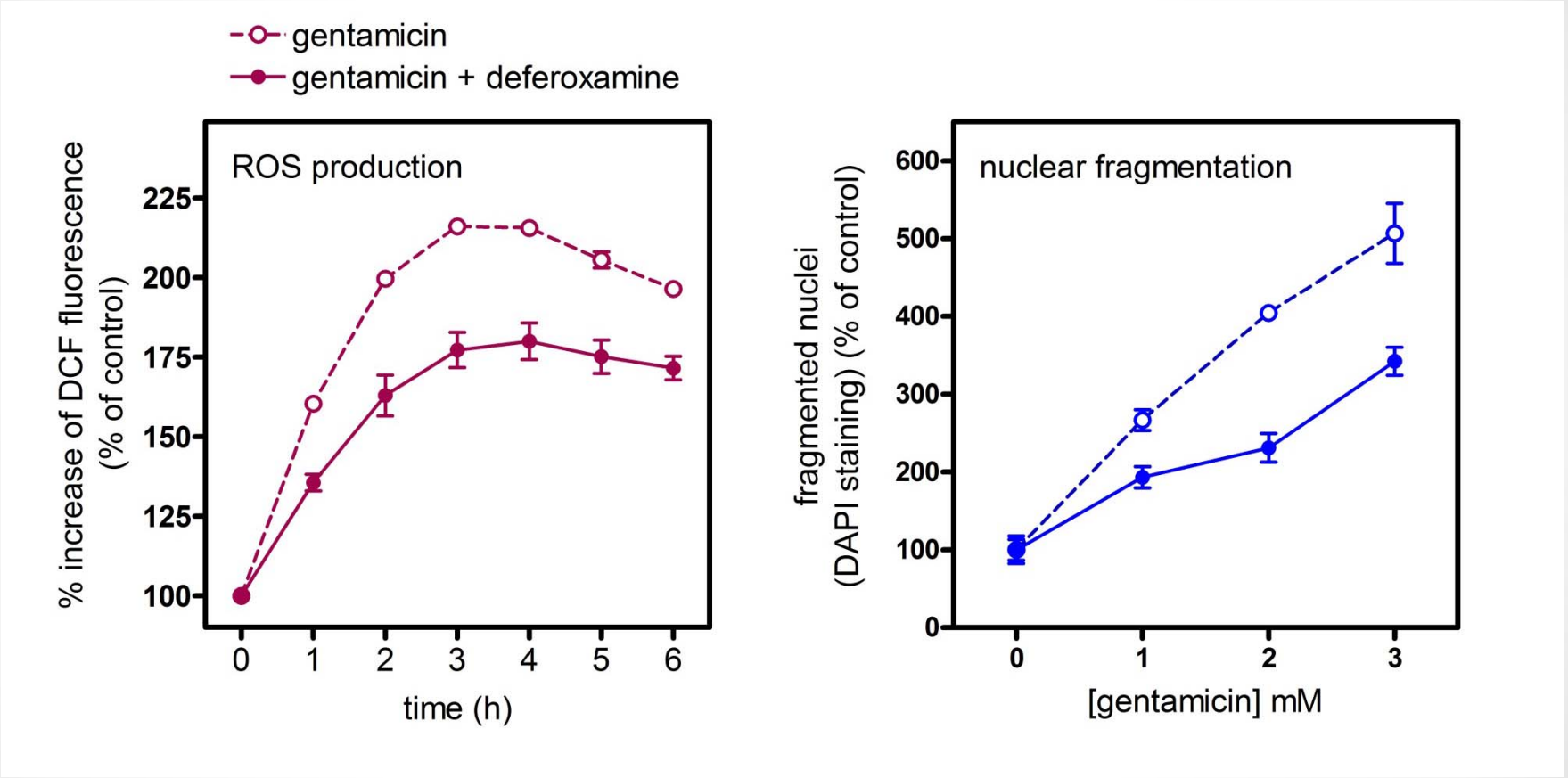


Protection afforded by anti-oxidant molecules



Partial protective effect of catalase (1.000 U/ml) and N-acetylcysteine (1 mM) against GEN-induced ROS production and apoptosis induction (nuclear fragmentation)

Implication of iron evaluated with the lysosomal iron chelator deferoxamine (DFO)



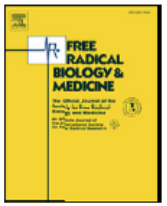
DFO partially reduces GEN-induced ROS production and apoptosis.

Free Radical Biology & Medicine 51 (2011) 1656–1665

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed



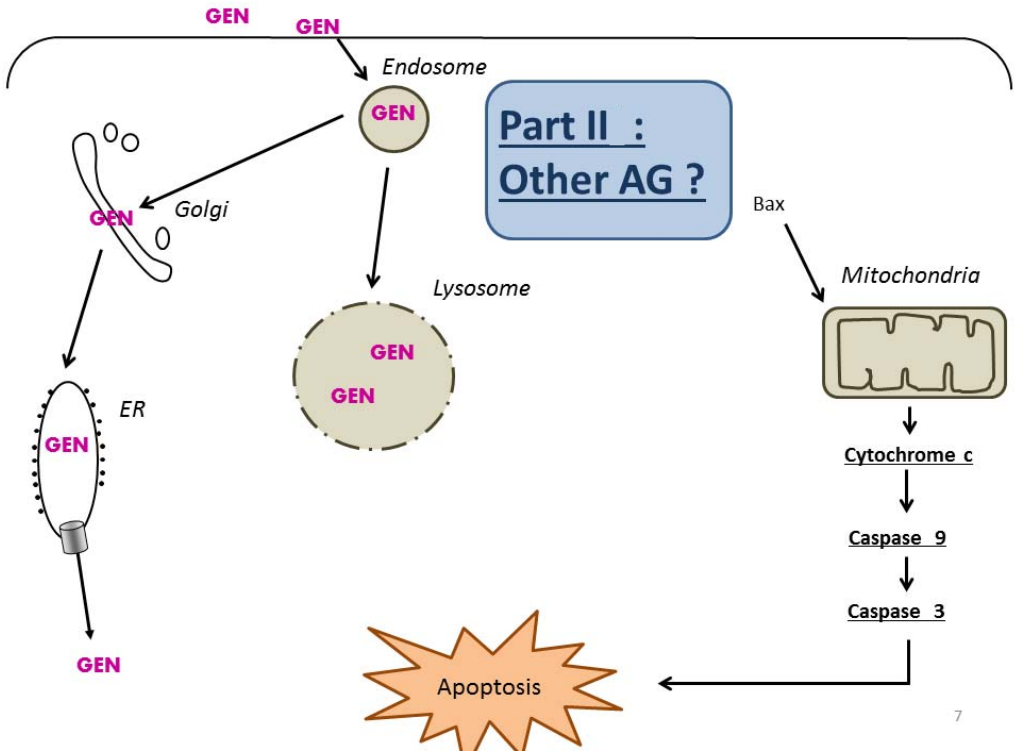
Original Contribution

Role of oxidative stress in lysosomal membrane permeabilization and apoptosis induced by gentamicin, an aminoglycoside antibiotic

Sophie Denamur [a,1], Donatienne Tyteca [b,1], Jacqueline Marchand-Brynaert [c], Françoise Van Bambeke [a], Paul M. Tulkens [a], Pierre J. Courtoy [b,1], Marie-Paule Mingeot-Leclercq [a,*,1]

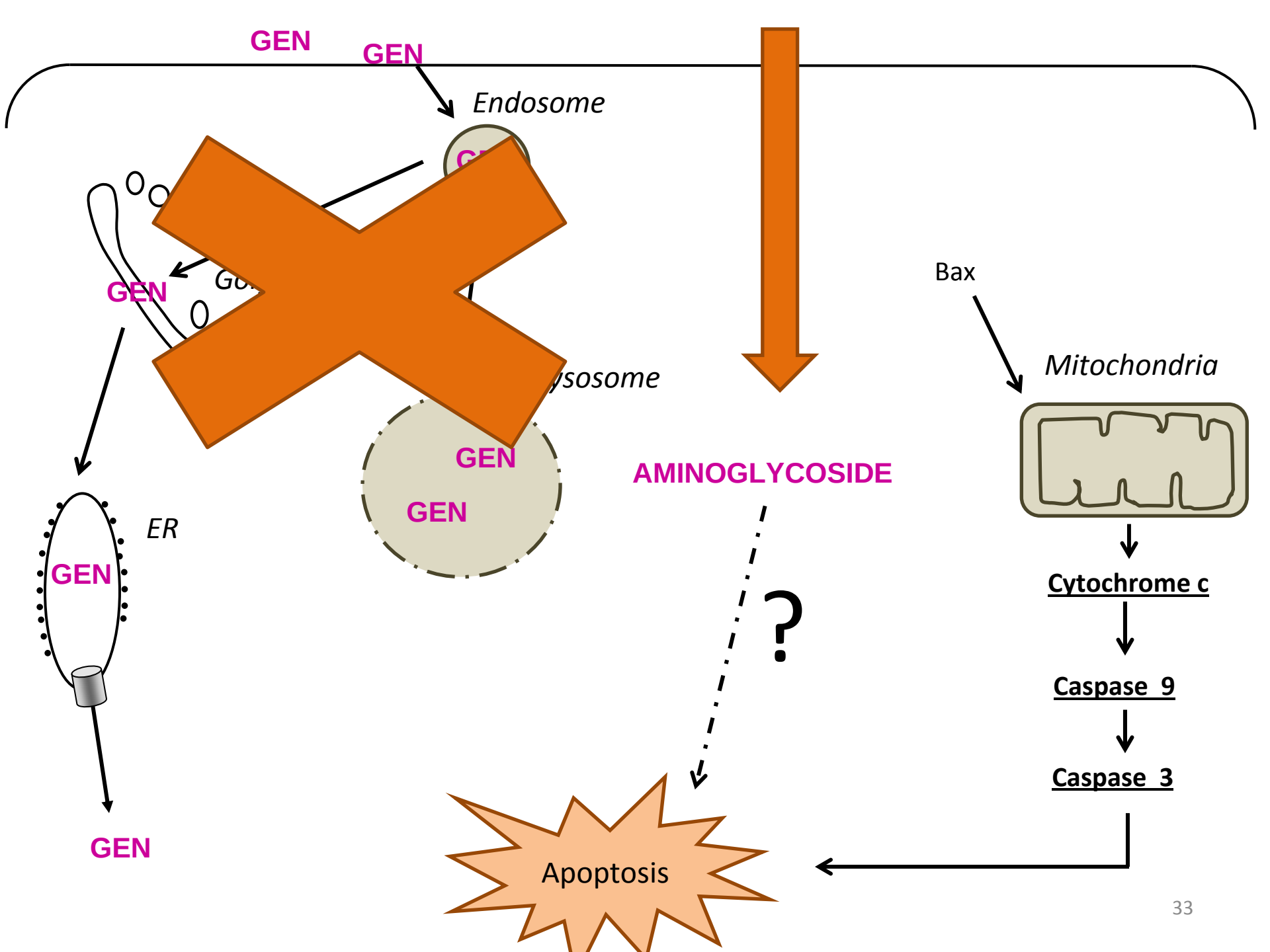
- Gentamicin induces lysosomal ROS production, lysosomal membrane permeabilization and apoptosis

- Lysosomal iron chelator and antioxidants afford partial protective effect against these events



Part II

COULD WE GENERALIZE THE POTENTIAL EFFECT OF CYTOSOLIC GENTAMICIN TO OTHER AMINOGLYCOSIDES ?


GEN
GEN
Endosome
GEN
GEN
GEN
GEN
GEN
ER
GEN
AMINOGLYCOSIDE
?
Bax
Mitochondria
Cytochrome c
Caspase 9
Caspase 3
Apoptosis

Choice of molecules

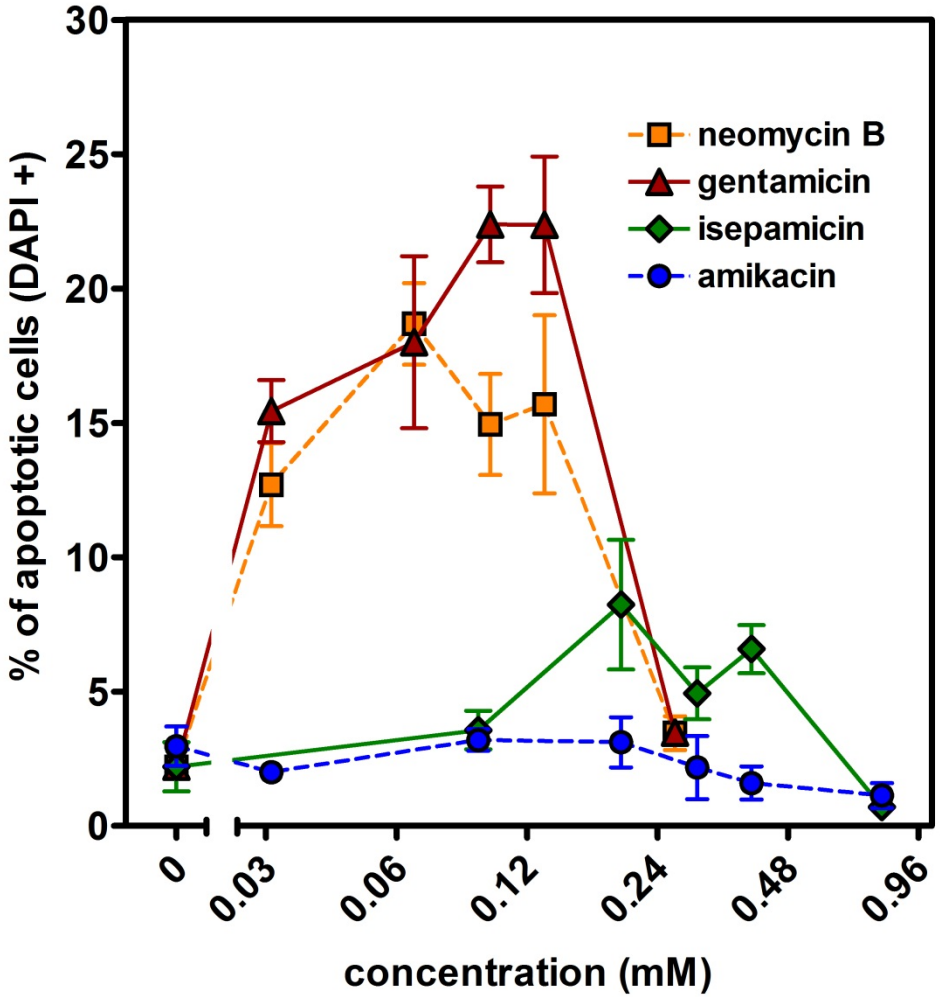
- 2 nephrotoxic aminoglycosides :

  GENTAMICIN and NEOMYCIN B

- 2 less nephrotoxic aminoglycosides :

  AMIKACIN and ISEPAMICIN

DAPI - Electroporated cells



Less nephrotoxic aminoglycosides induce less apoptosis

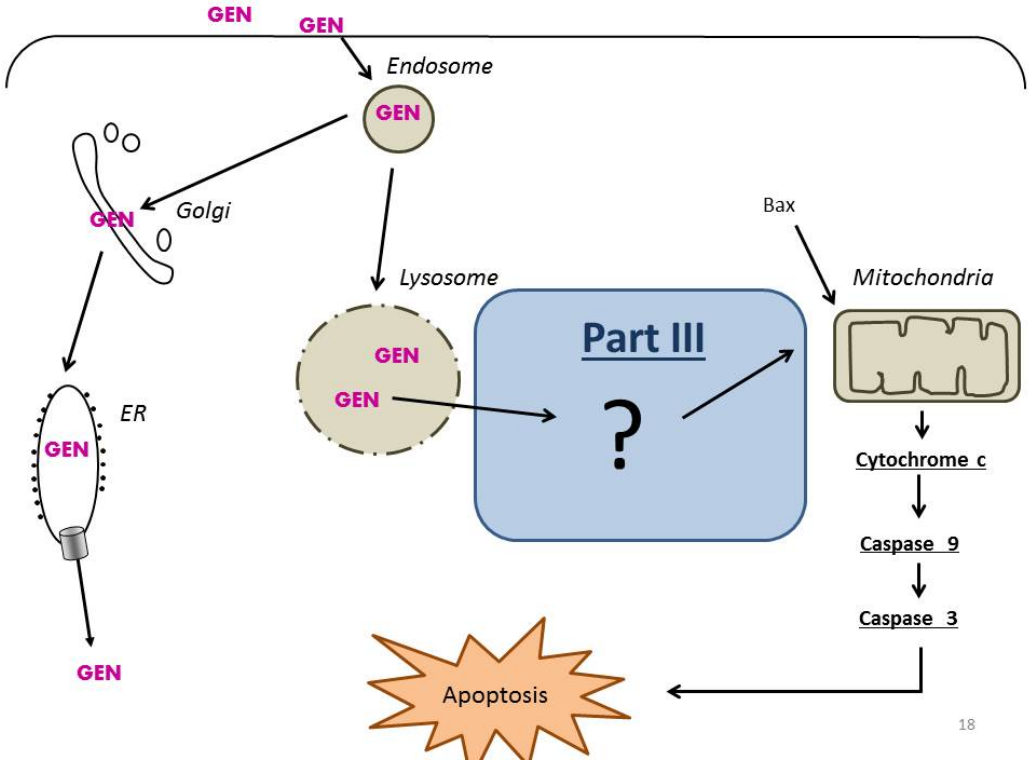
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2008, p. 2236–2238 Vol. 52, No. 6
0066-4804/08/$08.00+0 doi:10.1128/AAC.01680-07

Apoptosis Induced by Aminoglycosides in LLC-PK1 Cells: Comparative Study of Neomycin, Gentamicin, Amikacin, and Isepamicin Using Electroporation▽†

Sophie Denamur, Françoise Van Bambeke, Marie-Paule Mingeot-Leclercq, and Paul M. Tulkens*
Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, B-1200 Brussels, Belgium

Received 30 December 2007/Returned for modification 22 March 2008/Accepted 26 March 2008

- Importance of apoptosis in aminoglycoside-induced nephrotoxicity

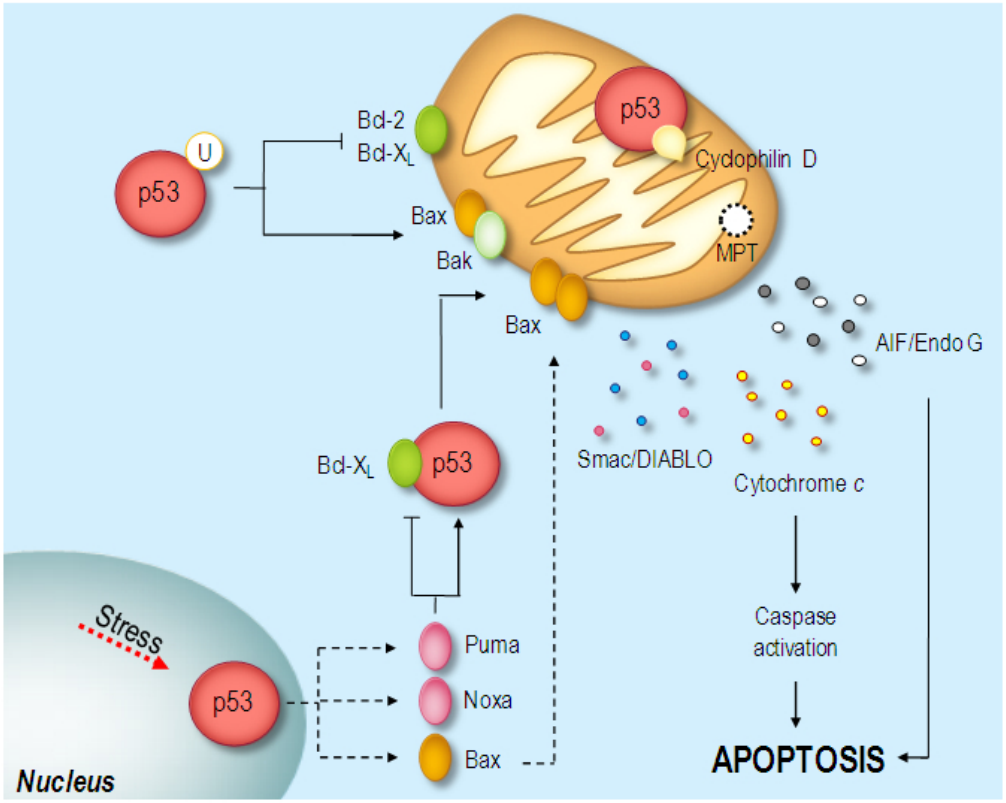


Part III

P53 SIGNALING PATHWAY, PROTEASOME IN GENTAMICIN-INDUCED APOPTOSIS

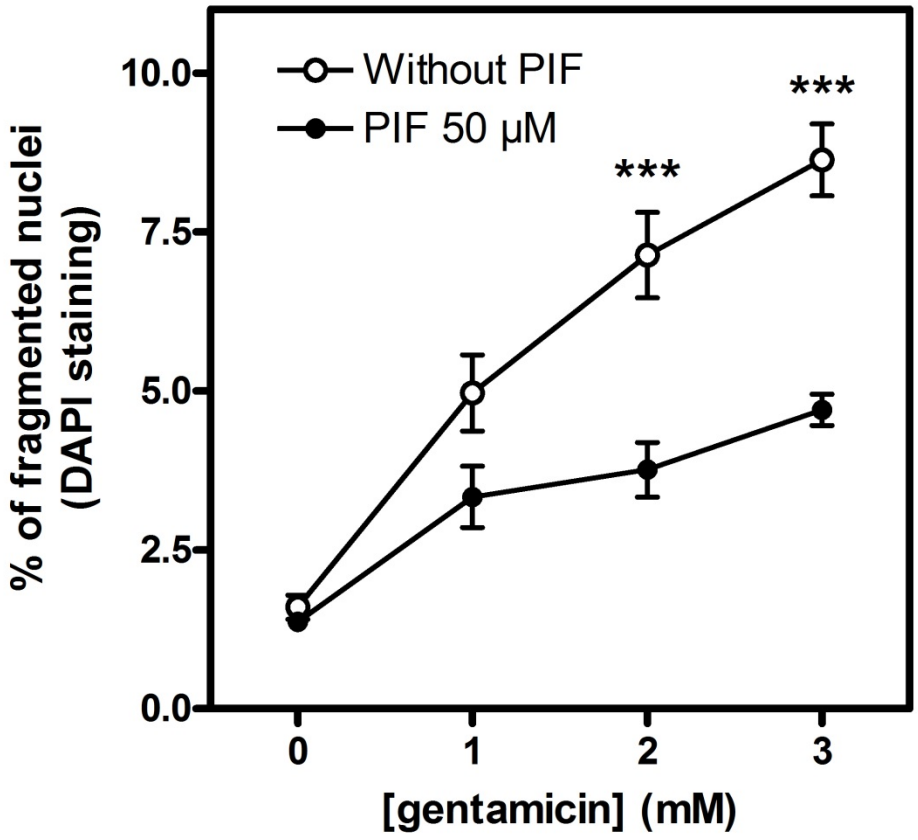
Is p53 signaling pathway implicated in gentamicin-induced apoptosis ?

- p53 is a transcription factor with an important role in apoptosis induction…



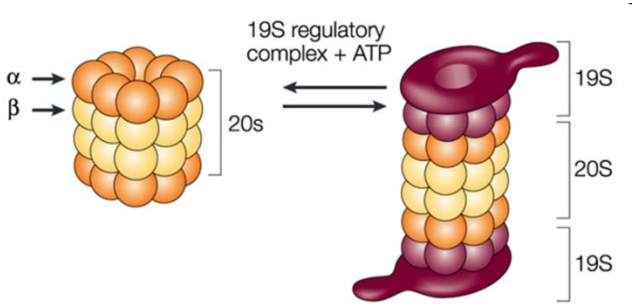
Amaral et al., 2010

Is p53 signaling pathway implicated in gentamicin-induced apoptosis ?



Pifithrin α, a p53 inhibitor, affords a partial protective effect against gentamicin-induced apoptosis

A role of proteasome ?



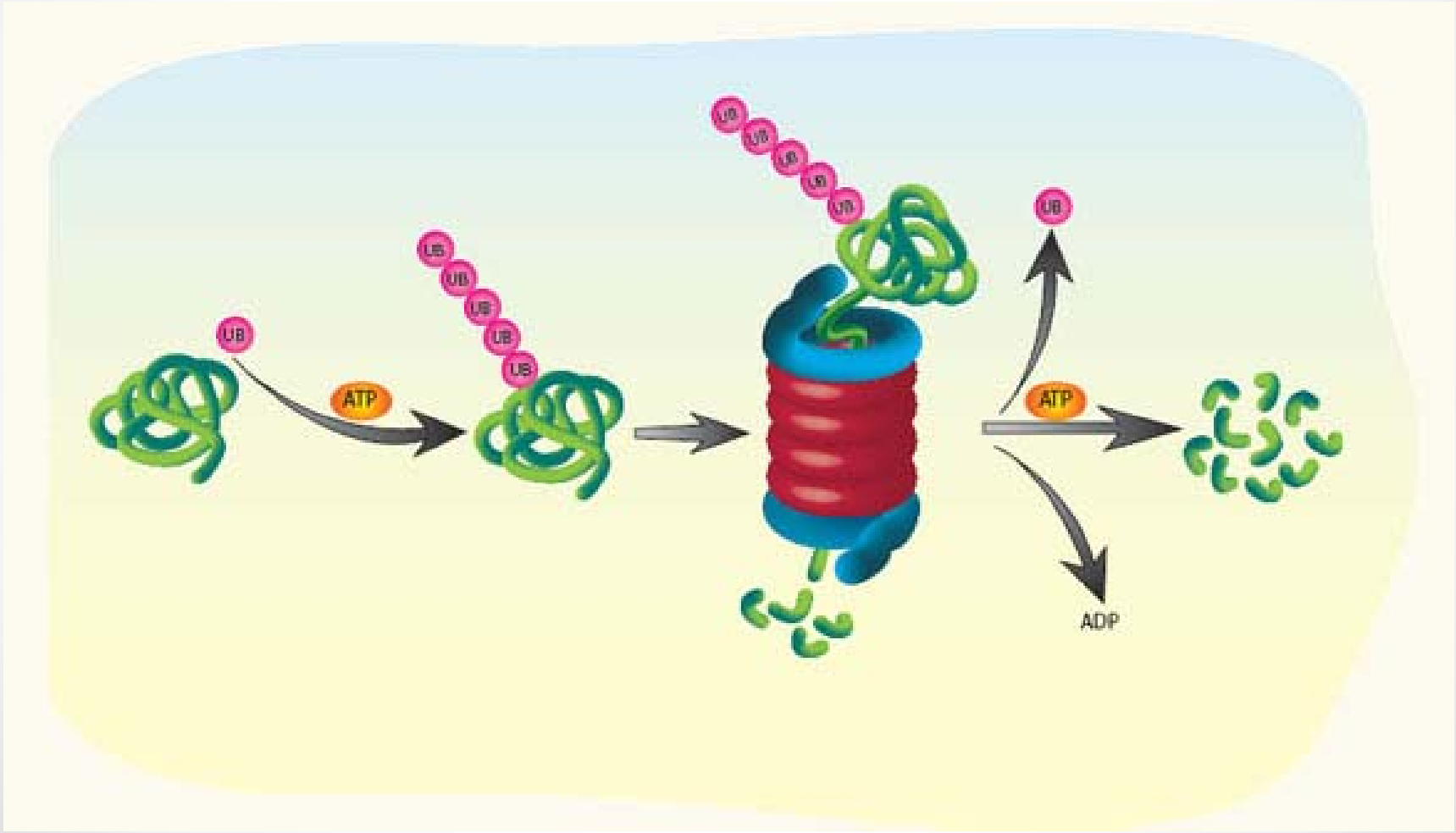
Adams, Nature Reviews Cancer 4 (2004) 349-360

Nature Reviews | Cancer

- Non-lysosomal degradation of proteins, important role in regulation of many cellular processes.

- Large multi-subunit protein complex comprised of a peptide degrading 20S core cylinder capped at both ends by a 19S regulatory cap.
  - β1 : caspase-like activity : cleaves after acidic residues
  - β2 : trypsin-like activity : cleaves after basic residues
  - β5 : chymotrypsin-like activity : cleaves after hydrophobic residues

Ubiquitin-proteasome system



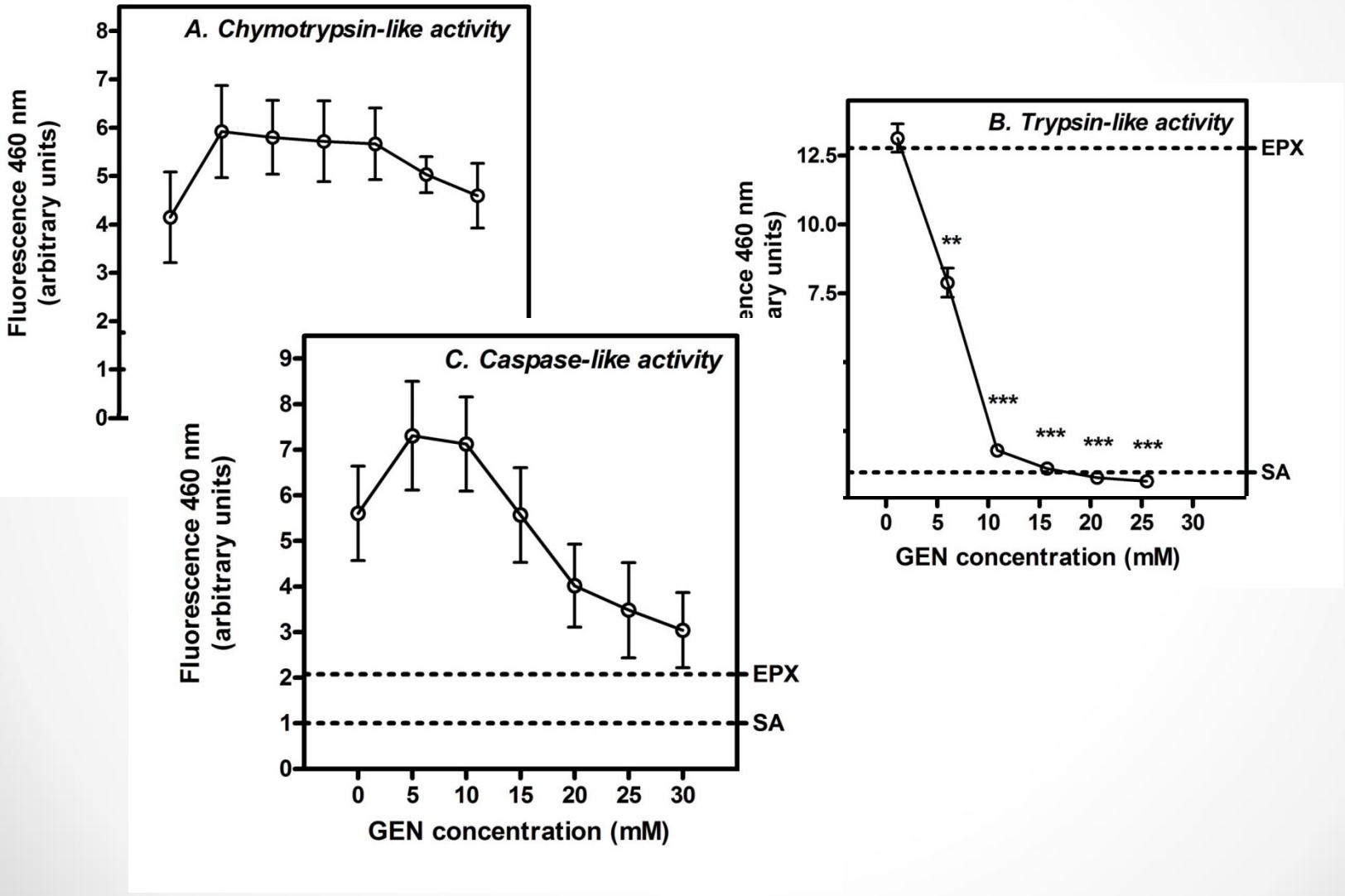
Inhibition of proteasome by gentamicin?

- Increase in the cell content of pro-apoptotic Bax protein and Ub-Bax after incubation with GEN (Servais et al., 2006)

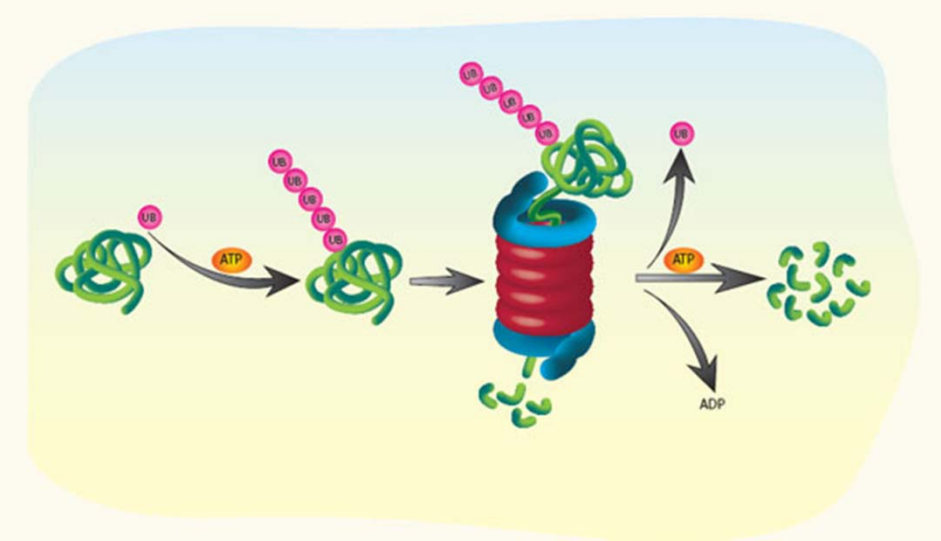
- Binding of GEN to β9-subunit of proteasome (Horibe et al., 2004)

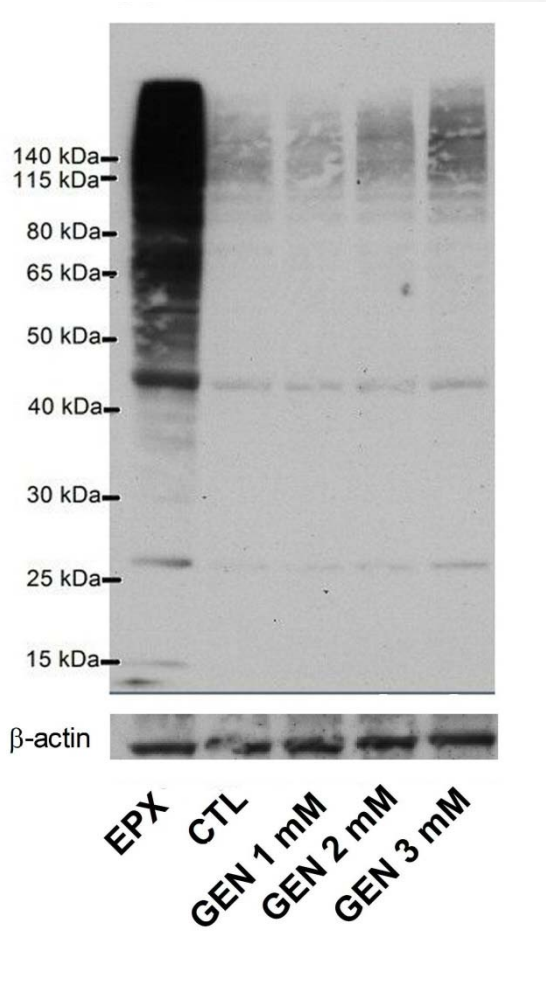
⇒ Does gentamicin inhibit the proteasome ?

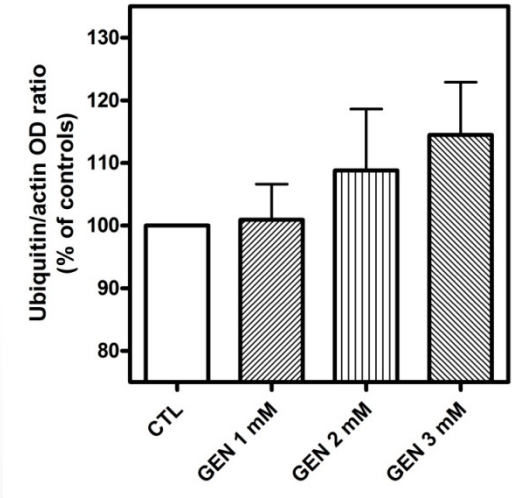
Effect of gentamicin on proteasome catalytic activities in cellular lysates



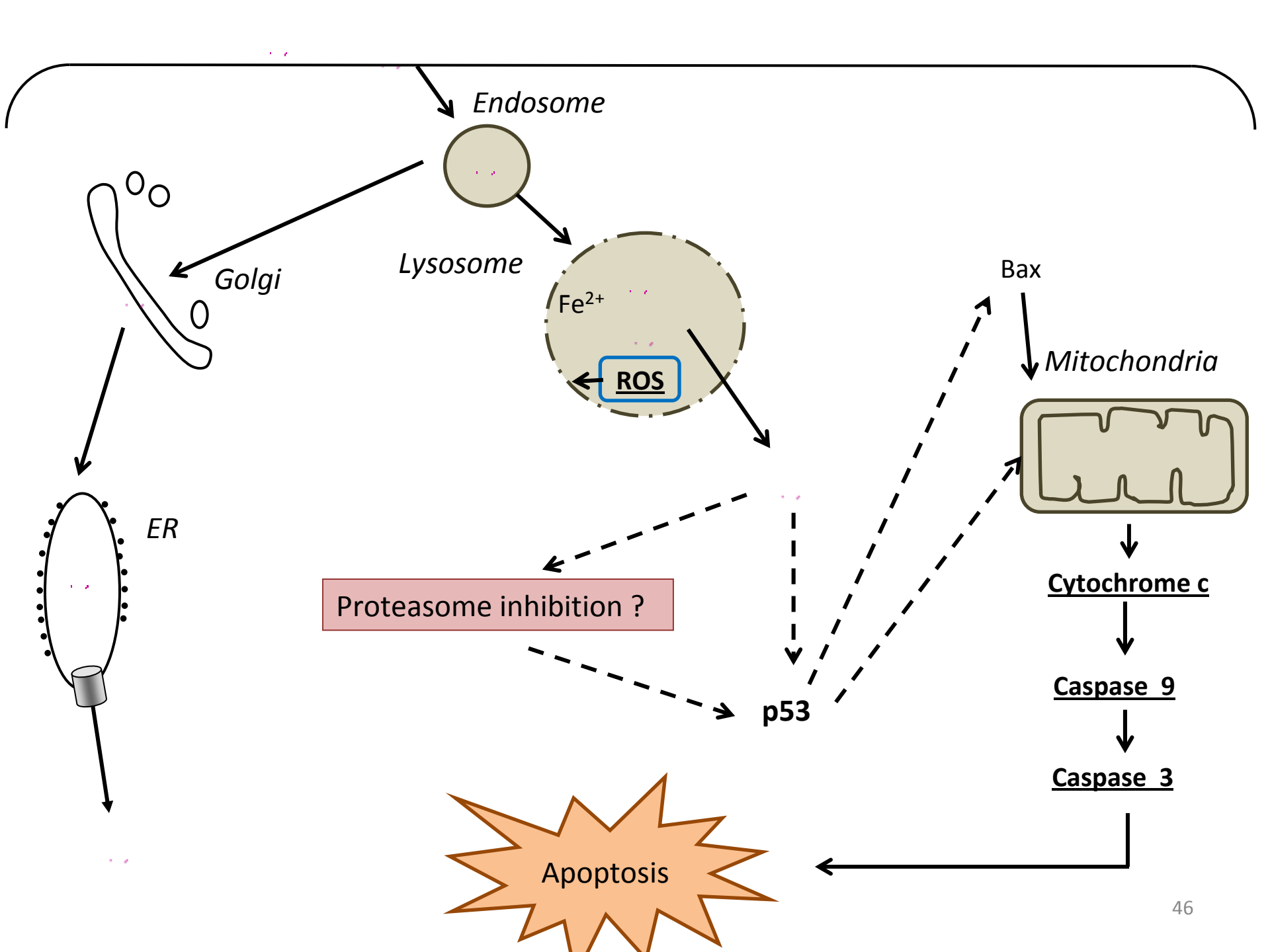
Impact on ubiquitinated proteins degradation





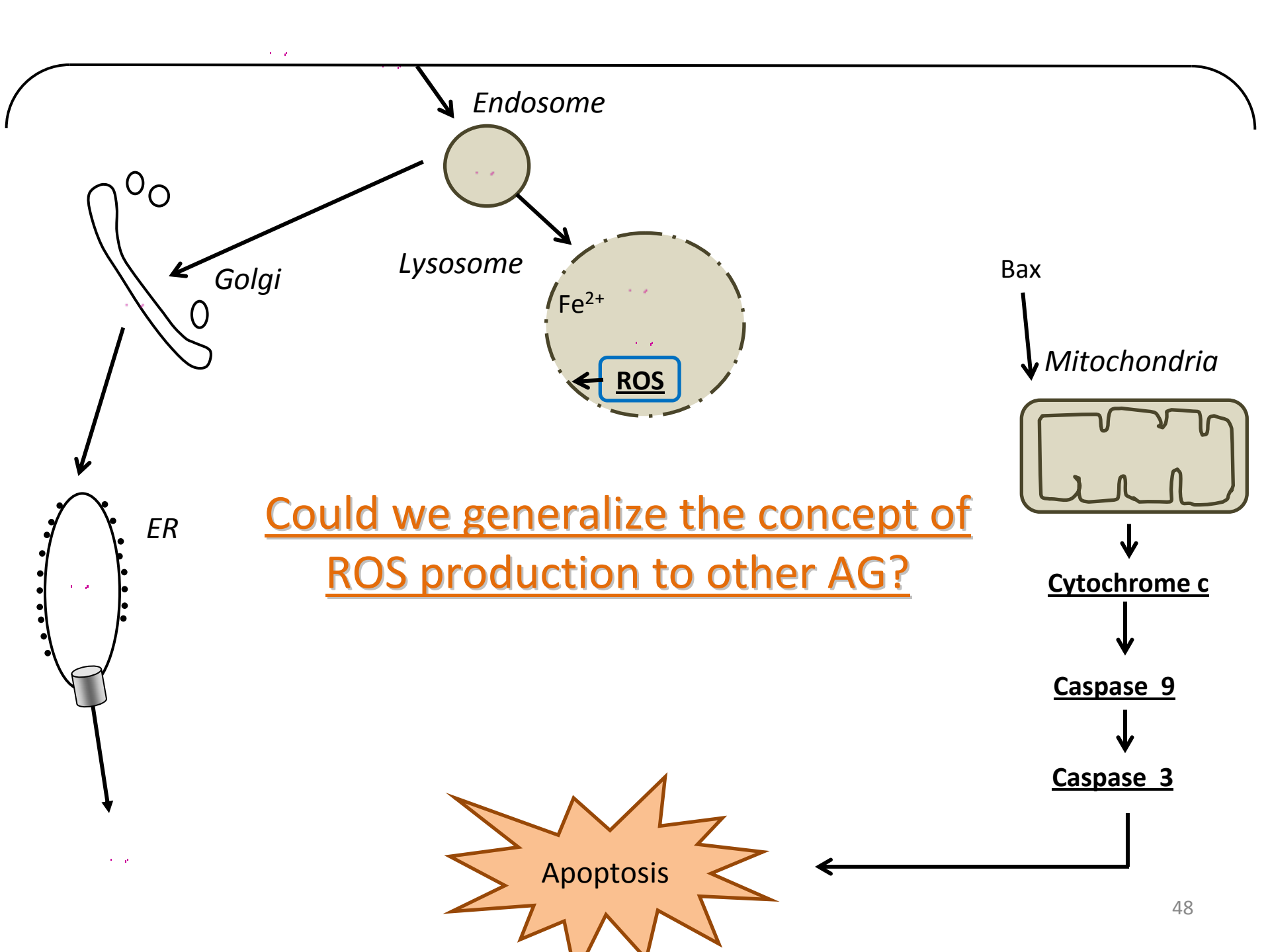


Conclusions

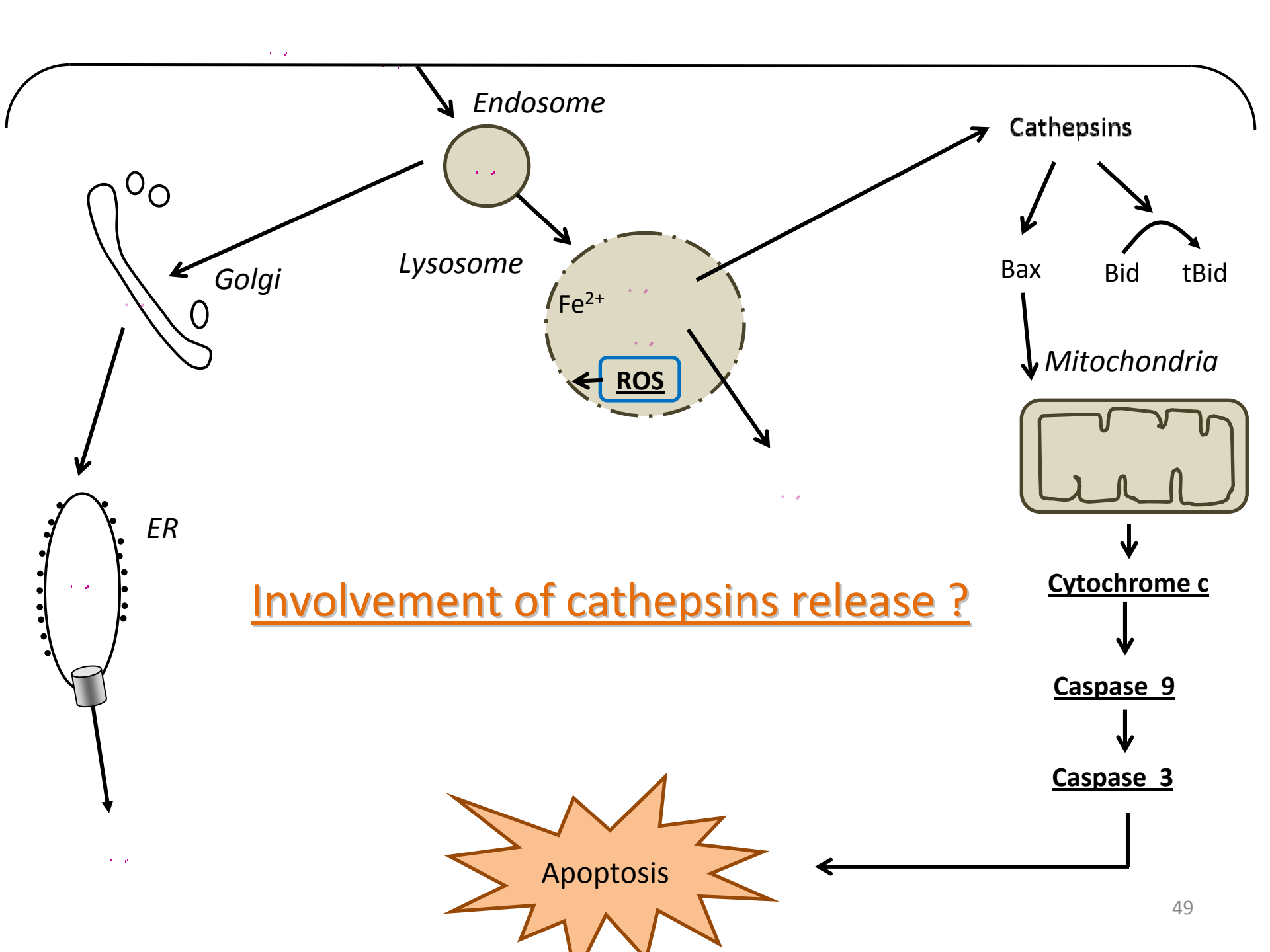

Endosome
Golgi
Lysosome
Fe2+
ROS
Bax
Mitochondria
ER
Proteasome inhibition ?
p53
Cytochrome c
Caspase 9
Caspase 3
Apoptosis

Perspectives

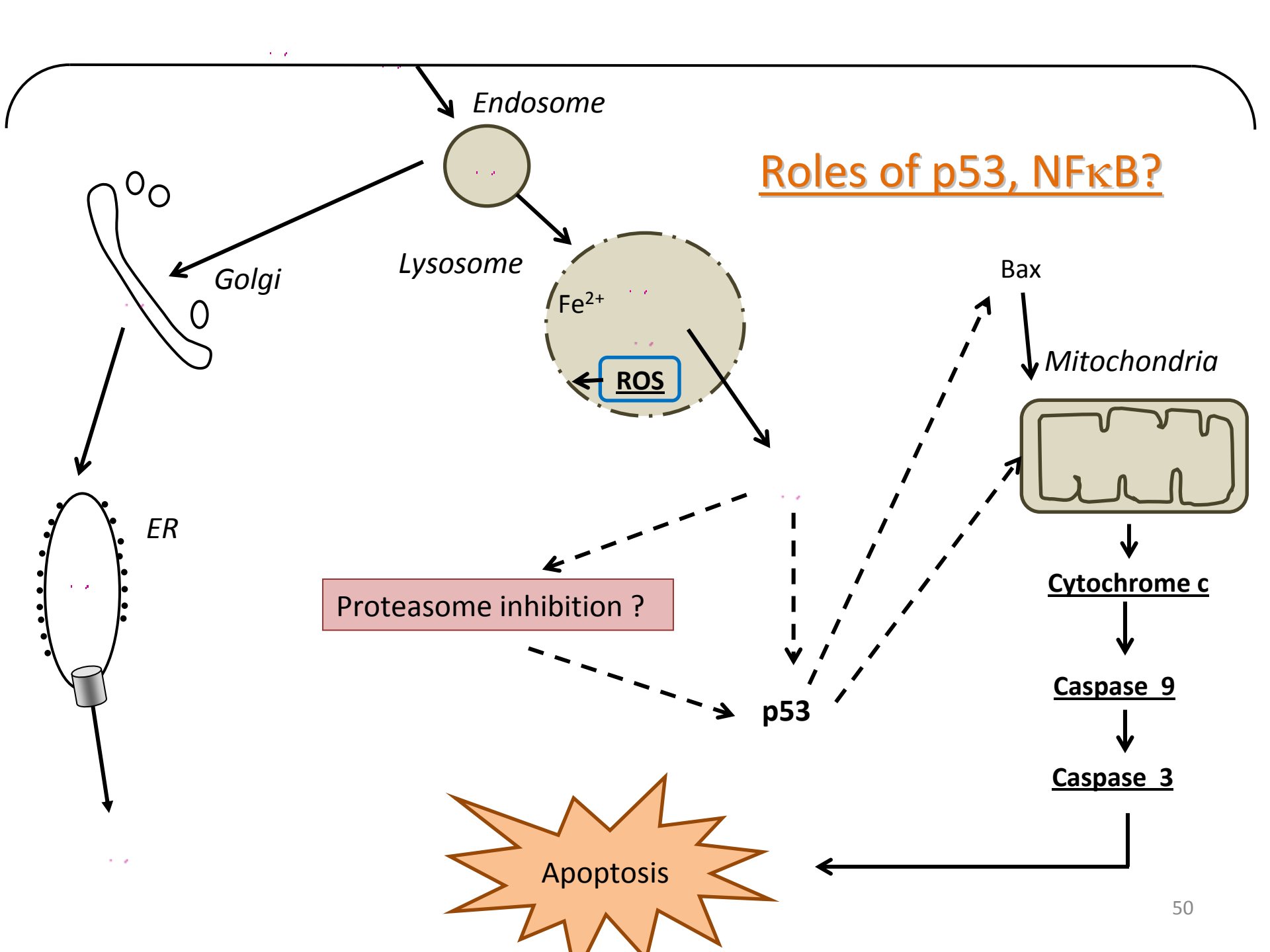
...



Could we generalize the concept of ROS production to other AG?



Involvement of cathepsins release ?


Endosome
Roles of p53, NFκB?
Golgi
Lysosome
Fe2+
ROS
Bax
Mitochondria
ER
Proteasome inhibition ?
p53
Cytochrome c
Caspase 9
Caspase 3
Apoptosis

Nephrotoxicity of aminoglycosides

Thank you for your attention

Acknowledgments

- Pr. V. Préat, B. Gallez, Pr., Pr. P. Courtoy, Pr. P. Buc Calderon, Pr. M. De Broe
- Pr. K. Kagedal, Pr. J.M. Lopez-Novoa, Dr. H. Appelqvist
- Pr. M-P. Mingeot, Pr. P.M. Tulkens
- Dr. D. Tyteca, Pr. P. Courtoy
- M-C. Cambier, M. Vergauwen
- V. Rucchin, K-A. Delongie, Q. Willot, L. Boland
- All the new and old FACM members
- Assistants
- Family