

APOPTOSIS: An overview

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INTRODUCTION

+ Cell death by injury

- Mechanical damage
- Exposure to toxic chemicals

+ Cell death by suicide

- Internal signals
- External signals

Conted.....

- Apoptosis or programmed cell death, is carefully coordinated collapse of cell, protein degradation , DNA fragmentation followed by rapid engulfment of corpses by neighbouring cells. (Tommi, 2002)
- Essential part of life for every multicellular organism from worms to humans. (Faddy *et al.*,1992)
- Apoptosis plays a major role from embryonic development to senescence.

Why should a cell commit suicide?

+ Apoptosis is needed for proper development

Examples:

The resorption of the tadpole tail

The formation of the fingers and toes of the fetus

The sloughing off of the inner lining of the uterus

The formation of the proper connections between neurons in the brain

+ Apoptosis is needed to destroy cells

Examples:

Cells infected with viruses

Cells of the immune system

Cells with DNA damage

Cancer cells

What makes a cell decide to commit suicide?

✚ Withdrawal of positive signals

examples :

growth factors for neurons
Interleukin-2 (IL-2)

✚ Receipt of negative signals

examples :

increased levels of oxidants within the cell
damage to DNA by oxidants

death activators :

Tumor necrosis factor alpha (TNF- α)

Lymphotoxin (TNF- β)

Fas ligand (FasL)

History of cell death / apoptosis research

- #1800s Numerous observation of cell death
- #1908 Mechnikov wins Nobel prize (phagocytosis)
- #1930-40 Studies of metamorphosis
- #1948-49 Cell death in chick limb & exploration of NGF
- #1955 Beginning of studies of lysosomes
- #1964-66 Necrosis & PCD described
- #1971 Term apoptosis coined
- #1977 Cell death genes in *C. elegans*
- #1980-82 DNA ladder observed & ced-3 identified
- #1989-91 Apoptosis genes identified, including bcl-2, fas/apo1 & p53, ced-3 sequenced

Necrosis vs. Apoptosis

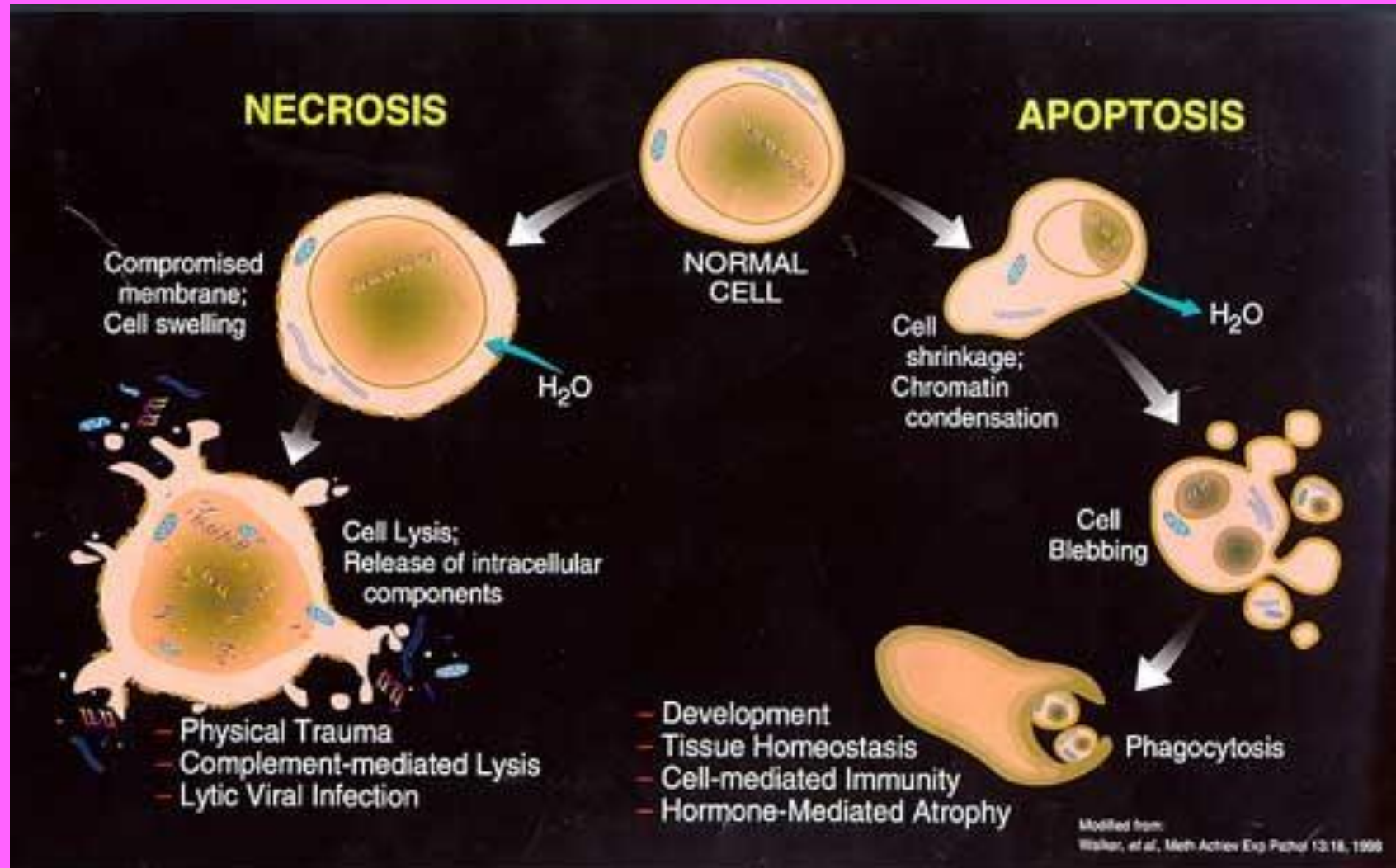
Necrosis

- Cellular swelling
- Membranes are broken
- ATP is depleted
- Cell lyses, eliciting an inflammatory reaction
- DNA fragmentation is random, or smeared
- In vivo, whole areas of the tissue are affected

Apoptosis

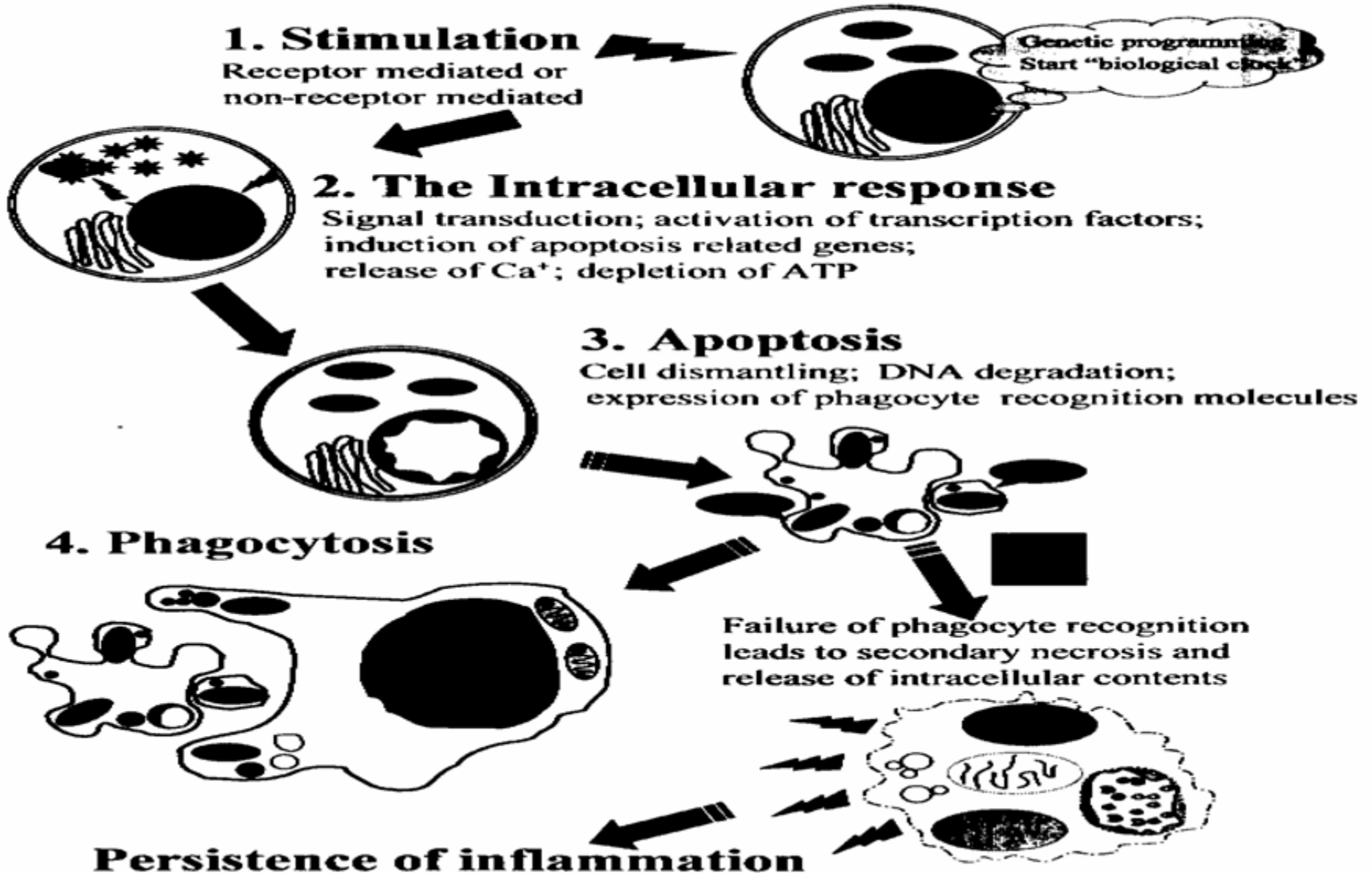
- Cellular condensation
- Membranes remain intact
- Requires ATP
- Cell is phagocytosed, no tissue reaction
- Ladder-like DNA fragmentation
- In vivo, individual cells appear affected

NECROSIS Vs APOPTOSIS

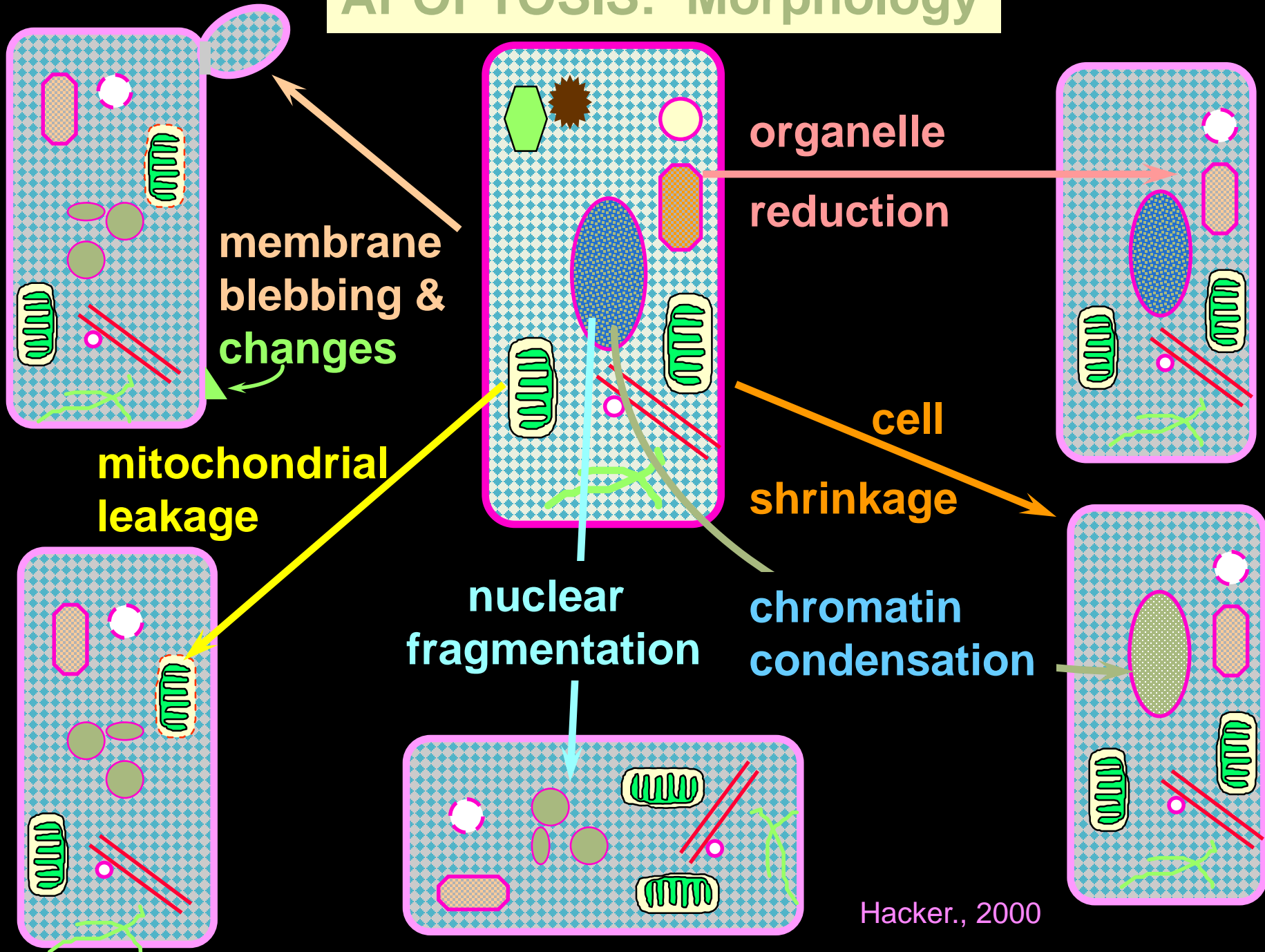


STAGES OF APOPTOSIS

Apoptosis



APOPTOSIS: Morphology



APOPTOSIS: Morphological events

- ◆ cell shrinkage

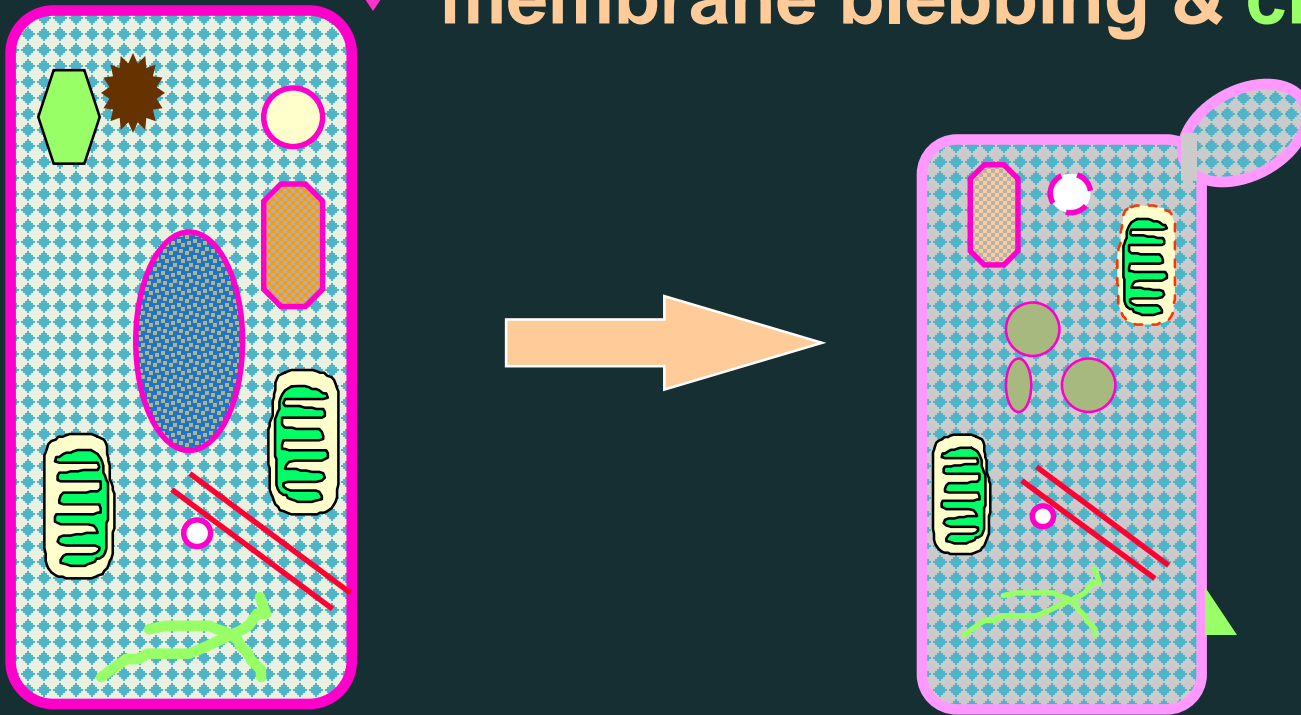
- ◆ organelle reduction

- ◆ mitochondrial leakage

- ◆ chromatin condensation

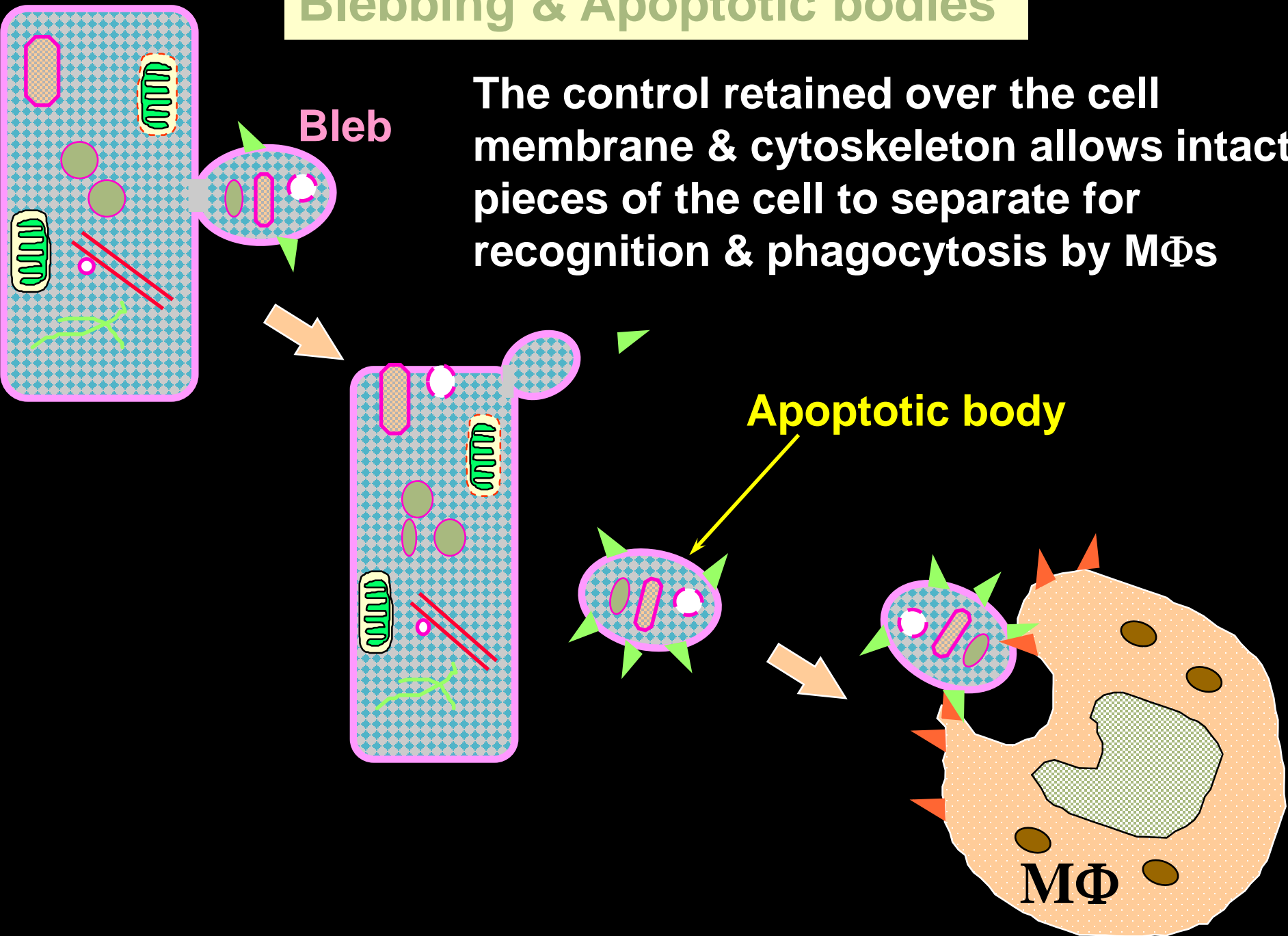
- ◆ nuclear fragmentation

- ◆ membrane blebbing & changes



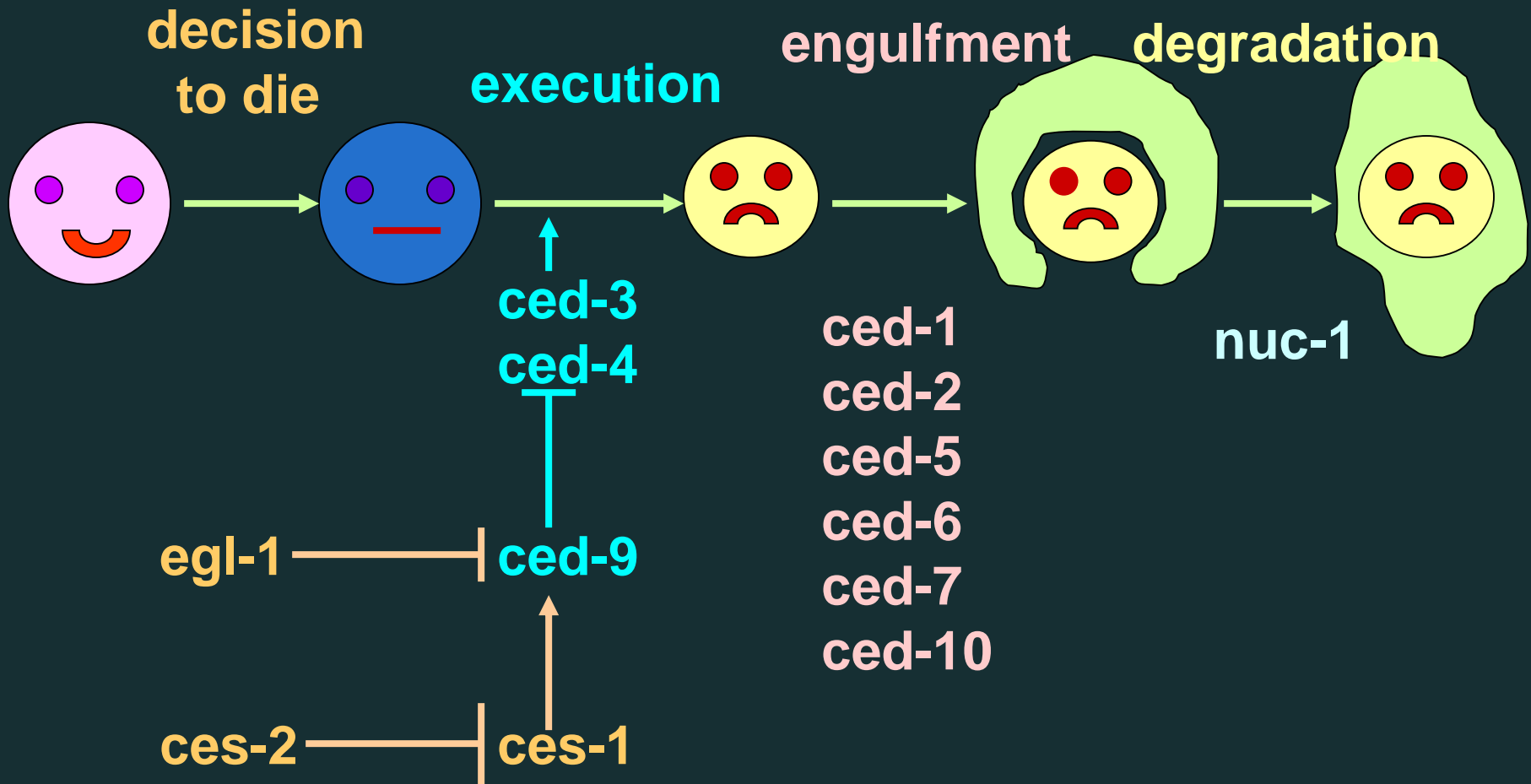
Blebbing & Apoptotic bodies

The control retained over the cell membrane & cytoskeleton allows intact pieces of the cell to separate for recognition & phagocytosis by MΦs



Caenorhabditis elegans

1090 cells → 131 cells → apoptosis



Apoptosis: Pathways

“Extrinsic Pathway”

Death
Ligands

Death
Receptors

Initiator
Caspase 8

Effector
Caspase 3

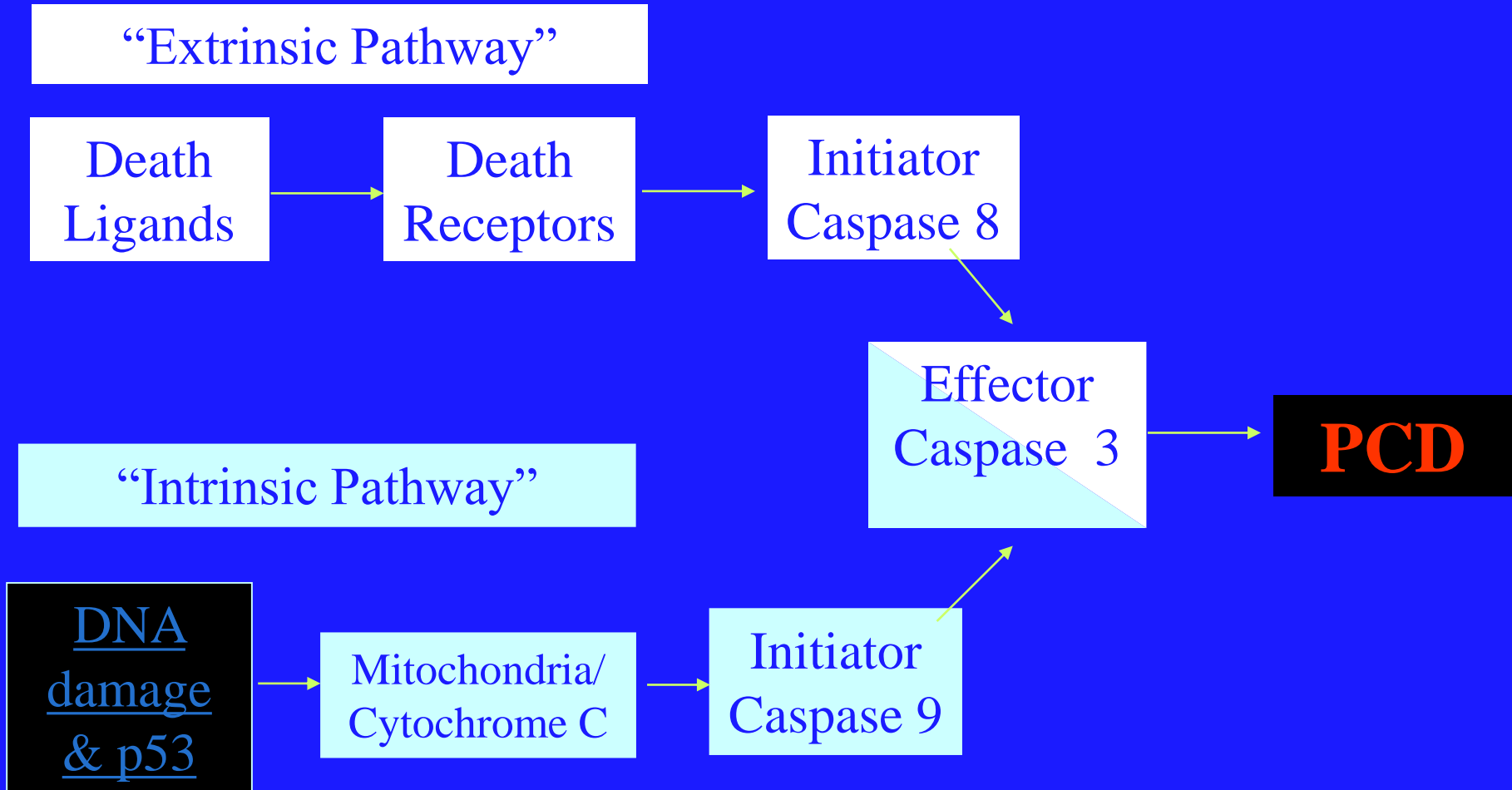
PCD

“Intrinsic Pathway”

DNA
damage
& p53

Mitochondria/
Cytochrome C

Initiator
Caspase 9



MAJOR PLAYERS IN APOPTOSIS

- Caspases
- **Adaptor proteins**
- **TNF & TNFR family**
- Bcl-2 family

Ligand-induced cell death

Ligand

Receptor

FasL

Fas (CD95)

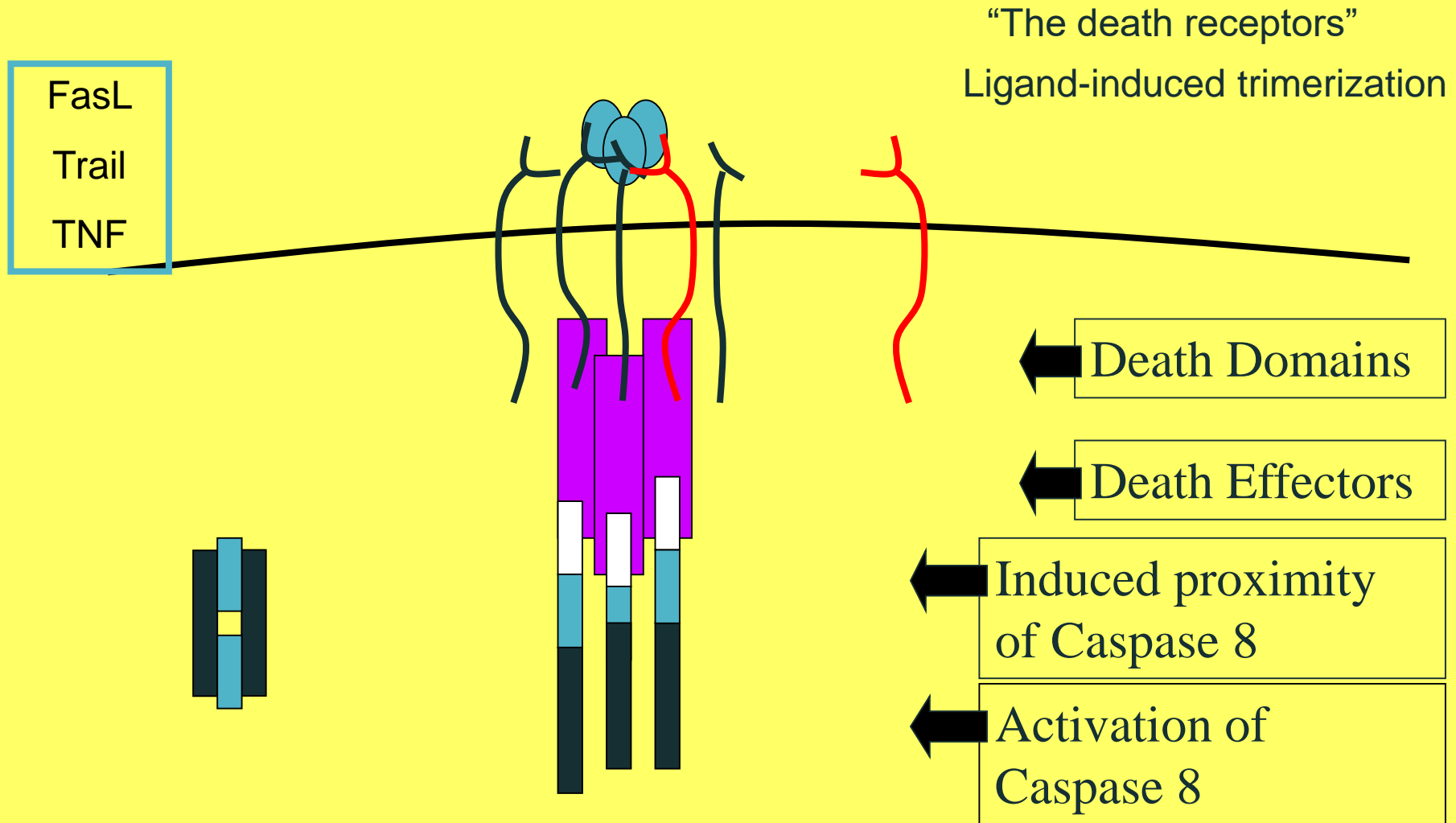
TNF

TNF-R

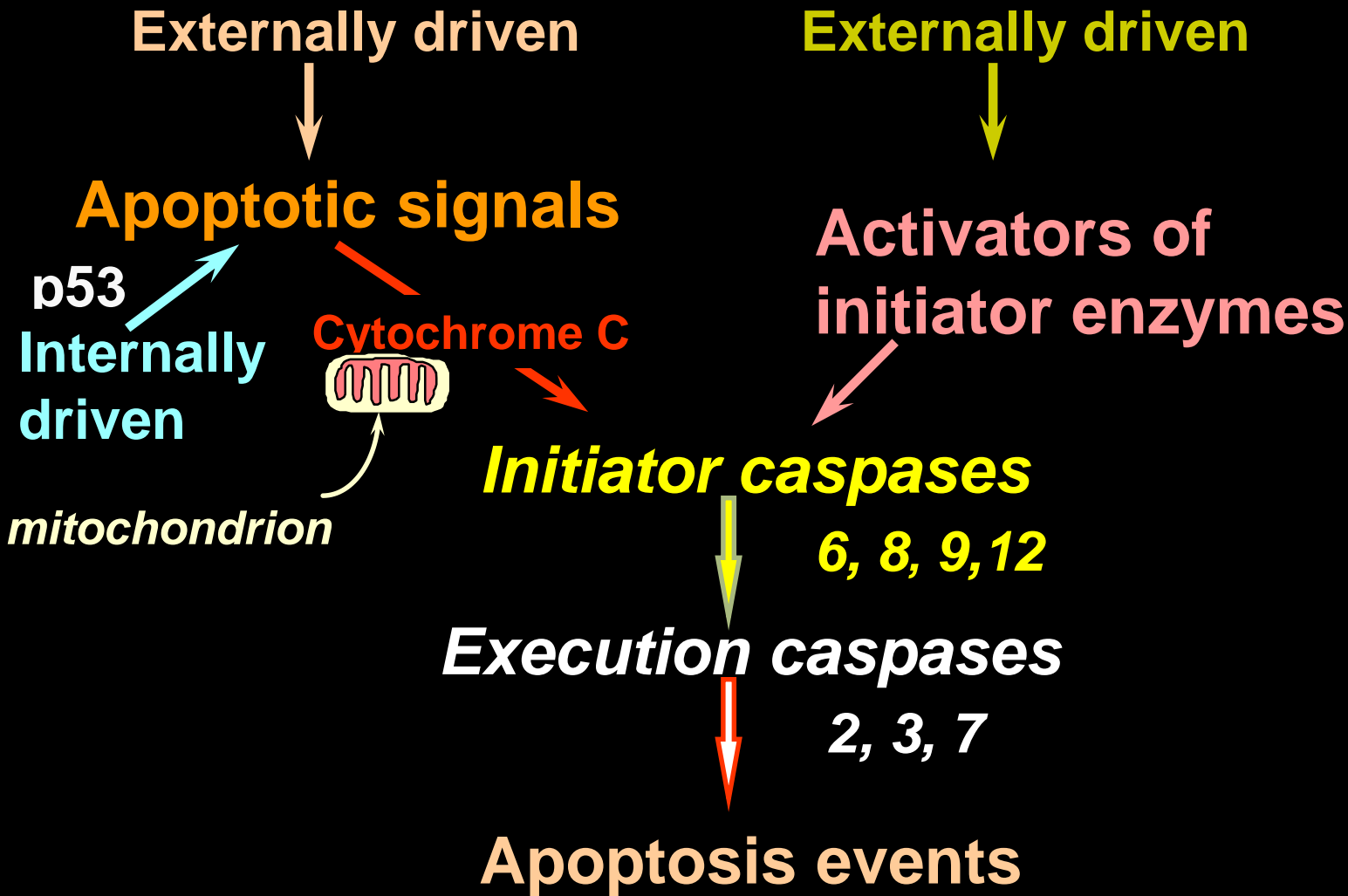
TRAIL

DR4 (Trail-R)

Ligand-induced cell death

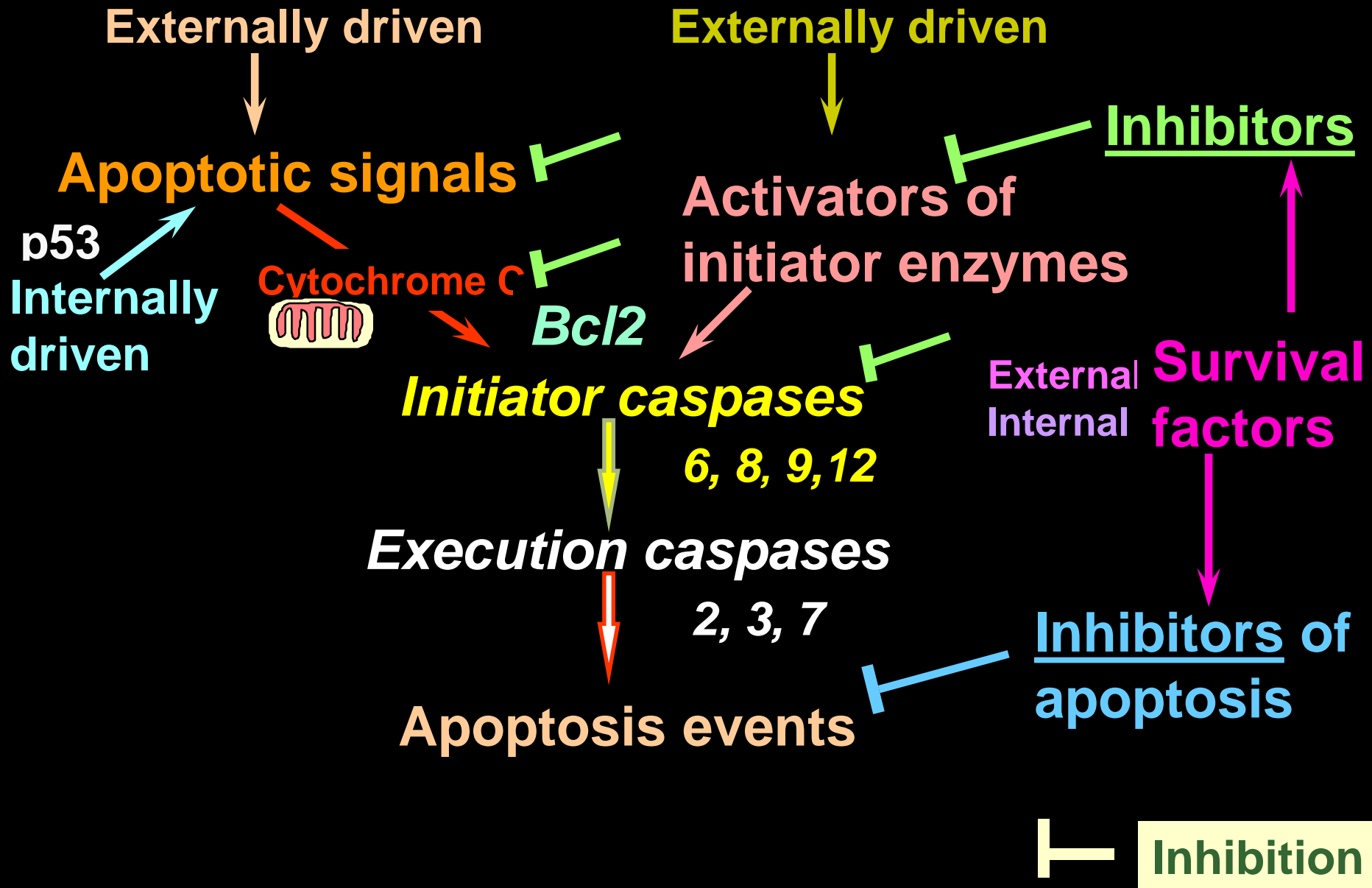


APOPTOSIS: Signaling & Control pathways I

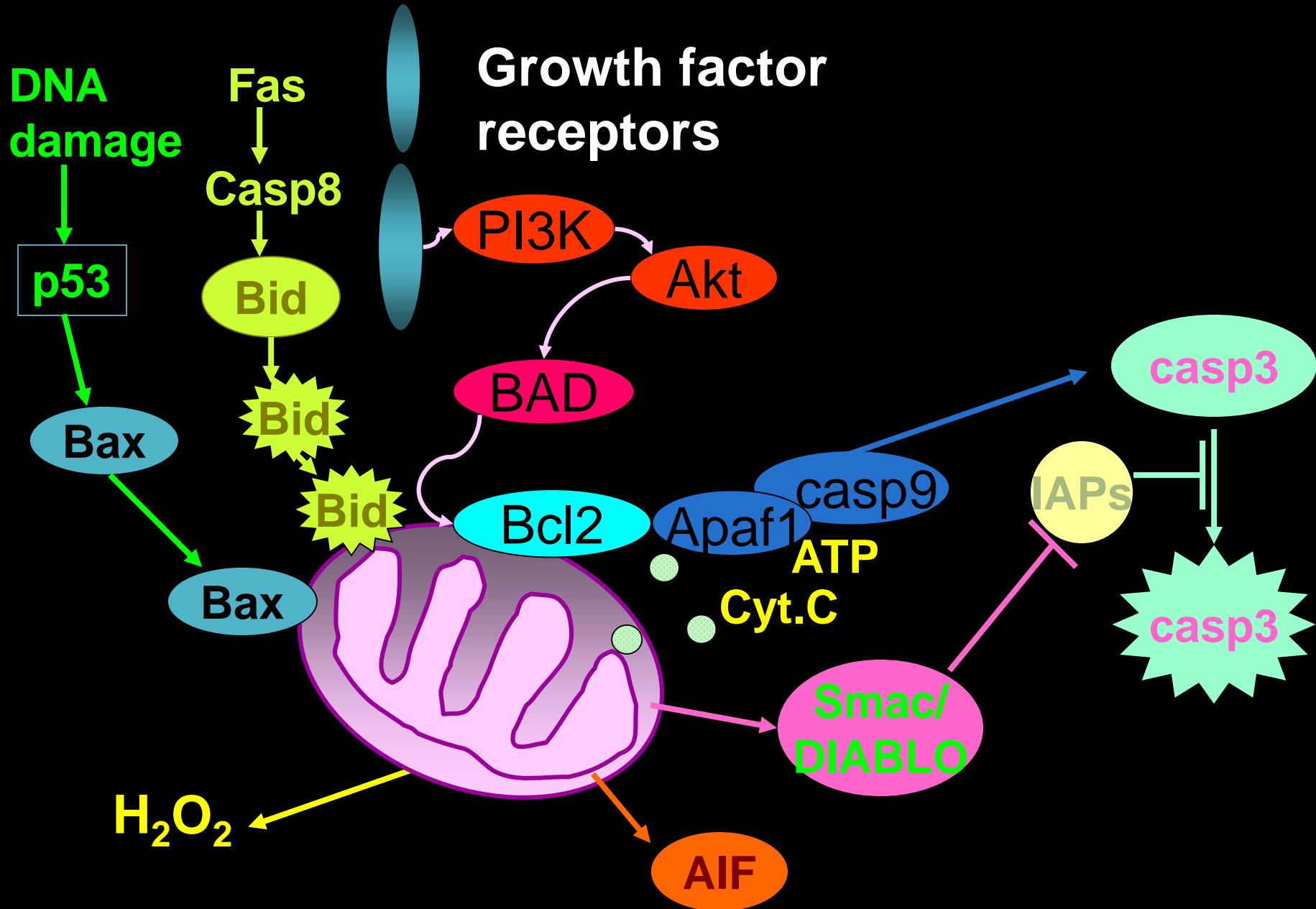


Activation

APOPTOSIS: Signaling & Control pathways II



The mitochondrial pathway



REGULATION OF APOPTOSIS

✚ Stimuli → apoptosis → selection of targets
(Rich *et al.*, 2000)

✚ Apoptosis by conflicting signals that scramble the normal status of cell
(Canlon & Raff, 1999)

✚ Apoptotic stimuli → cytokines, death factors (FasL)
(Tabibzadeh *et al.*, 1999)

✚ DNA breaks → p53 is activated → arrest cell cycle or activate self destruction
(Blain & Vousden, 2001)

Importance of Apoptosis

- Important in normal physiology / development
 - **Development**: Immune systems maturation, Morphogenesis, Neural development
 - **Adult**: Immune privilege, DNA Damage and wound repair.
- Excess apoptosis
 - Neurodegenerative diseases
- Deficient apoptosis
 - Cancer
 - Autoimmunity

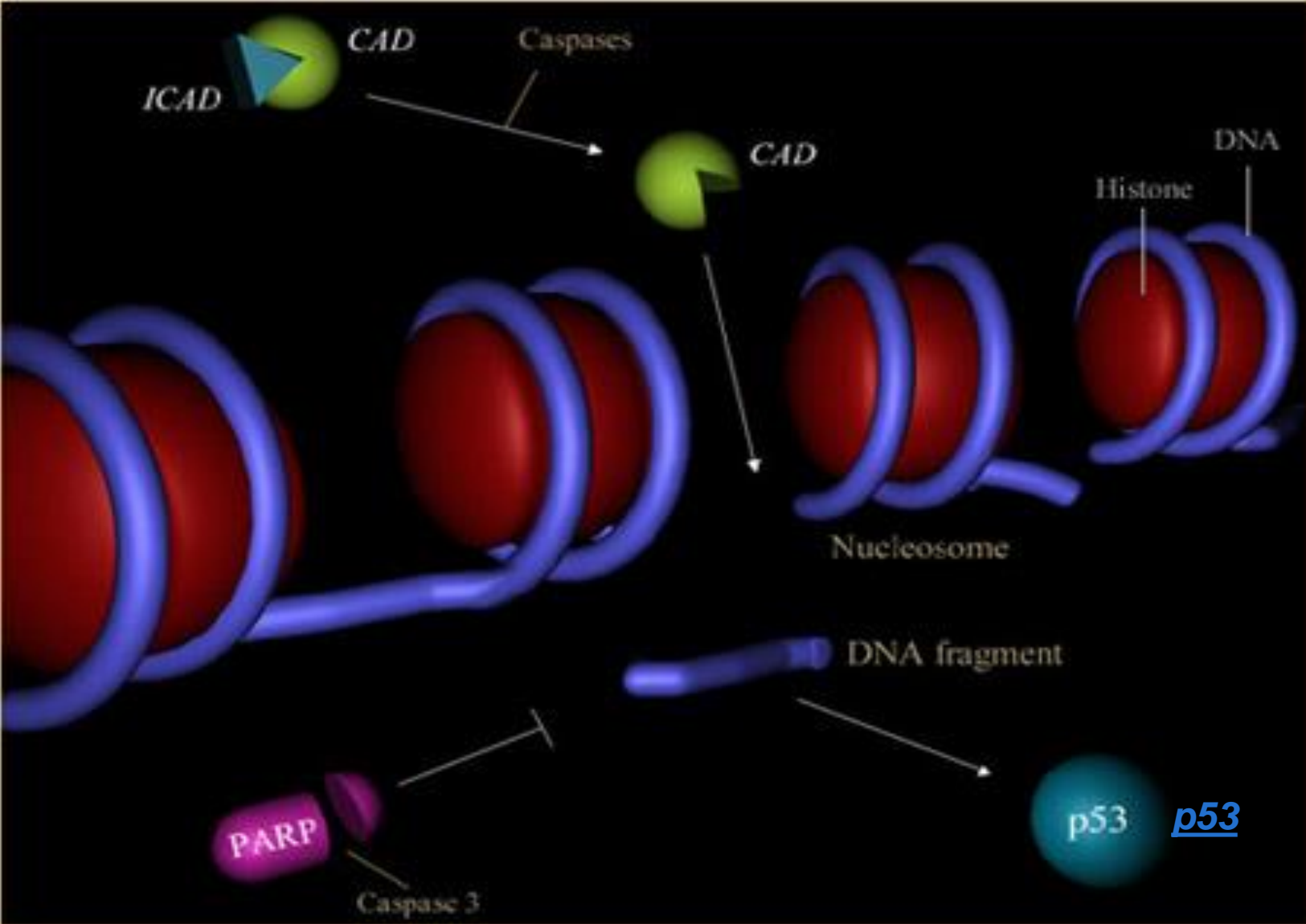
FUTURE PERSPECTIVES

- ✚ The biological roles of newly identified death receptors and ligands need to be studied
- ✚ Need to know whether defects in these ligands and receptors contribute to disease

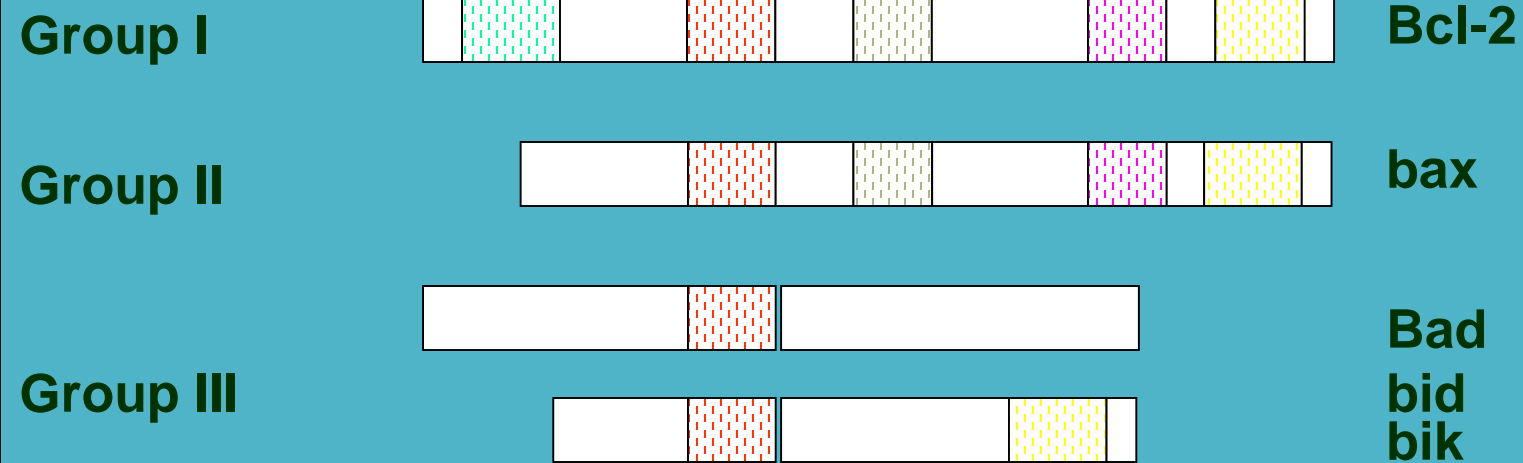
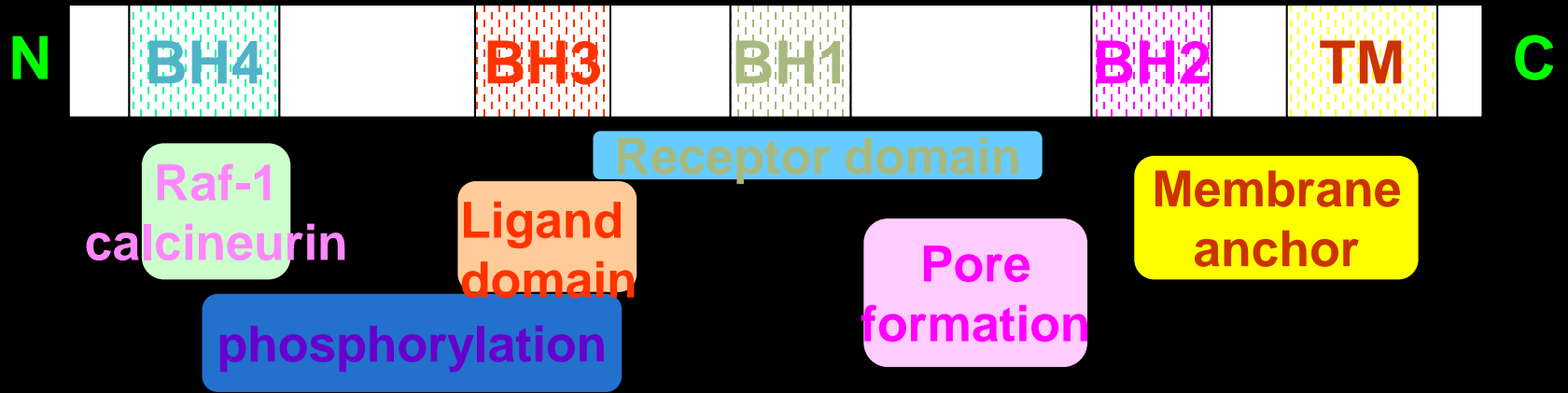
CONCLUSION

- # an important process of cell death
- # can be initiated extrinsically through death ligands (e.g. TRAIL, FasL) activating initiator caspase 8 through induced proximity.
- # can be initiated intrinsically through DNA damage (via cytochrome c) activating initiator caspase 9 through *oligomerization*.
- # Initiator caspases 8 and 9 cleave and activate effector caspase 3, which leads to cell death.

DNA DAMAGE



The bcl-2 family



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P53 & Apoptosis

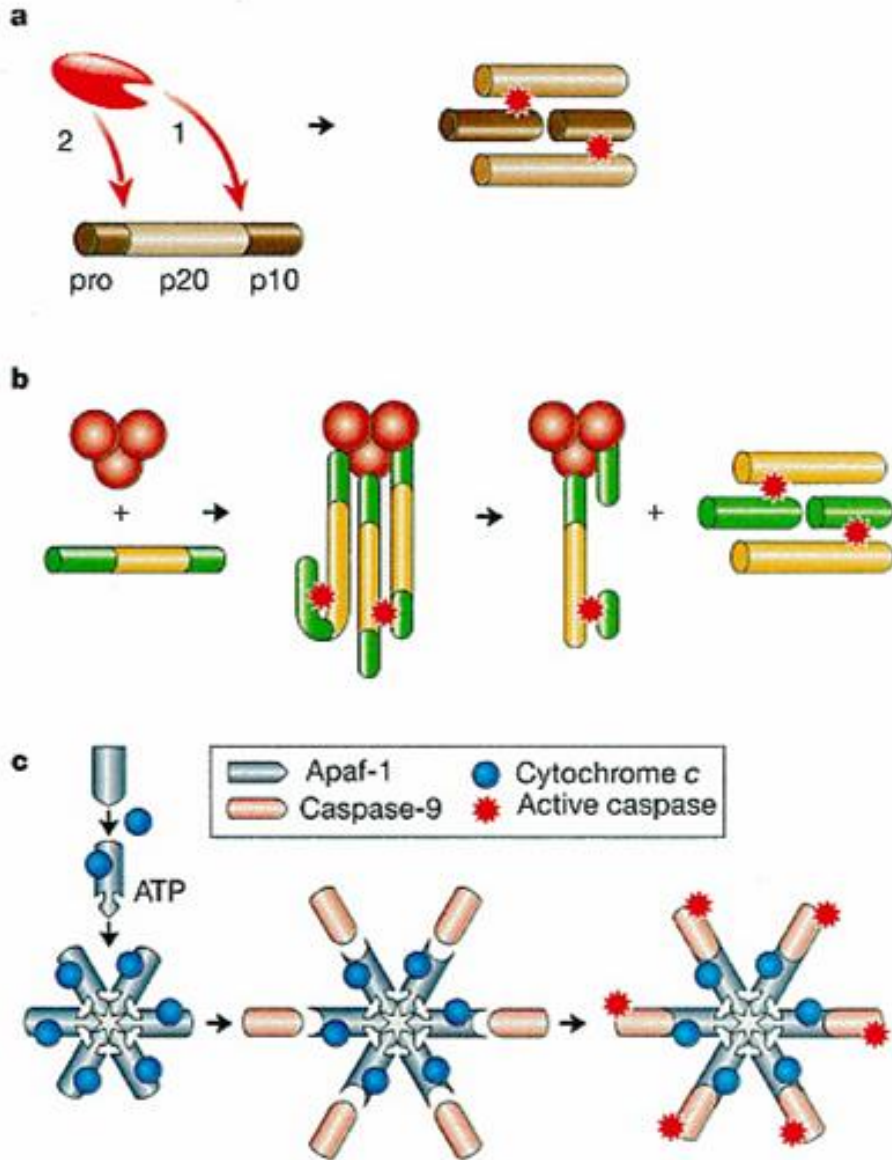
p53 first arrests cell growth between G1 → S

This allows for DNA repair during delay

If the damage is too extensive then p53 induces gene activation leading to apoptosis (programmed cell death)

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3 mechanisms of caspase activation



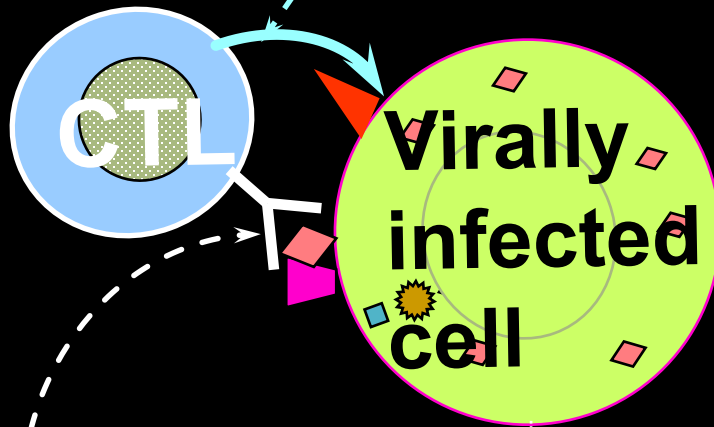
a. Proteolytic cleavage e.g.
pro-caspase 3

b. Induced proximity, e.g.
pro-caspase 8

c. Oligomerization, e.g. cyt c,
Apaf-1 & caspase 9

Apoptosis signal to kill infected cells

Cytolytic lymphocyte/CTL (& natural killer lymphocyte) presents **Fas ligand/CD178** on its surface to tell the infected cell to die



Fas ligand



Externally driven

Apoptotic signals

Cytochrome c

Initiator caspases

Execution caspases

Apoptosis events

The **immunological synapse** holds the cells much tighter together than shown here

Fas/ CD95 is the 'death receptor'