Programmed Cell Death

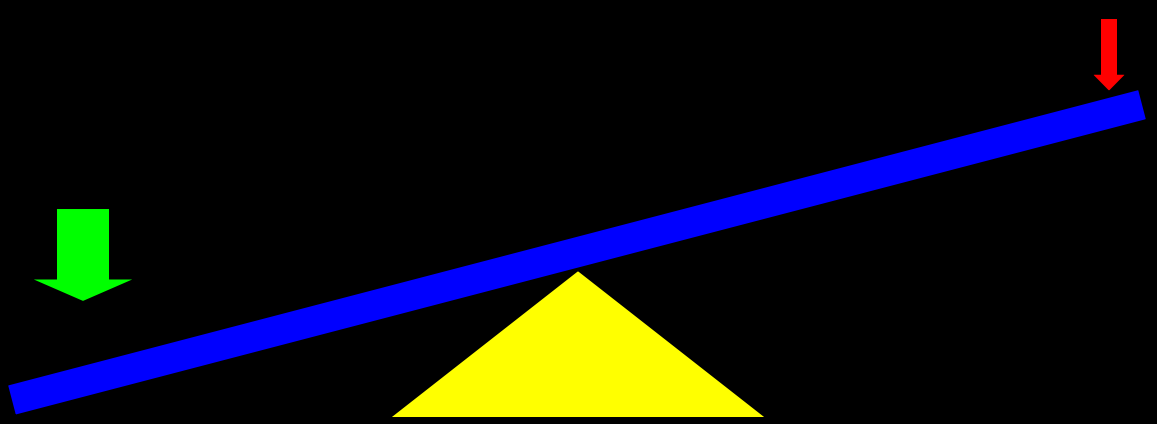
GSK Core Course 2025

Emily Cheng, M.D., Ph.D.
Human Oncology & Pathogenesis Program
Memorial Sloan Kettering Cancer Center

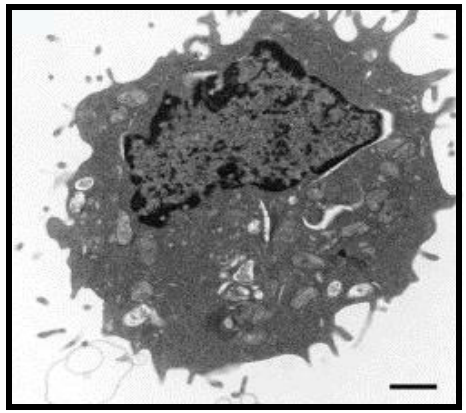
Caner
Autoimmunity

Proliferation

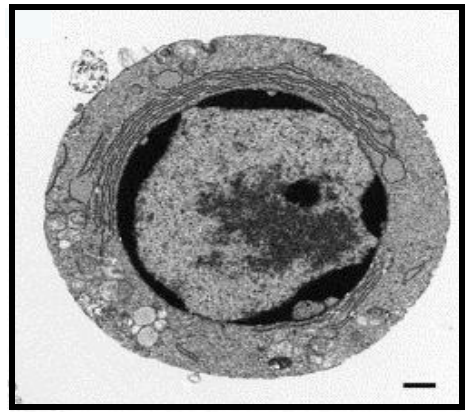
Death



Three Types of Cell Death



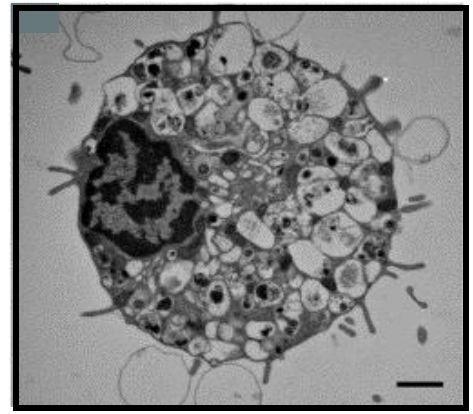
Normal



Apoptosis
(non-lytic cell death)

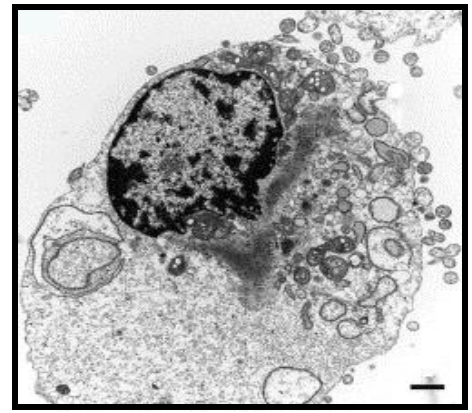
BCL-2 Family Proteins

Caspase-Dependent



Autophagy
(an adaptive response to nutrient deprivation)

ATG genes



Necrosis
(lytic cell death)

TNF-Induced Necroptosis (RIPK1, RIPK3, MLKL)

Inflammatory Caspase-Induced Pyroptosis (Casp1, Casp11/4/5, GSDMD)

Calcium-Mediated Mitochondrial Permeability Transition (Cyclophilin D)

Kerr, et. al, Br. J. Cancer 1972; 26, 239-257

Schweichel, et. al. Teratology 1973; 7:253-266

Edinger Al, Thompson CB. 2004. *Curr Opin Cell Biol*. 16(6):663-9.

Programmed Cell Death (Apoptosis)

- Evolutionarily conserved genetic pathway of cell suicide.
- Characteristics: cytoplasmic shrinkage, membrane blebbing, chromatin condensation, internucleosomal DNA fragmentation, phosphatidylserine exposure, and fragmentation into membrane-enclosed apoptotic bodies.
- Essential for the development and maintenance of tissue homeostasis in multicellular organisms.
- Deregulation contributes to many disease processes.

1st Landmark Discovery

Cloning of Bcl-2 from t(14;18) in follicular lymphoma

Stanley Korsmeyer (Cell 1985, 41:899-906)

Carlo Croce (Science. 1984, 226:1097-9)

Michael Cleary (Cell. 1986, 47:19-28.)

2nd Landmark Discovery

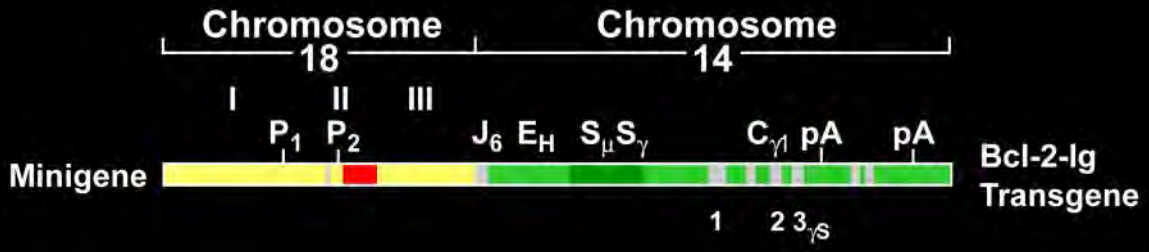
Defining Bcl-2 as a novel class of oncogene by inhibiting apoptosis

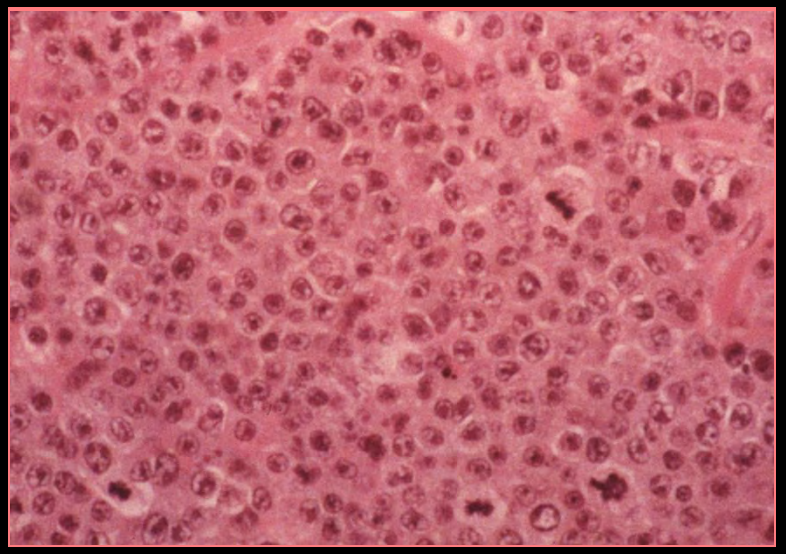
Suzanne Cory and Jerry Adams

(Nature. 1988, 335:440-2.)

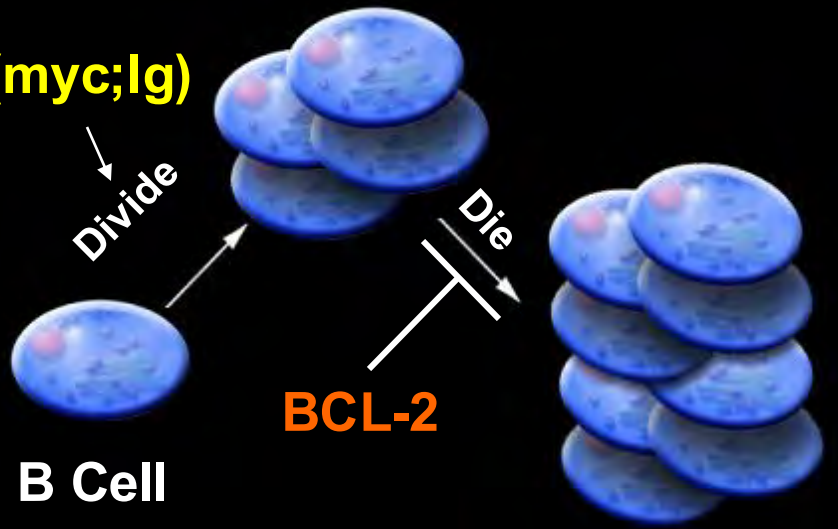
Stanley Korsmeyer (Cell. 1989, 57:79-88.)

Model of Human Lymphoma





High Grade Lymphoma



3rd Landmark Discovery

Cloning of CED-3 and Caspases

H. Robert Horvitz & Junying Yuan

(Cell. 1993, 75:641-52.)

4th Landmark Discovery

Identification of Apoptosome

Cytochrome c/dATP/Apaf-1/Caspase 9

Linking BCL-2 to cytochrome c translocation

Xiaodong Wang

(Cell. 1996, 86:147-57; Cell. 1997, 90:405-13; Cell. 1997, 91:479-89)

(Science. 1997, 275:1129-32)

Bcl-2 Apaf-1 Caspases

5th Landmark Discovery

BAX and BAK are essential effectors controlling cytochrome c translocation

Stanley Korsmeyer
Craig Thompson

(Science. 2001, 292:727-30
Mol Cell. 2001, 8:705-11
Genes Dev. 2001, 15:1481-6)

Venclexta (venetoclax) is the first FDA-approved treatment that targets the BCL-2 protein

The NEW ENGLAND JOURNAL of MEDICINE

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Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia

Andrew W. Roberts, M.B., B.S., Ph.D., Matthew S. Davids, M.D., John M. Pagel, M.D., Ph.D., Brad S. Kahl, M.D., Soham D. Puvvada, M.D., John F. Gerecitano, M.D., Ph.D., Thomas J. Kipps, M.D., Ph.D., Mary Ann Anderson, M.B., B.S., Jennifer R. Brown, M.D., Ph.D., Lori Gressick, B.S., Shekman Wong, Ph.D., Martin Dunbar, Dr.P.H., Ming Zhu, Ph.D., Monali B. Desai, M.D., M.P.H., Elisa Cerri, M.D., Sari Heitner Enschede, M.D., Rod A. Humerickhouse, M.D., Ph.D., William G. Wierda, M.D., Ph.D., and John F. Seymour, M.B., B.S., Ph.D.

79% response rate for relapsed or refractory CLL
20% complete remissions

The U.S. Food and Drug Administration approved Venclexta (venetoclax) for the treatment of patients with chronic lymphocytic leukemia (CLL) who have a chromosomal abnormality called 17p deletion and who have been treated with at least one prior therapy (April 11, 2016).

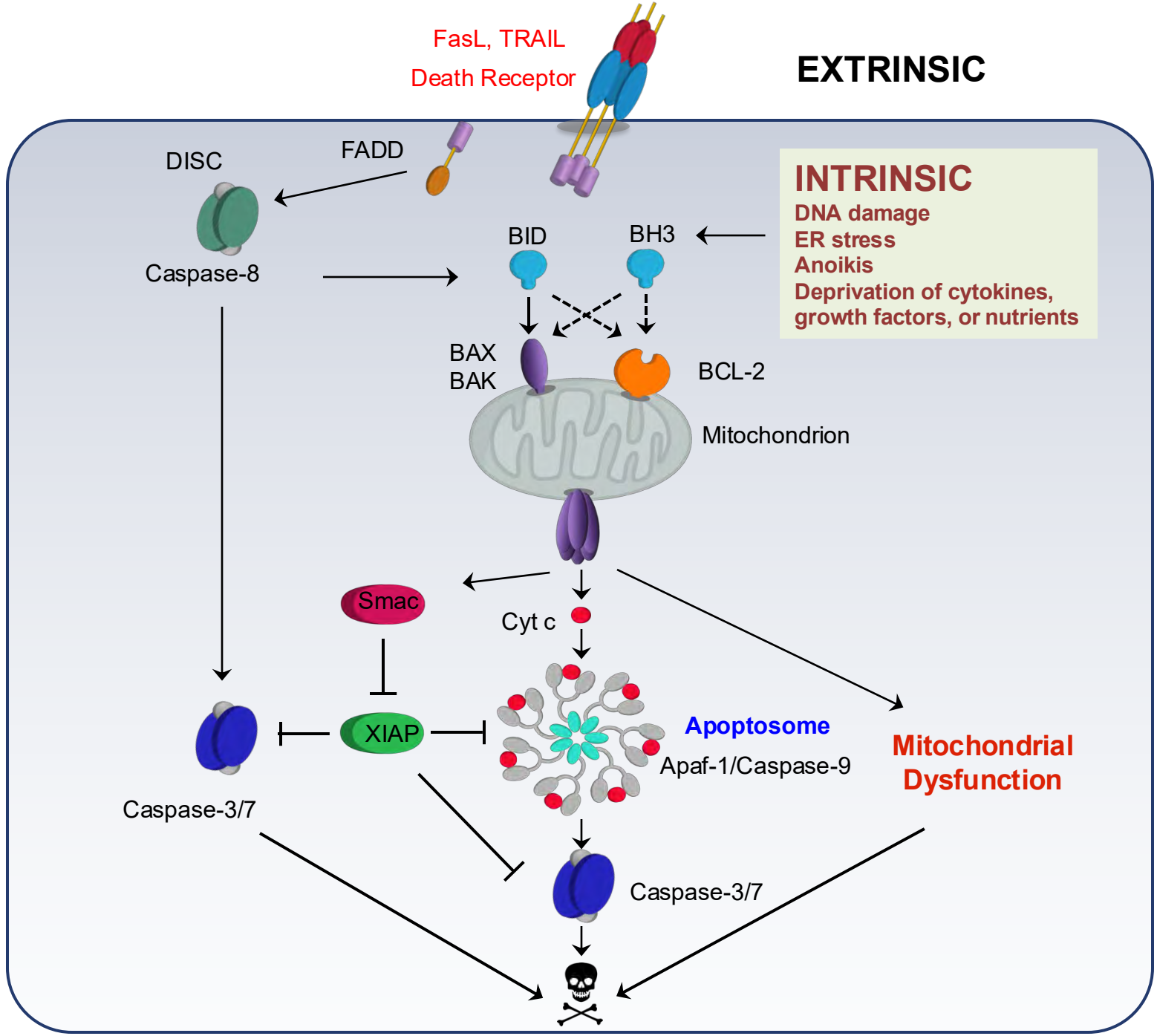
Extrinsic Apoptotic Pathway (Death Receptors)

FAS ligand – FAS
TRAIL – DR4 and DR5
TNF alpha – TNFR1

Intrinsic Apoptotic Pathway (Mitochondrial Apoptosis)

(Death signals initiated from inside the cells)

DNA damage
ER stress
Cytokine deprivation
Growth factor deprivation
Nutrient deprivation
Anoikis


FasL, TRAIL
Death Receptor
EXTRINSIC
DISC
FADD
Caspase-8
INTRINSIC
DNA damage
ER stress
Anoikis
Deprivation of cytokines, growth factors, or nutrients
BID
BH3
BAX
BAK
BCL-2
Mitochondrion
Smac
Cyt c
XIAP
Apoptosome
Apaf-1/Caspase-9
Mitochondrial Dysfunction
Caspase-3/7
Caspase-3/7

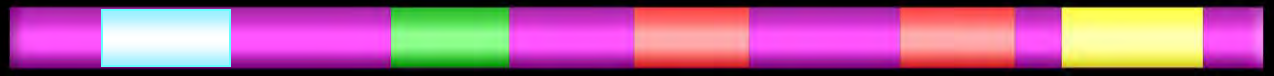
BCL-2 Family: 3 Subfamilies

Anti-Death "Multi-Domain"

BCL-2
BCL-X_L
MCL-1

Pro-Death "Multi-Domain"

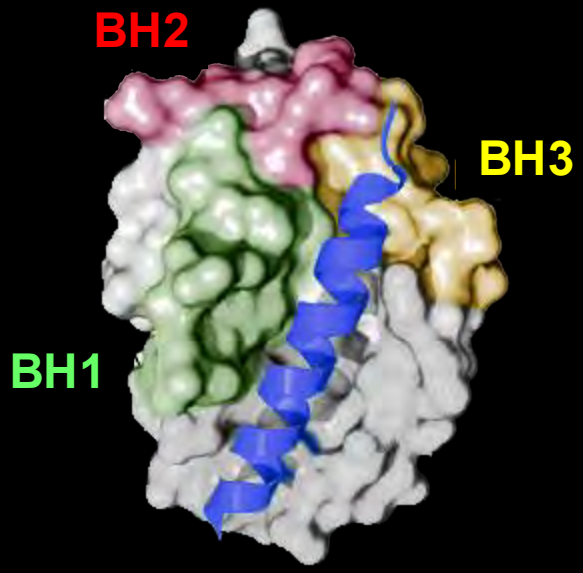
BAX
BAK



Pro-Death "BH3-Only"

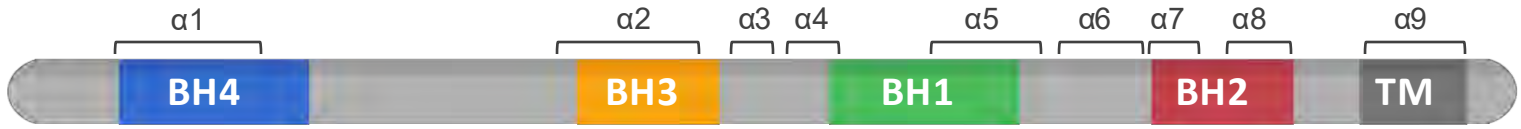
BID
BIM
PUMA

NOXA, BAD, BIK, HRK, BNIP3, NIX

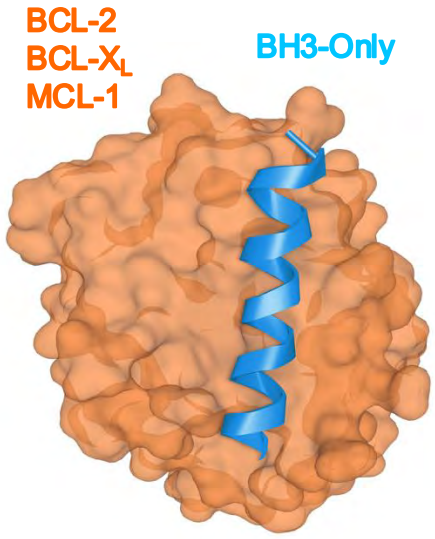


BH3 Helix in Groove

BH3-in-Groove: Structural Basis of Heterodimerization between BH3s and Multidomain Members as well as Homodimerization of BAX/BAK

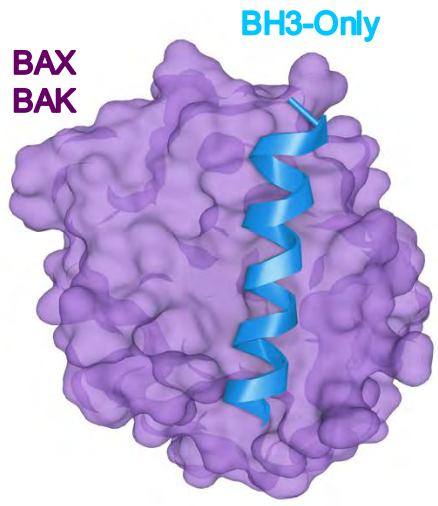


A



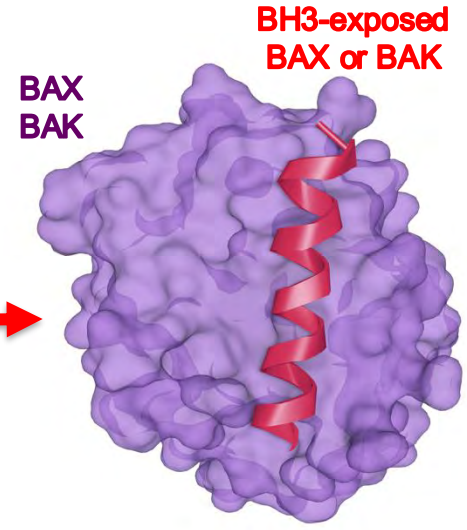
Inhibitory
Stable

B



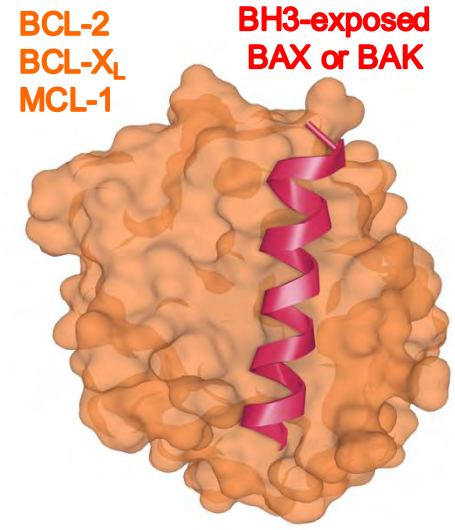
Stimulatory
Dynamic

C



Death Machinery

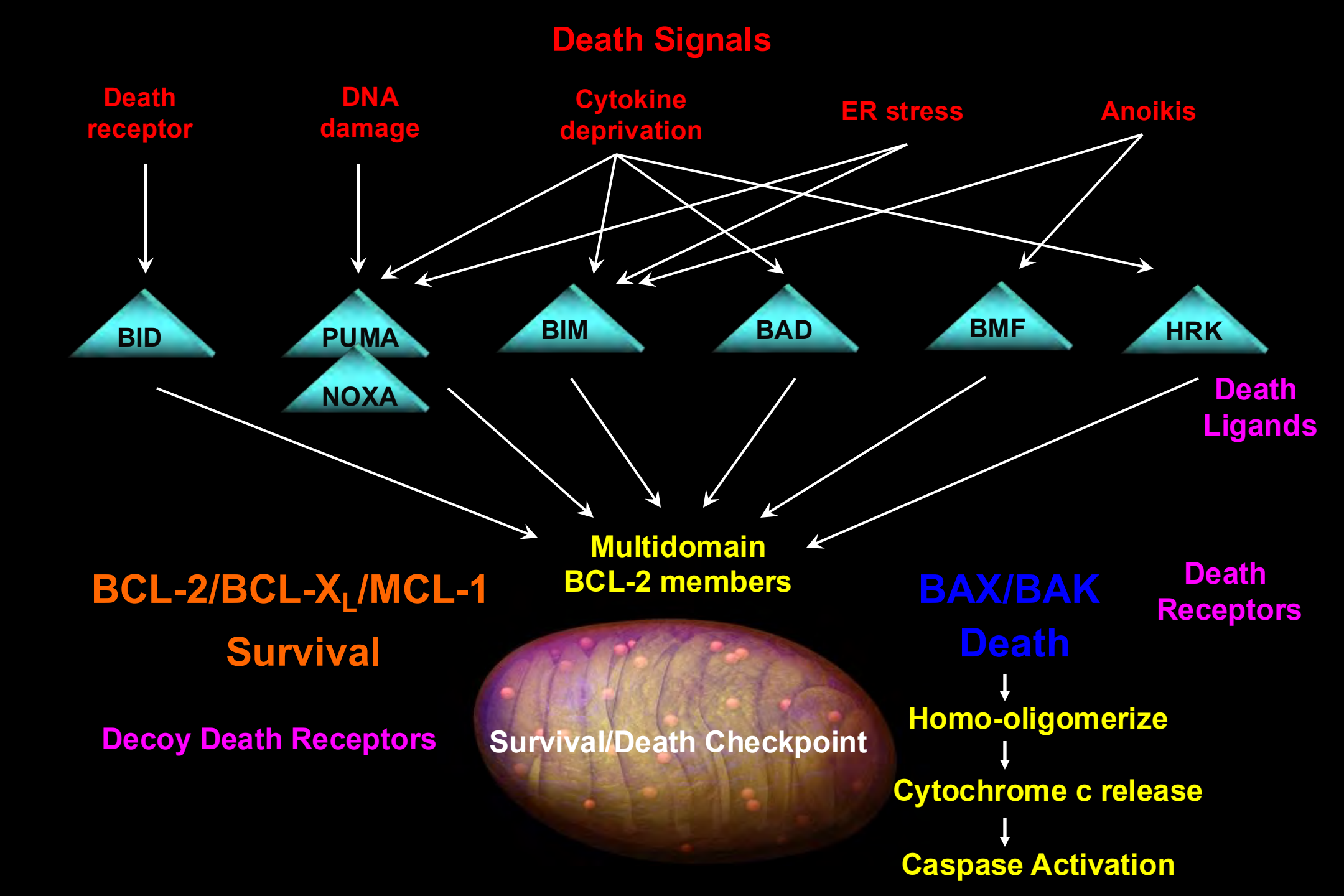
D



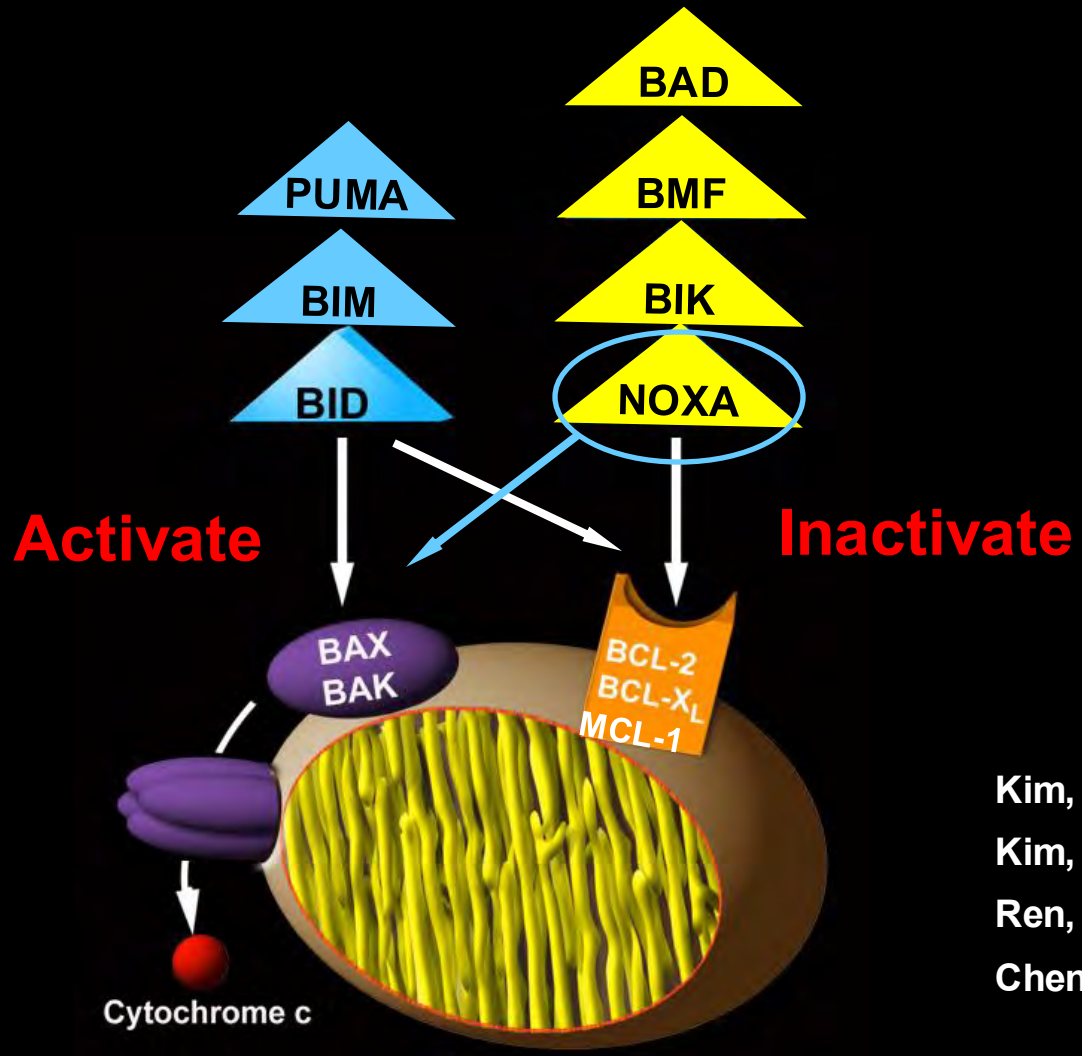
Inhibitory
Stable

Roles for BH3-Only Molecules

- Sentinels for cellular damage
- Interconnect signal transduction and the checkpoint of BCL-2 “multidomain” members
- Link between private and common apoptotic pathways
- Activity controlled by transcriptional regulation or post-translational modification


Death Signals
Death receptor
DNA damage
Cytokine deprivation
ER stress
Anoikis
BID
PUMA
NOXA
BIM
BAD
BMF
HRK
Death Ligands
Multidomain BCL-2 members
BCL-2/BCL-XL/MCL-1
Survival
Decoy Death Receptors
Survival/Death Checkpoint
BAX/BAK
Death
Death Receptors
Homo-oligomerize
Cytochrome c release
Caspase Activation

A "Two Class" Model of BH3-Only Molecules



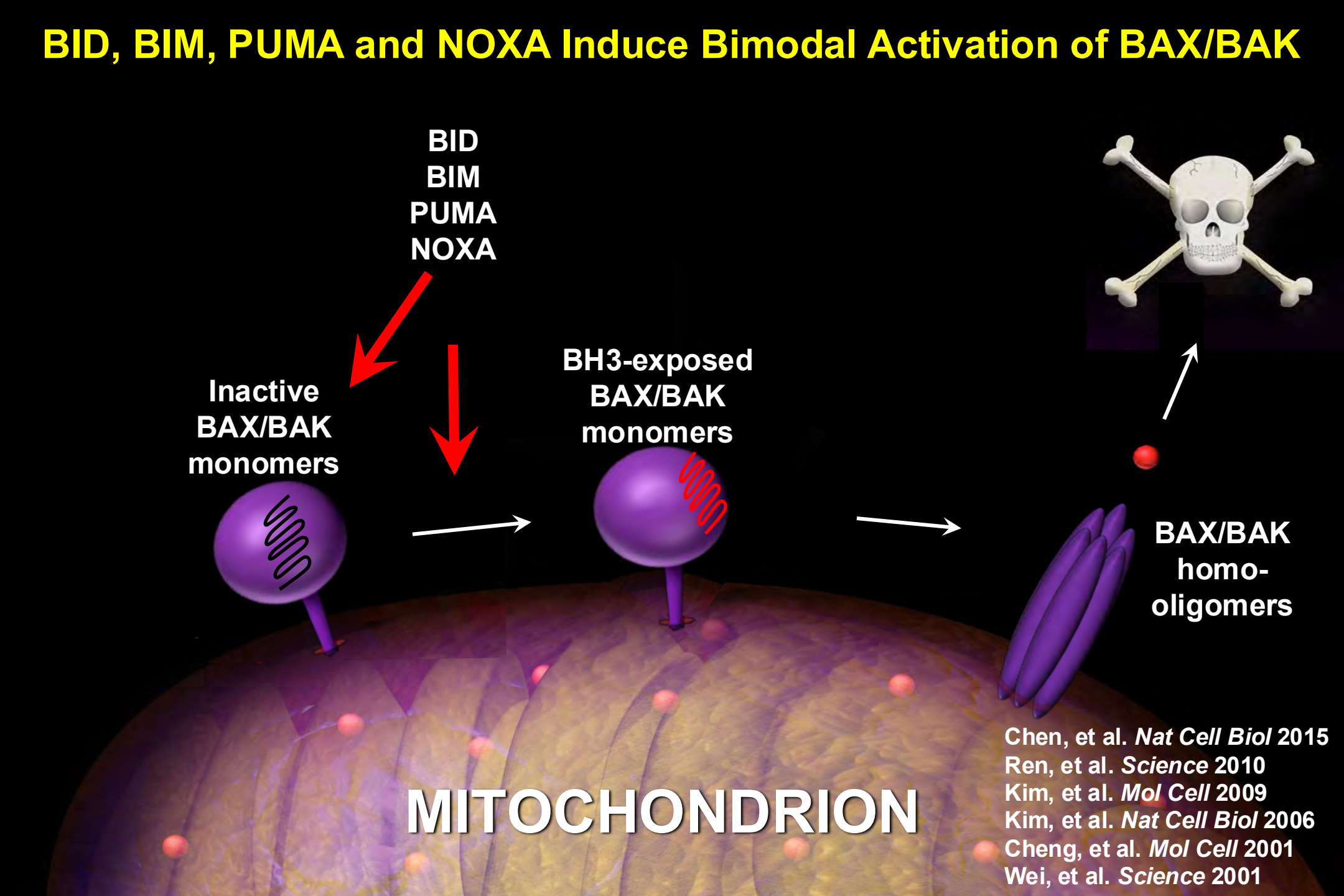
Kim, et al. *Nat Cell Biol* 2006

Kim, et al. *Molecular Cell* 2009

Ren, et al. *Science* 2010

Chen, et al. *Nat Cell Biol* 2015

BID, BIM, PUMA and NOXA Induce Bimodal Activation of BAX/BAK



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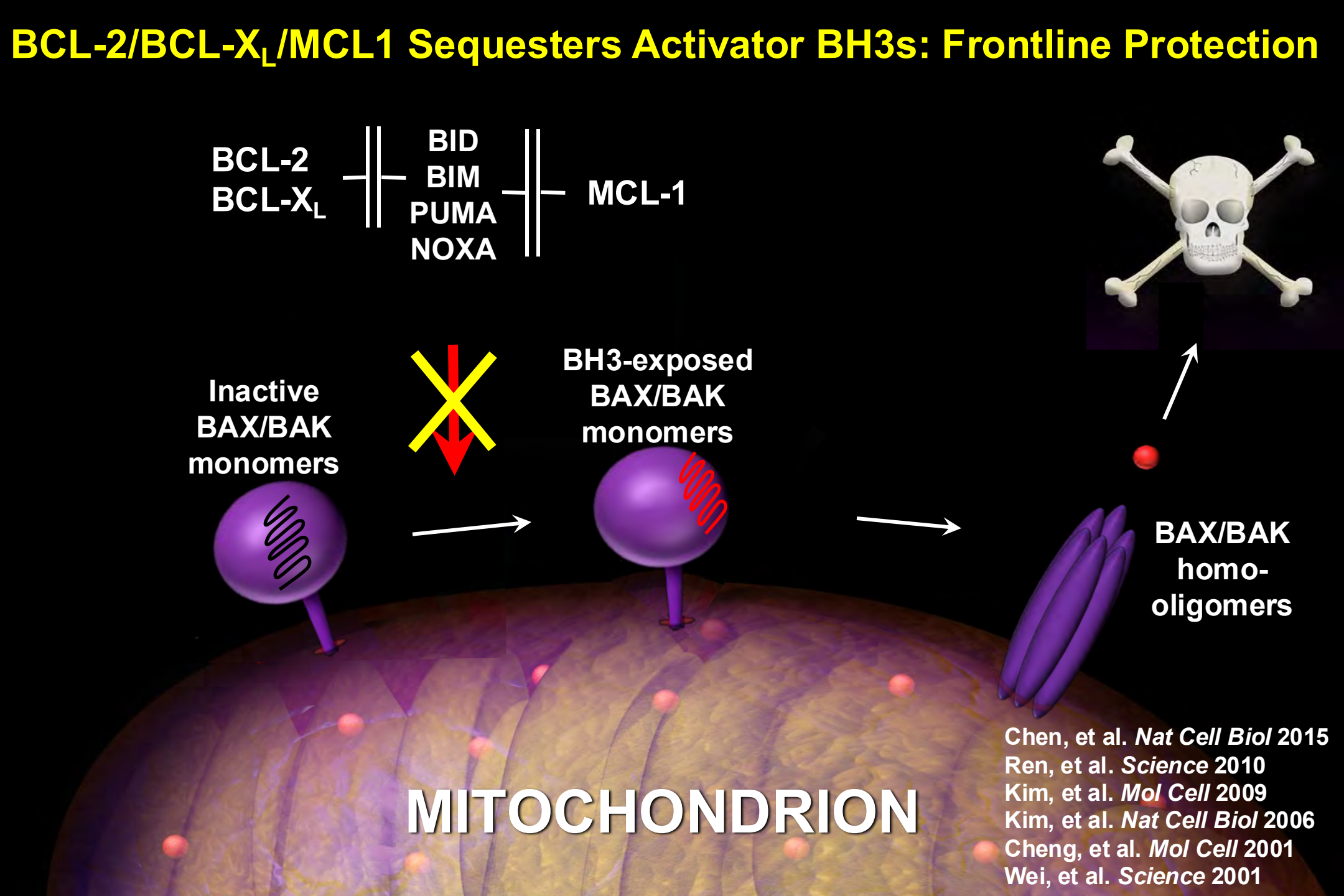
Kim, et al. *Nat Cell Biol* 2006
Cheng, et al. *Mol Cell* 2001
Wei, et al. *Science* 2001

BCL-2/BCL-X_L/MCL1 Sequesters Activator BH3s: Frontline Protection



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Cheng, et al. *Mol Cell* 2001
Wei, et al. *Science* 2001

Inactivator BH3s Prevent BCL-2/BCL-X_L/MCL1 from Sequestering Activator BH3s

BAD
BMF
BIK
HRK

BCL-2
BCL-X_L

BID
BIM
PUMA
NOXA

MCL-1

NOXA

Inactive BAX/BAK monomers

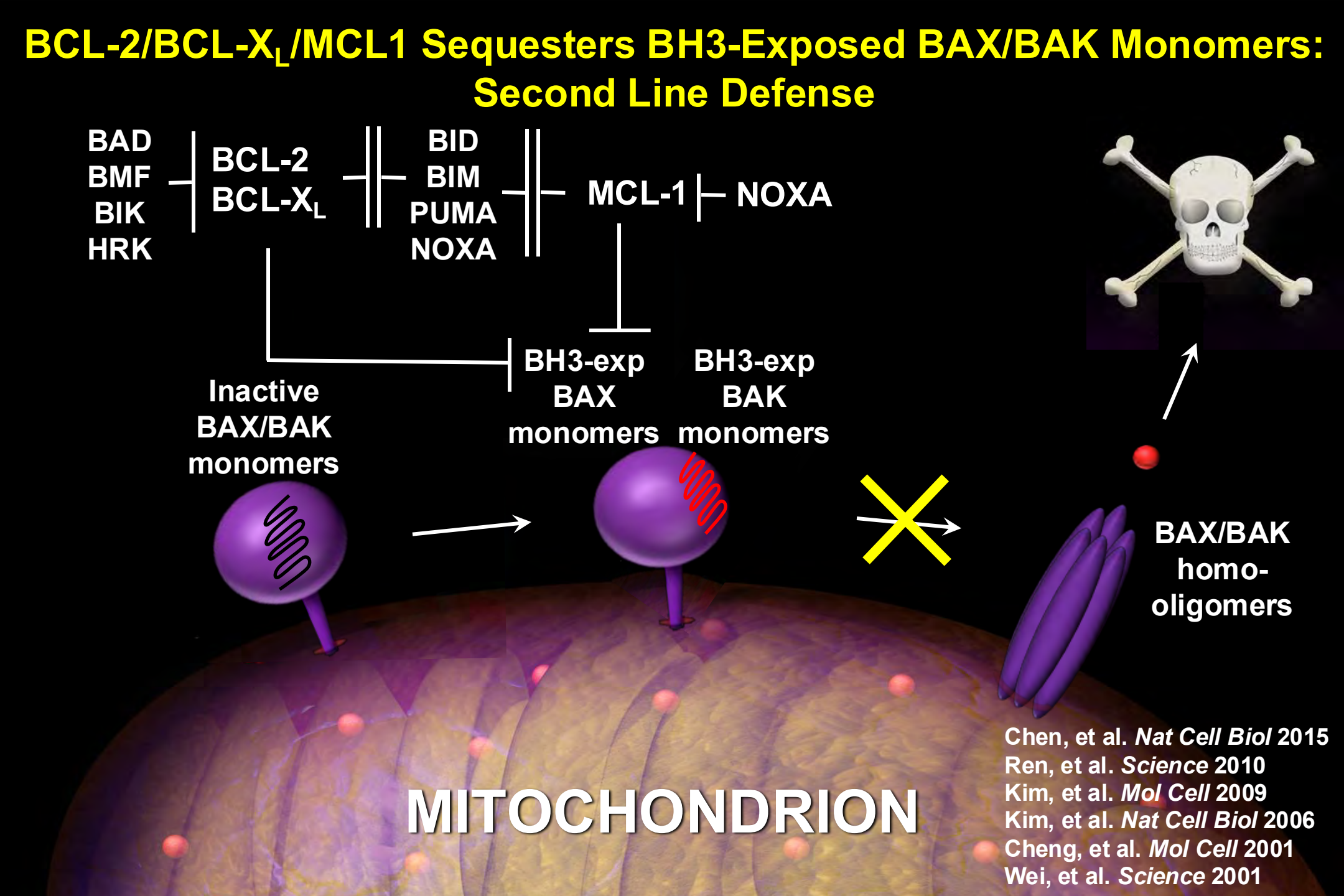
BH3-exposed BAX/BAK monomers

BAX/BAK homo-oligomers

MITOCHONDRION

Chen, et al. *Nat Cell Biol* 2015
Ren, et al. *Science* 2010
Kim, et al. *Mol Cell* 2009
Kim, et al. *Nat Cell Biol* 2006
Cheng, et al. *Mol Cell* 2001
Wei, et al. *Science* 2001

BCL-2/BCL-X_L/MCL1 Sequesters BH3-Exposed BAX/BAK Monomers: Second Line Defense



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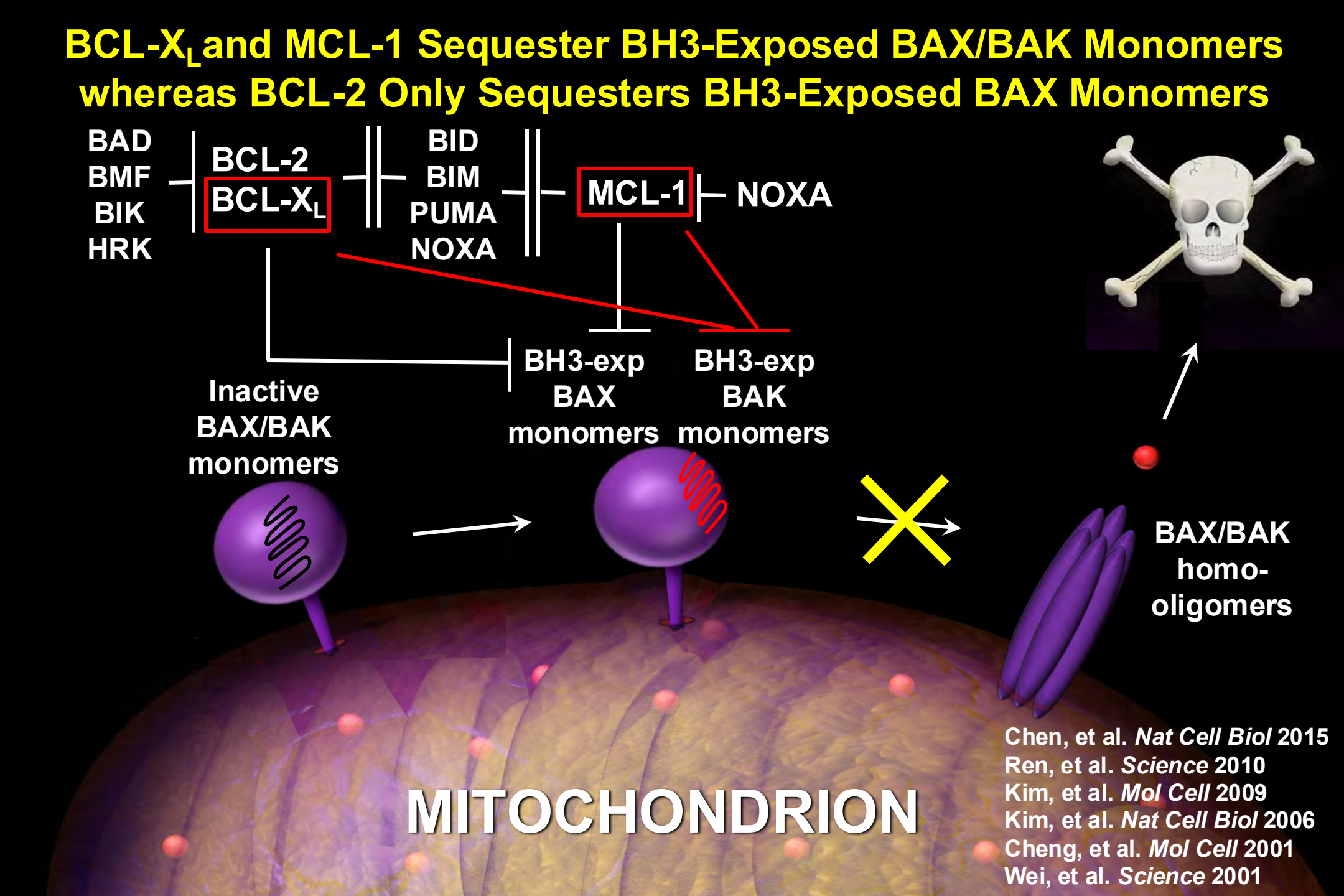
Kim, et al. *Mol Cell* 2009
Kim, et al. *Nat Cell Biol* 2006
Cheng, et al. *Mol Cell* 2001
Wei, et al. *Science* 2001

BCL-X_L and MCL-1 Sequester BH3-Exposed BAX/BAK Monomers whereas BCL-2 Only Sequesters BH3-Exposed BAX Monomers



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Cheng, et al. *Mol Cell* 2001
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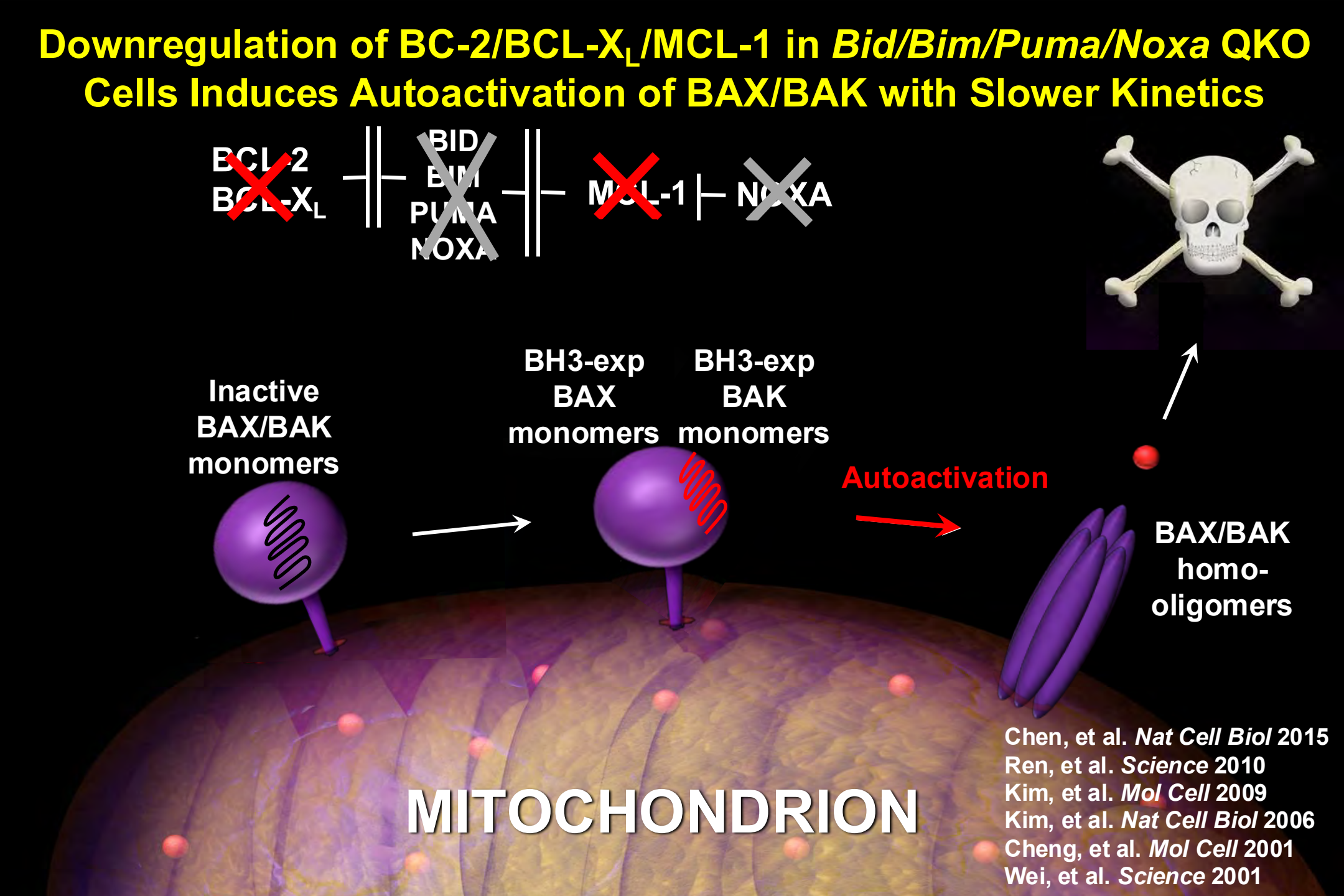
Downregulation of BC-2/BCL-X_L/MCL-1 in *Bid/Bim/Puma/Noxa* QKO Cells Induces Autoactivation of BAX/BAK with Slower Kinetics



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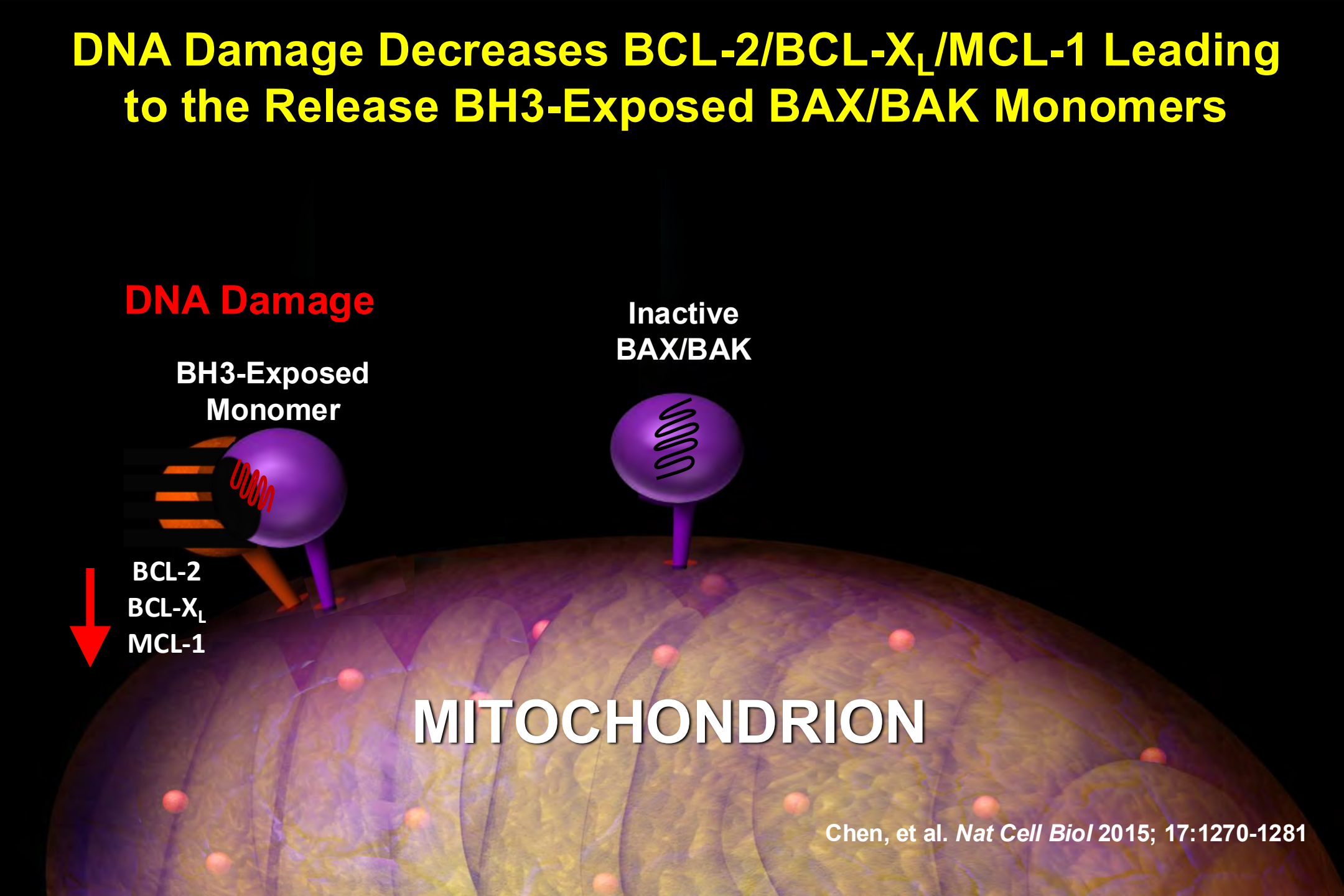
BCL-2/BCL-X_L/MCL1 Sequesters BH3-Exposed BAX/BAK Monomers: Second Line Defense

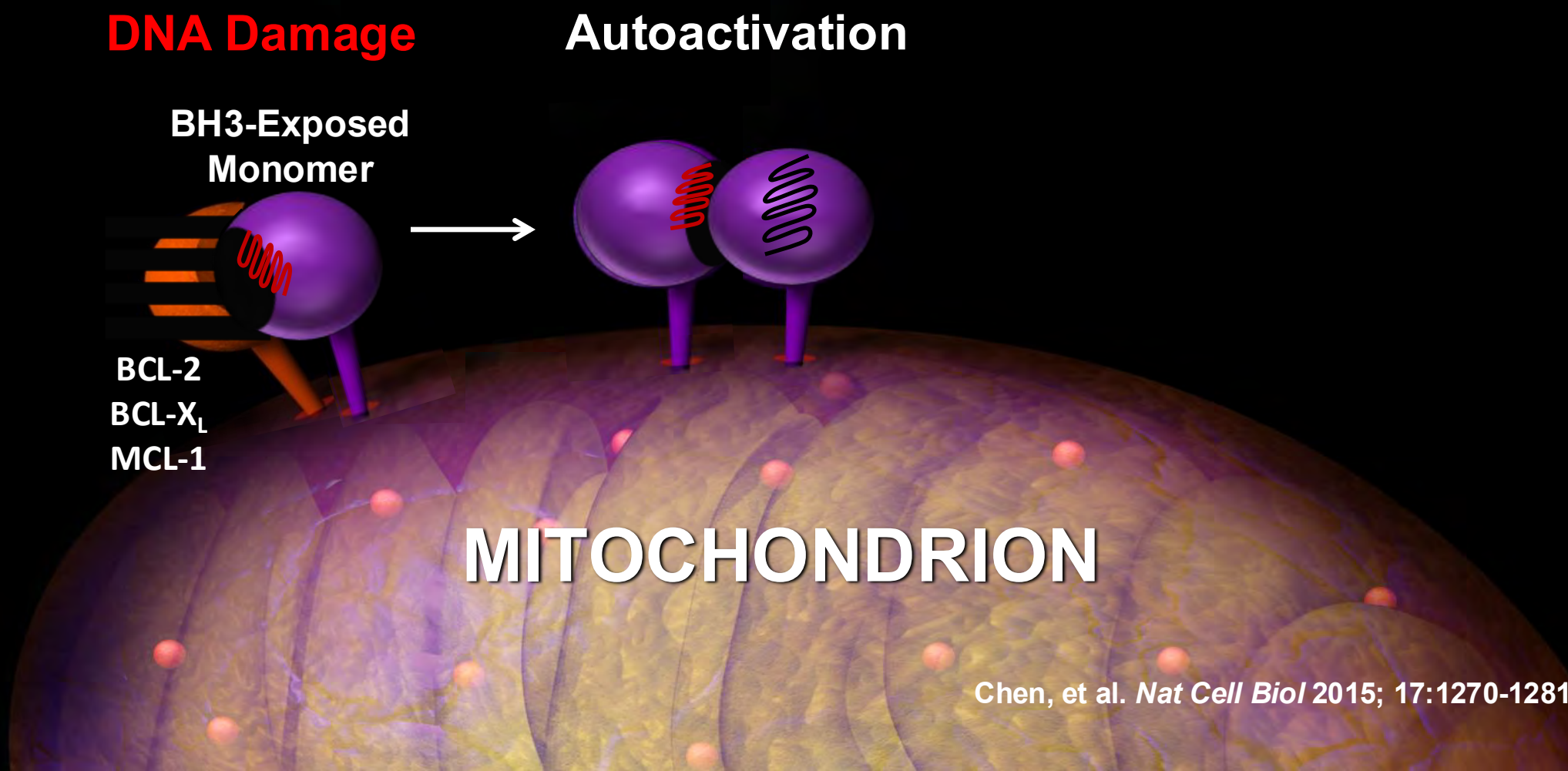
Chen, et al. *Nat Cell Biol* 2015; 17:1270-1281

DNA Damage Decreases BCL-2/BCL-X_L/MCL-1 Leading to the Release BH3-Exposed BAX/BAK Monomers



Chen, et al. *Nat Cell Biol* 2015; 17:1270-1281

BH3-Exposed BAX/BAK Monomers Can Activate Inactive BAX/BAK Monomers



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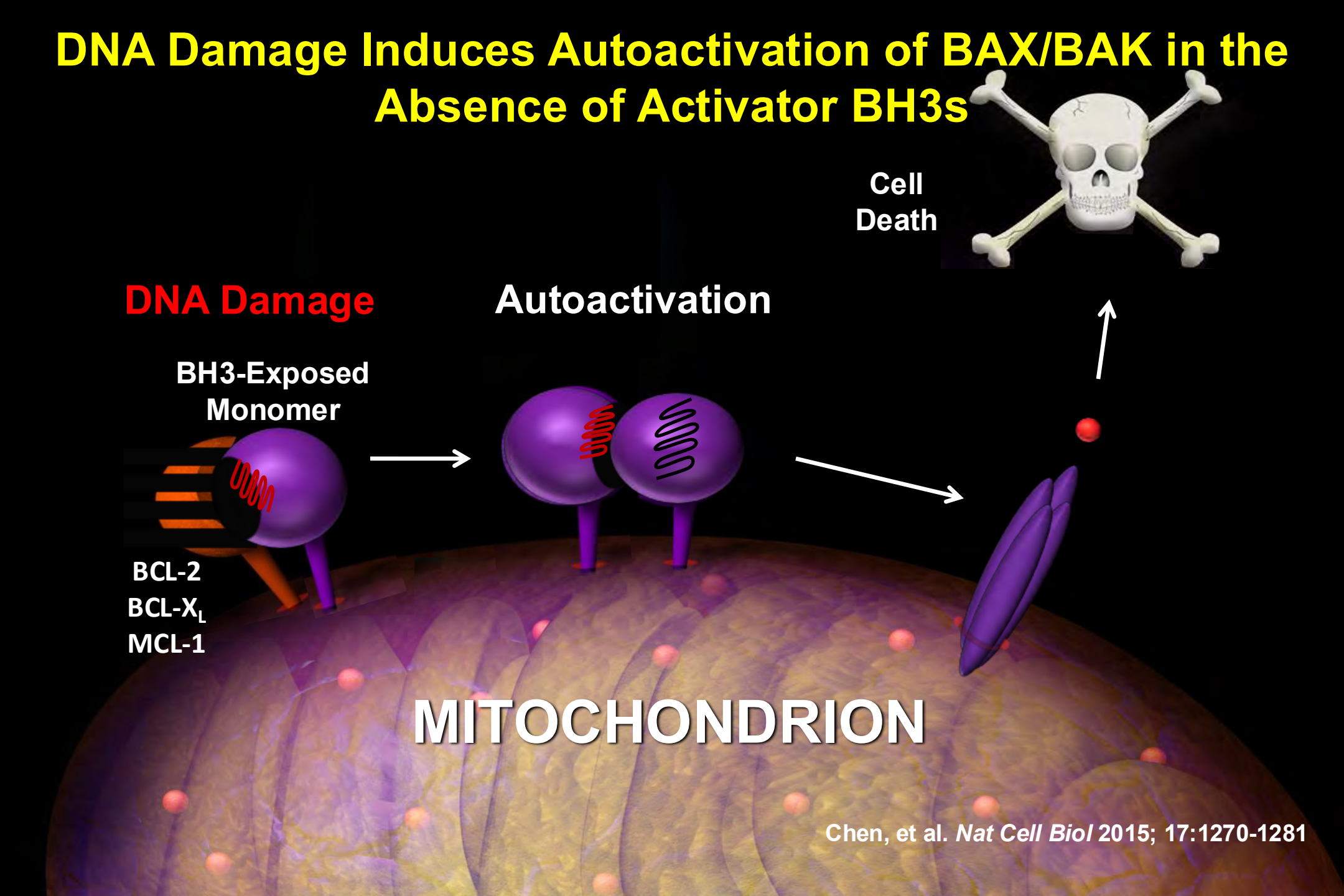
DNA Damage Induces Autoactivation of BAX/BAK in the Absence of Activator BH3s



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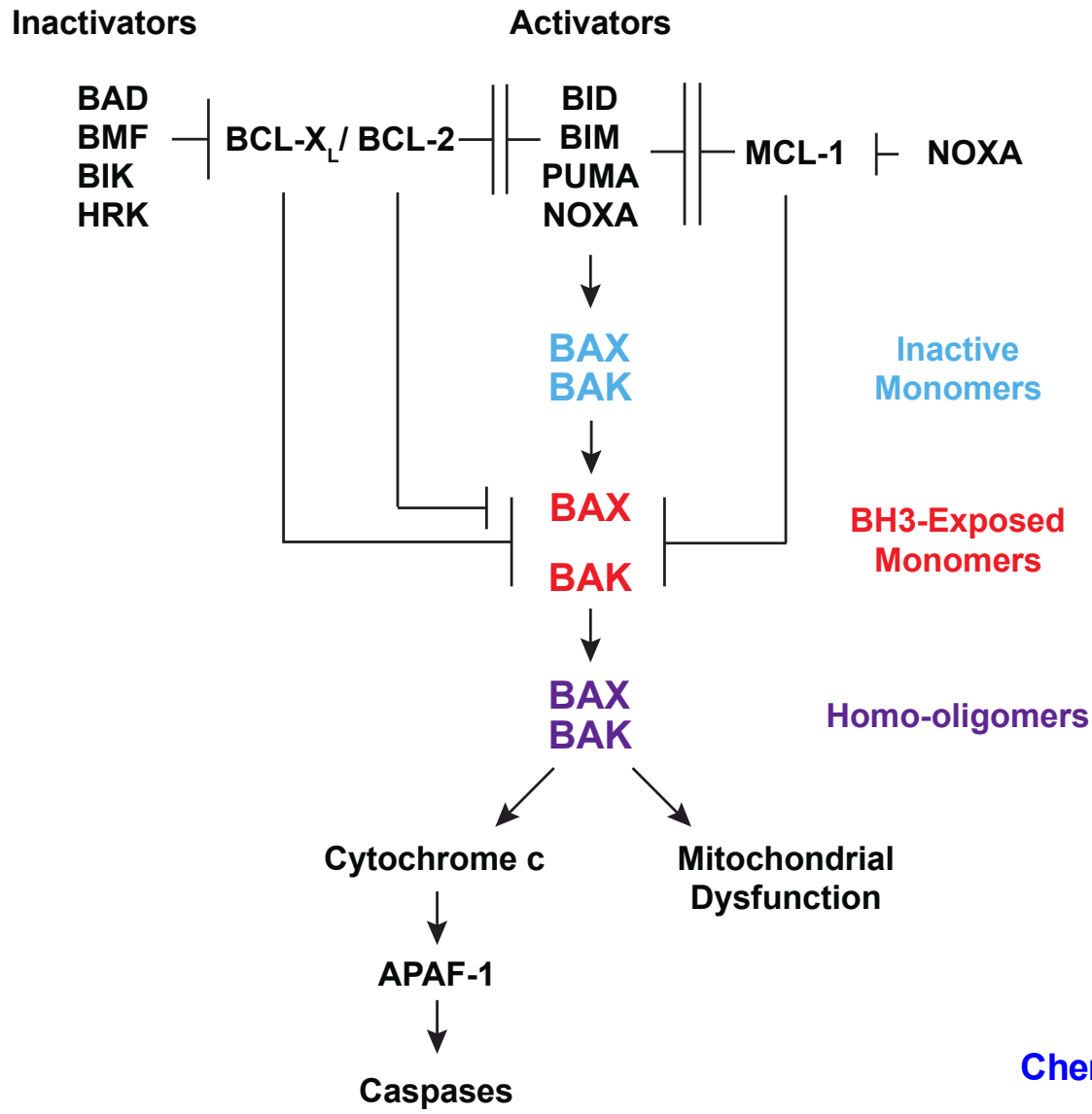
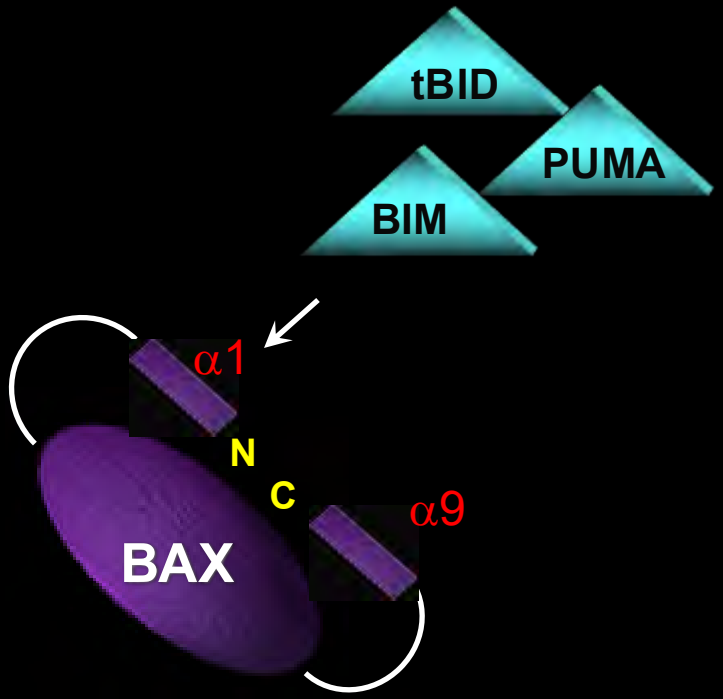
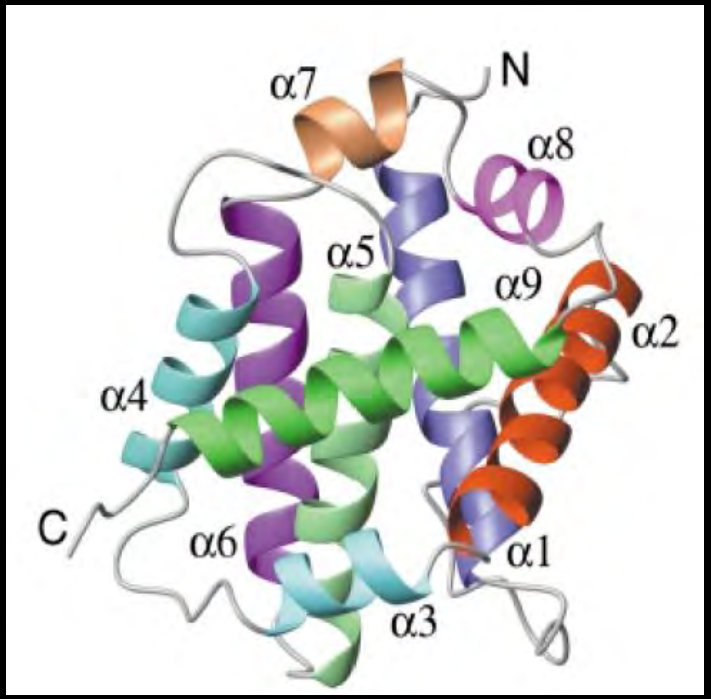
Interconnected Hierarchical Model of Cell Death Regulation by the BCL-2 Family



Chen, et al. *Nat Cell Biol* 2015

Stepwise Activation of BAX by tBID/BIM/PUMA

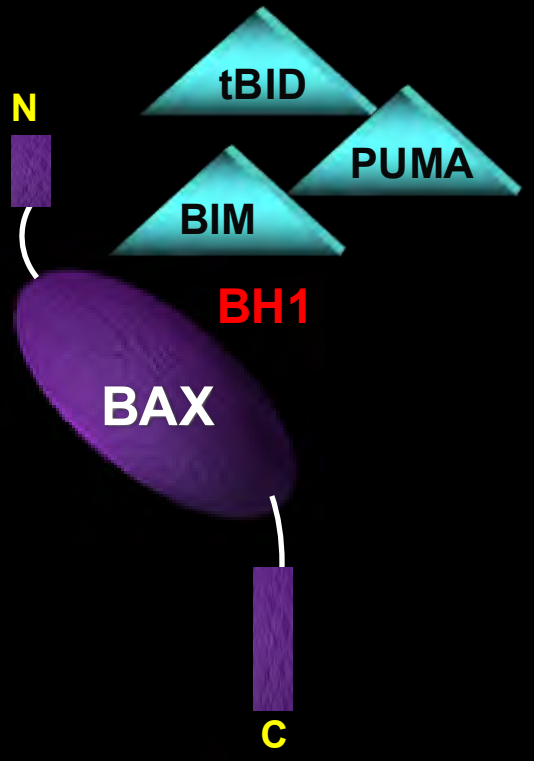




Suzuki et al., 2000 Cell 103:645-654

Molecular Cell 2009, 36:487-99.

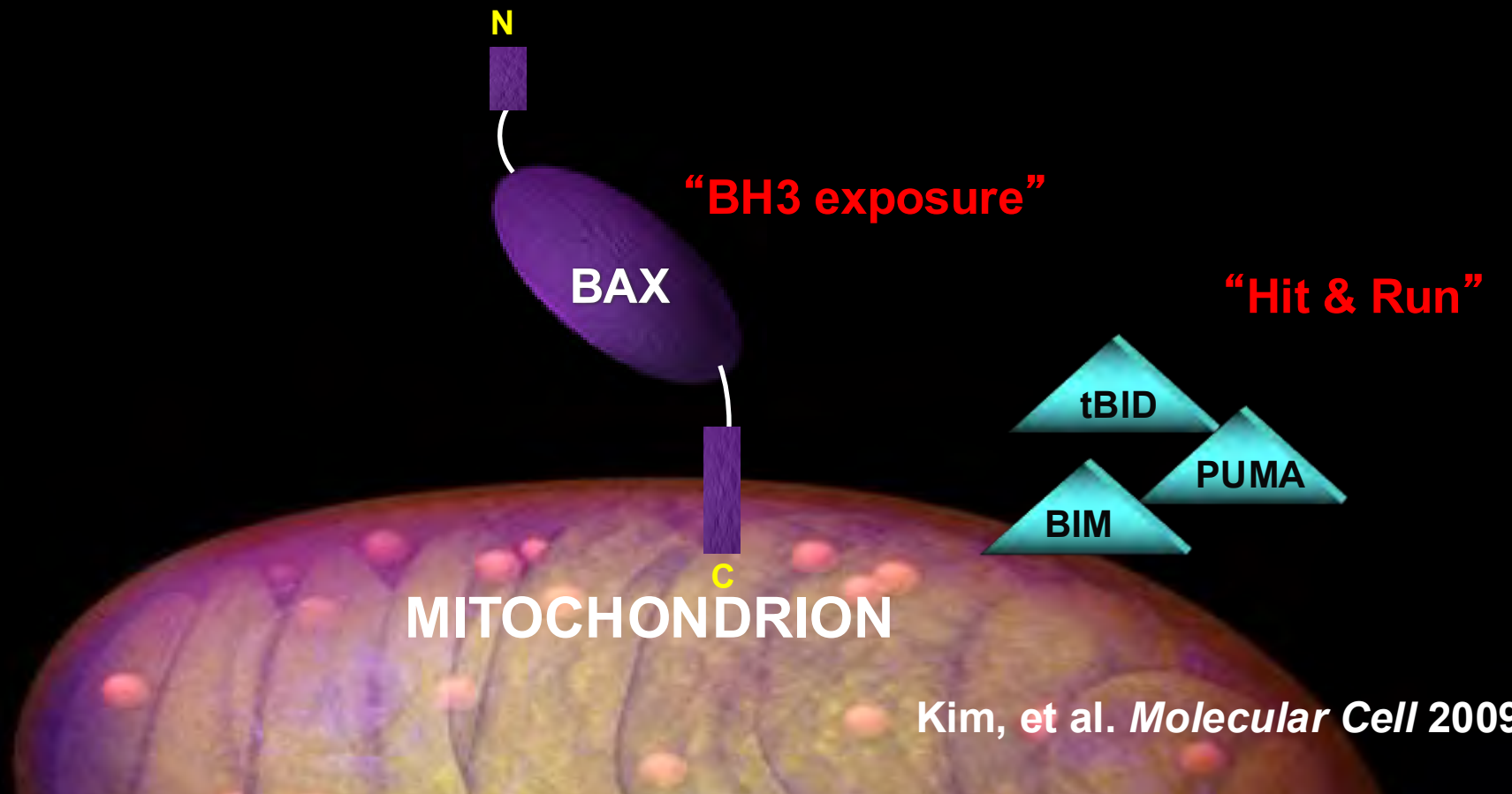
Stepwise Activation of BAX by tBID/BIM/PUMA



MITOCHONDRION

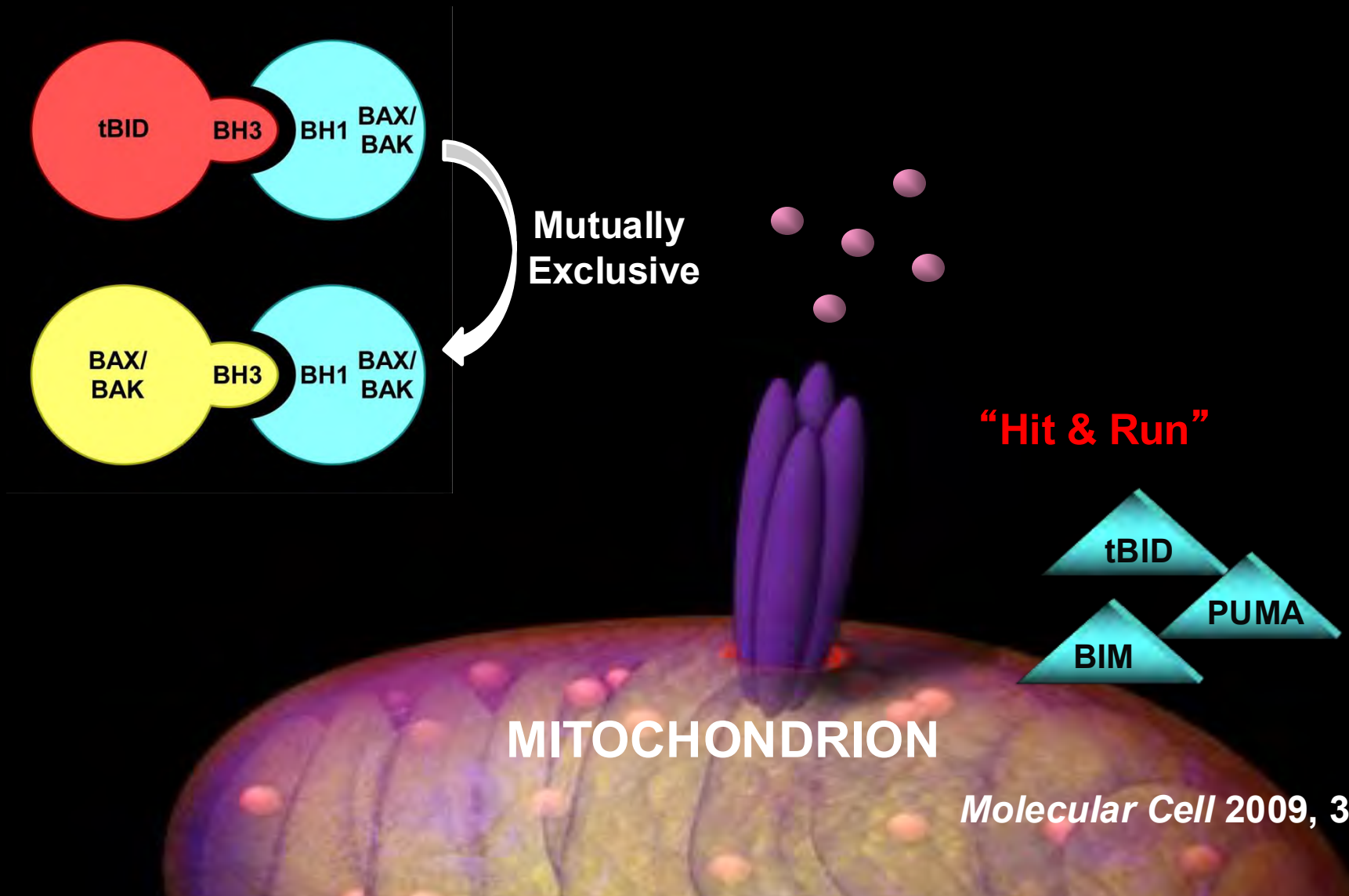
Molecular Cell 2009, 36:487-99.

Stepwise Activation of BAX by tBID/BIM/PUMA



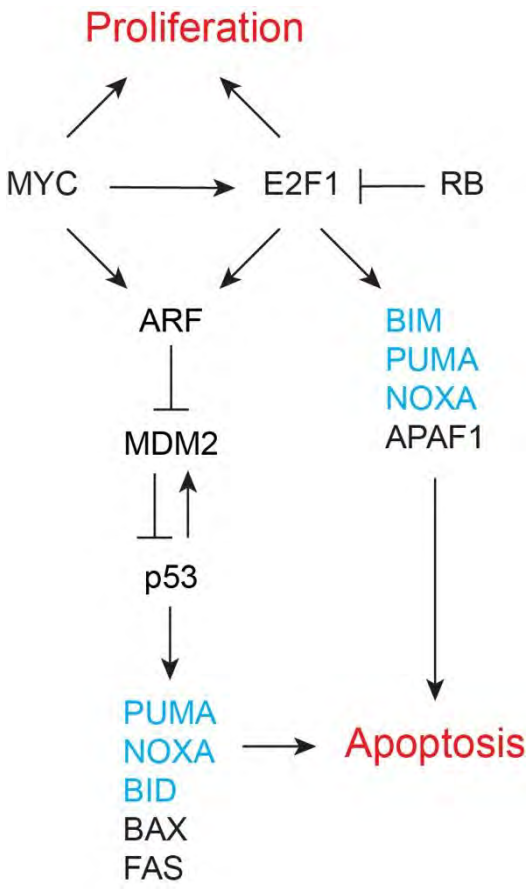
Kim, et al. *Molecular Cell* 2009

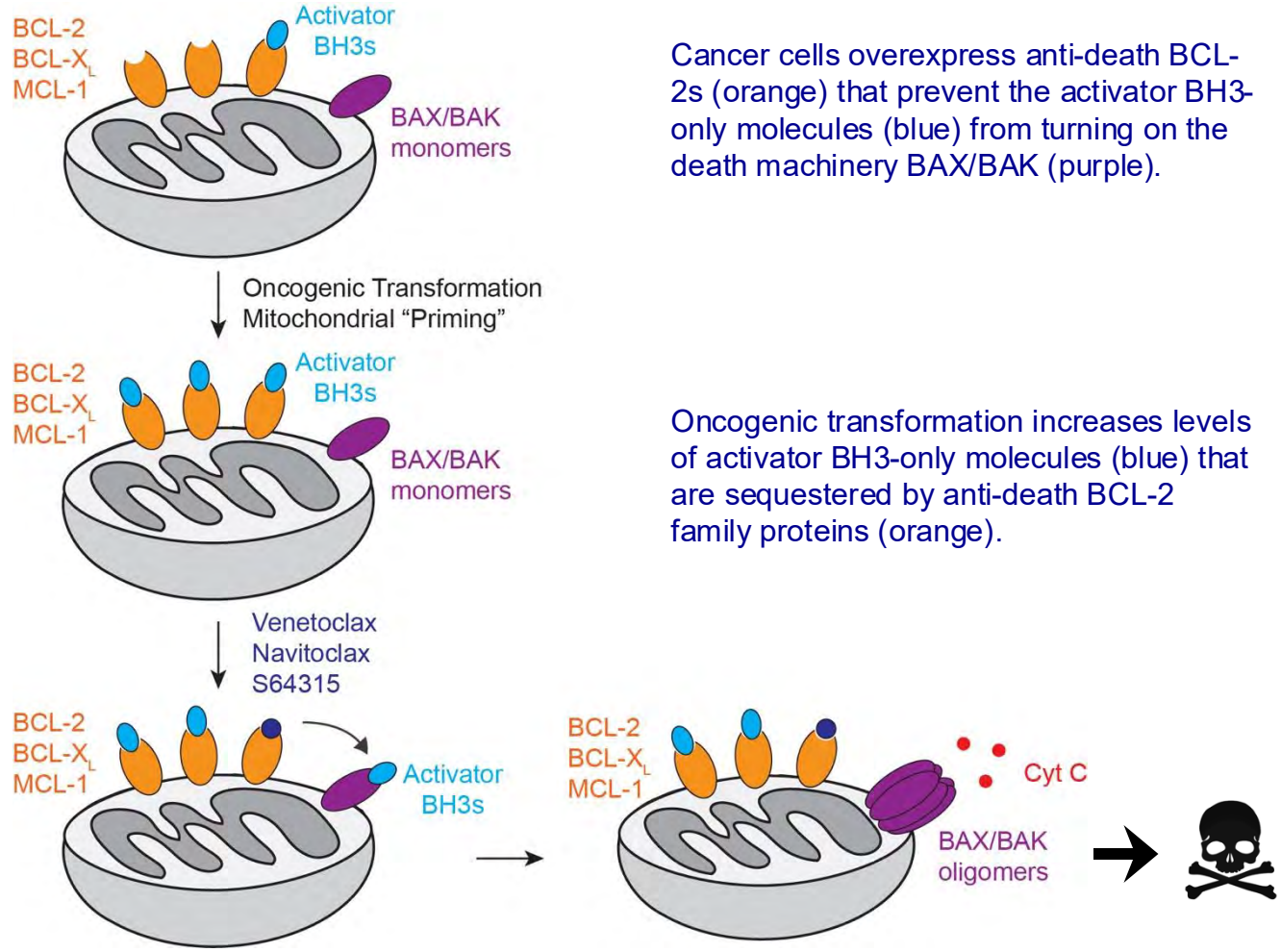
Stepwise Activation of BAX by tBID/BIM/PUMA



Molecular Cell 2009, 36:487-99.

Cancer Cells Are Primed to Undergo Apoptosis upon Inhibition of Anti-Death BCL-2 Family Proteins



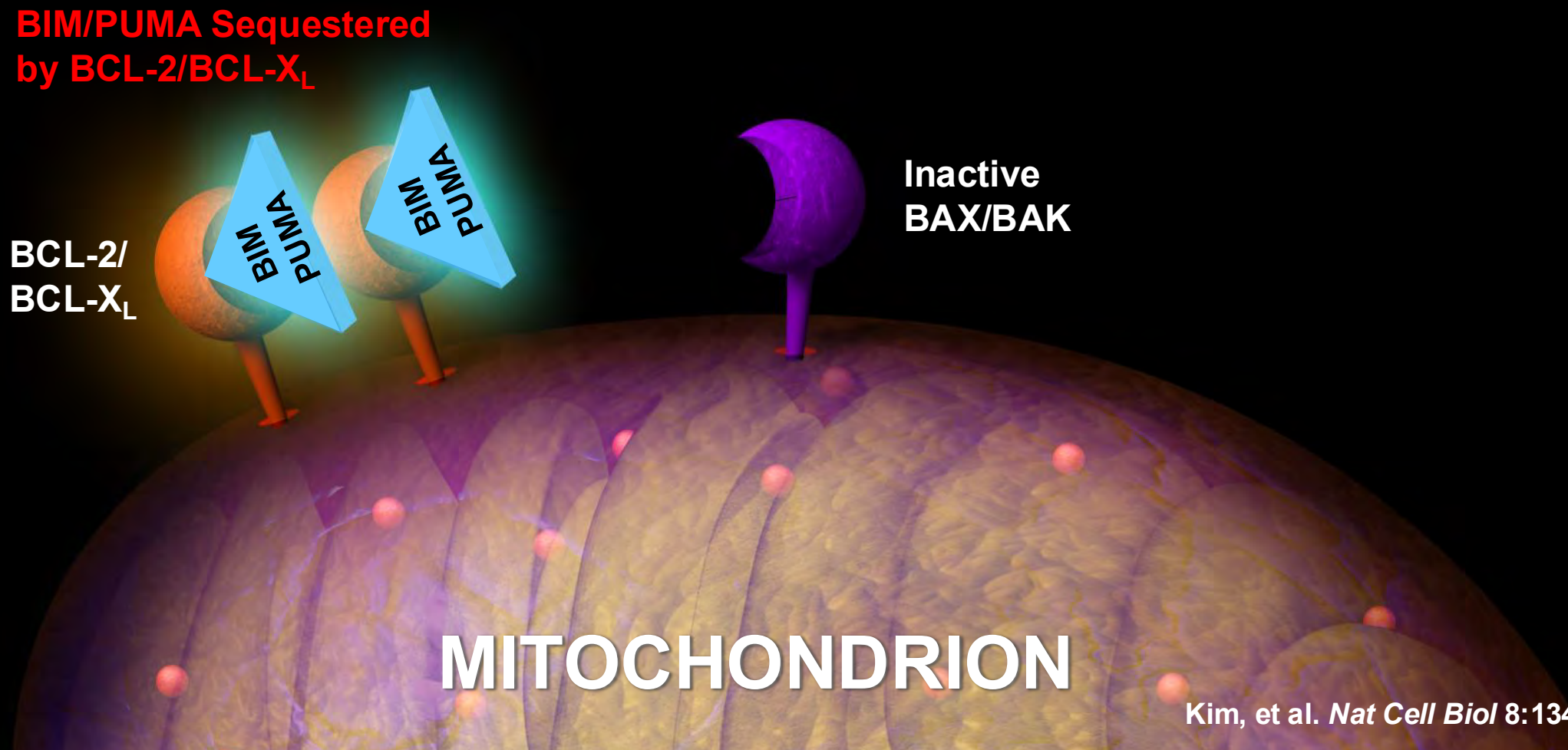


Cancer cells overexpress anti-death BCL-2s (orange) that prevent the activator BH3-only molecules (blue) from turning on the death machinery BAX/BAK (purple).

Oncogenic transformation increases levels of activator BH3-only molecules (blue) that are sequestered by anti-death BCL-2 family proteins (orange).

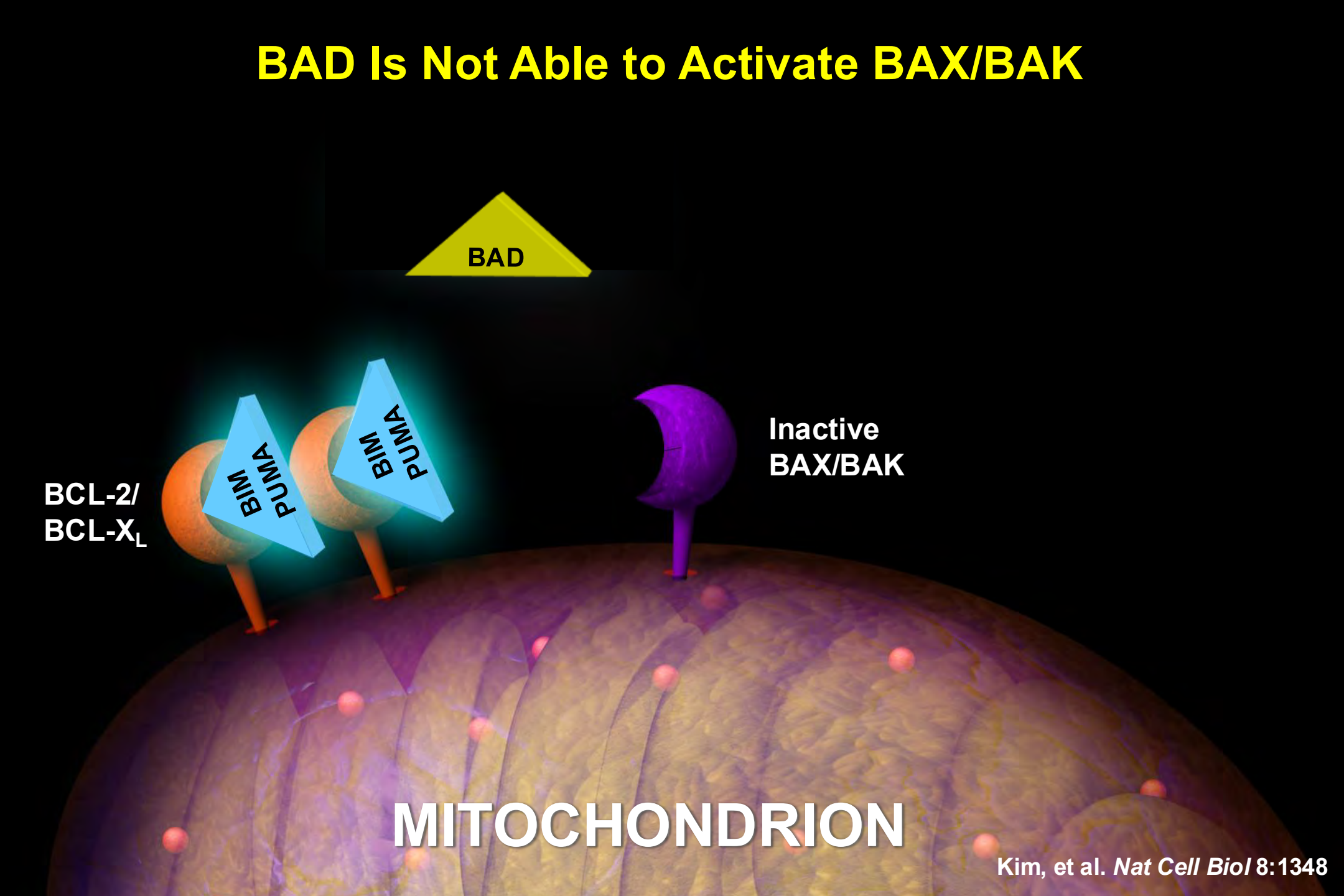
BCL-2 family inhibitors release the activator BH3-only molecules (blue) from anti-death proteins (orange), leading to BAX/BAK death machinery activation and cancer cell death.

BCL-2/BCL-X_L Inhibits Apoptosis by Sequestering Activator BH3-only BIM/PUMA



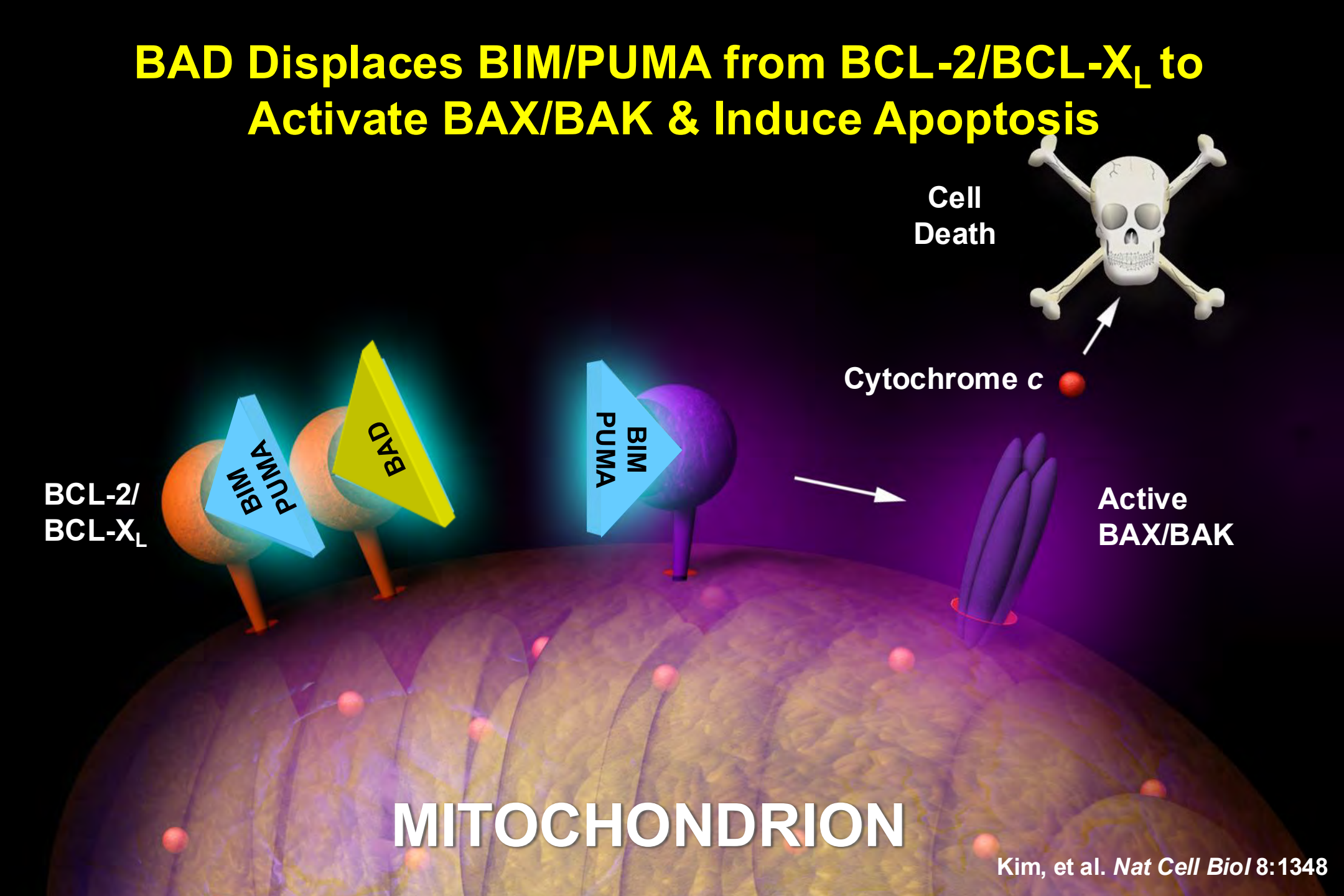
Kim, et al. *Nat Cell Biol* 8:1348

BAD Is Not Able to Activate BAX/BAK



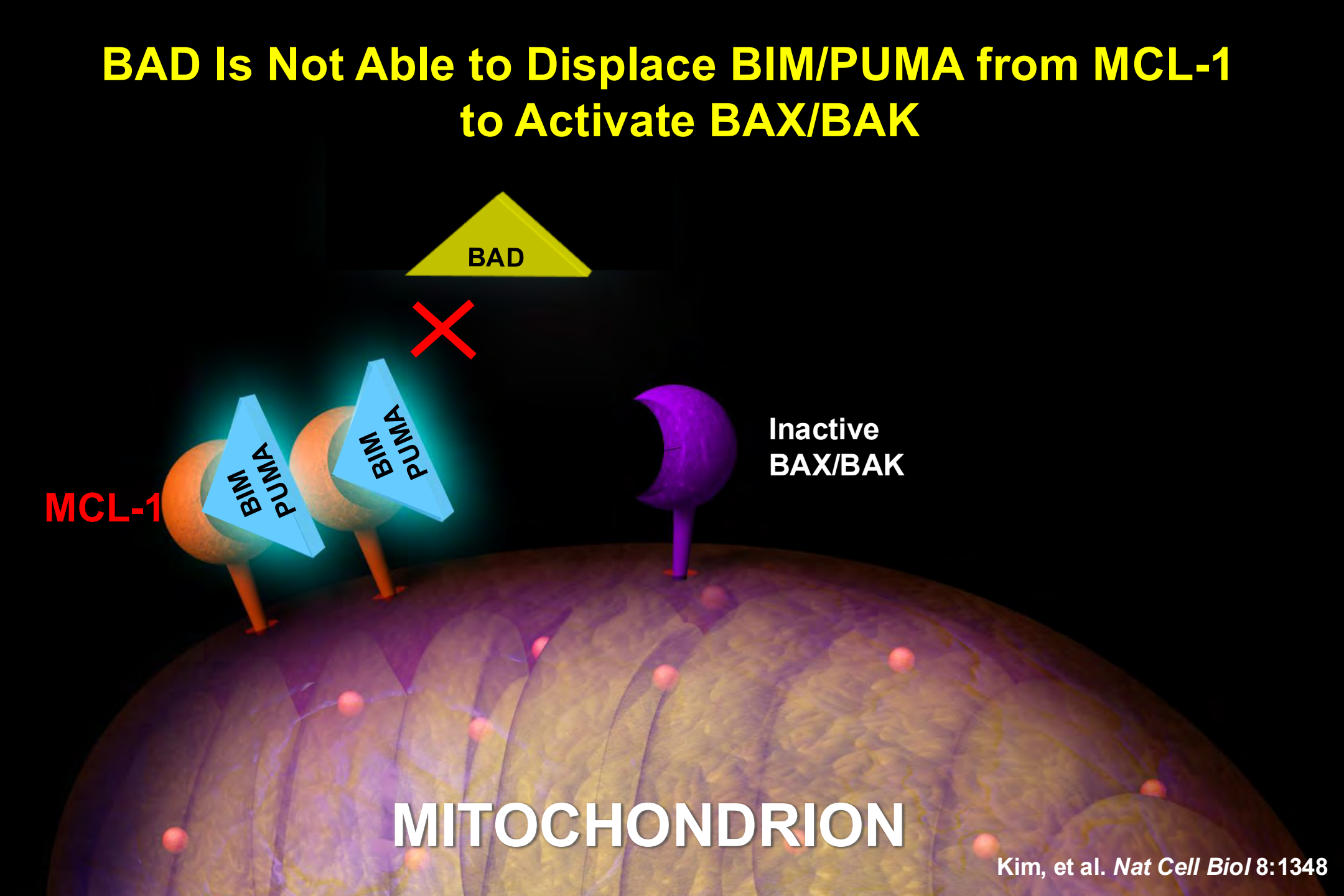
Kim, et al. *Nat Cell Biol* 8:1348

BAD Displaces BIM/PUMA from BCL-2/BCL-X_L to Activate BAX/BAK & Induce Apoptosis



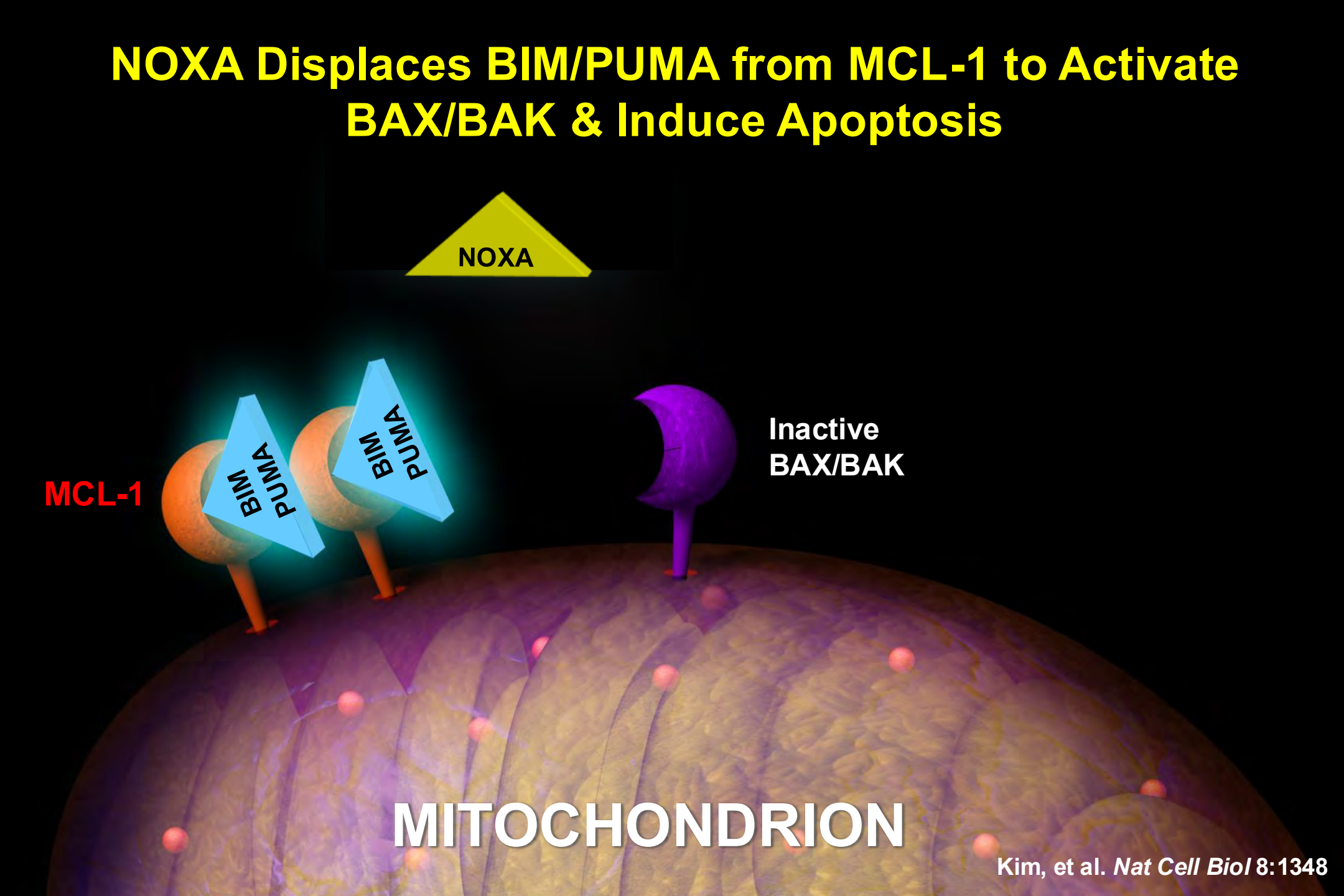
Kim, et al. *Nat Cell Biol* 8:1348

BAD Is Not Able to Displace BIM/PUMA from MCL-1 to Activate BAX/BAK



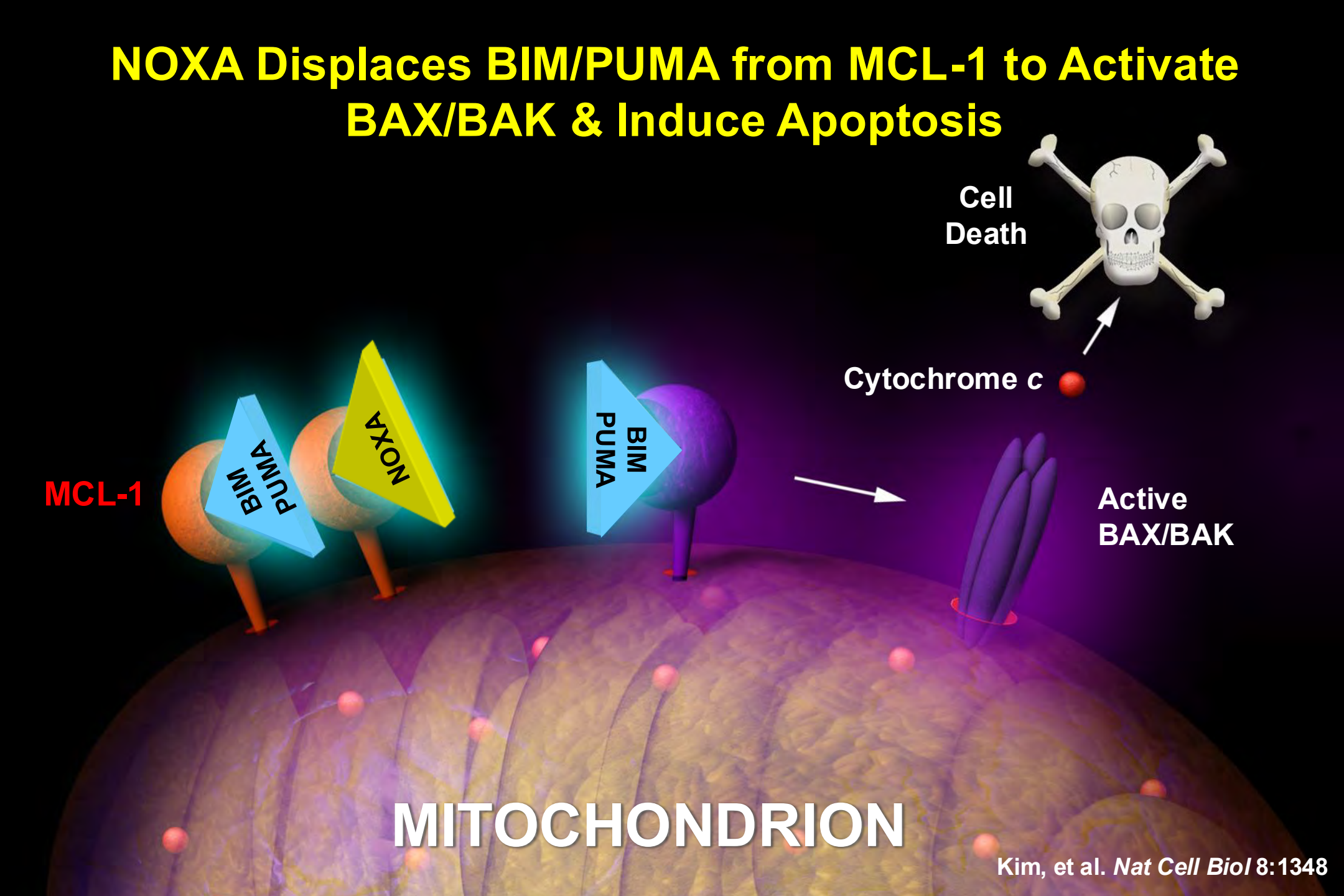
Kim, et al. *Nat Cell Biol* 8:1348

NOXA Displaces BIM/PUMA from MCL-1 to Activate BAX/BAK & Induce Apoptosis



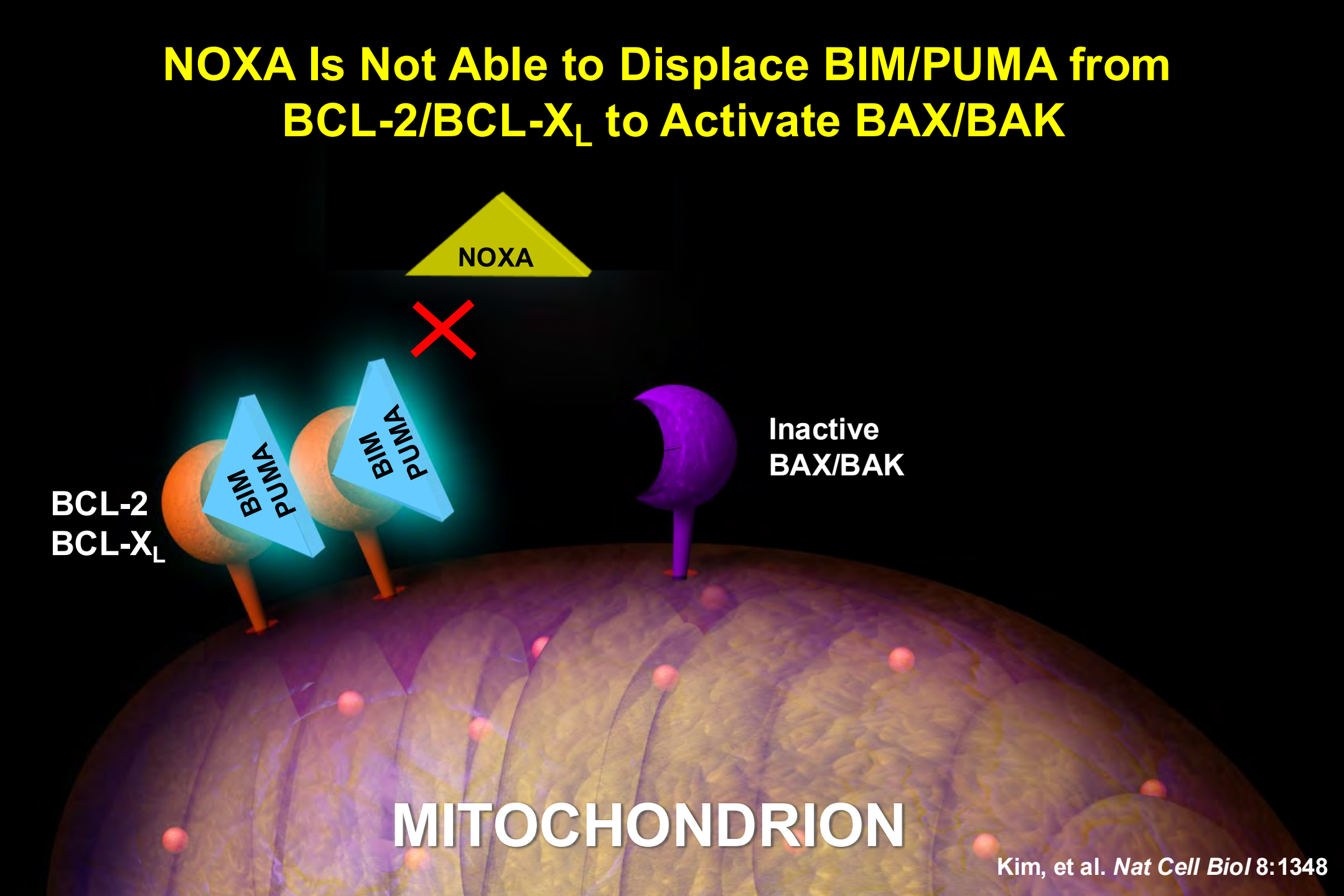
Kim, et al. *Nat Cell Biol* 8:1348

NOXA Displaces BIM/PUMA from MCL-1 to Activate BAX/BAK & Induce Apoptosis



Kim, et al. *Nat Cell Biol* 8:1348

NOXA Is Not Able to Displace BIM/PUMA from BCL-2/BCL-X_L to Activate BAX/BAK



Kim, et al. *Nat Cell Biol* 8:1348

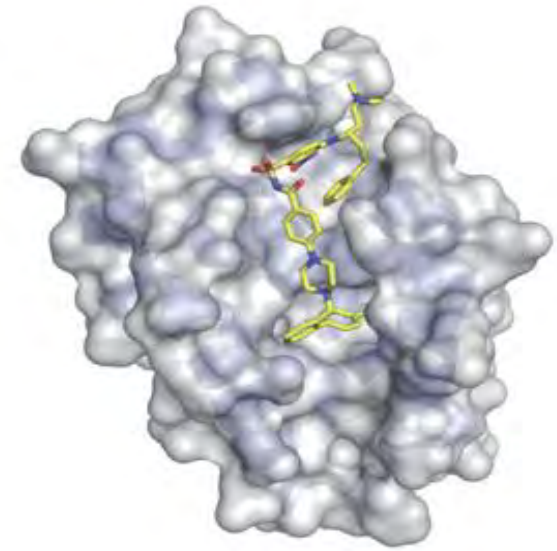
Development of Anti-Death BCL-2 Family Inhibitors for Cancer Therapy

Abbott Laboratories (AbbVie)

"SAR by NMR" | *In vitro* binding analysis | Medicinal chemistry

Drug	BCL-2	BCL-X_L	MCL-1
ABT-737	**K_i < 1 nM**	**K_i < 0.5 nM**	K_i > 1 μM
ABT-263	**K_i < 1 nM**	**K_i < 0.5 nM**	K_i = 550±40 nM
ABT-199	**K_i < 0.01 nM**	K_i = 48 nM	K_i > 444 nM

BCL-X_L-ABT-737 complex



ABT-263 (NAVITOCLAX)

- Orally bioavailable
- Dose-limiting, on-target thrombocytopenia

ABT-199 (VENETOCLAX)

- Selective, platelet sparing BCL-2 inhibitor
- ~80% response rate in CLL

2016 - Approved for the treatment of relapsed/refractory CLL (17p deletion)

S64315 (MIK665)

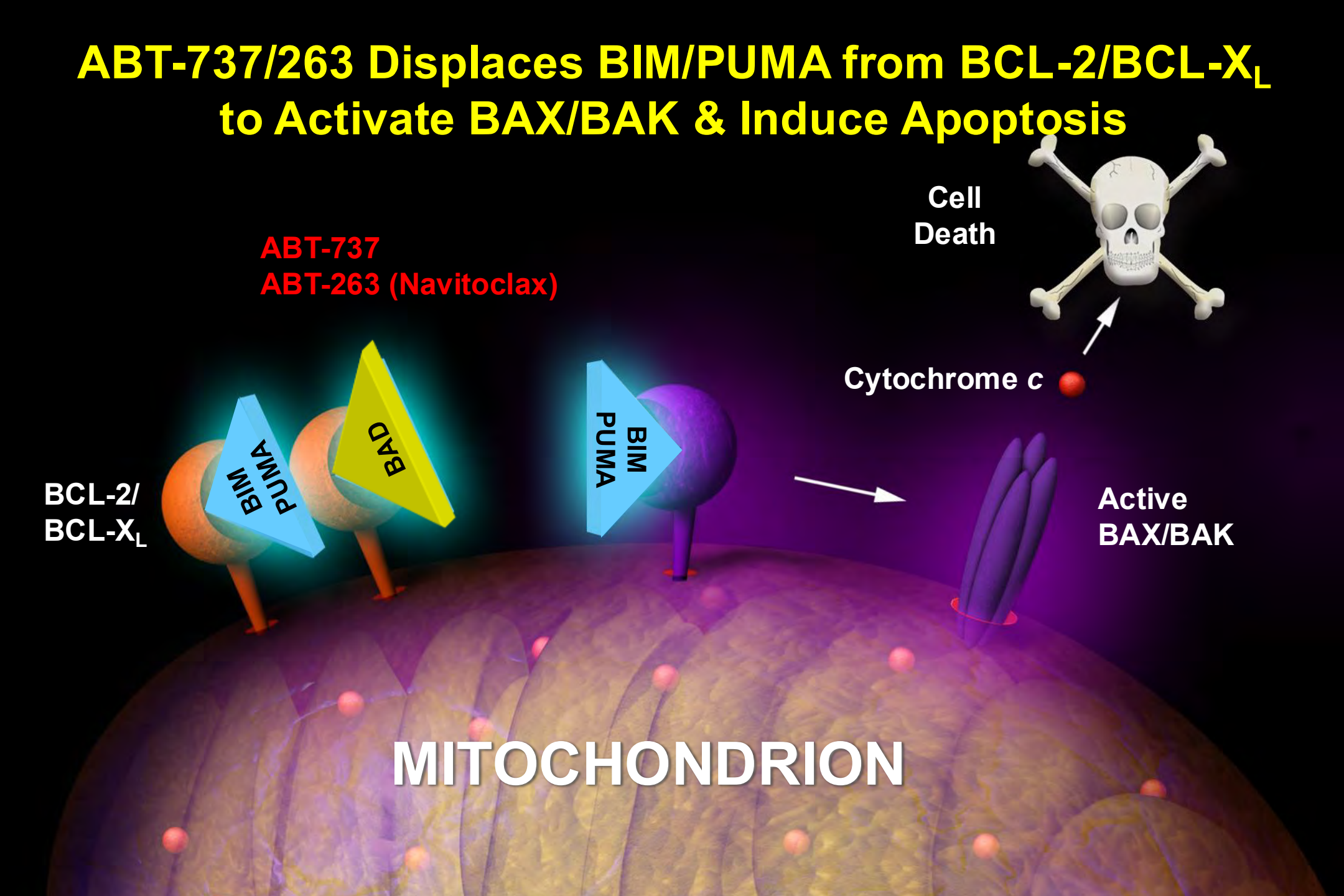
PRT1419

- Selective MCL-1 inhibitors in Clinical trials

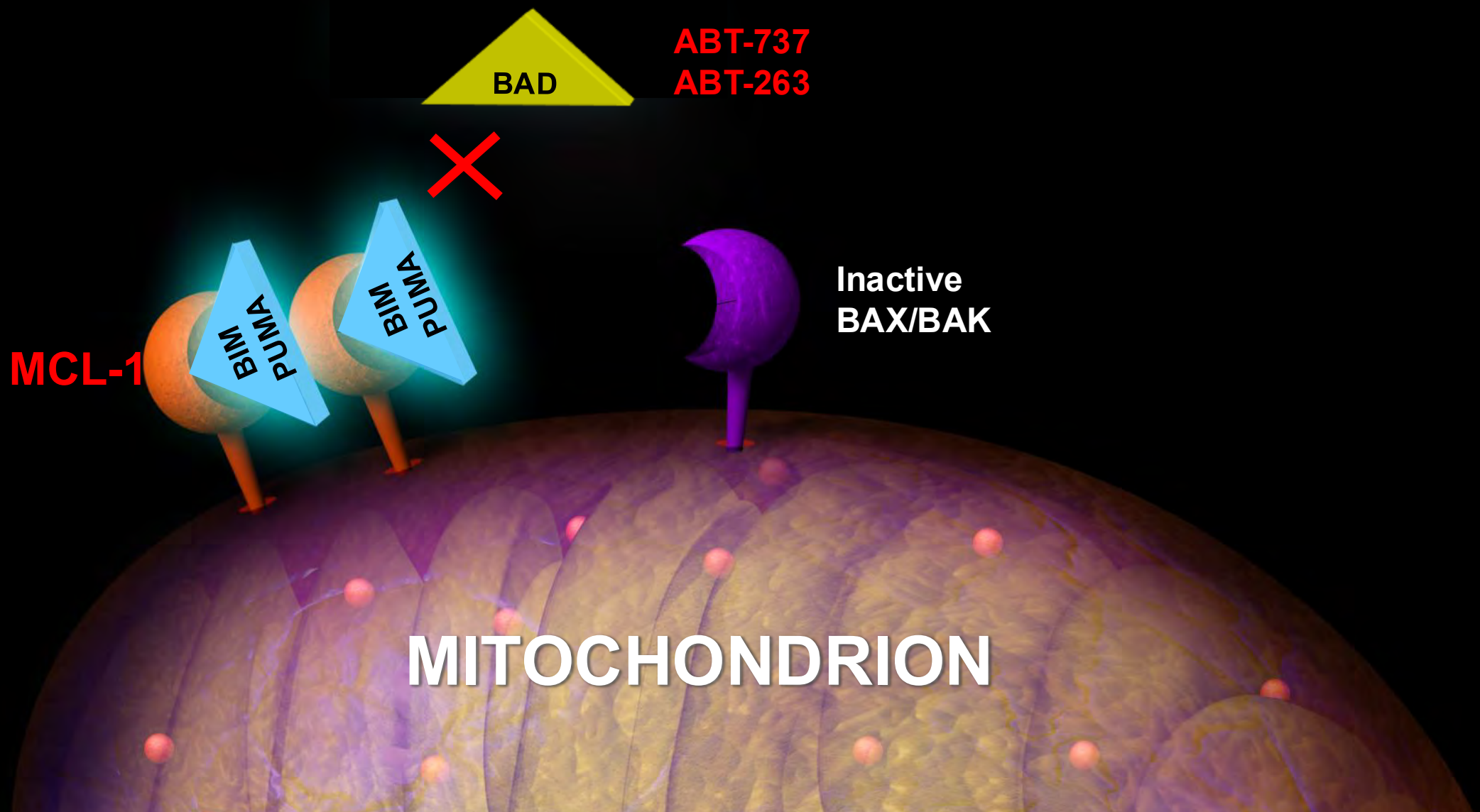
AMG-176 (terminated)
ABBV-467 (terminated)
AZD5991 (terminated)
AMG-397 (terminated)

Oltersdorf, T. et al. *Nature.* **2005** | Sours, A.J. *Nat Med.* **2013** | Roberts, A.W. et al. N Engl J Med **2016**

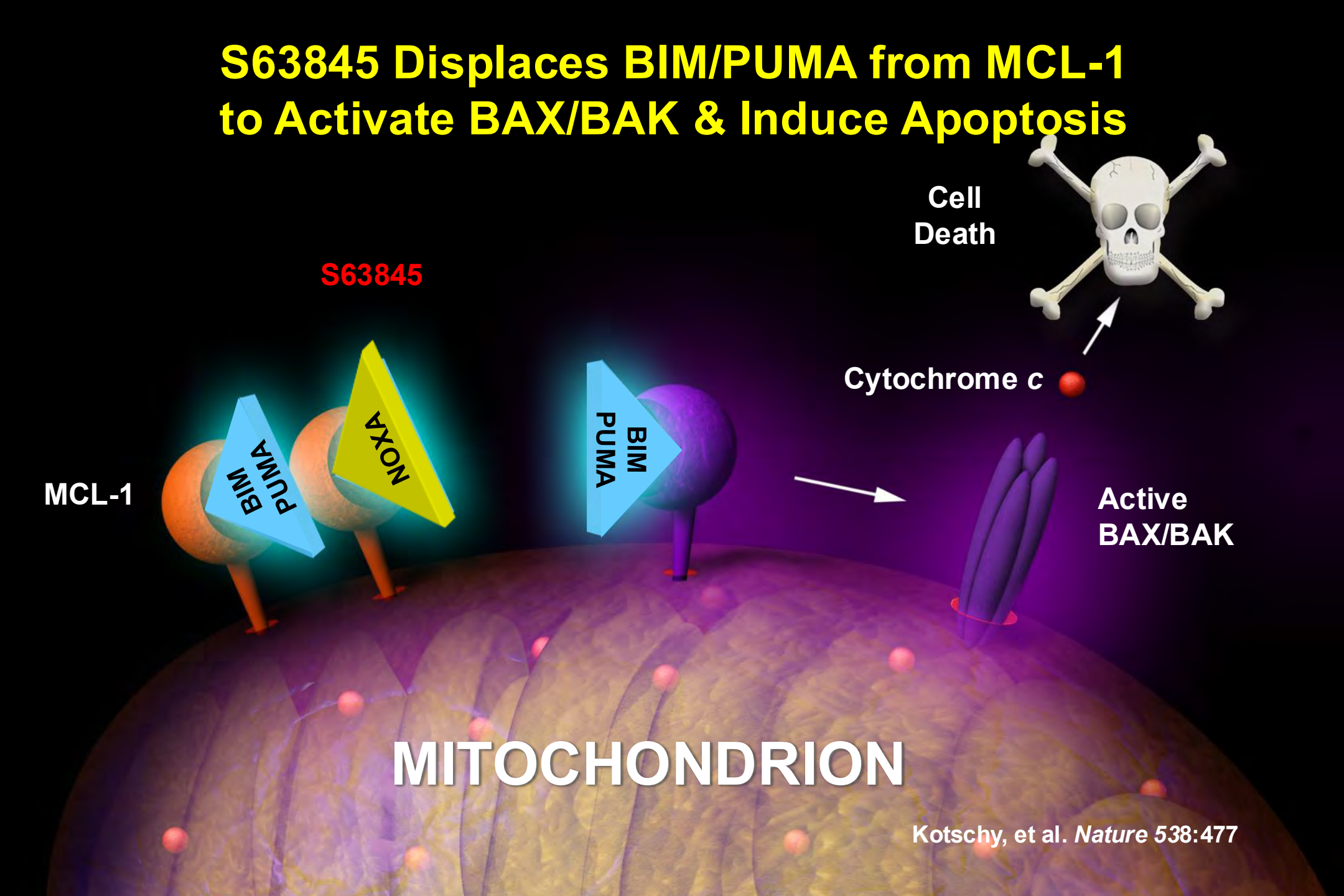
ABT-737/263 Displaces BIM/PUMA from BCL-2/BCL-X_L to Activate BAX/BAK & Induce Apoptosis



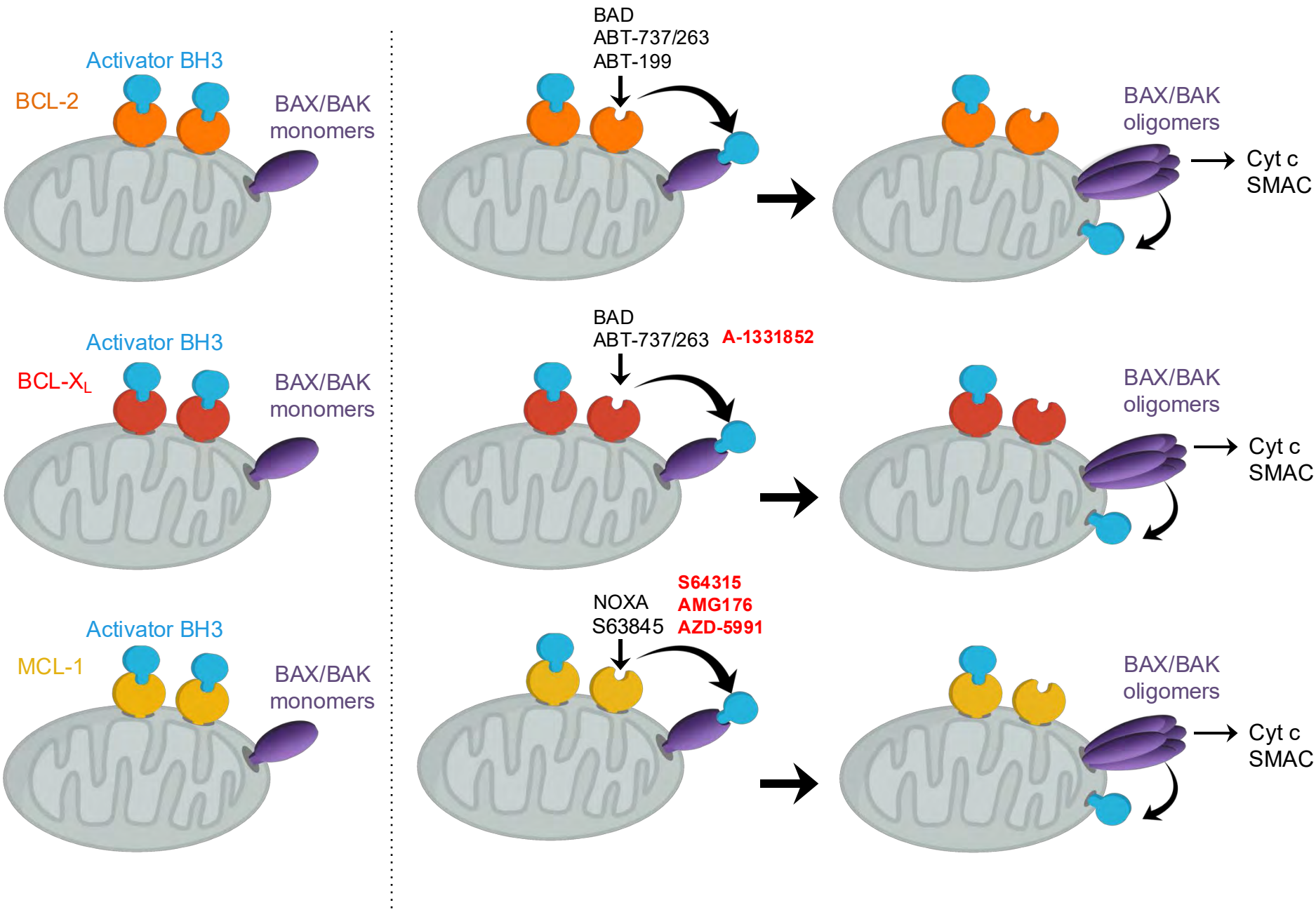
ABT-737/263 Is Not Able to Displace BIM/PUMA from MCL-1 to Activate BAX/BAK



S63845 Displaces BIM/PUMA from MCL-1 to Activate BAX/BAK & Induce Apoptosis

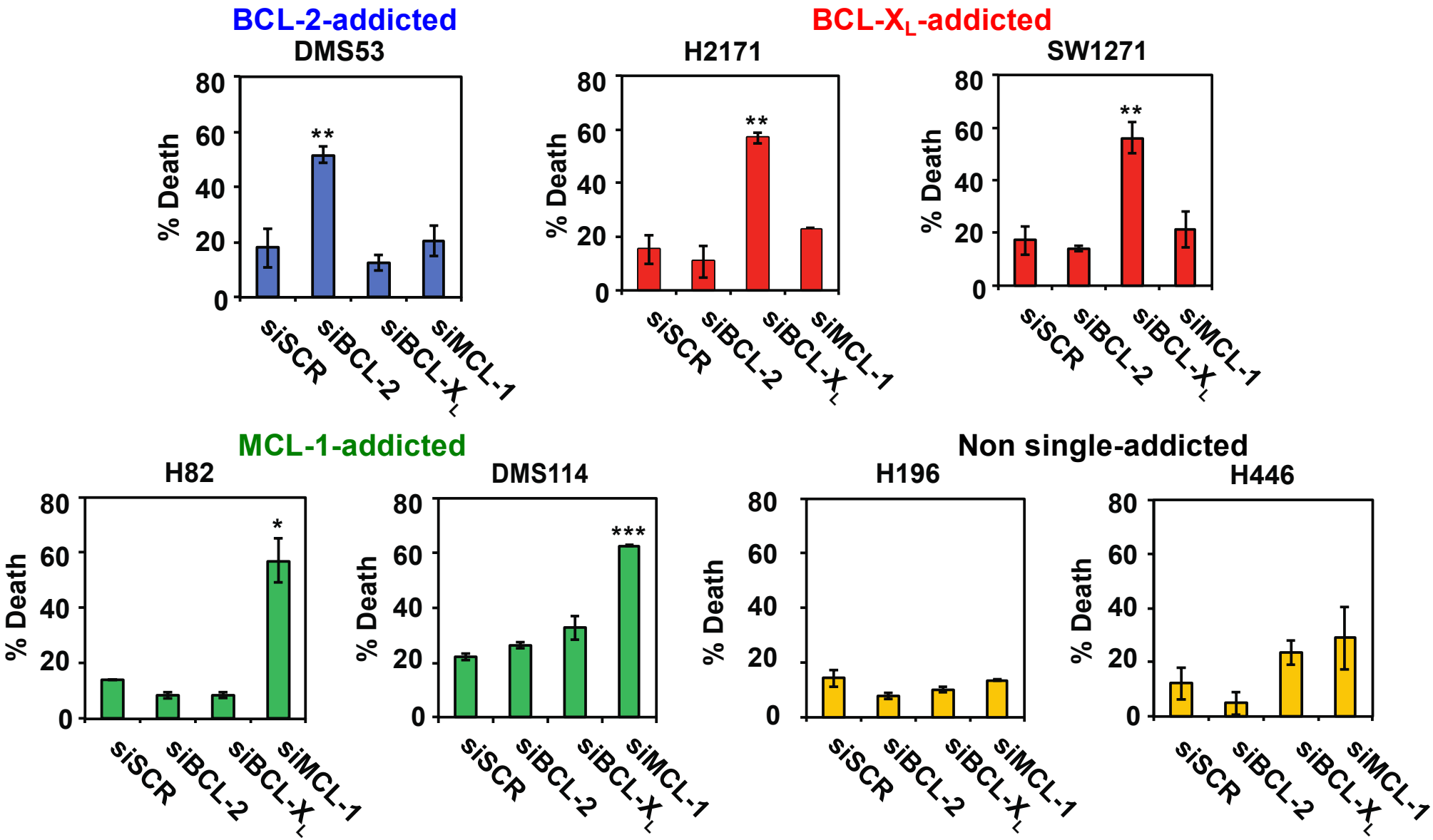


Kotschy, et al. *Nature 538*:477


Activator BH3
BCL-2
BAX/BAK
monomers
BAD
ABT-737/263
ABT-199
BAX/BAK
oligomers
Cyt c
SMAC
Activator BH3
BCL-XL
BAX/BAK
monomers
BAD
ABT-737/263
A-1331852
BAX/BAK
oligomers
Cyt c
SMAC
Activator BH3
MCL-1
BAX/BAK
monomers
NOXA
S63845
S64315
AMG176
AZD-5991
BAX/BAK
oligomers
Cyt c
SMAC

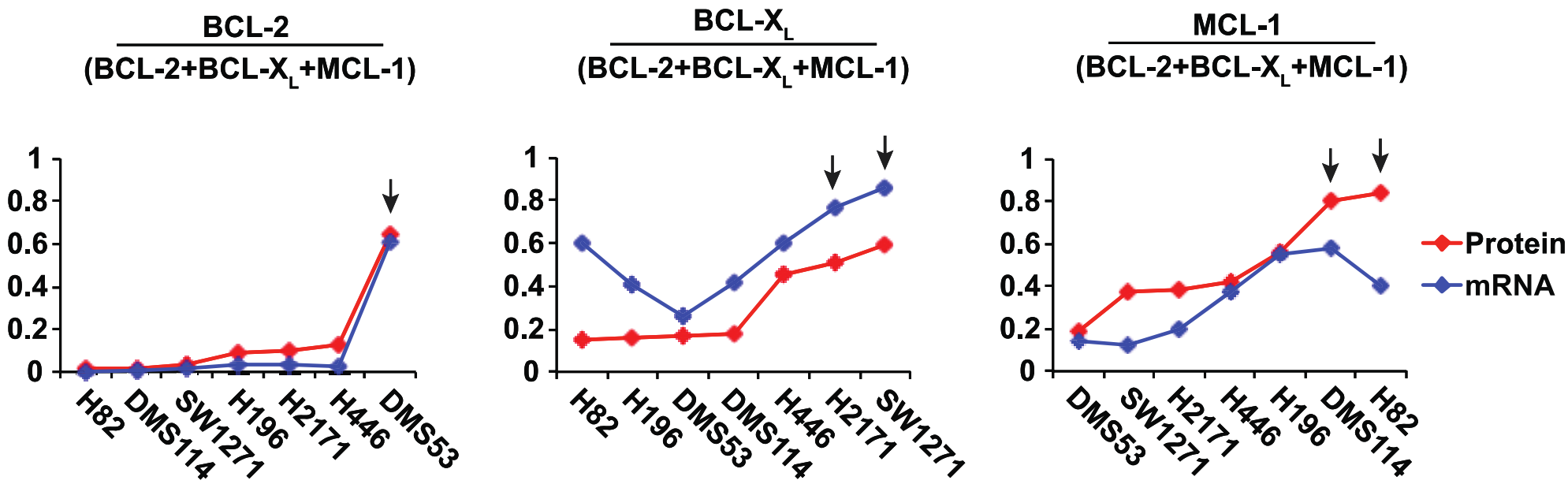
How to identify patients who will respond to ABT-263, ABT-199, or S63845?

SCLC Cell Lines Display Differential Addiction to BCL-2, BCL-X_L or MCL-1 for Survival



Inoue-Yamauchi, et al. *Nat Commun 8:16078*

Differential Addiction to BCL-2, BCL-X_L or MCL-1 Can Be Determined by the Protein Ratios in SCLC



Inoue-Yamauchi, et al. *Nat Commun 8:16078*

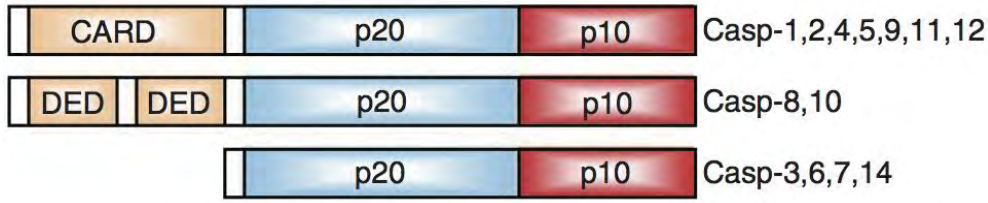
Caspases

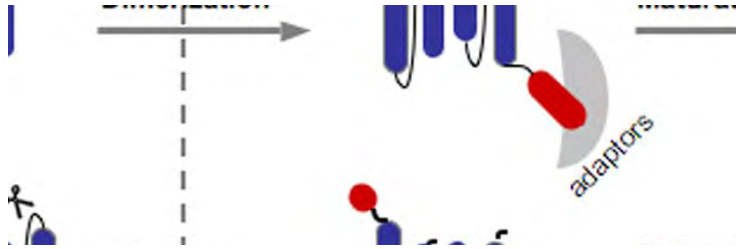
12 Caspases in human: CASP1-10, 12 & 14 (keratinocyte differentiation)

human CASP4/5 = mouse Casp11 or bovine Casp13

Function-based caspase subfamilies

Initiator	Initiator	Effector or Executioner
Inflammation/Pyroptosis	Apoptosis	Apoptosis
Casp-1 (Inflammasome)	Casp-2 (Piddosome)	Casp-3
Casp-4 (human)	Casp-9 (Apoptosome)	Casp-6
Casp-5 (human)	Casp-8 & 10 (DISC)	Casp-7
Casp-11 (mouse)		
Casp-12		





pase subfamilies

Initiator	*Effector*
Apoptosis	*Apoptosis*
casp-2	casp-3
casp-8	casp-6
casp-9	casp-7
casp-10	
casp-12	

c

Substrate specificities

P4-P3-P2-P1

casp-1	W-E-H-D	large P4
casp-4	(W/L)-E-H-D	

Caspase Activation Mechanisms

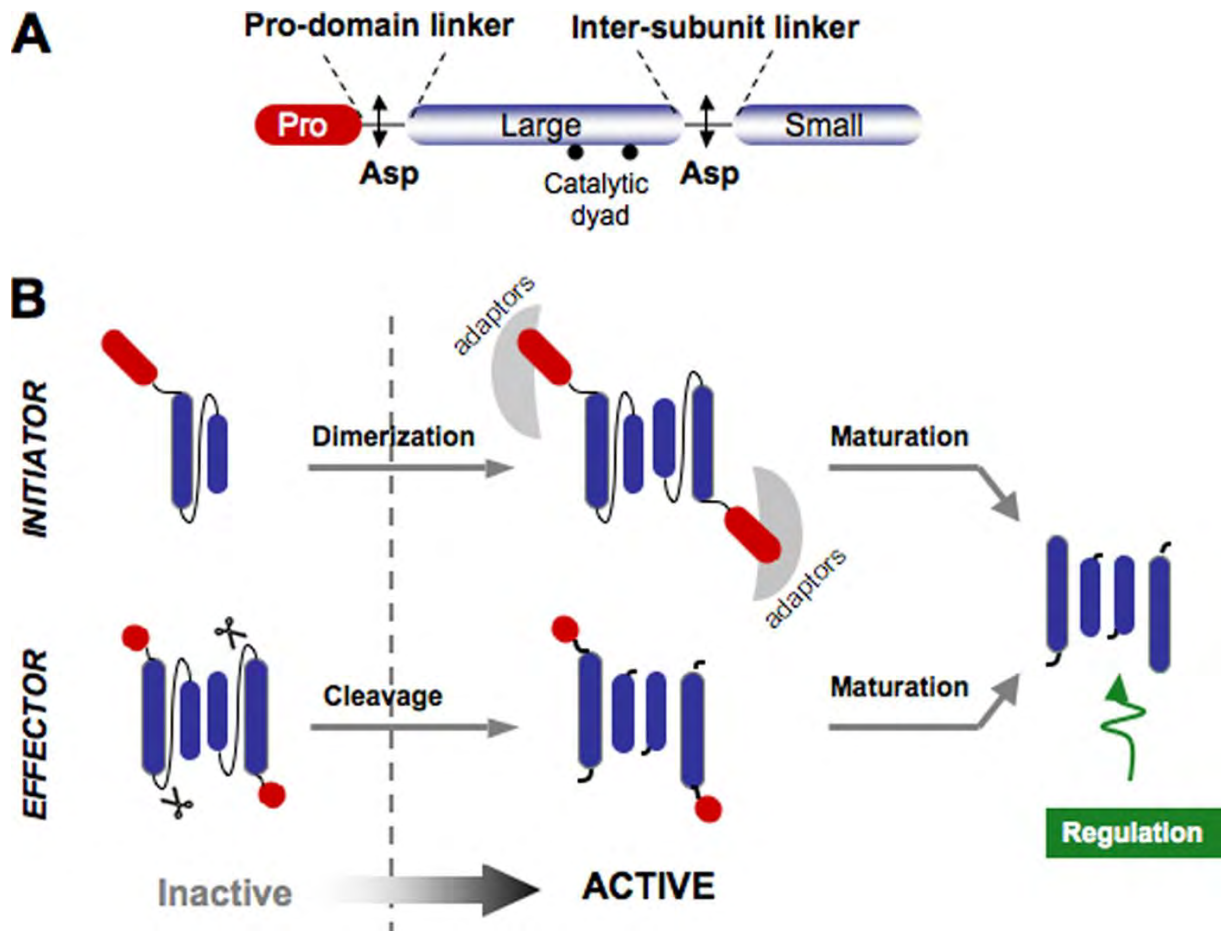
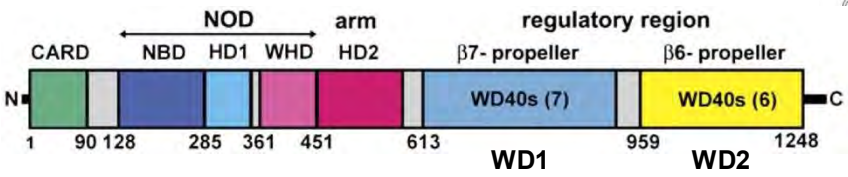


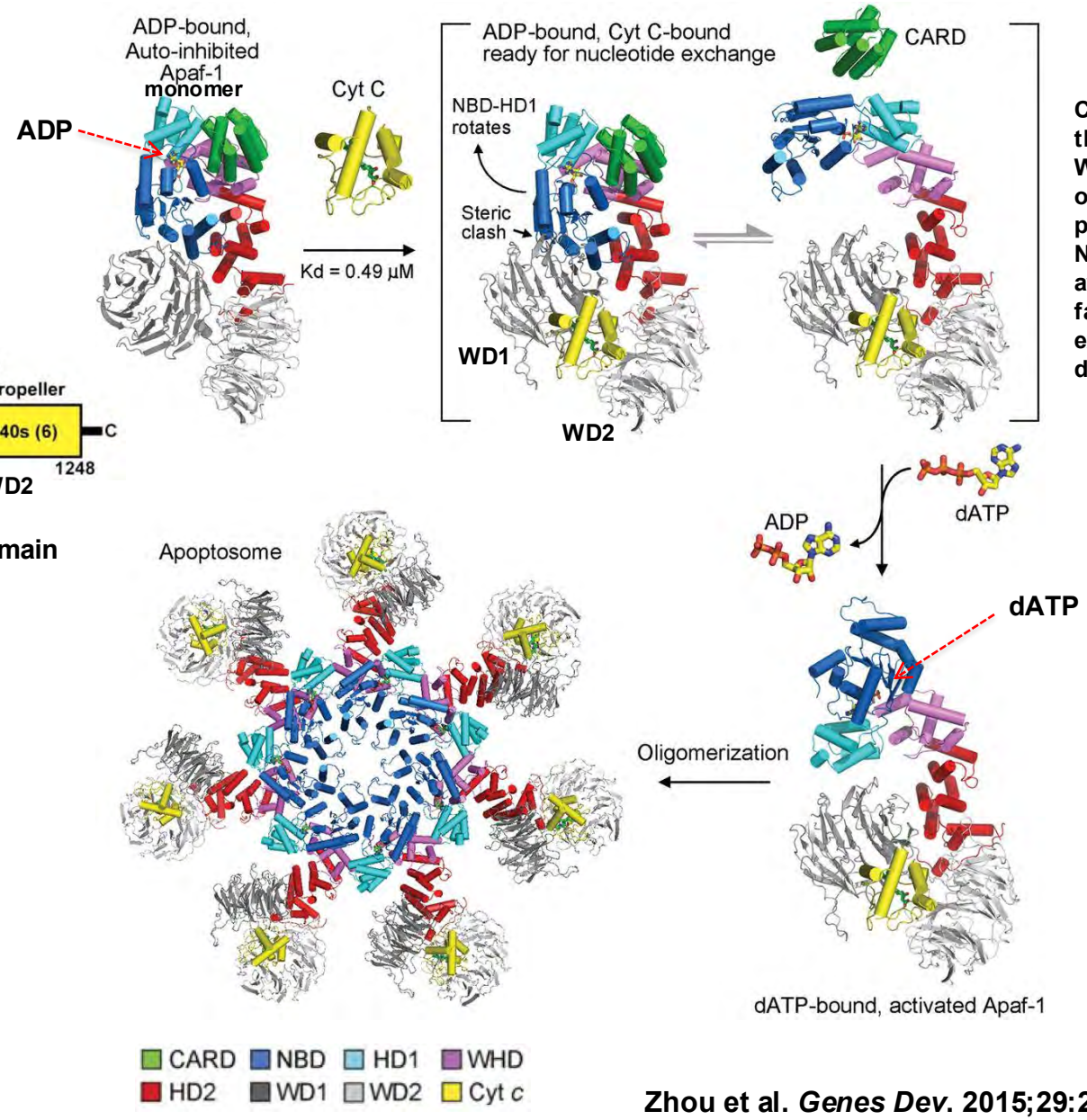
FIGURE 1. *A*, caspase organization. A prodomain precedes the catalytic domain, composed of two covalently linked subunits. Sites for (auto)proteolysis at Asp residues are indicated. *B*, activation mechanisms. Initiators are monomers that activate by prodomain-mediated dimerization. Executioners are dimers that activate by cleavage of intersubunit linkers. Following activation, additional proteolytic events mature the caspases to more stable forms, prone to regulation.

***JBC*, 284:21777-21781**

Mechanism of Apaf-1 Activation and Apoptosome Assembly



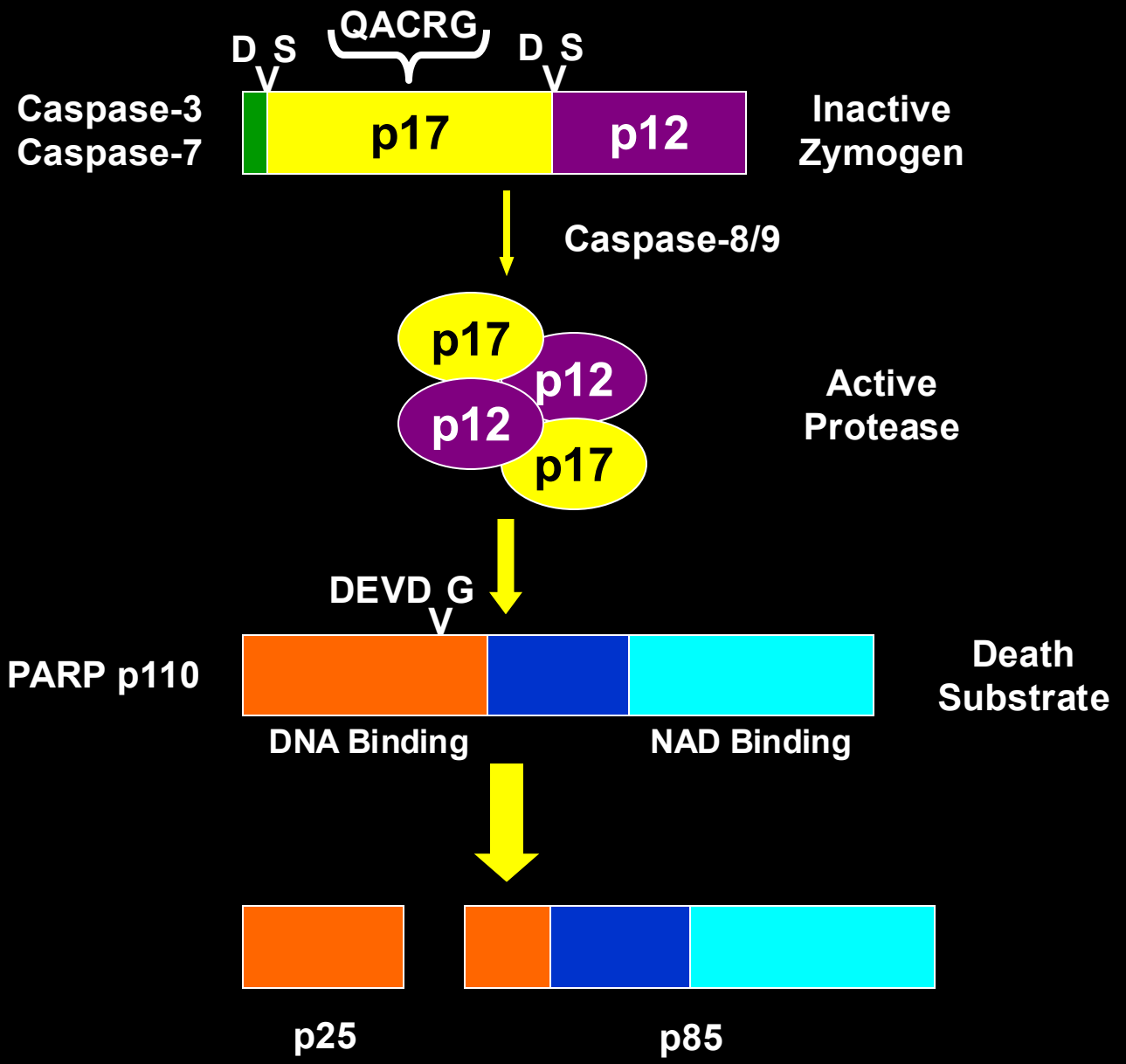
NOD: Nucleotide-binding Oligomerization Domain



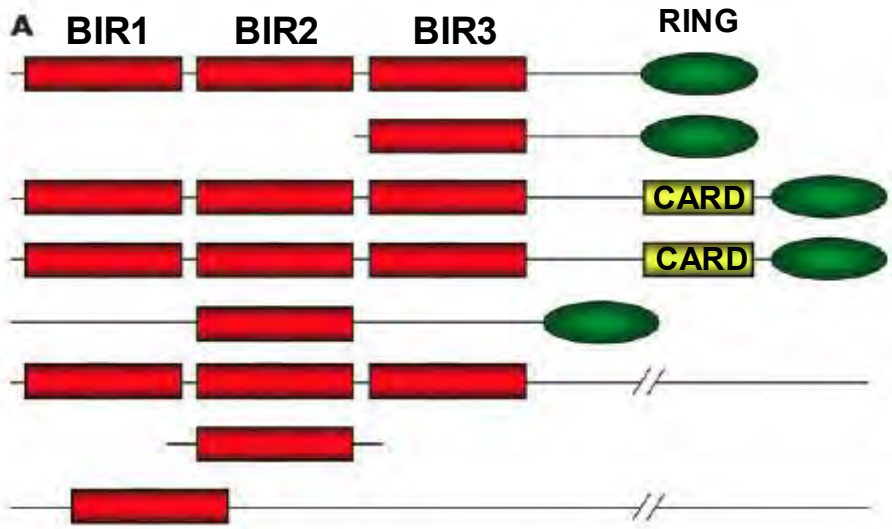
CytC binds to the WD1 and WD2 domains of Apaf-1, pushing the NBD–HD1 away to facilitate ADP exchange with dATP or ATP.

Zhou et al. *Genes Dev.* 2015;29:2349-2361

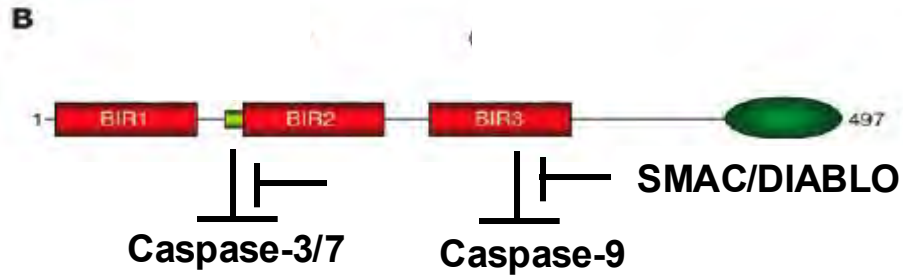
Effector or Executioner Caspases

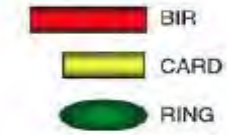


The IAP Family Members



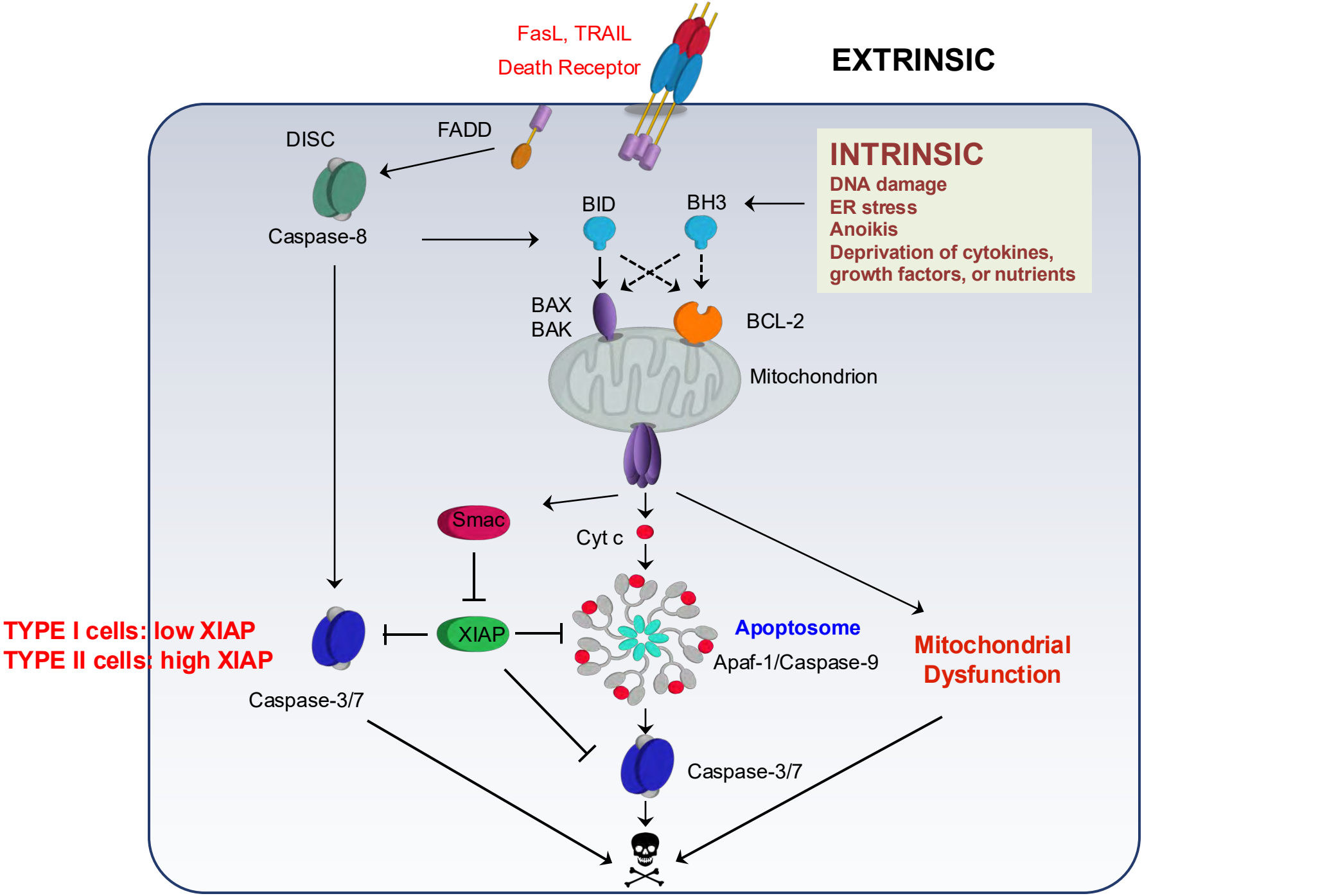
	Caspase binding	SMAC binding
XIAP (BIRC4)	+	+
ILP-2 (BIRC8)		
cIAP1 (BIRC2)	–	+
cIAP2 (BIRC3)	–	+
Livin (BIRC7)	–	+
NAIP (BIRC1)		
Survivin (BIRC5)		
Bruce (BIRC6)		

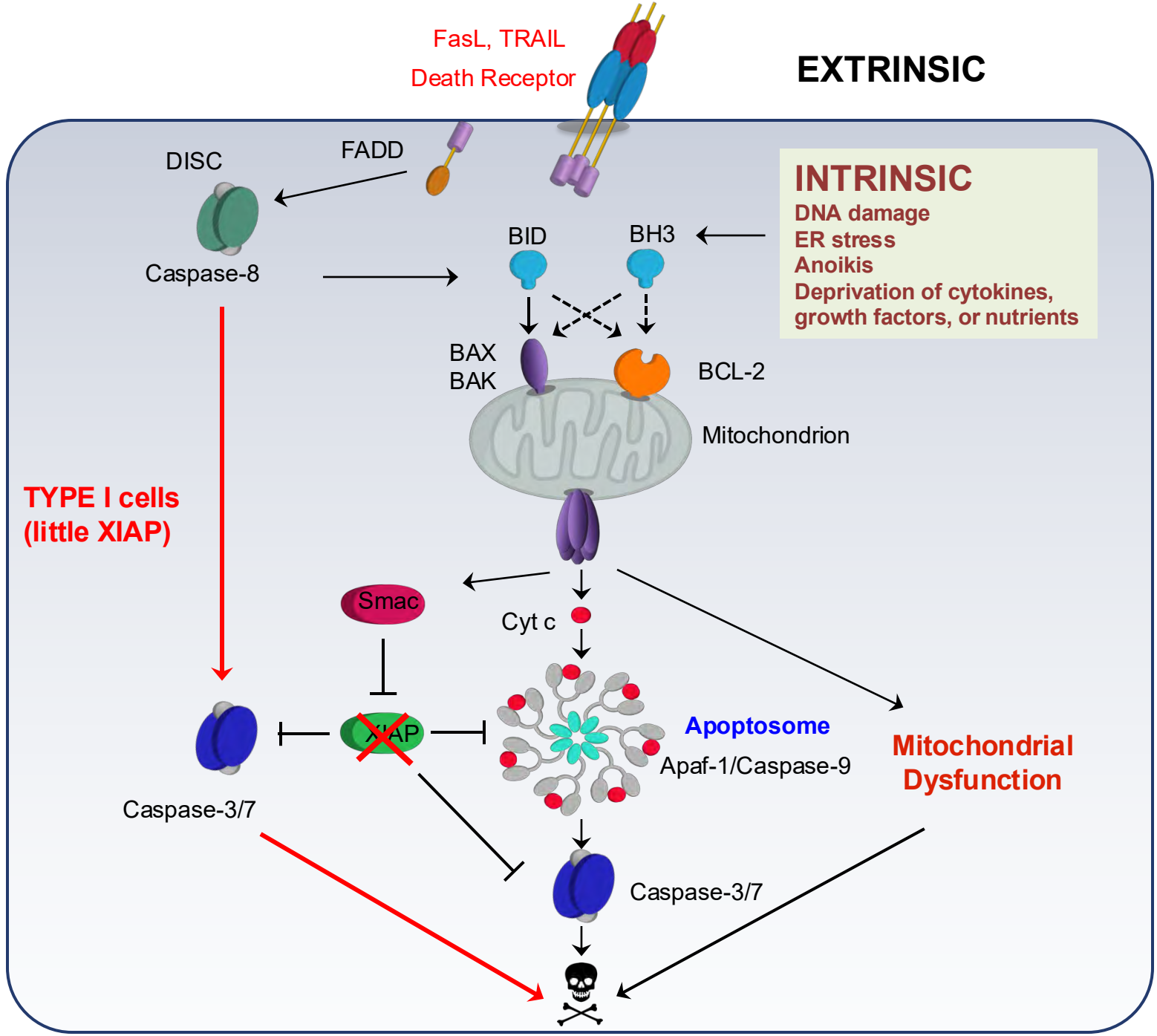


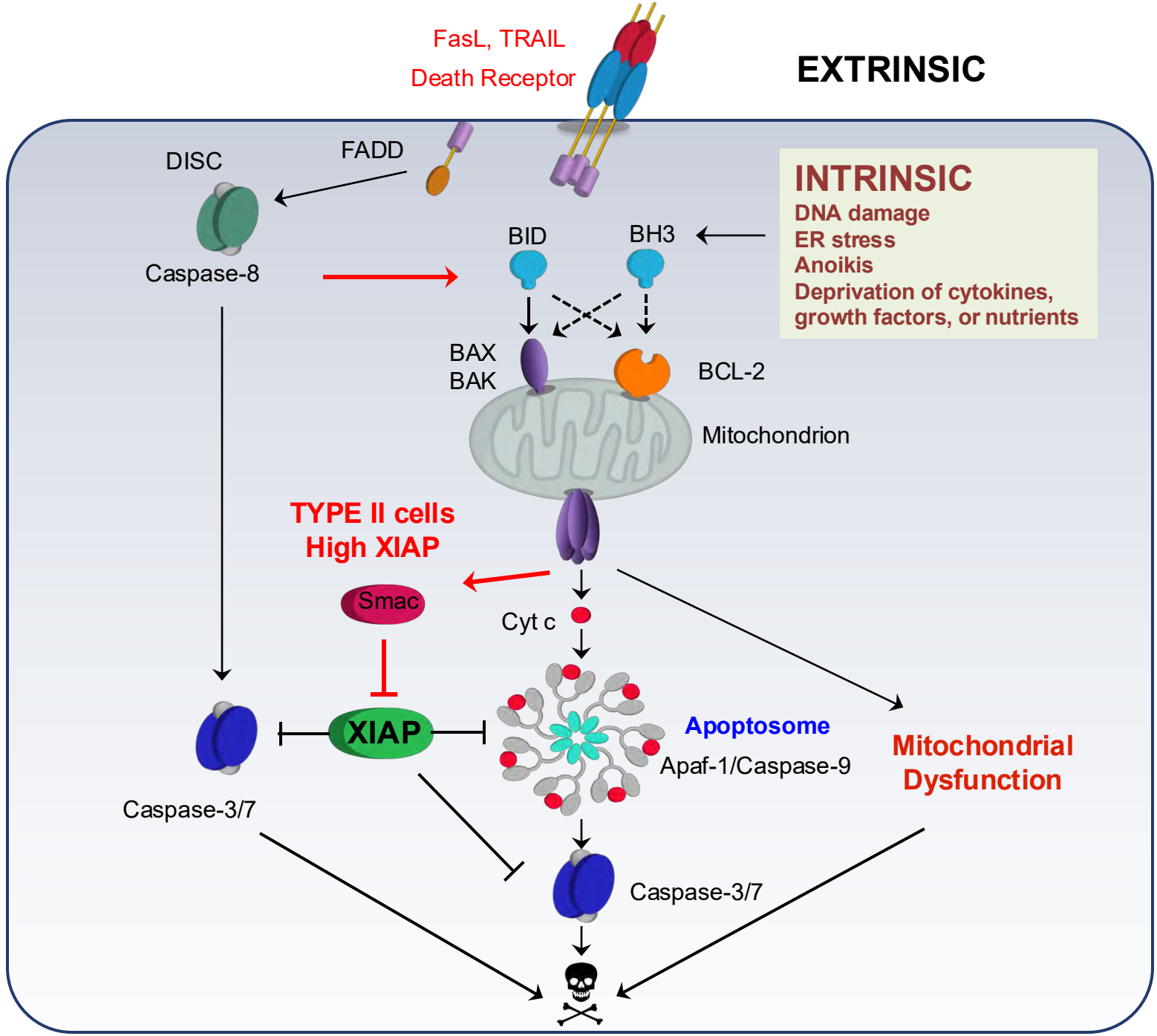


(XIAP prevents substrate binding to Casp3/7 & 9)

IAP-Binding Motif (IBM): AVPI

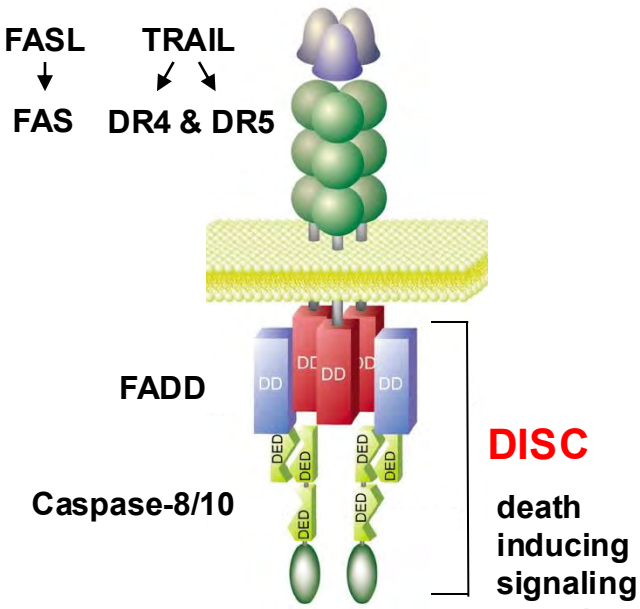

FasL, TRAIL
Death Receptor
EXTRINSIC
DISC
FADD
Caspase-8
INTRINSIC
DNA damage
ER stress
Anoikis
Deprivation of cytokines, growth factors, or nutrients
BID
BH3
BAX
BAK
BCL-2
Mitochondrion
Smac
Cyt c
TYPE I cells: low XIAP
TYPE II cells: high XIAP
XIAP
Apoptosome
Apaf-1/Caspase-9
Mitochondrial Dysfunction
Caspase-3/7
Caspase-3/7

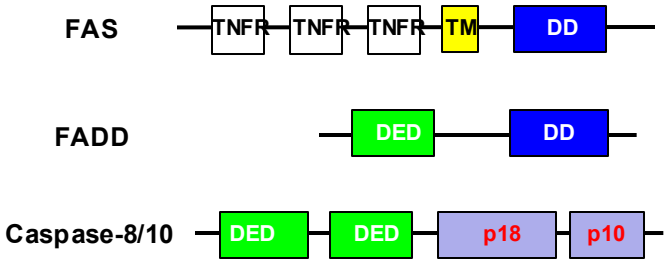

FasL, TRAIL
Death Receptor
EXTRINSIC
DISC
FADD
Caspase-8
INTRINSIC
DNA damage
ER stress
Anoikis
Deprivation of cytokines, growth factors, or nutrients
BID
BH3
BAX
BAK
BCL-2
Mitochondrion
TYPE I cells
(little XIAP)
Smac
Cyt c
XIAP
Apoptosome
Apaf-1/Caspase-9
Mitochondrial Dysfunction
Caspase-3/7
Caspase-3/7


FasL, TRAIL
Death Receptor
EXTRINSIC
DISC
FADD
Caspase-8
INTRINSIC
DNA damage
ER stress
Anoikis
Deprivation of cytokines, growth factors, or nutrients
BID
BH3
BAX
BAK
BCL-2
Mitochondrion
TYPE II cells
High XIAP
Smac
Cyt c
XIAP
Apoptosome
Apaf-1/Caspase-9
Mitochondrial Dysfunction
Caspase-3/7
Caspase-3/7

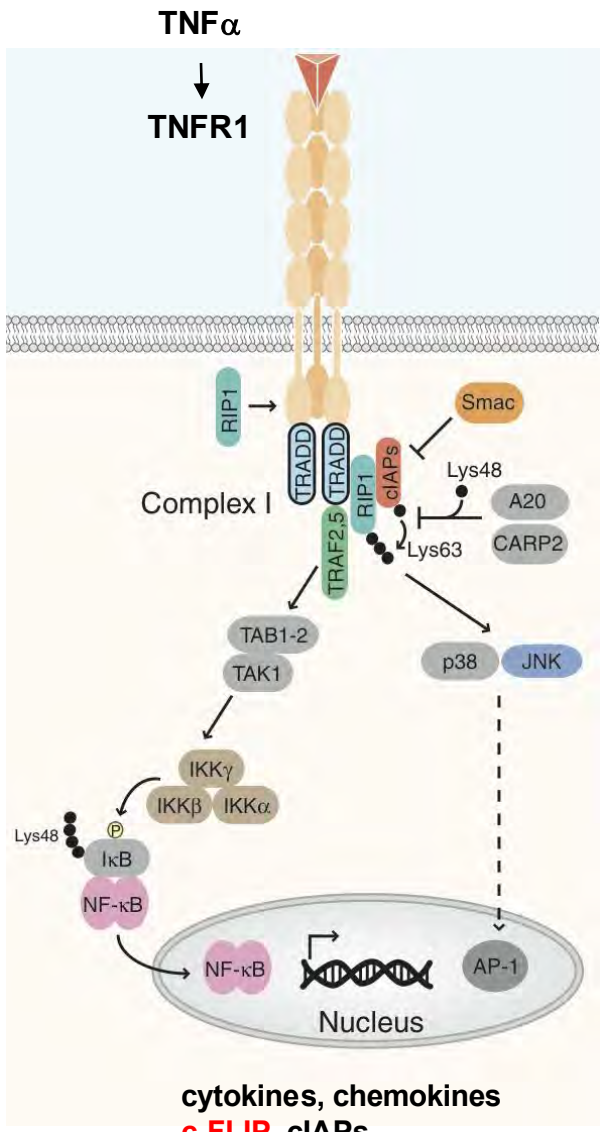
Death Receptor Signaling

Pro-apoptotic

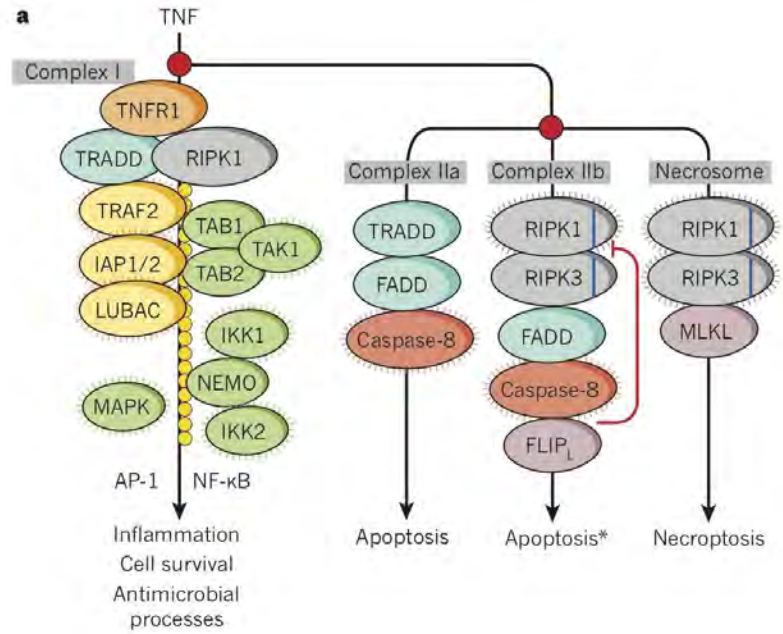




Pro-inflammatory



cytokines, chemokines
c-FLIP, cIAPs

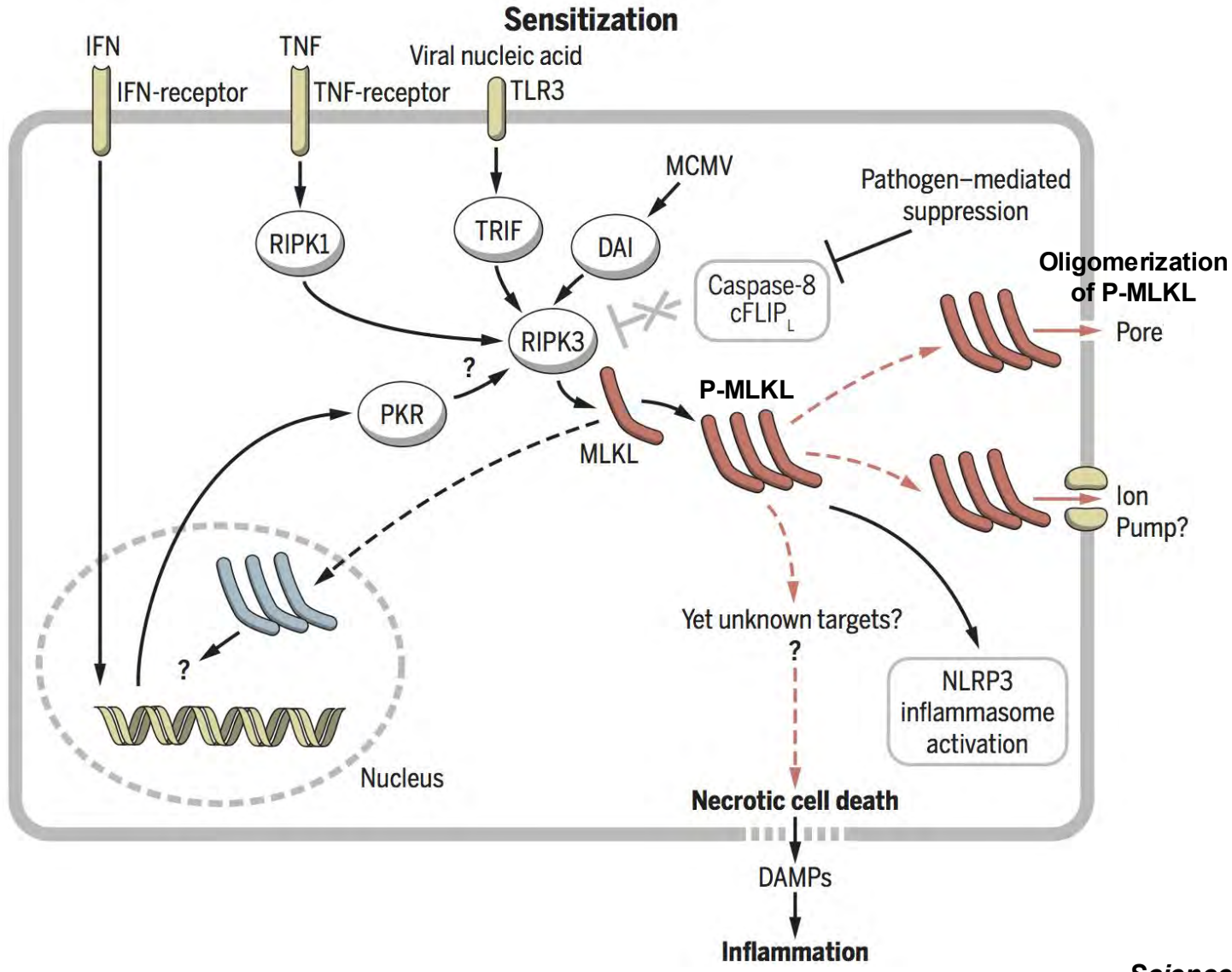


Cell, 116: 205–219

Nature Immunology, 10: 348-355

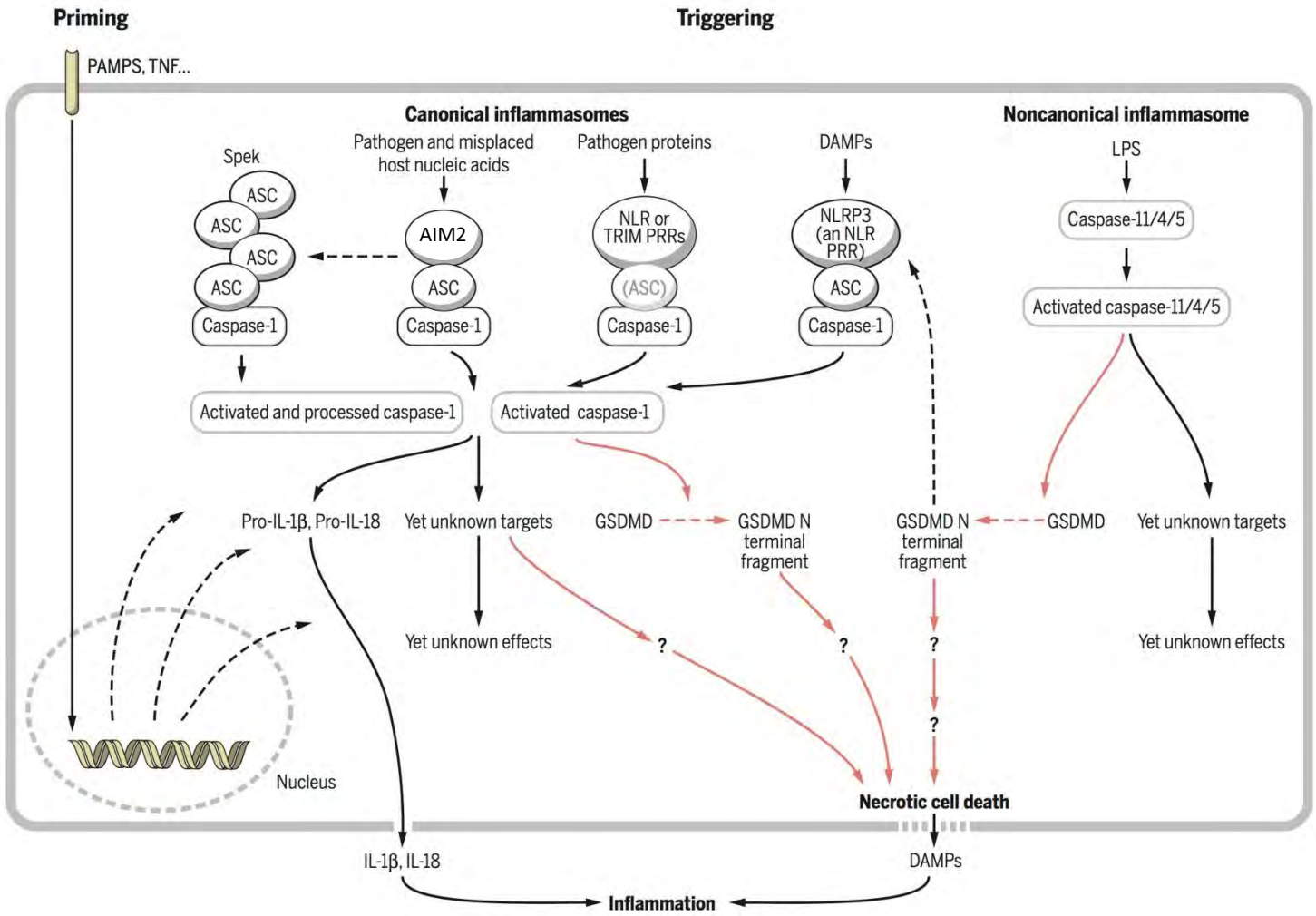
Nature, 517: 311-320

Necroptosis

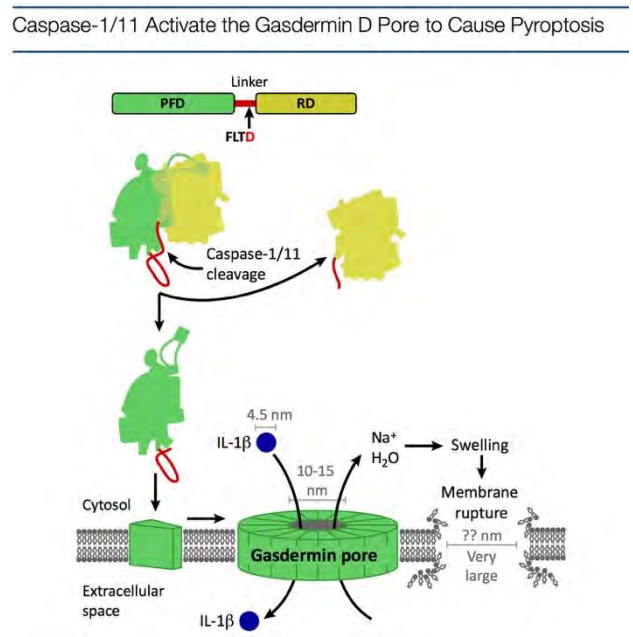


Science, 352 : aaf2154

Pyroptosis

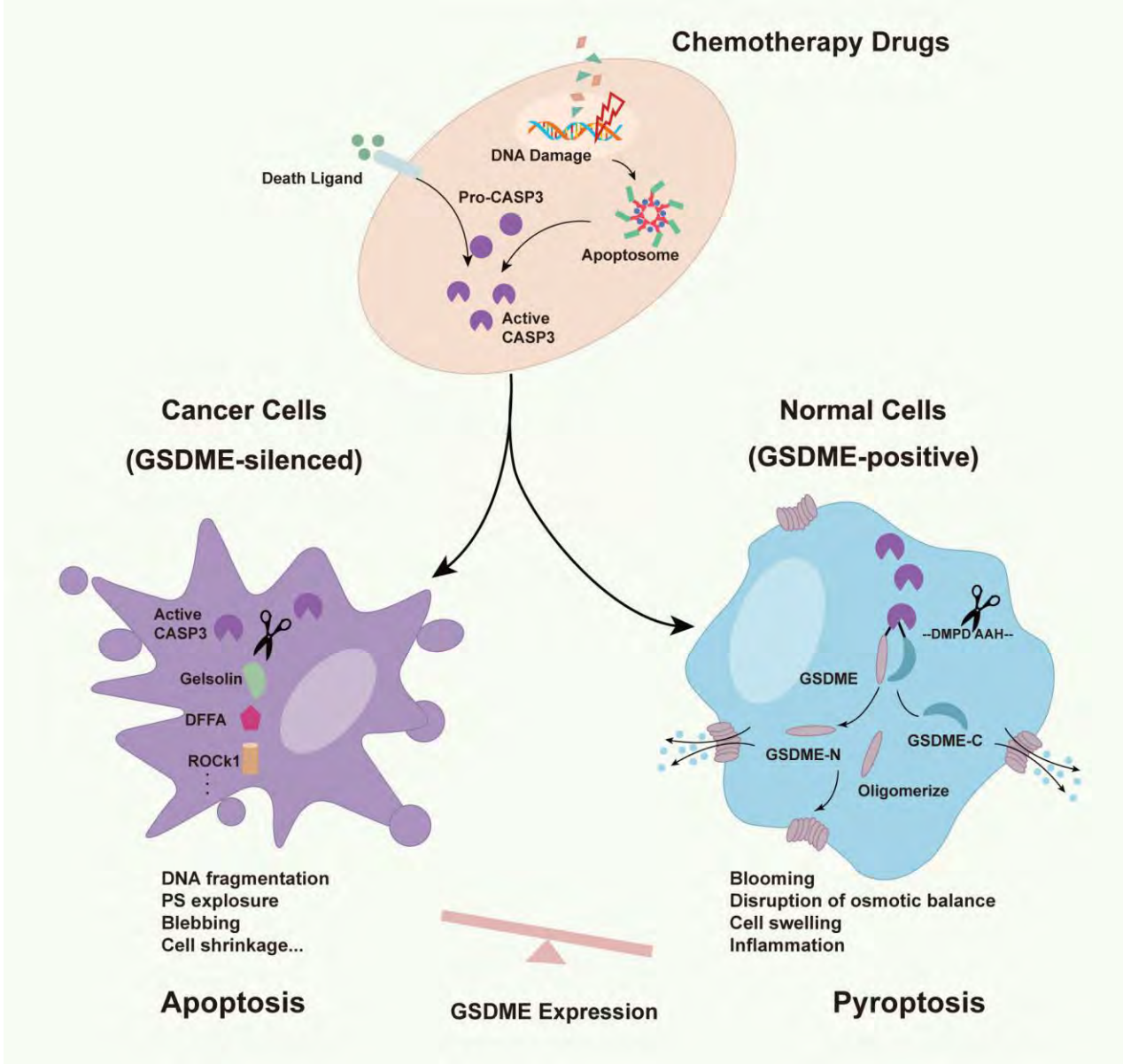


Science, 352 : aaf2154



Trends in Cell Biology, 27 : 673-684
Nature, 557 : 62–67

Caspase3-Mediated Cleavage of GSDME Converts Apoptosis to Pyroptosis

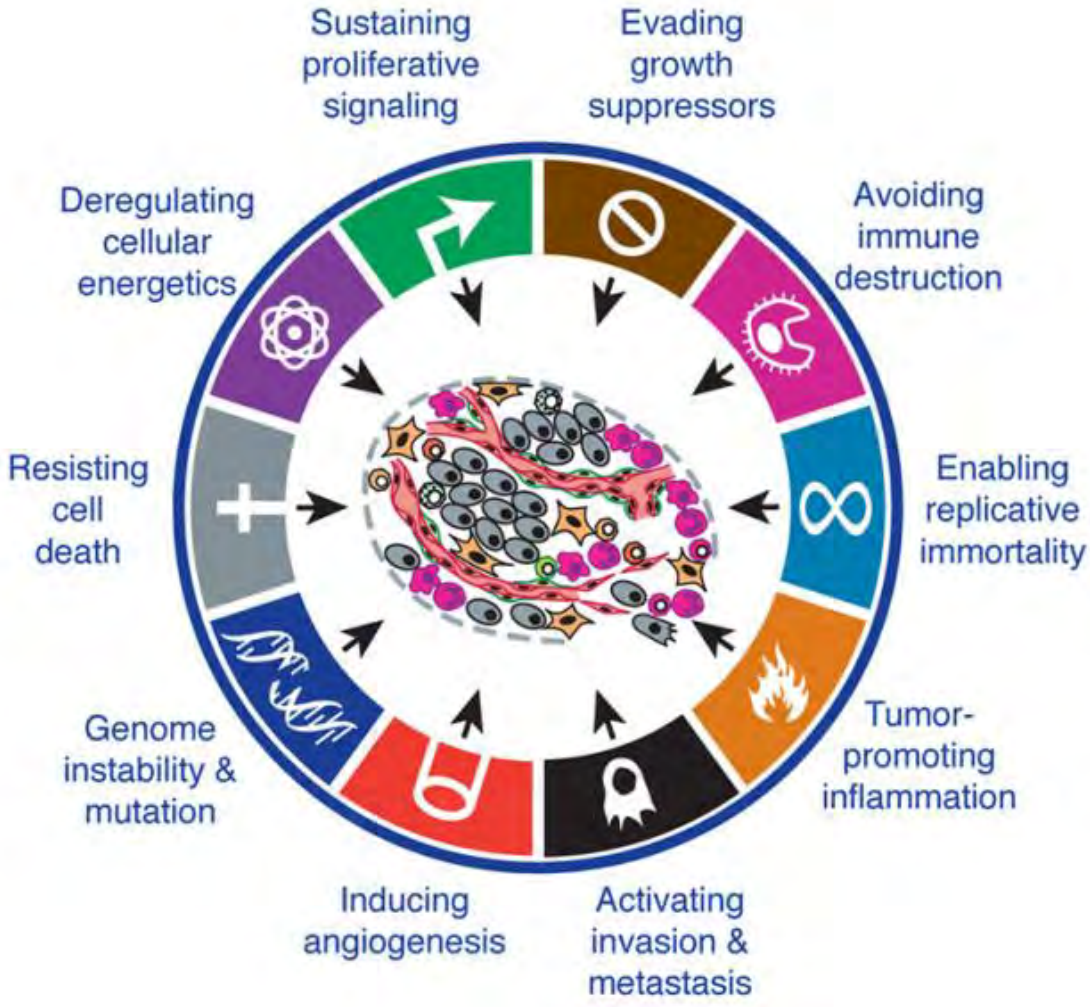


Nature, 547: 99–103

Apoptosis and Cancer

Resisting Cell Death: A Hallmark of Cancer

The Hallmarks of Cancer



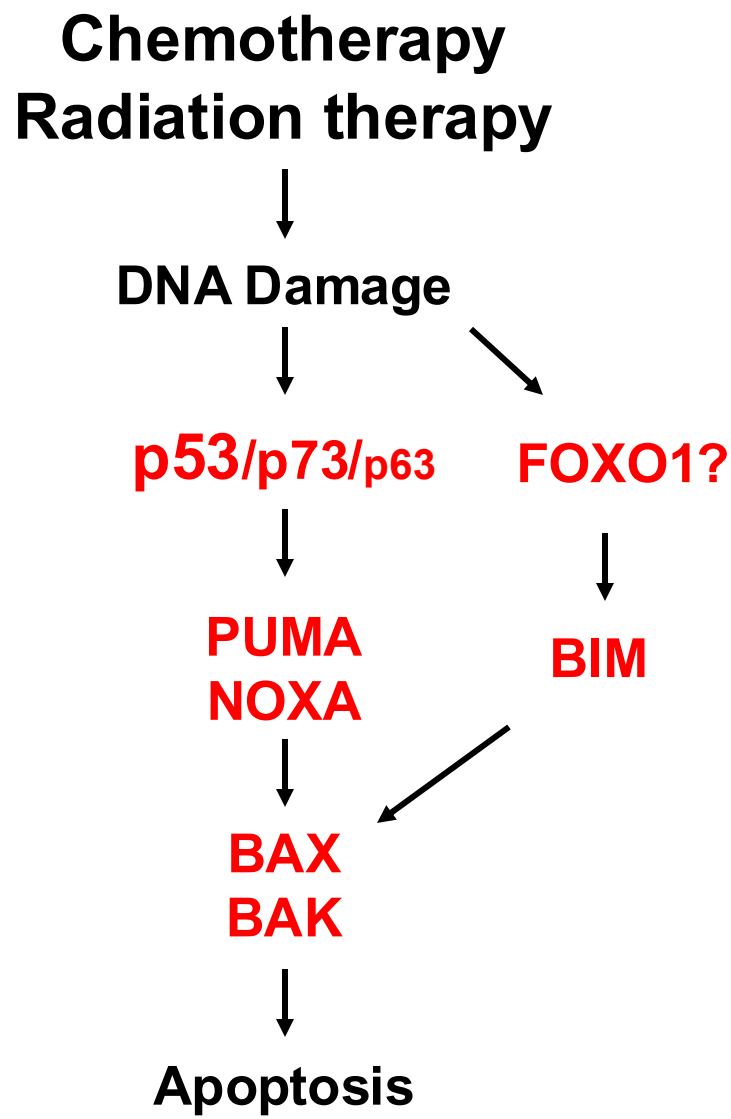
Hanahan & Weinberg. *Cell* 2011, 144:646-674

Impairment of Apoptosis is Central to Cancer Development

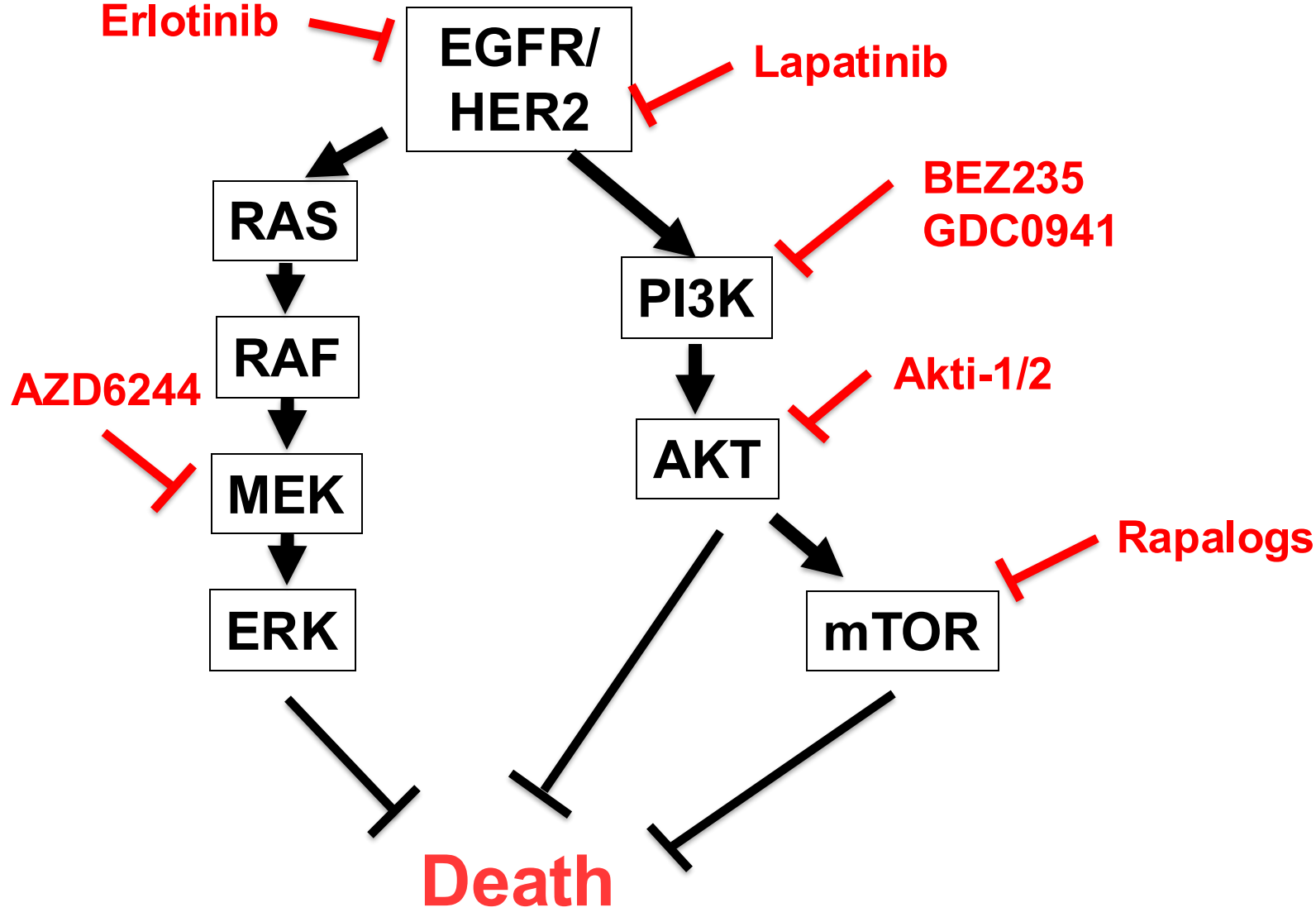
- Many oncogenes that drive cell division also trigger apoptosis (e.g. Myc, E1a, E2F1, Cyclin D)
- Allow neoplastic cells to survive even in the absence of exogenous survival factors
- Allow time for accumulative genetic alterations
- Permit survival of genetically unstable cells
- Provide protection from hypoxia and oxidative stress as tumor mass expands
- Facilitate metastasis by allowing epithelial cells to survive without attachment to extracellular matrix (anoikis)

Apoptosis is Critical for Cancer Treatment

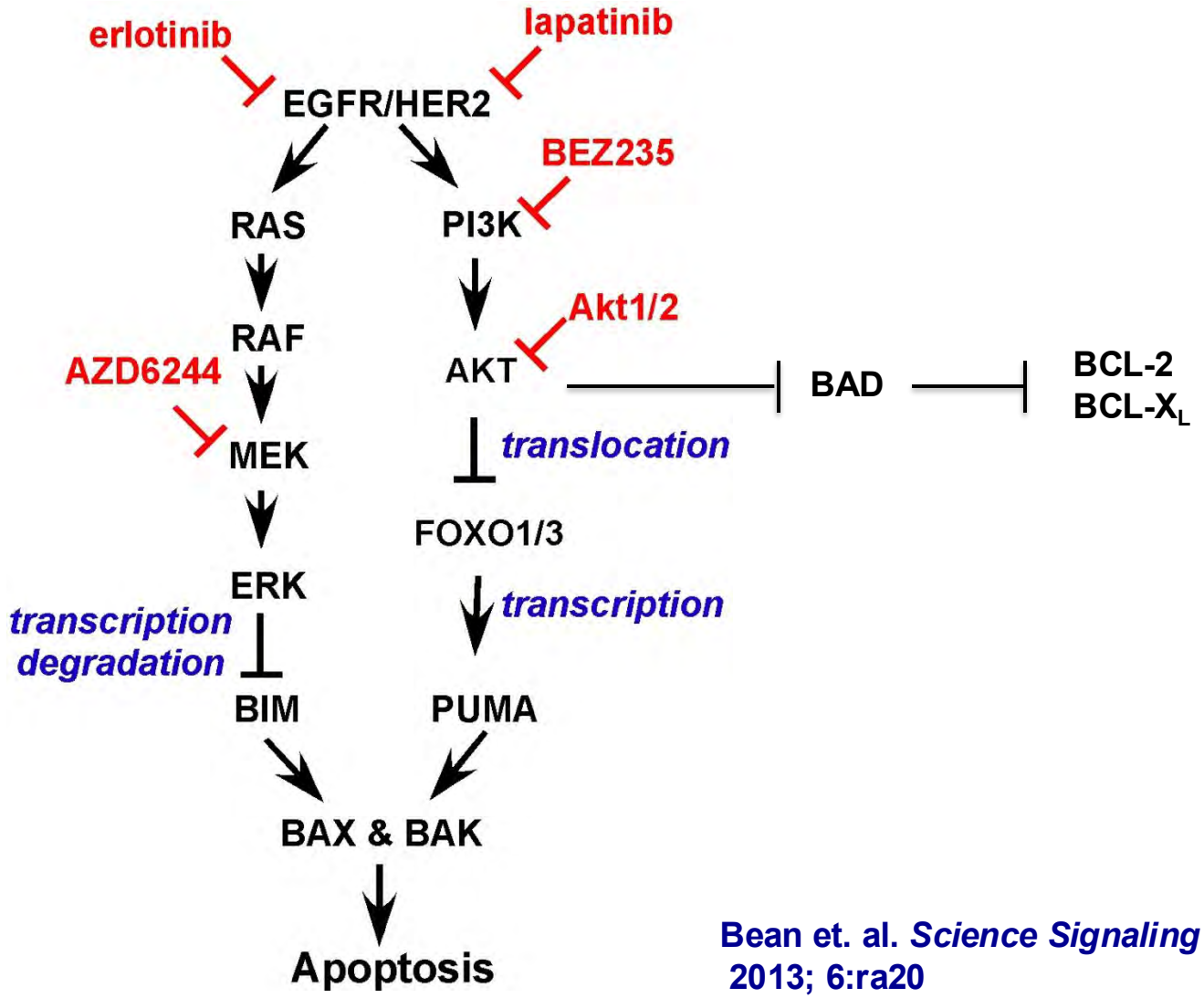
- Most anti-cancer therapeutics induces apoptosis
- Apoptotic defects contribute to chemo- and radio-resistance
- Novel anti-cancer therapeutics targeted on apoptotic pathways


Chemotherapy
Radiation therapy
DNA Damage
p53/p73/p63
FOXO1?
PUMA
NOXA
BIM
BAX
BAK
Apoptosis

Oncogene Addiction: Targeted Cancer Therapy

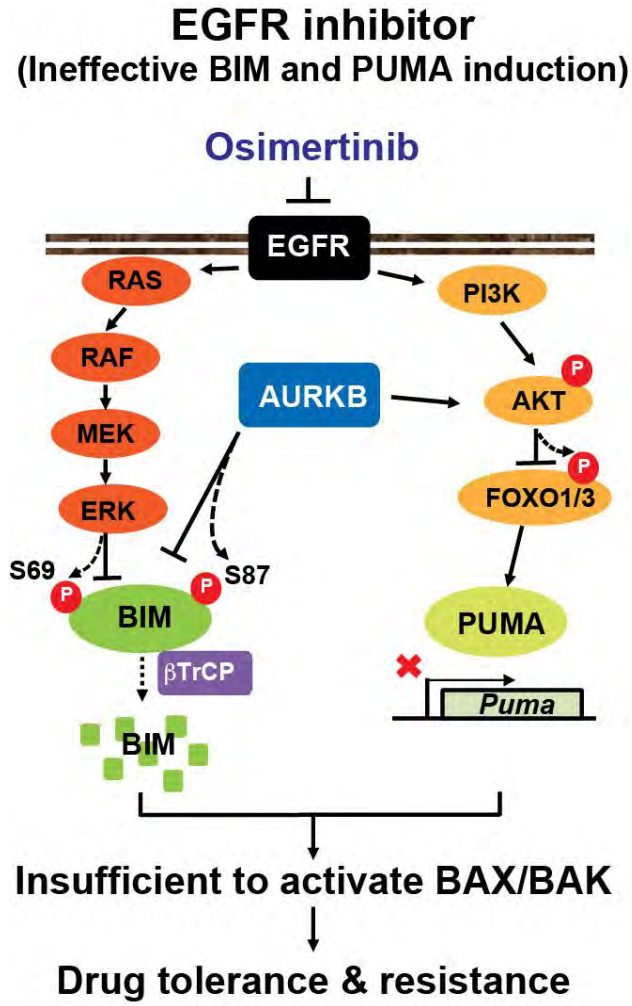


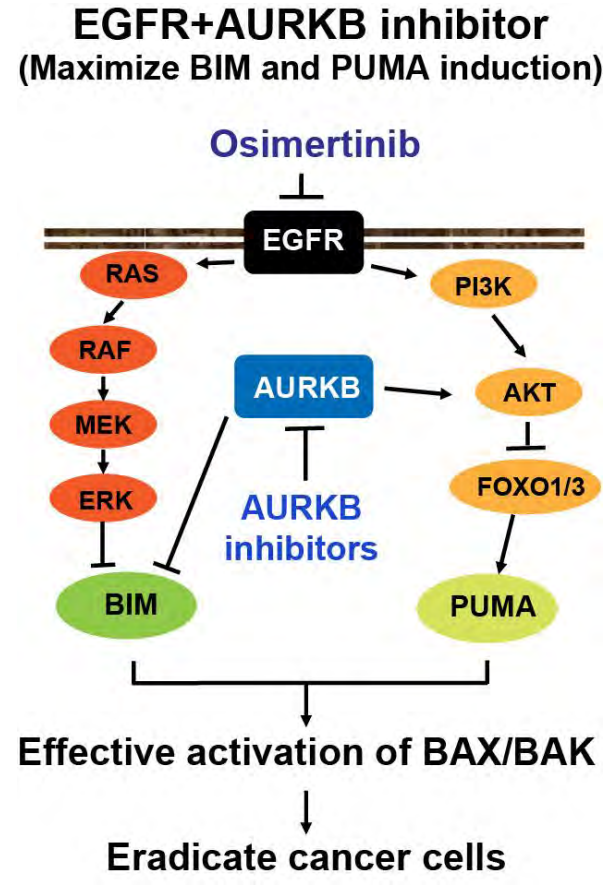
BIM and PUMA Are Apoptotic Effectors of Oncogenic Kinase Inhibitors



Bean et. al. *Science Signaling* 2013; 6:ra20

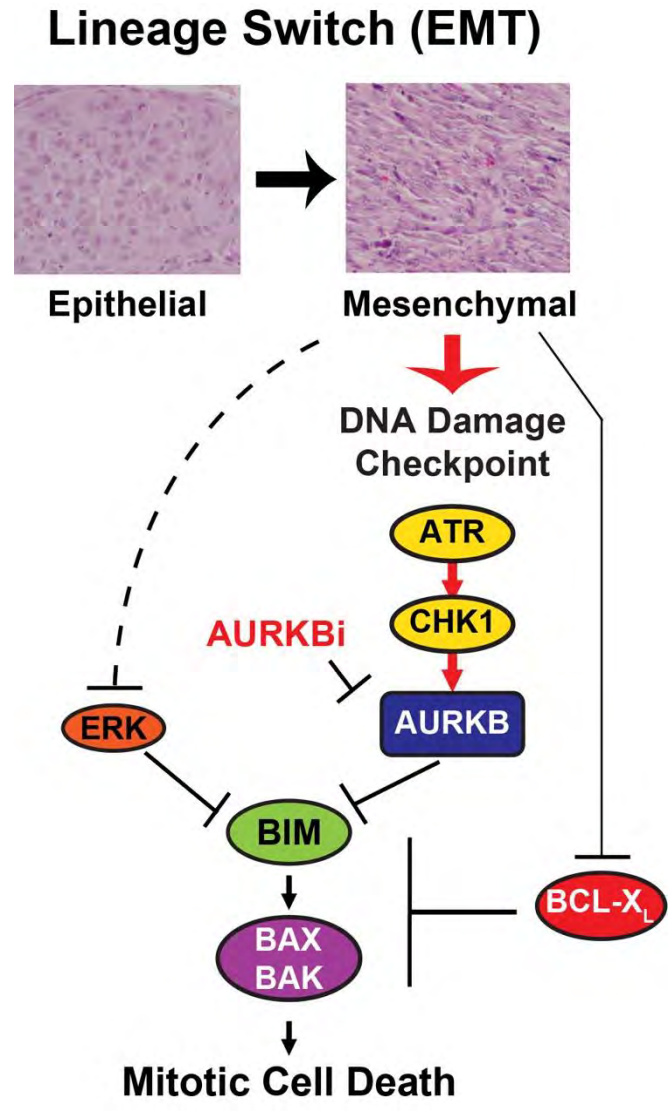
Targeting Aurora B Kinase Prevents and Overcomes Resistance to EGFR Inhibitors in Lung Cancer





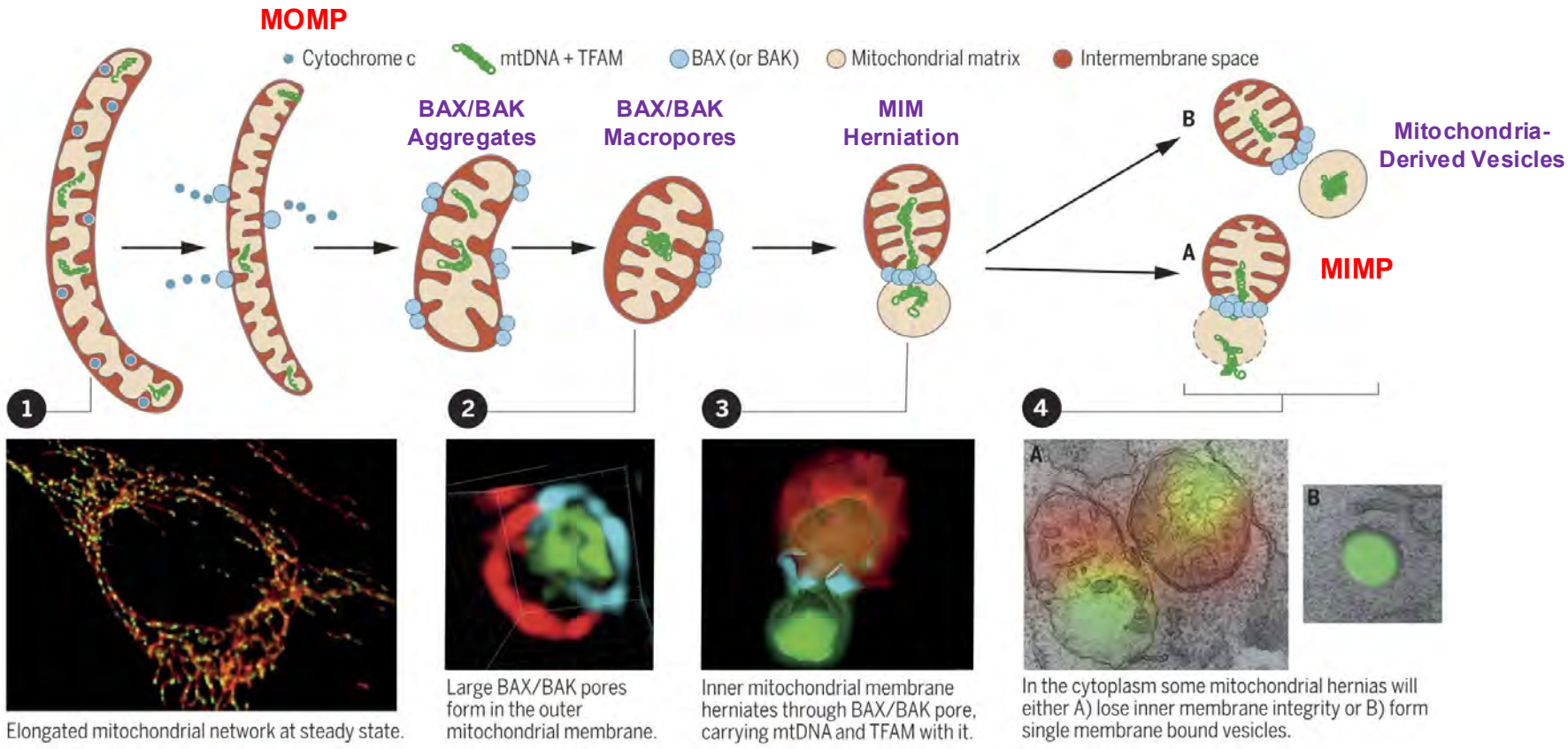
Tanaka et. al. *Cancer Cell* 2021; 39: 1245-1261

EMT activates ATR-CHK1-Aurora B and thereby engenders hypersensitivity to the respective kinase inhibitors by activating BIM-mediated mitotic cell death



Tanaka et. al. *Cancer Cell* 2021; 39: 1245-1261

Temporal Sequences of BAX/BAK-Mediated Mitochondrial Permeabilization



McArthur et al., *Science* 359, 883 (2018)