Mitochondria in apoptosis

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The dual role of mitochondria in life and death

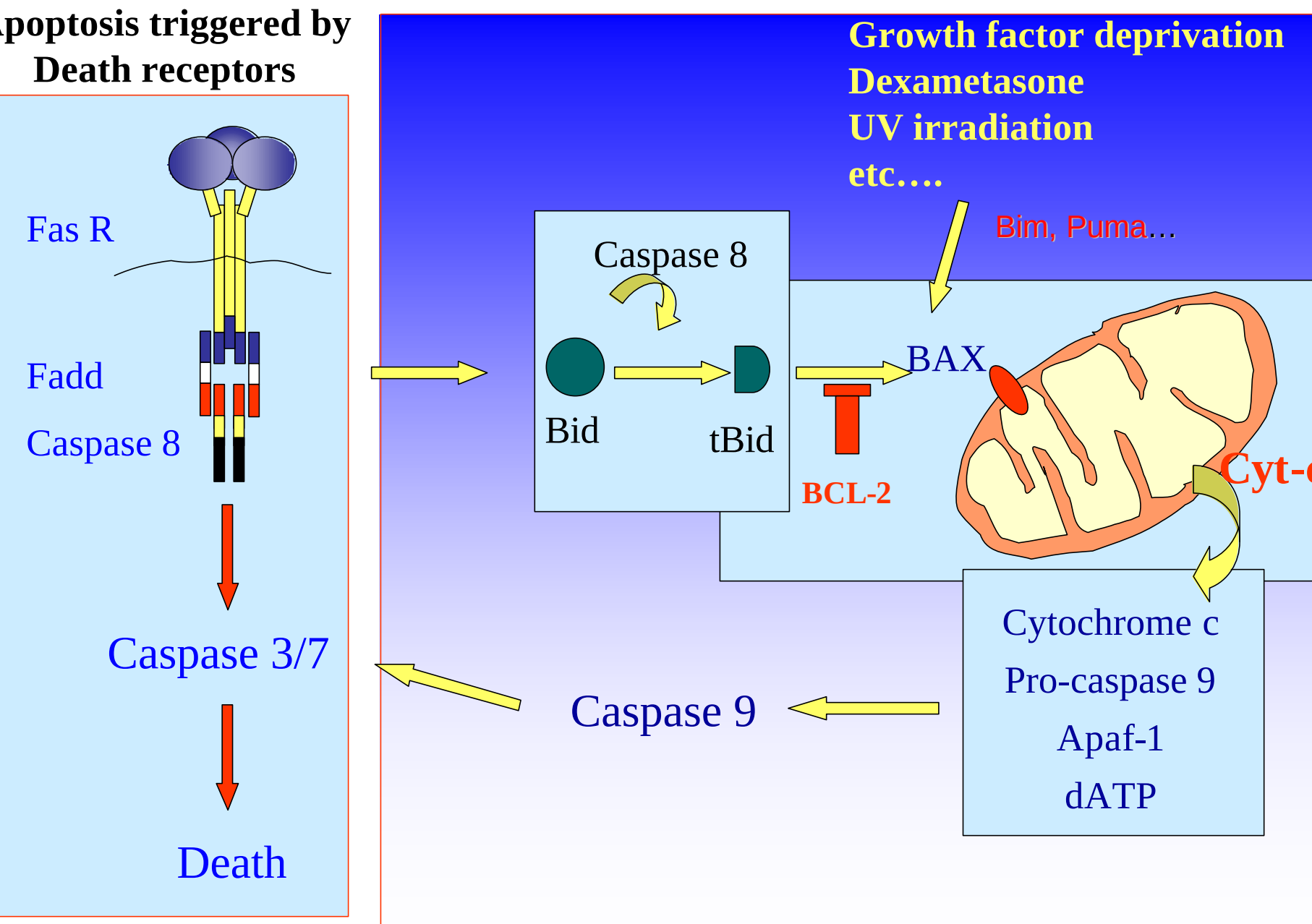
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

1) Oxydative phosphorylation
ATP production

2) Apoptosis

Frey and Mannella, Trends Biochem. Sci. 2000, 25:319-324

Signaling pathways



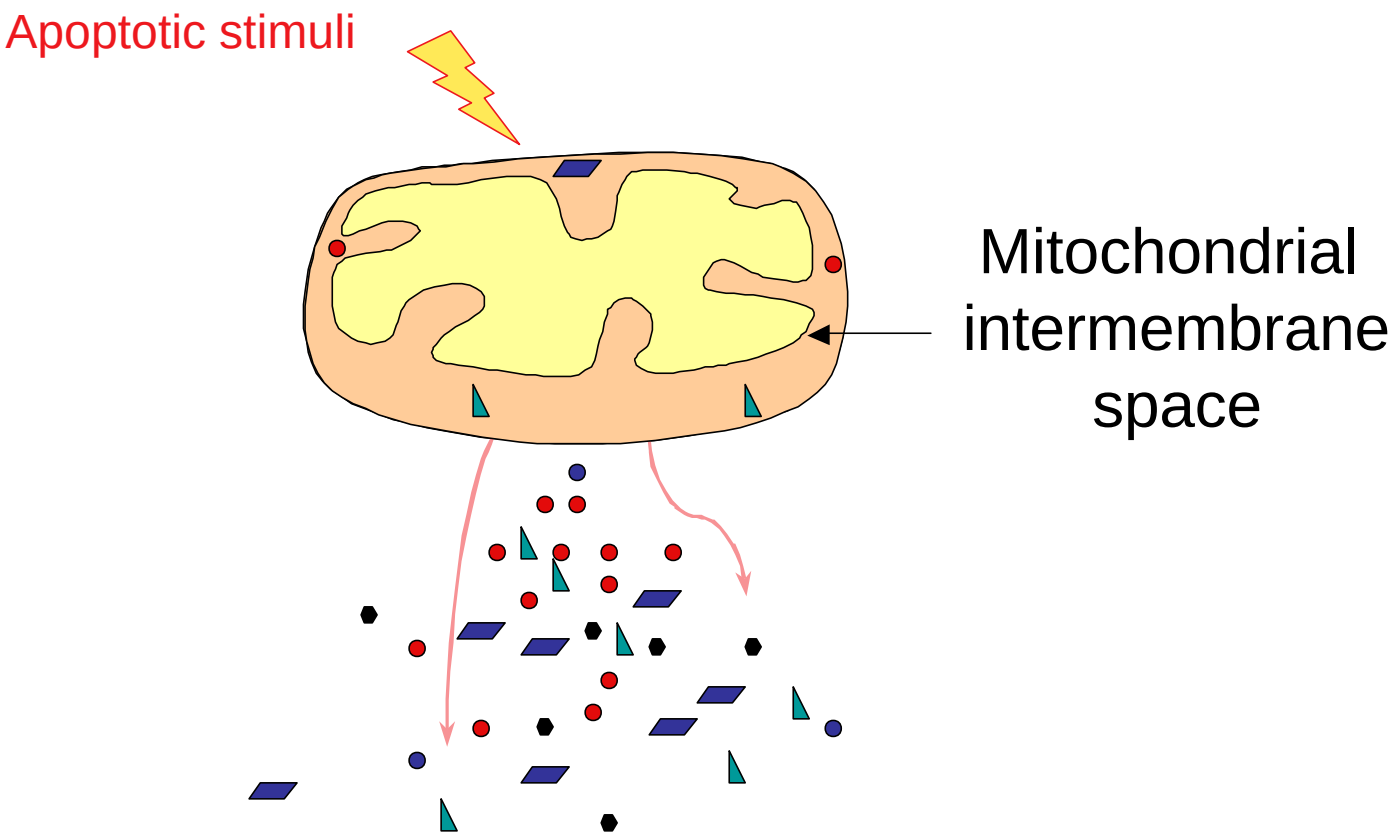
Early observations on the role of mitochondria in cell death

- Suppression of oxidative phosphorylation in radiation-induced cell death (Ashwell & Hickman, 1952)
- Suppression of oxidative phosphorylation is associated with appearance of pyknotic nuclei (van Bekkum *et al*,.1964)
- Impairment of mitochondrial electron transport is linked to controlled release of mitochondrial cytochrome *c* in radiosensitive tissues ('cytochrome *c* effect') (Scaife, 1964)
- Release of cytochrome *c* in radiosensitive tissues precedes the appearance of pyknotic bodies (Hanson *et al.,* 1965)
- Exogenous cytochrome *c* is able to restore oxidative phosphorylation in mitochondria isolated from radiosensitive tissues (van Bekkum *et al.,* 1967; Hanson *et al.,* 1969)

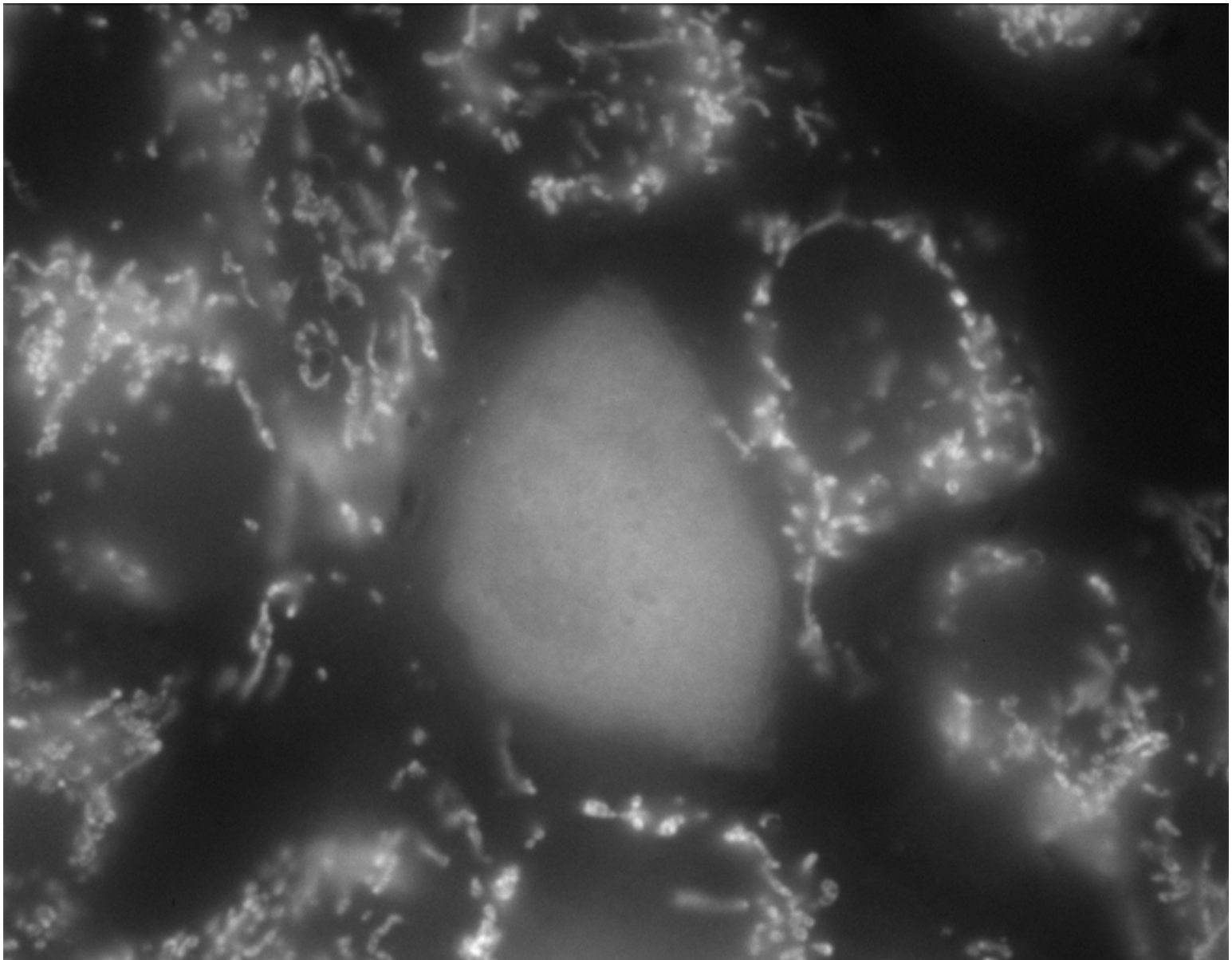
Early observations on the role of mitochondria in cell death

- Hockenbery DM, Oltvai ZN, Yin XM, Milliman CL, Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis.Cell. 1993 Oct 22;75(2):241-51.
- Hockenbery D, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature. 1990 Nov 22;348(6299):334-6.
- Hennet T, Bertoni G, Richter C, Peterhans E. Expression of BCL-2 protein enhances the survival of mouse fibrosarcoid cells in tumor necrosis factor-mediated cytotoxicity.Cancer Res. 1993 Mar 15;53(6):1456-60.
- Newmeyer DD, Farschon DM, Reed JC. Cell-free apoptosis in Xenopus egg extracts: inhibition by Bcl-2 and requirement for an organelle fraction enriched in mitochondria.Cell. 1994 Oct 21;79(2):353-64.
- Susin SA, Zamzami N, Castedo M, Hirsch T, Marchetti P, Macho A, Daugas E, Geuskens M, Kroemer G. Bcl-2 inhibits the mitochondrial release of an apoptogenic protease.J Exp Med. 1996 Oct 1;184(4):1331-41.
- Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c.Cell. 1996 Jul 12;86(1):147-57.

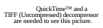
Mitochondria: the Pandora's box



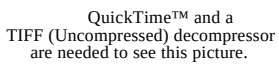
Release of cytochrome c during apoptosis



Analysis of cytochrome c release by Western blot

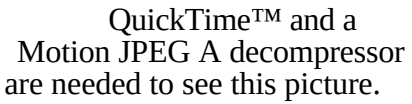


The release of cytochrome c precedes the loss of mitochondrial membrane potential

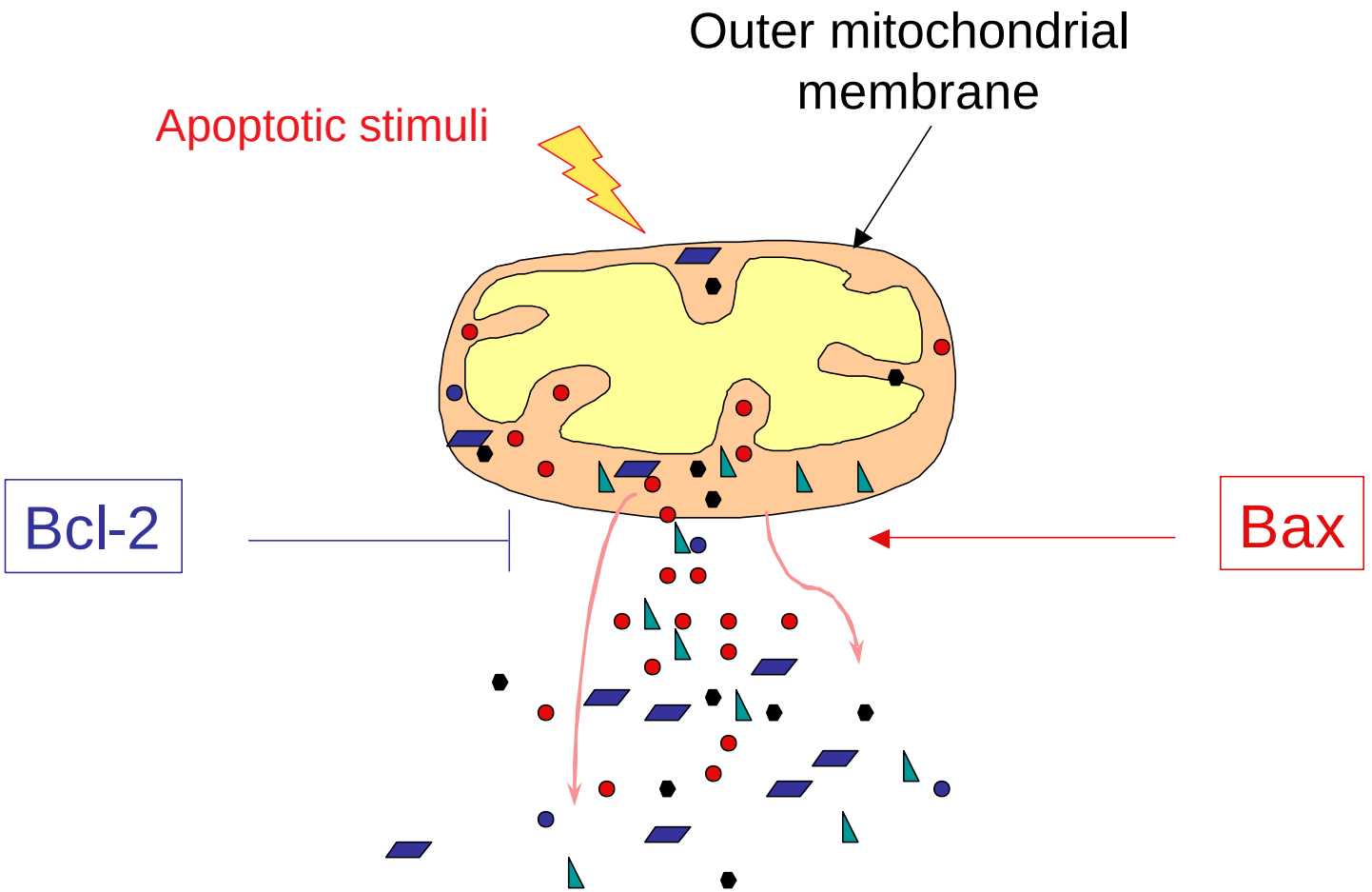


Goldstein et al. CDD. 2005

Release of cytochrome c



How does Pandora's box open?



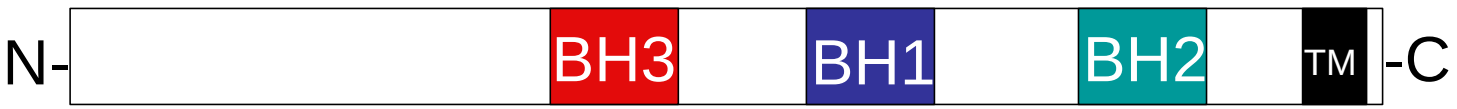
Bcl-2 family

Antiapoptotic proteins

Bcl-2
Bcl-xL
Bcl-w
Mcl-1
Etc…

Pro-Apoptotic proteins

Bax-like

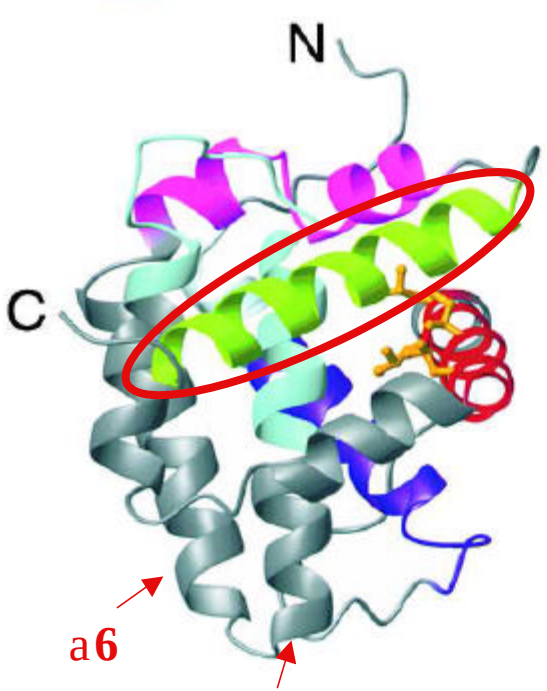


Bax
Bak
Bok

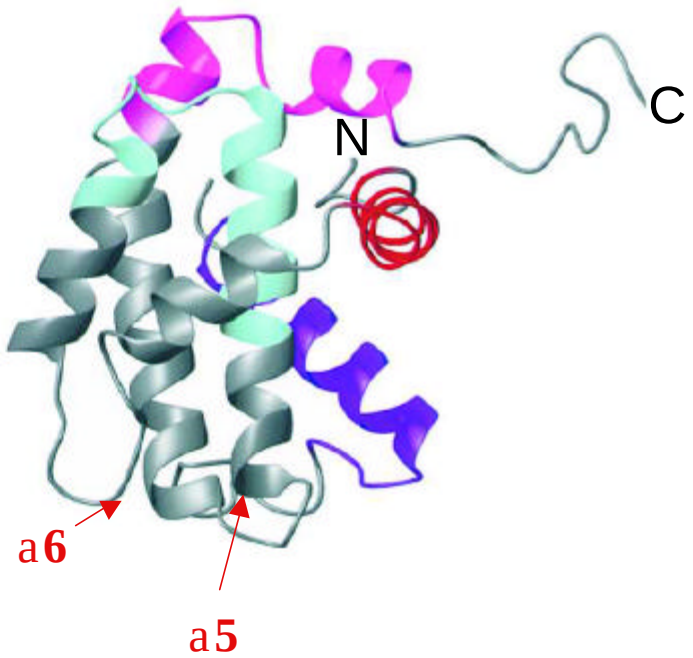
BH3-only proteins

Bid
Bad
Bim
Noxa
Puma
Etc…

Three dimensional structure



Bax



Bcl-x_L

An hydrophobic pocket for interactions with BH3 domains

QuickTime™ and a
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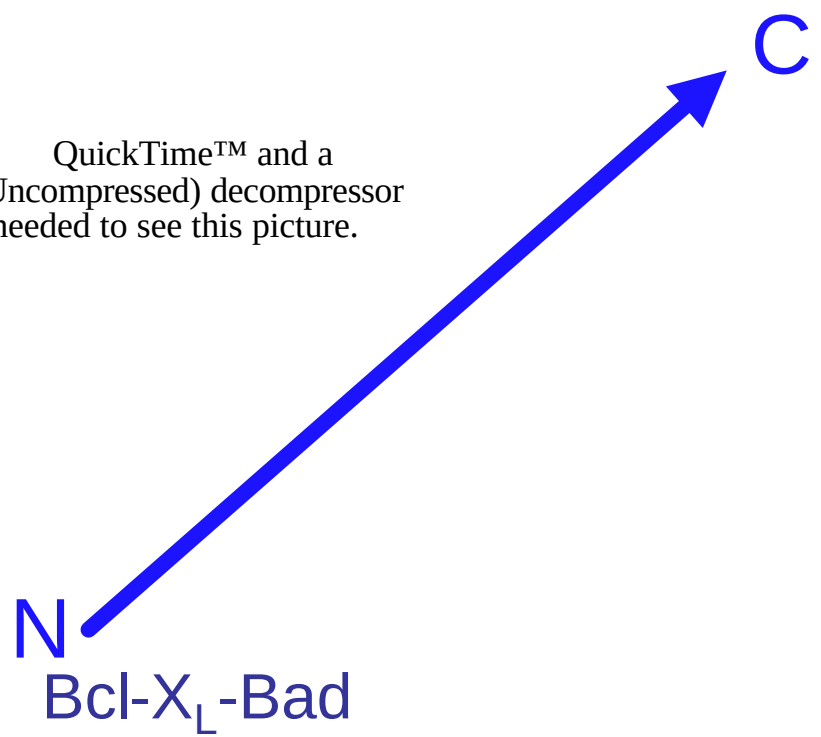
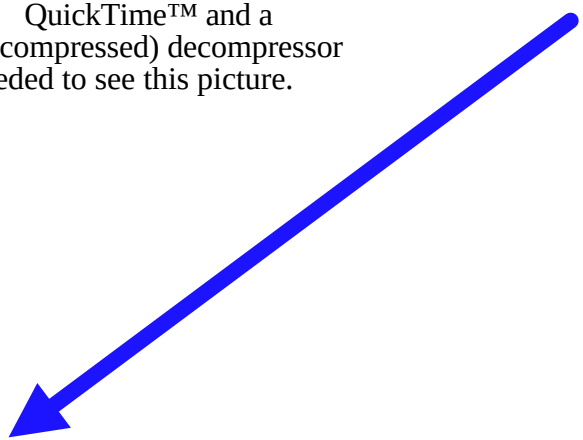
are needed to see this picture.

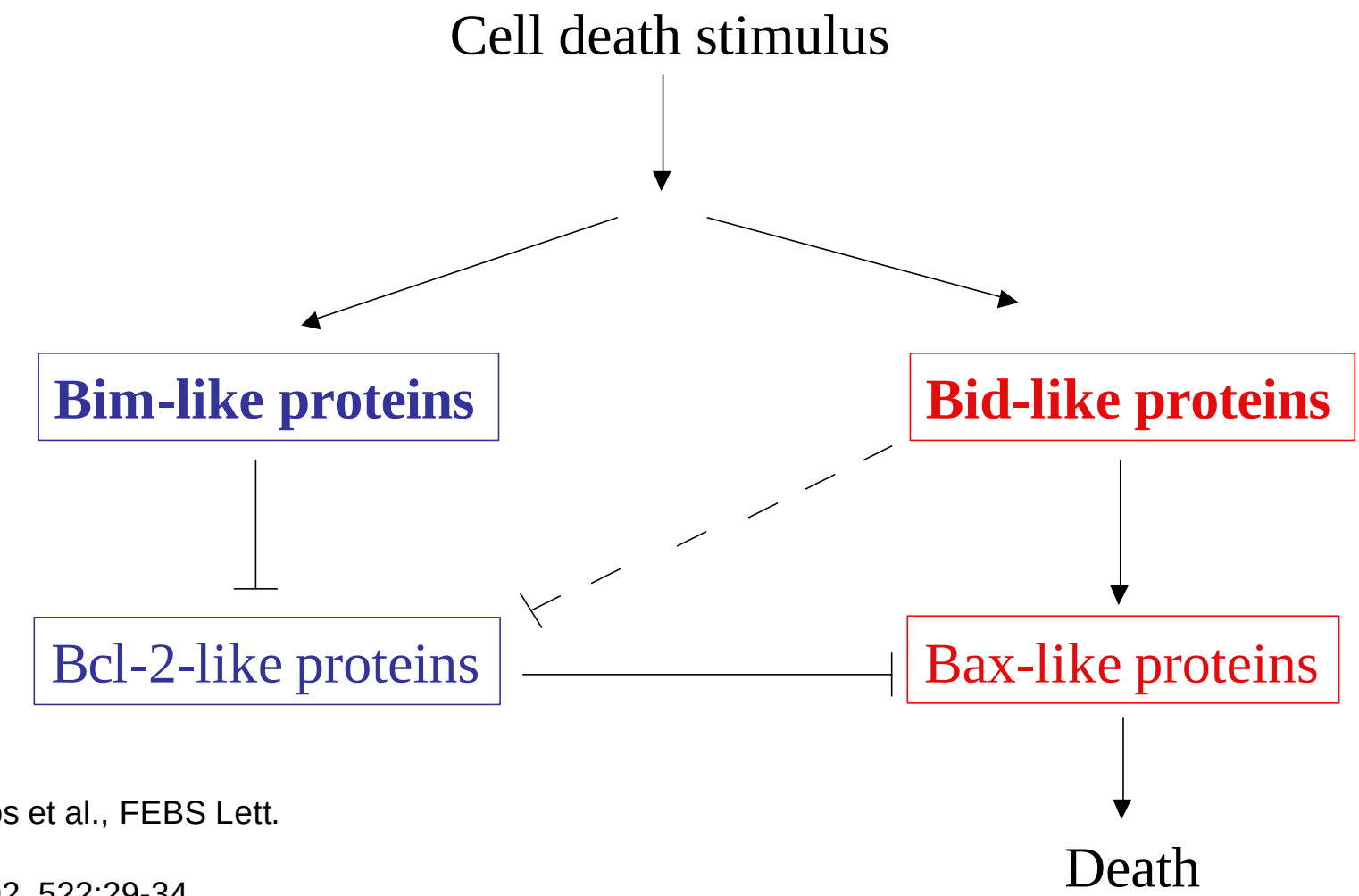


In Bax the hydrophobic pocket is occupied by the TM domain

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



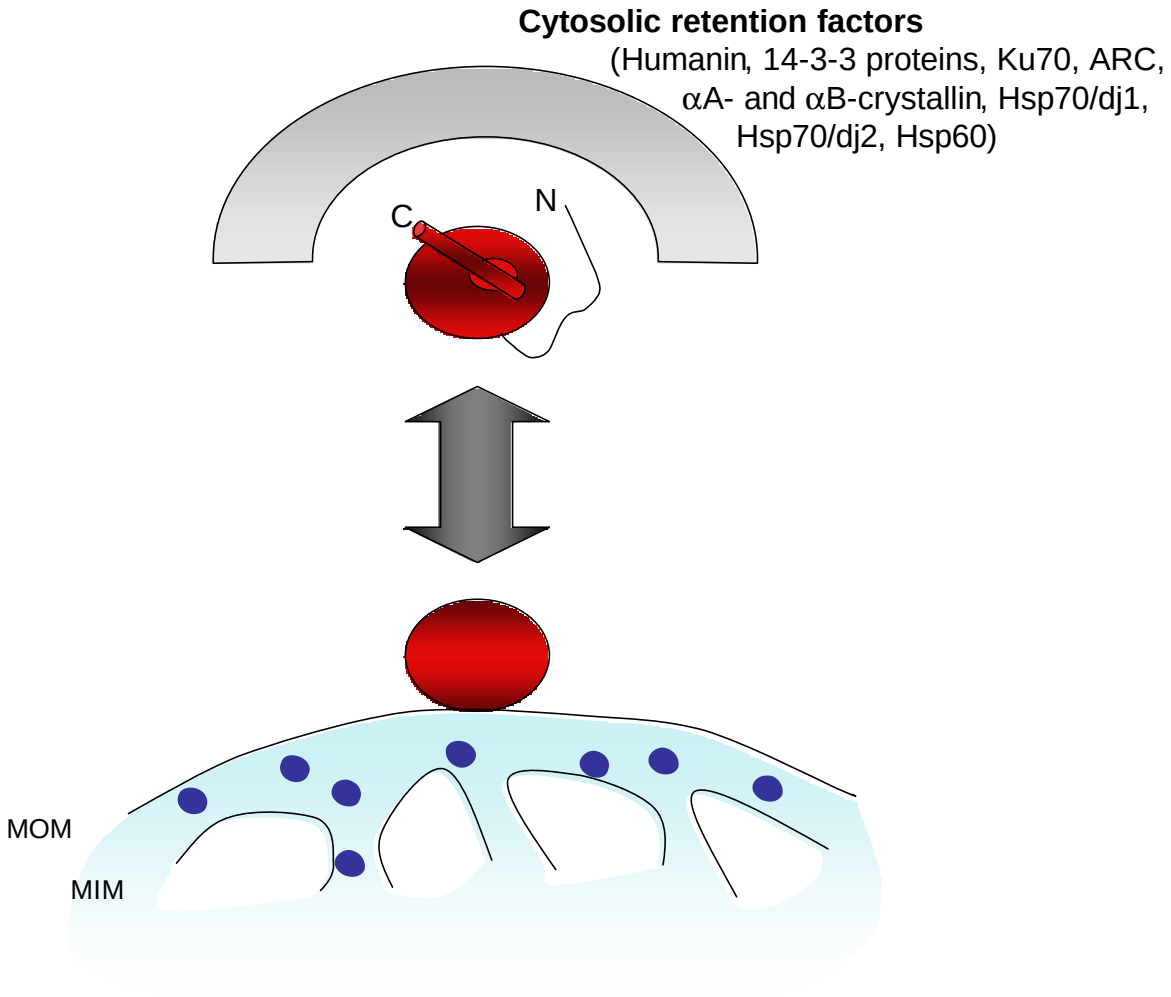
Two functional classes of BH3-only proteins



Terradillos et al., FEBS Lett.

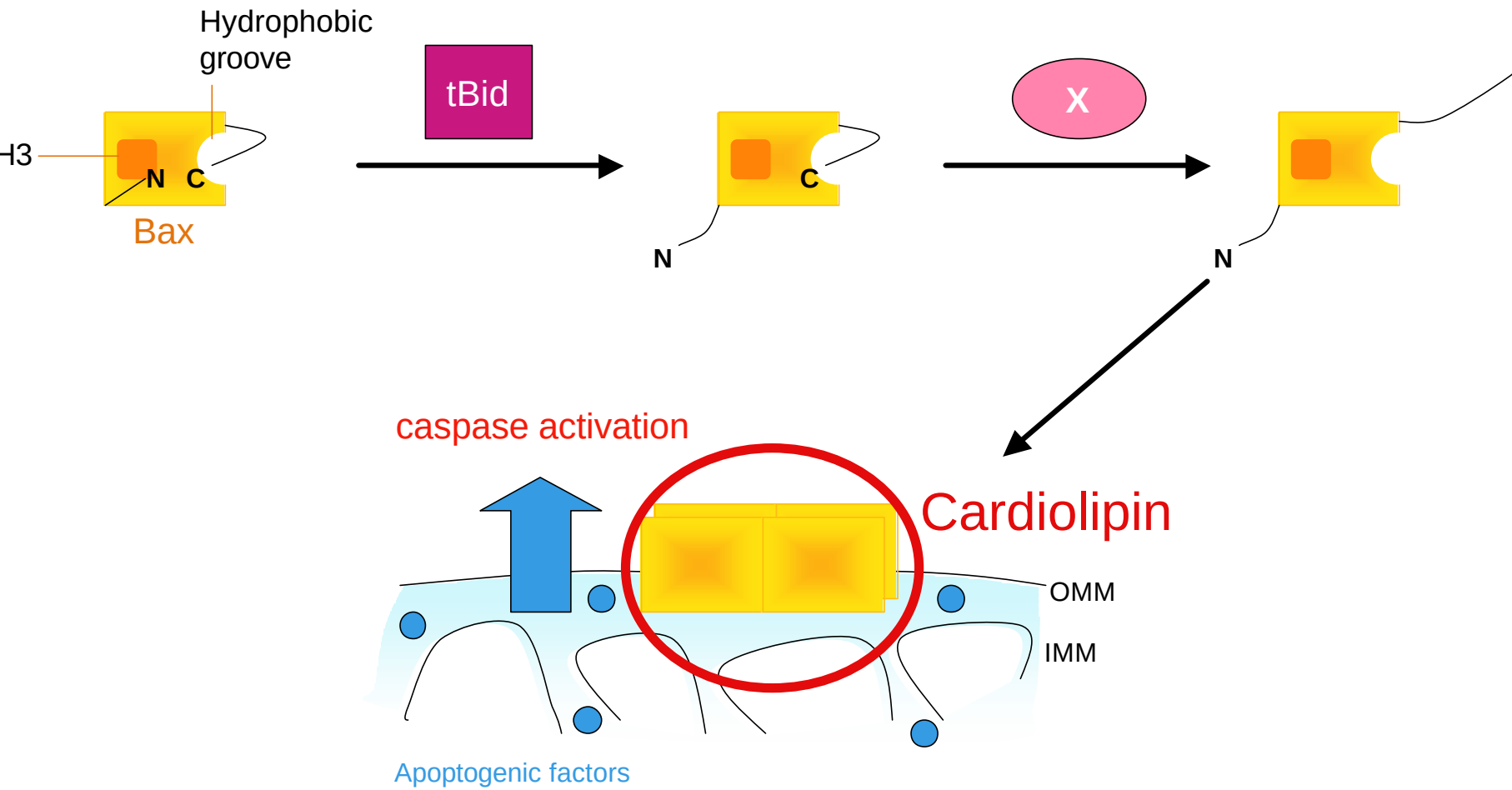
2002, 522:29-34.

Bax under resting conditions



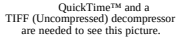
Lucken-Ardjomande and Martinou,
J Cell Sci. 2005, 118(Pt 3):473-83

Bax during apoptosis

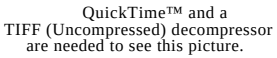


Lucken-Ardjomande and Martinou, C R Biol. 2005 Jul;328(:616-3

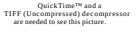
Exposure of N-terminal region



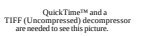
Bax insertion into membranes: resistance to alkali treatment



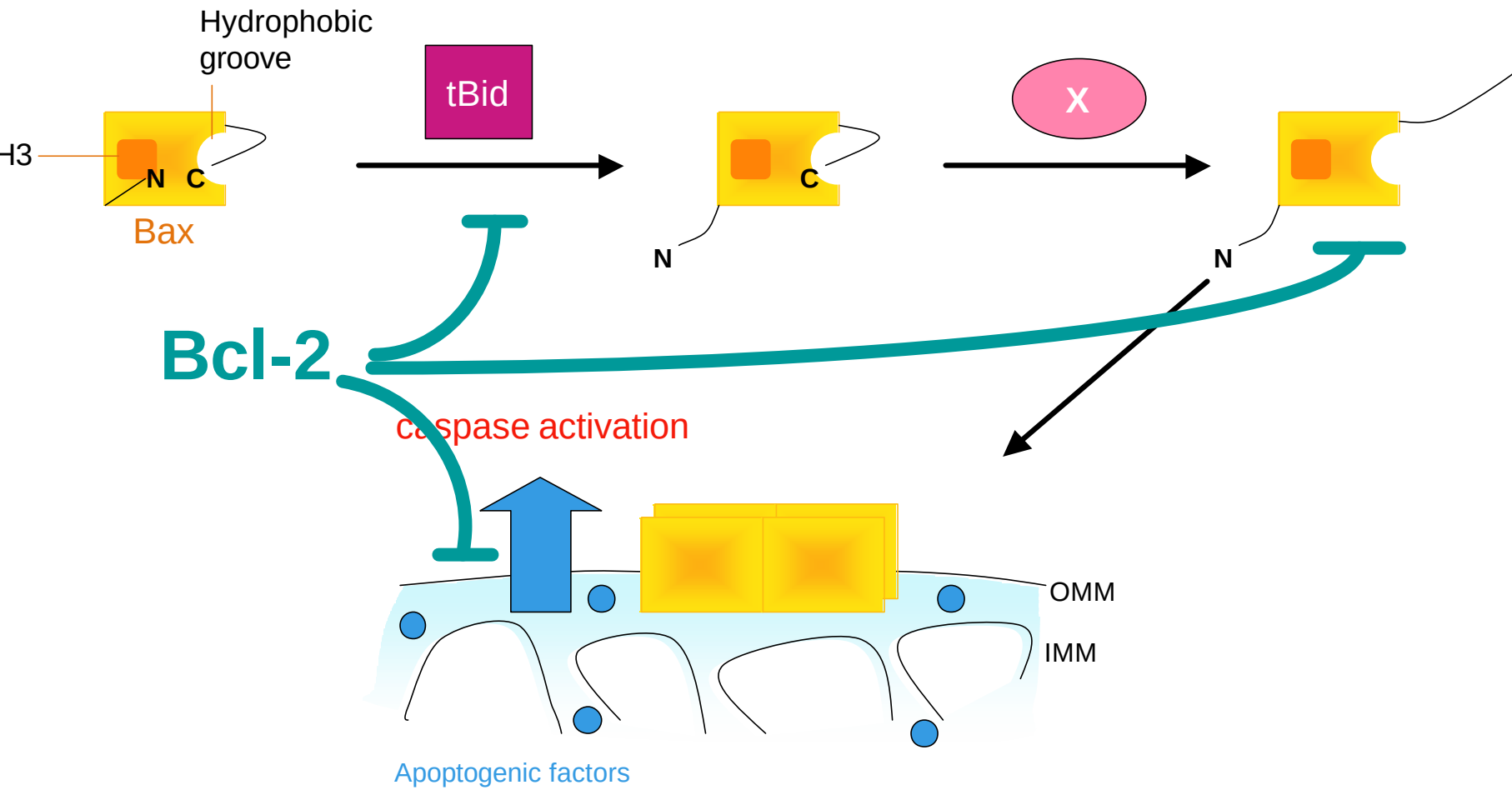
Gel filtration analysis of Bax complexes



Bax oligomers: the use of cross linkers



How do antiapoptotic proteins protect?



Conclusion 1

1) Bax, when oligomerized, permeabilizes the outer mitochondrial membrane. Irreversible except in neurons

2) Bax requires BH3-only proteins of the Bid-like subgroup

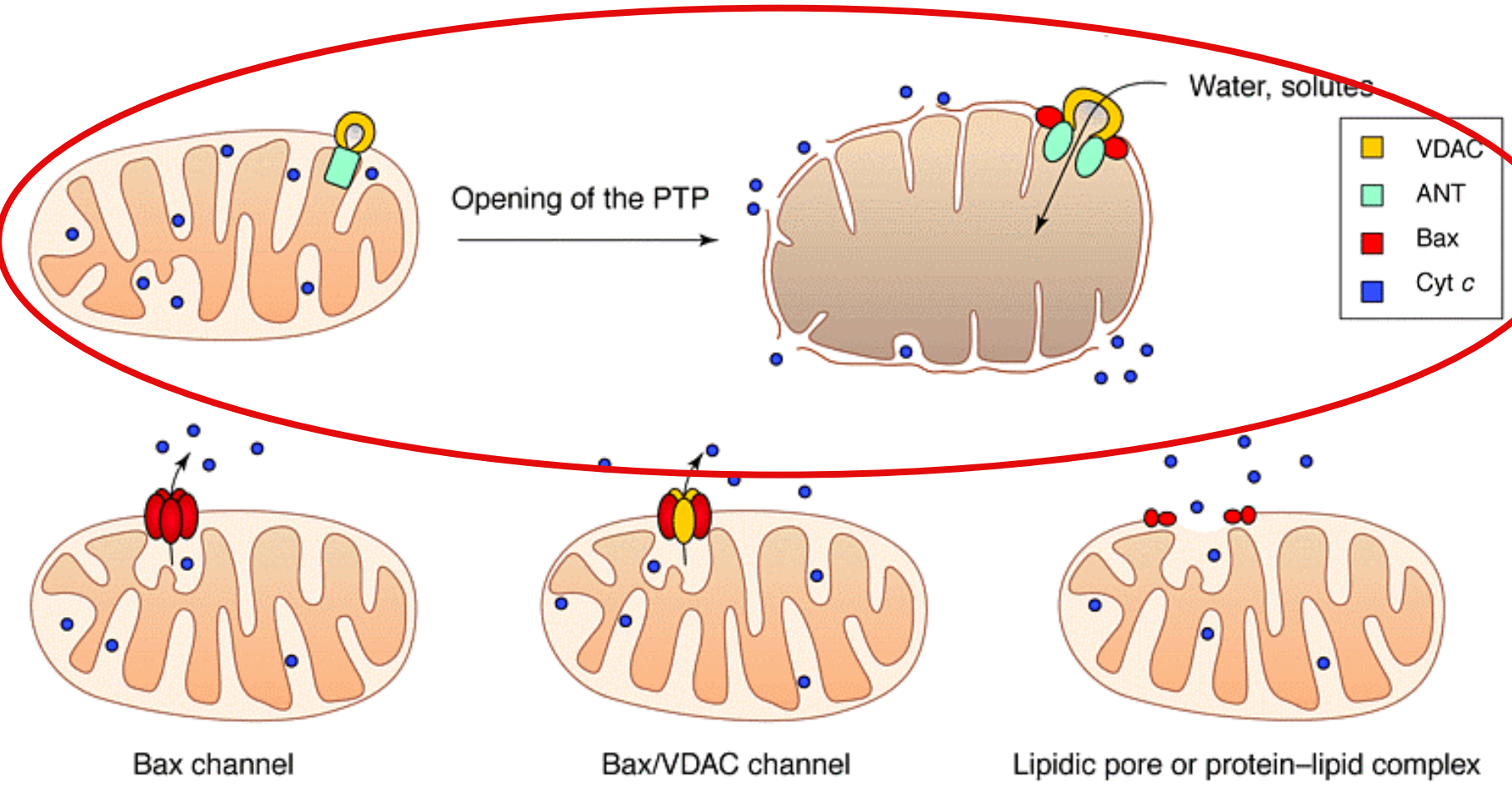
3) Specific phospholipids such as cardiolipin and other proteins (as yet unknown) are also required for Bax oligomerization

Two fundamental questions

- What are the mechanisms responsible for Bax/Bak activation?

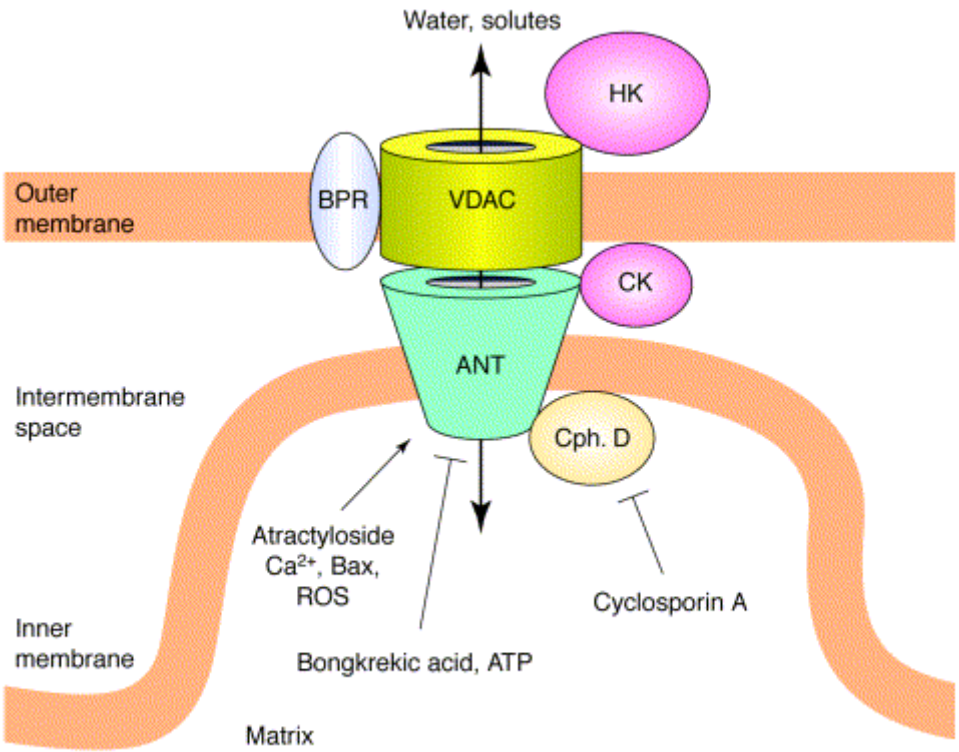
- By which mechanisms Bax like proteins render the outer mitochondrial membrane permeable?

Models for the release of cytochrome c



Desagher and Martinou,

The permeability transition pore



Swelling of mitochondria induced by opening of PTP

Ctrl

+Ca^{++}

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

+Bax

Opening of the PTP into question

1) PT opening can form in the absence of ANT

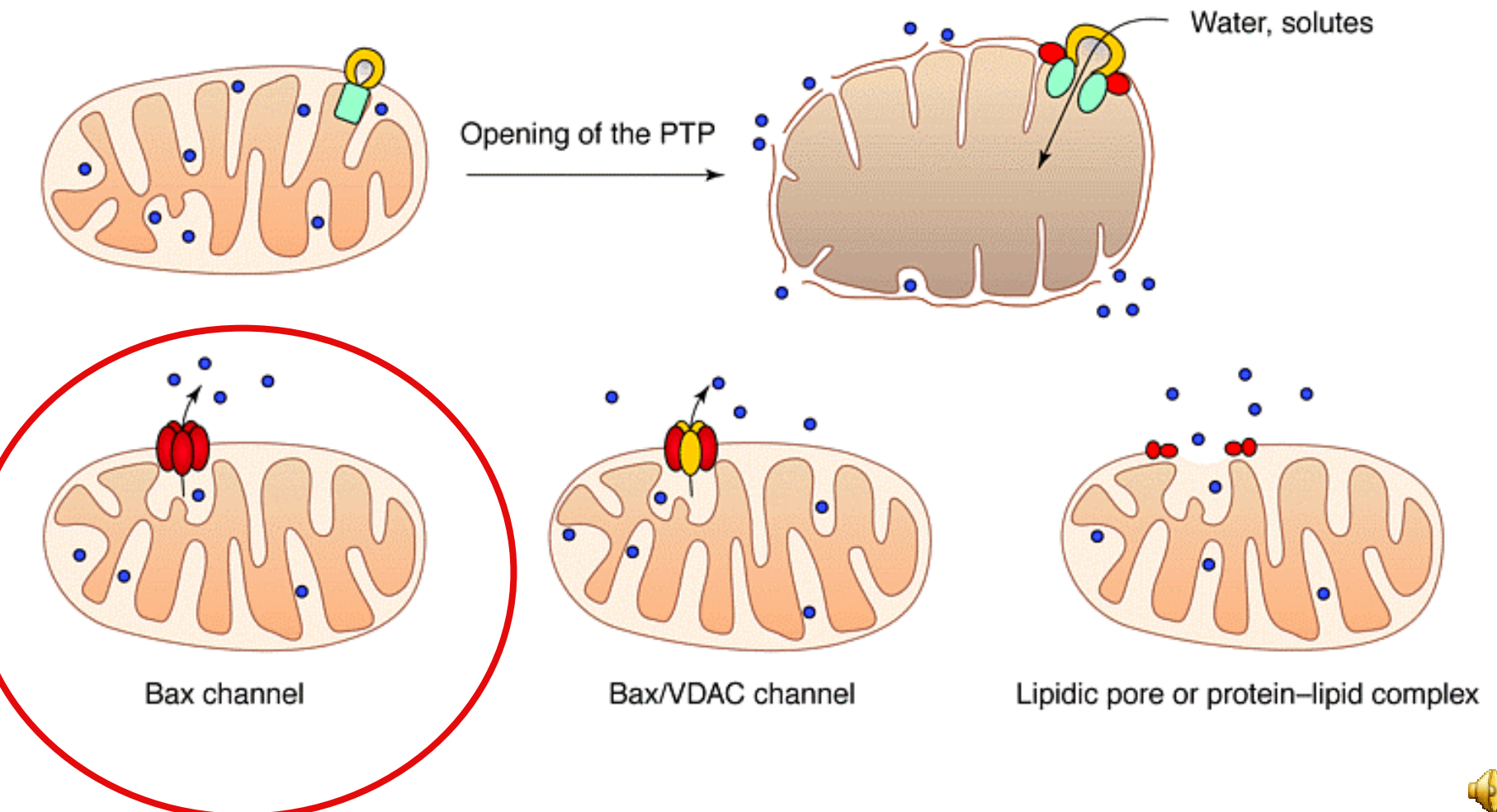
2) Bax can trigger apoptosis in the absence of major components of the PTP (ANT and Cyclophilin D)

What about VDAC?

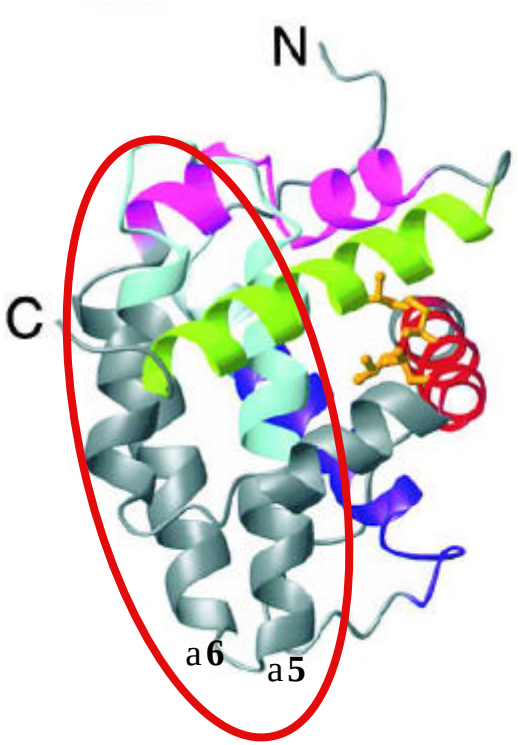
1) Bax can trigger VDAC opening in synthetic liposomes

2) Bax is inefficient in inducing cytochrome c release in yeast deficient in VDAC isoforms

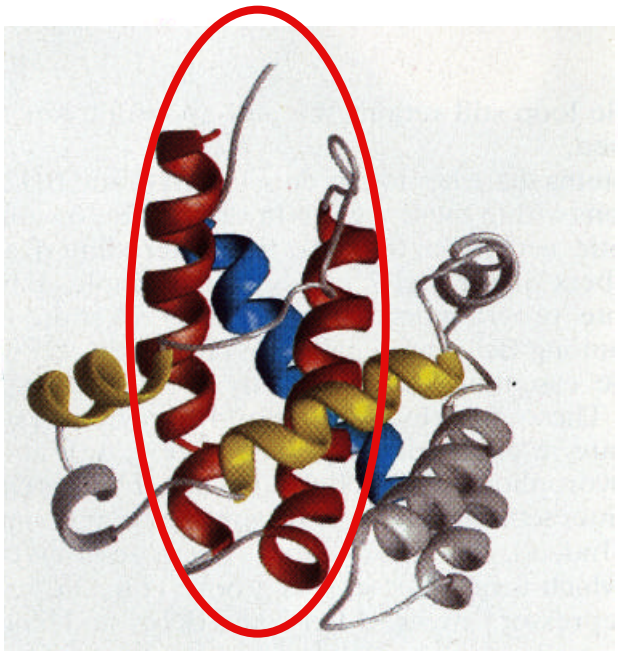
Models for the release of cytochrome c



Structural similarities between Bcl-2 family proteins and bacterial toxins



Bax



Pore forming
domain of colicins

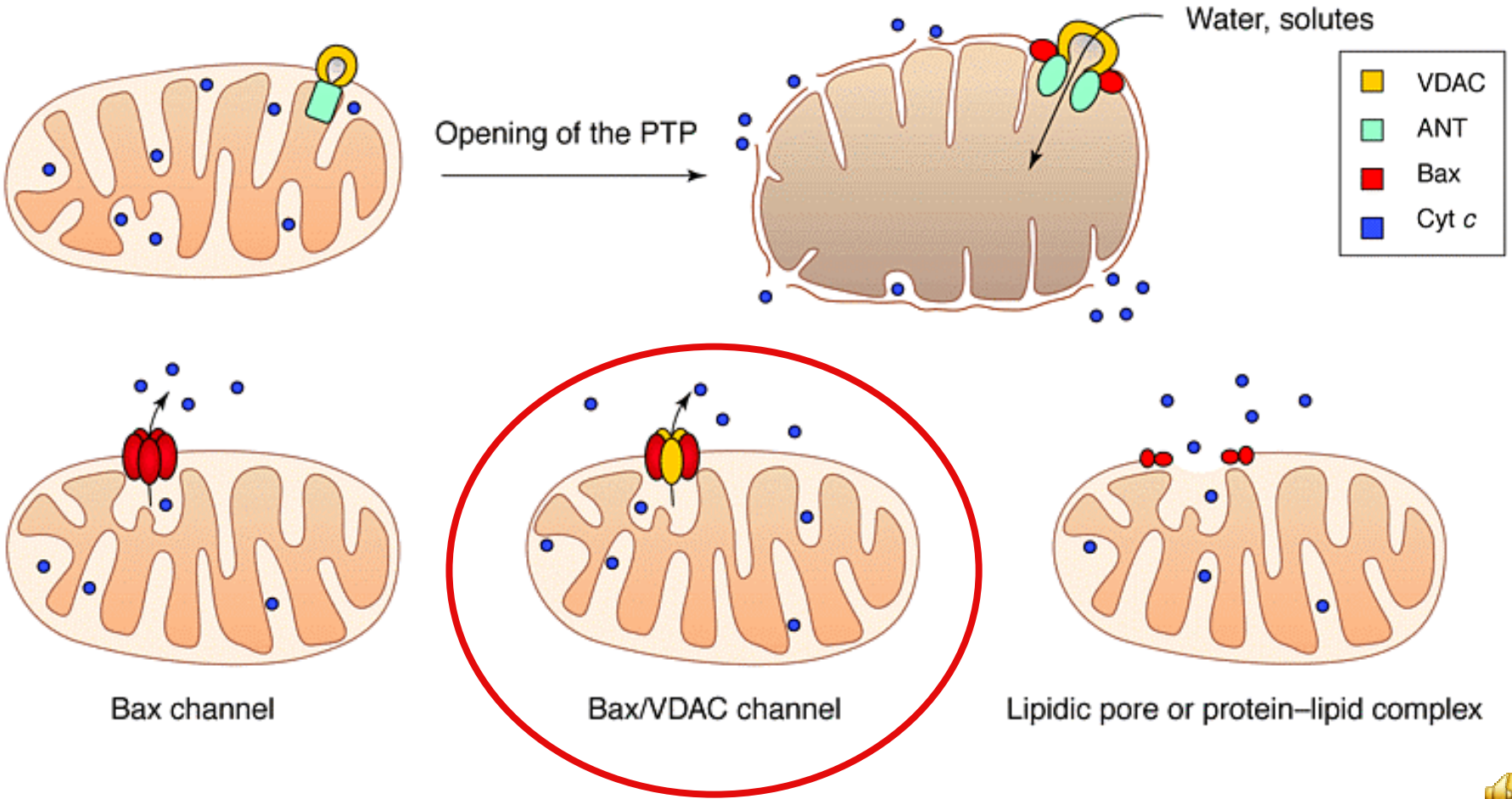
Bax channels in lipid planner bilayers

Bax pores

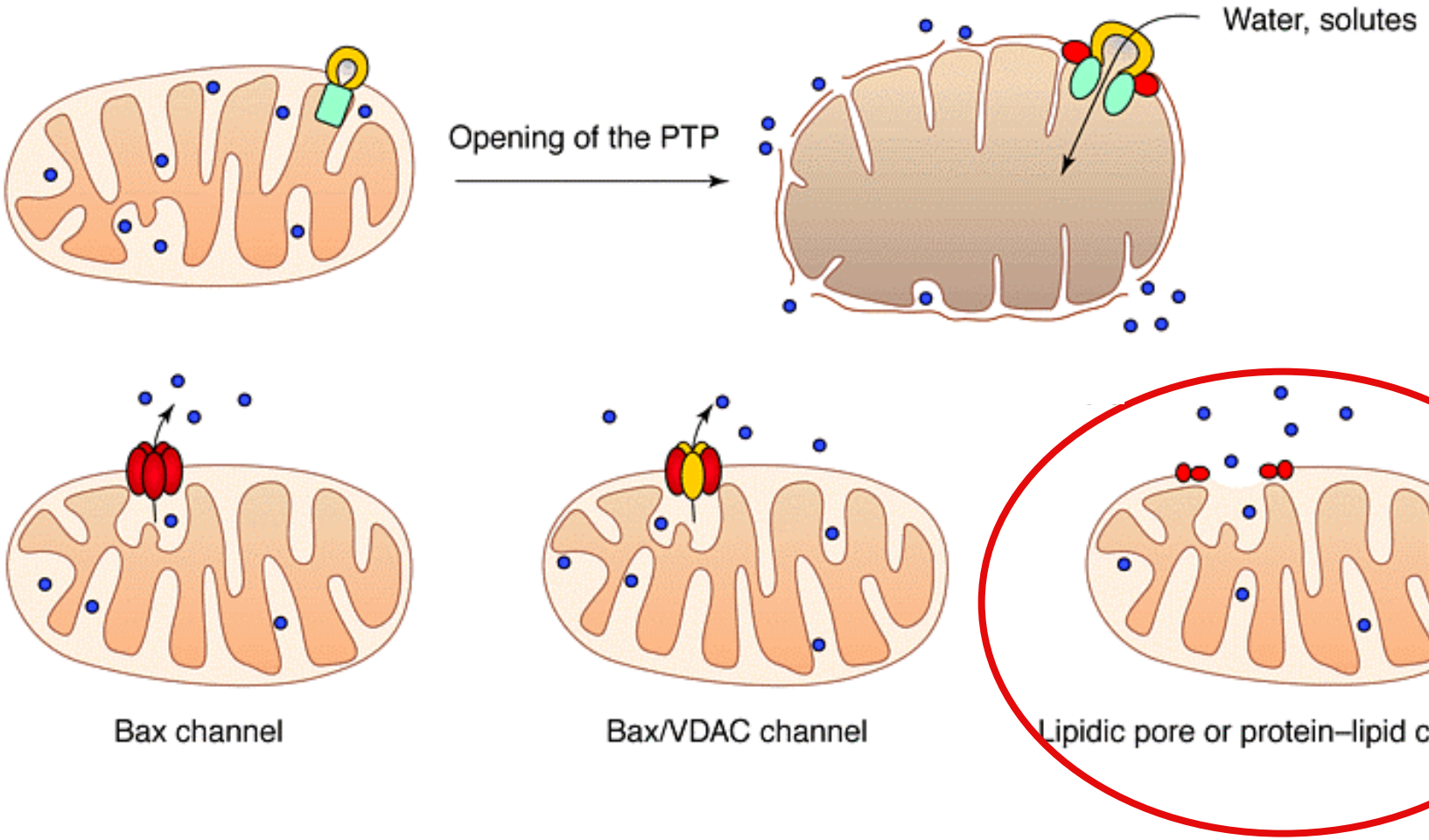
1) Bax can form pores in lipid planar bilayers and synthetic liposomes

2) Oligomerized Bax combined with tBid can form very large pores that can release 2000 kDa size dextran molecules

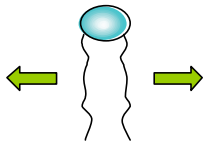
Models for the release of cytochrome c



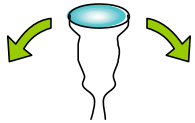
Lipids and mitochondrial membrane permeabilization



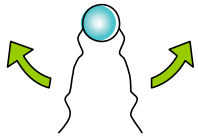
Structure of phospholipids



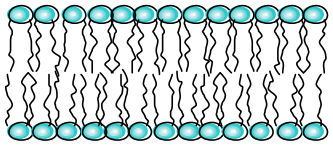
Flat
(ex: PC, PG, PS, PI,…)



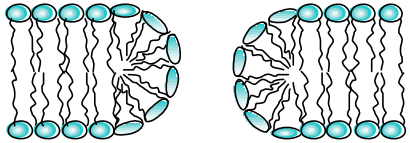
Positive curvature
(ex: lysophospholipids,…)



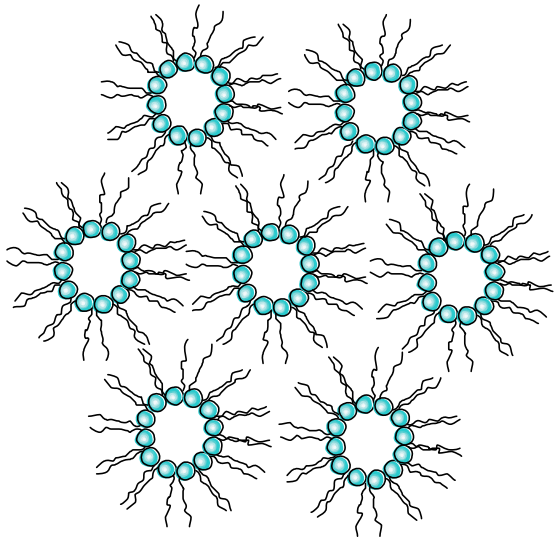
Negative curvature
(ex: PE, DAG,…)



Bilayer



Lipid pore



Hexagonal II phase

Lucken-Ardjomande and Martinou,

Bax lipidic pores

1) Bax pores in planar lipid bilayers are unstable and can result in membrane rupture

2) In contrast the pores formed by Bcl-xL are stable and do not result in membrane rupture

3) Formation of lipidic pores by Bax would be facilitated by lipids that impose a positive curvature to membranes

4) Formation of pores by Bcl-x_L is facilitated by lipids with a negative curvature

Summary

1) The mechanism of mitochondrial membrane permeabilization is still unclear

2) The PTP does not appear to be essential for mitochondrial membrane permeabilization

3) Modification of the lipid structure is the most likely explanation

Bax lipidic pores

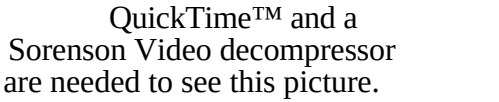
1) Bax pores in planar lipid bilayers are unstable and can result in membrane rupture

2) In contrast the pores formed by Bcl-xL are stable and do not result in membrane rupture

3) Formation of lipidic pores by Bax would be facilitated by lipids that impose a positive curvature to membranes

4) Formation of pores by Bcl-x_L is facilitated by lipids with a negative curvature

The role of mitochondrial fission in apoptosis


QuickTime™ and a
Sorenson Video decompressor
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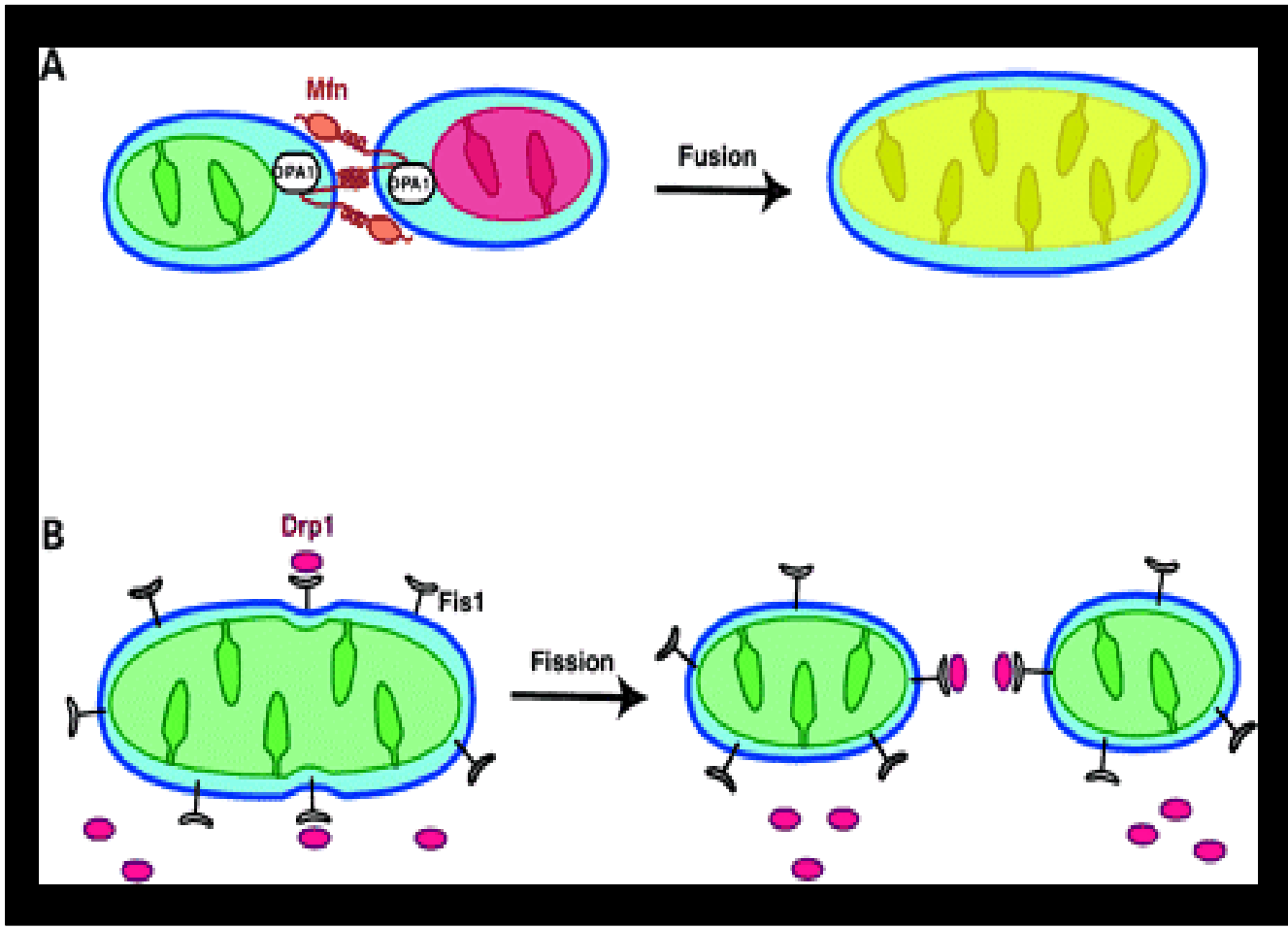
Mitochondria dynamics

Fusion proteins :

Mfn1&2
OPA1

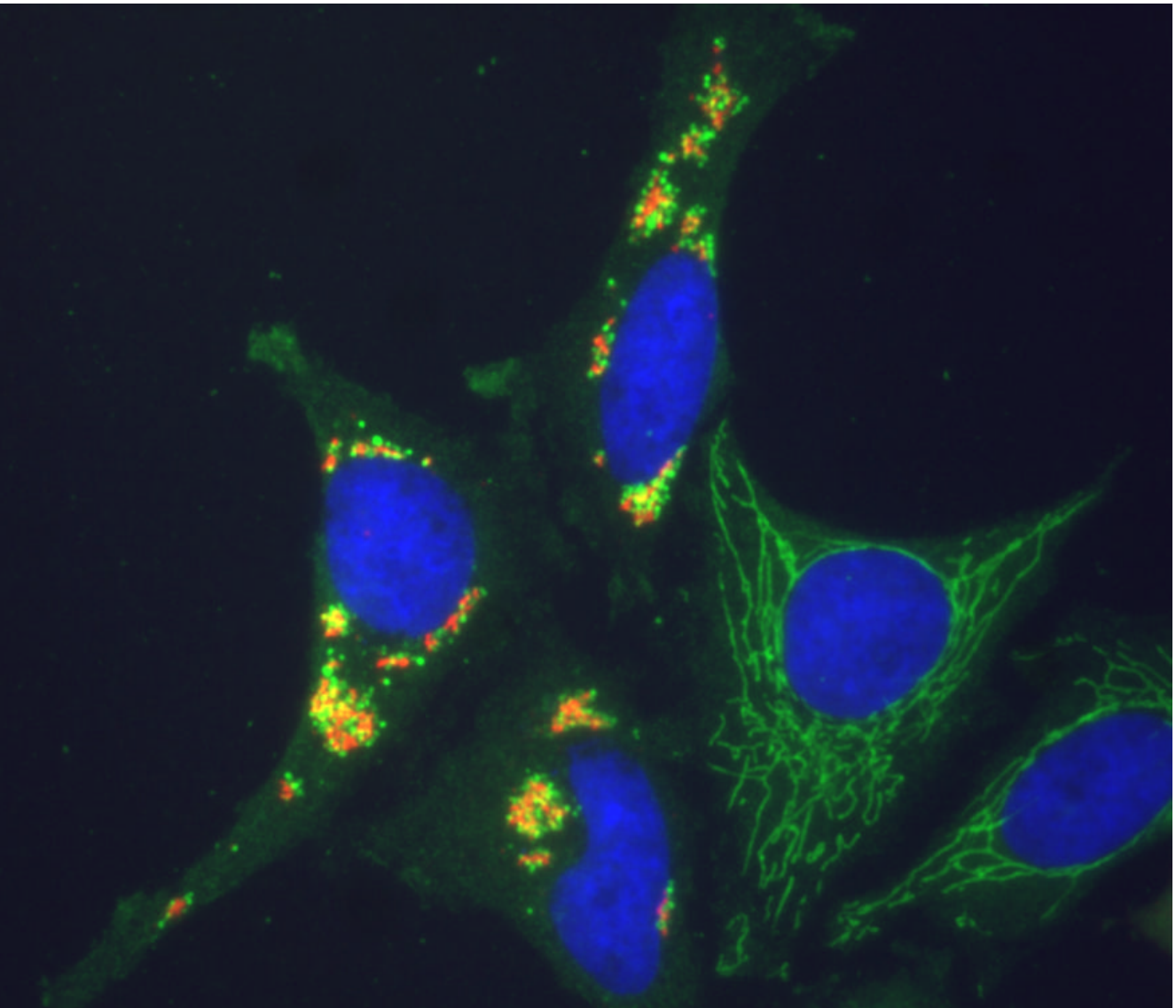
Fission proteins :

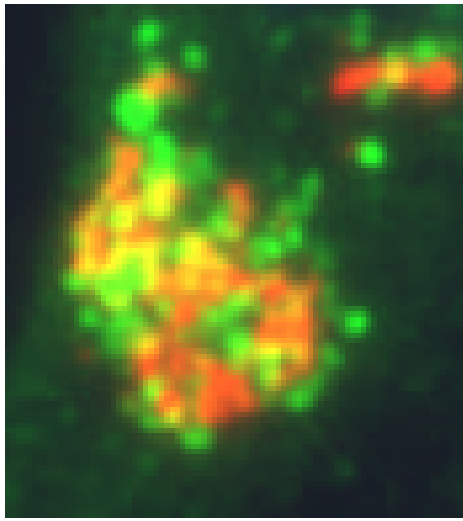
Drp1 (Dlp1)
hFis



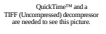
Chan D. Hum Mol genet 2005

mitochondrial fission

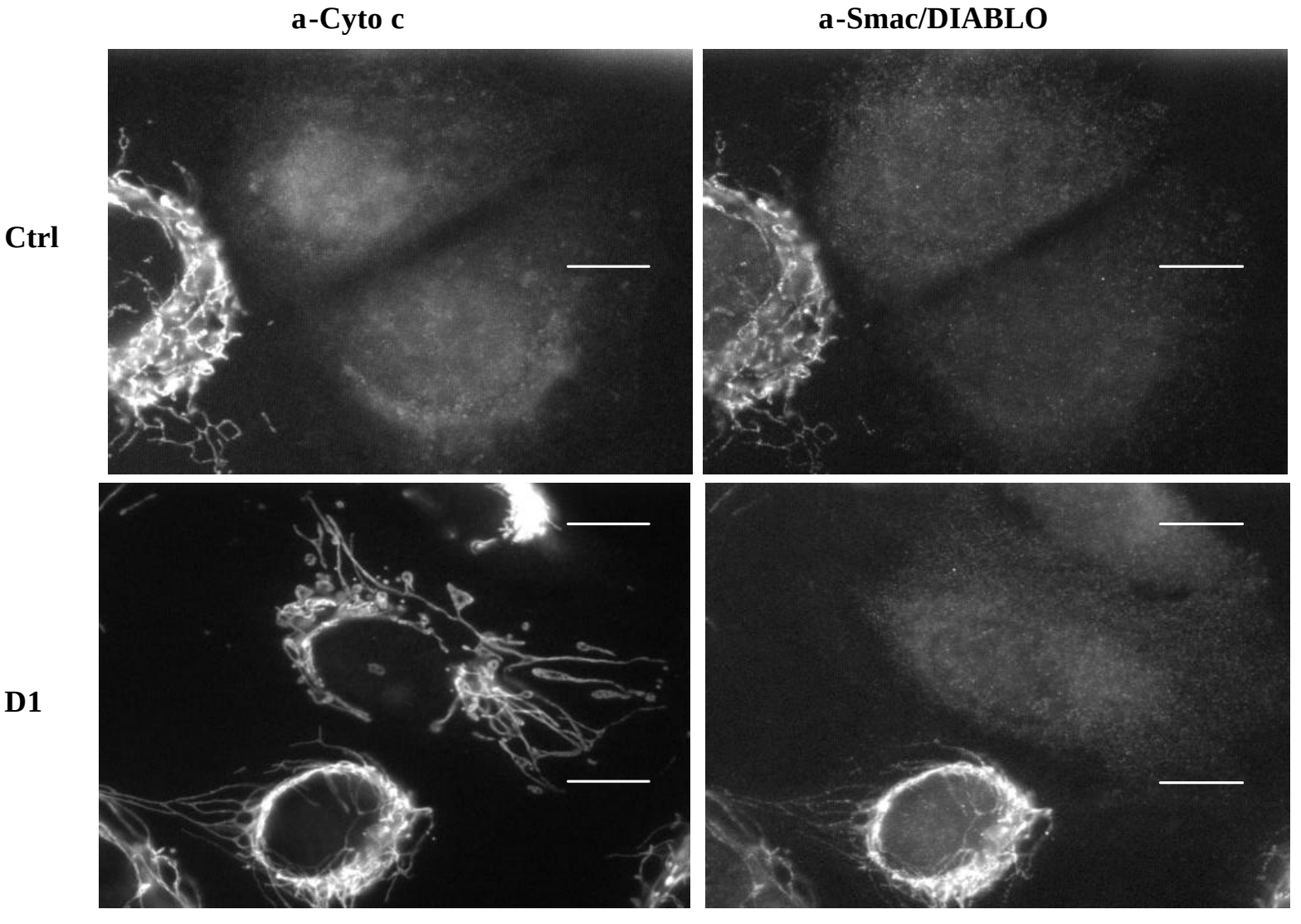




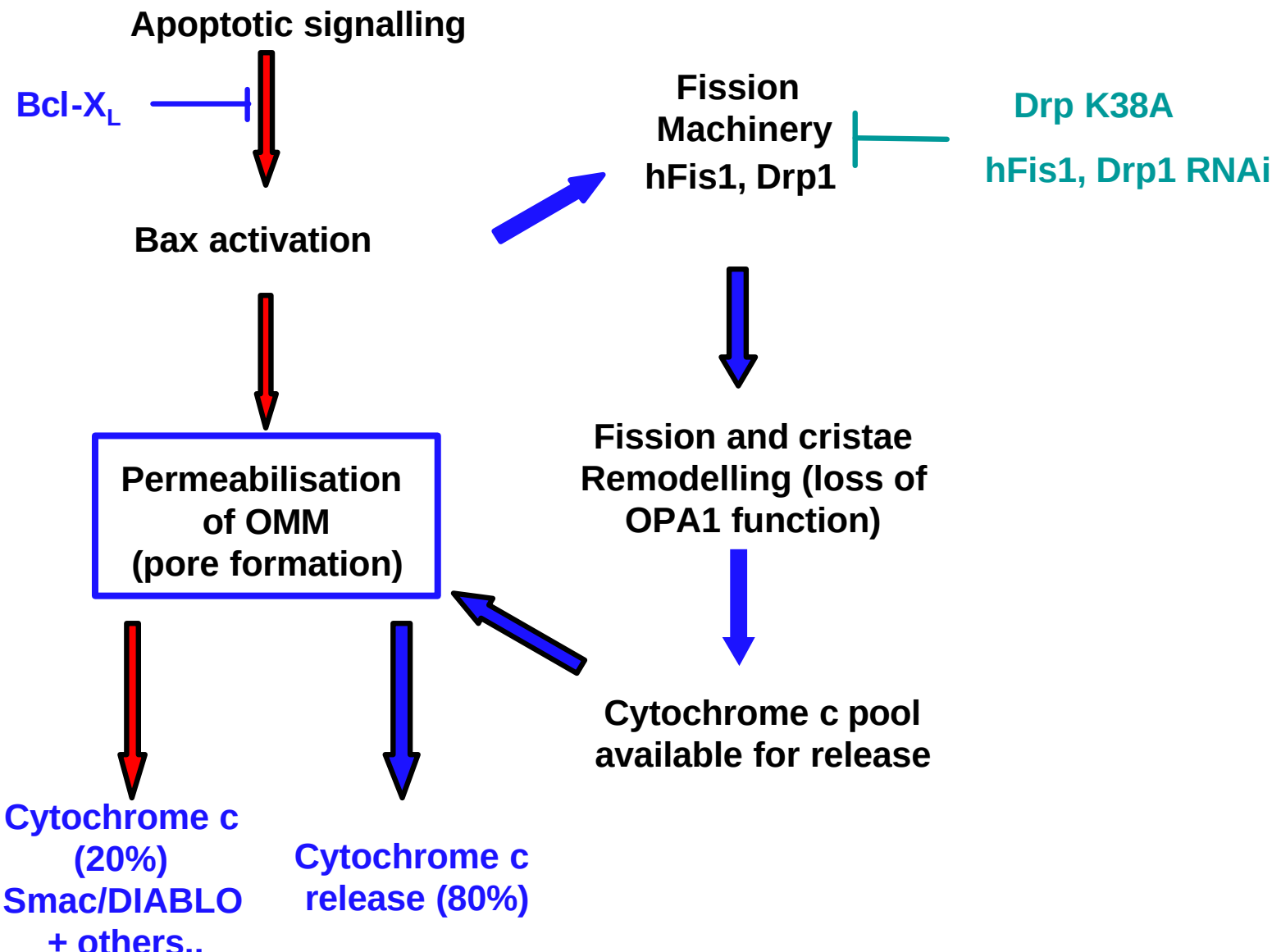
Bax triggers mitochondrial fission



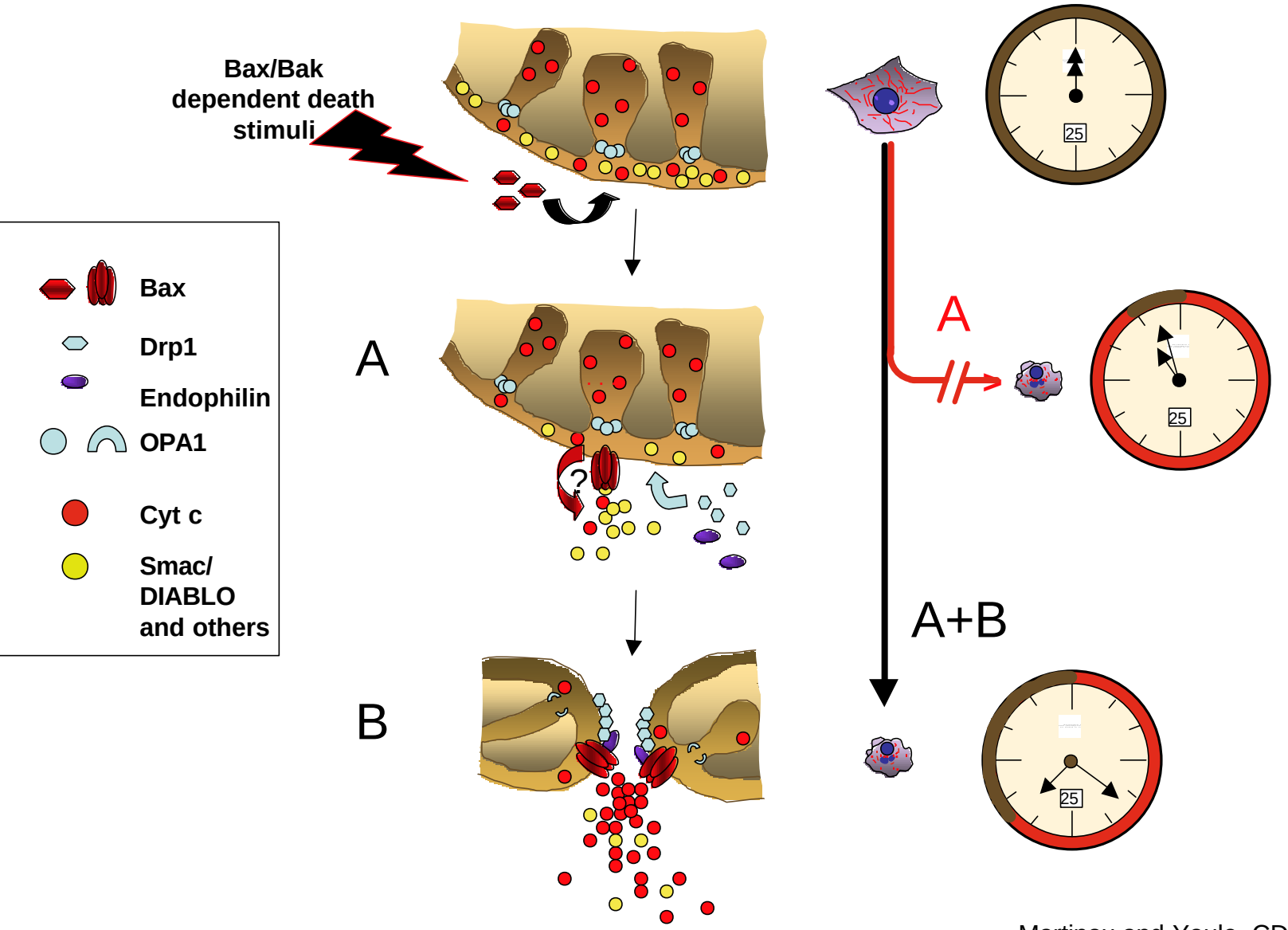
Differential release of cytochrome c and Smac/DIABLO



Mitochondrial fission and cytochrome c release



The role of mitochondrial fission during apoptosis



Martinou and Youle, CDD 2006

A different view on mitochondria

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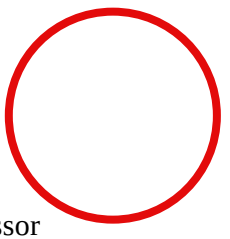

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Frey and Mannella, Trends Biochem. Sci. 2000, 25:319-324

Loss of OPA1 function and mobilization of the cytochrome c