

L'APOPTOSI

MORTE CELLULARE PROGRAMMATA

Importante per:

- *Il corretto sviluppo dell'organismo
- *L'omeostasi tissutale
- *Come risposta cellulare a stress o patogeni

Evento

Esempio

Normale sviluppo embrionale/fetale

Eliminazione della membrana interdigitale

Normale turnover cellulare

Villi intestinali

Metamorfosi

Regressione della coda degli anfibi

Citotossicità cellulo-mediata

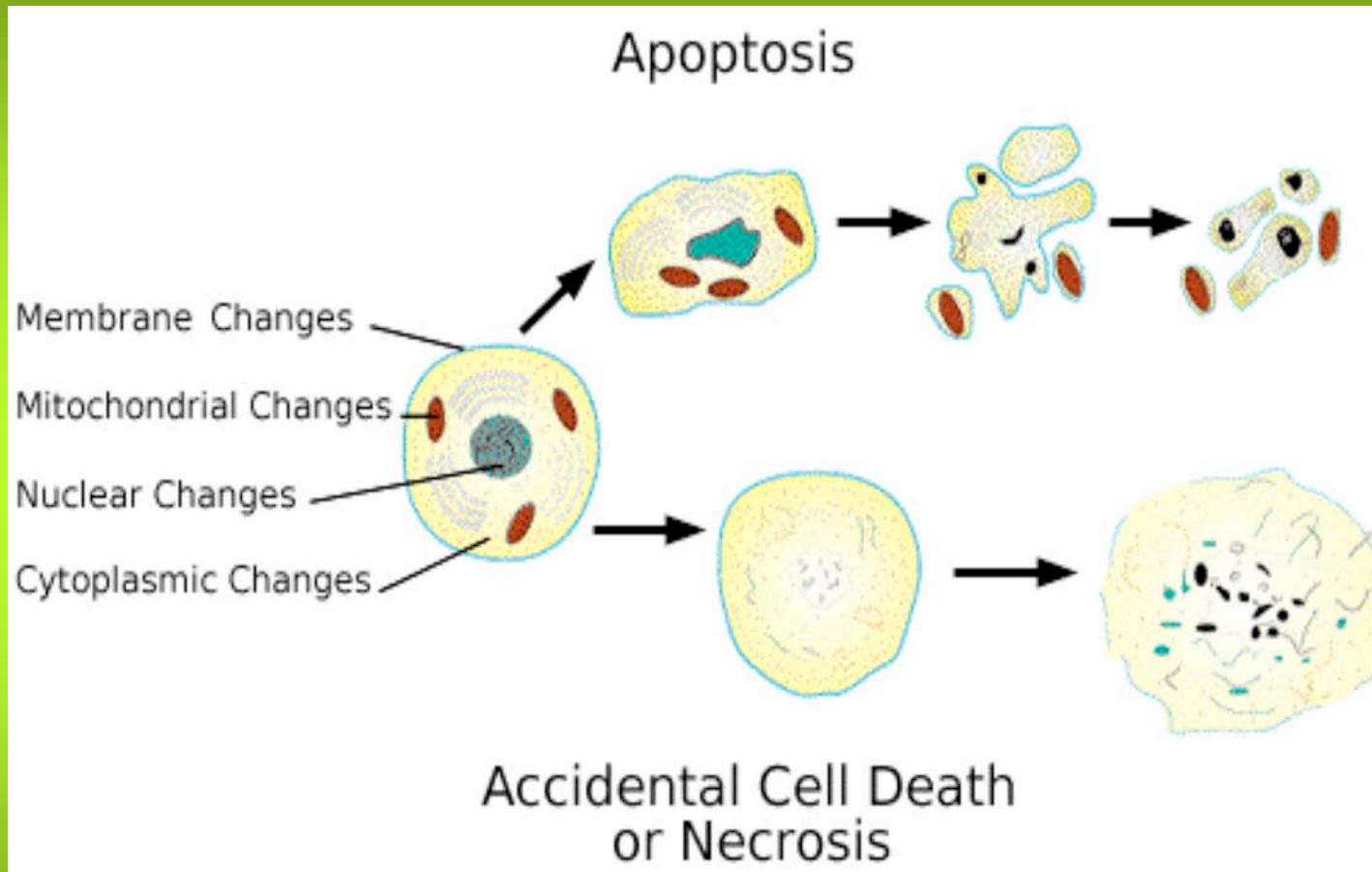
Uccisione T-cell mediata

Condizioni di crescita
sfavorevoli/patologiche

Deprivazione di fattori di crescita
Irradiazione
Esposizione a tossine

Apoptosi

Morte cellulare programmata



Necrosi

Morte cellulare incontrollata

Apoptosi

Morte cellulare
programmata

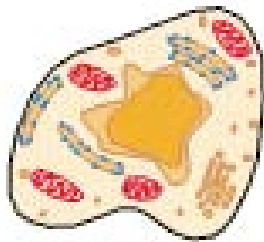
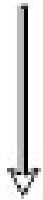
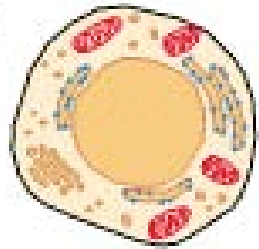
- *Perdita dei contatti cellulari
- * Condensazione citoplasmatica e riduzione del volume cellulare
- *Permeabilità normale della membrana
 - Cromatina condensata in tipici aggregati granulari
- *Frammentazione tipica della cromatina
 - *Frammentazione della cellula in corpi apoptotici senza rilascio di citoplasma
- *Esposizione della fosfatidilserina
- *Infiammazione assente

Necrosi

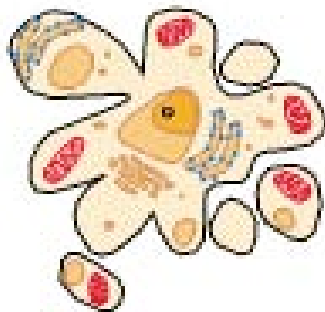
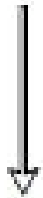
Morte cellulare
incontrollata

- *Rigonfiamento cellulare e di tutti i compartimenti
- *Permeabilità alterata della membrana
- *Lisi cellulare con rilascio di enzimi lisosomiali nel fluido extracellulare
- *Infiammazione

(a)



Mild convolution
Chromatin
compaction and
segregation
Condensation of
cytoplasm

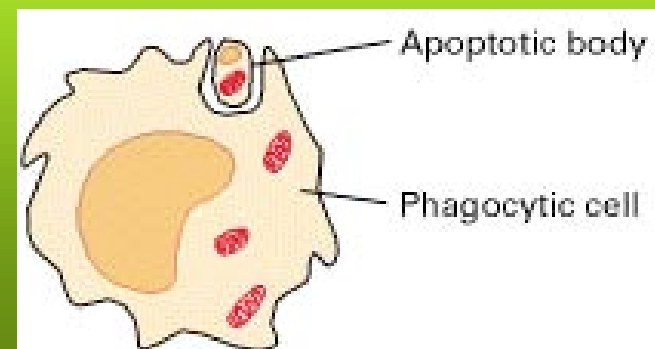


Nuclear
fragmentation
Blebbing
Cell fragmentation

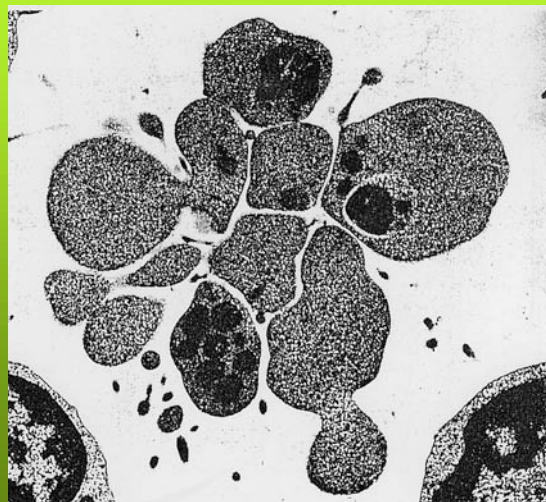
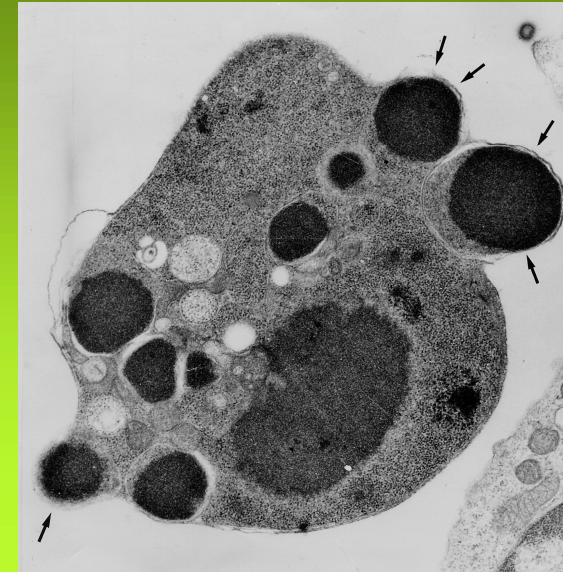
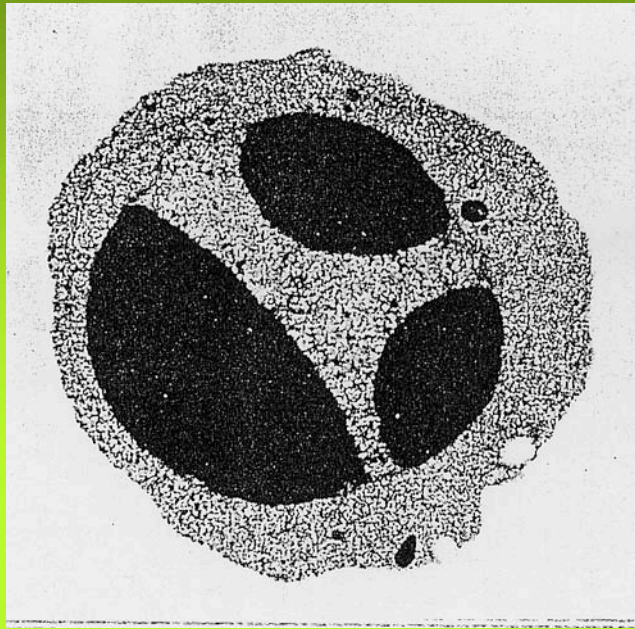
Apoptosi

Morte cellulare programmata

Fagocitosi



La cellula apoptotica si frammenta in corpi apoptotici
(Blebbing)

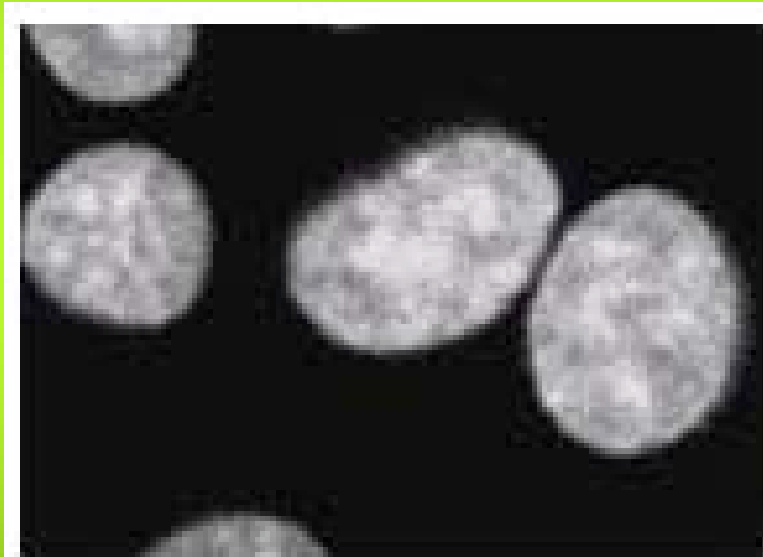


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Nuclei

Controllo

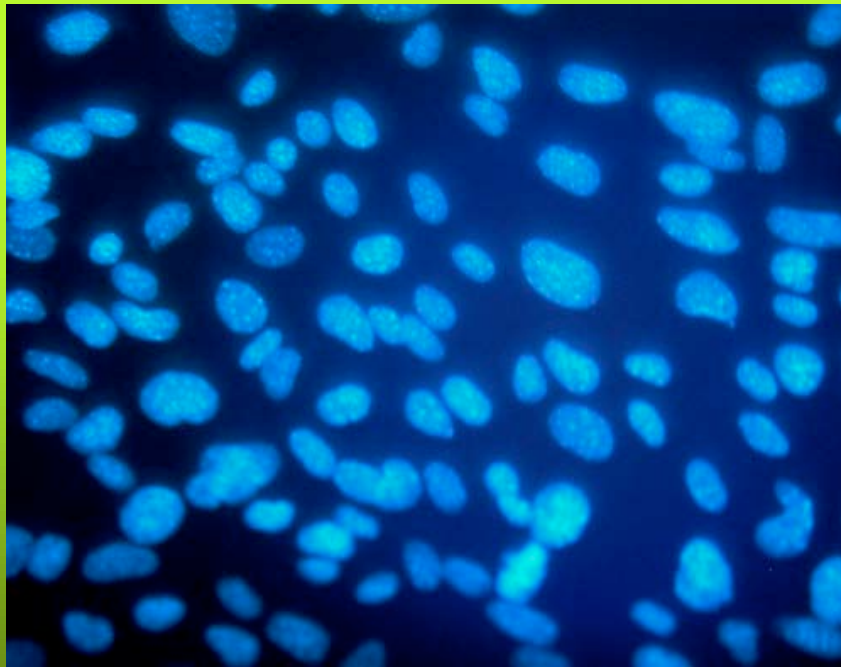


Apoptosi

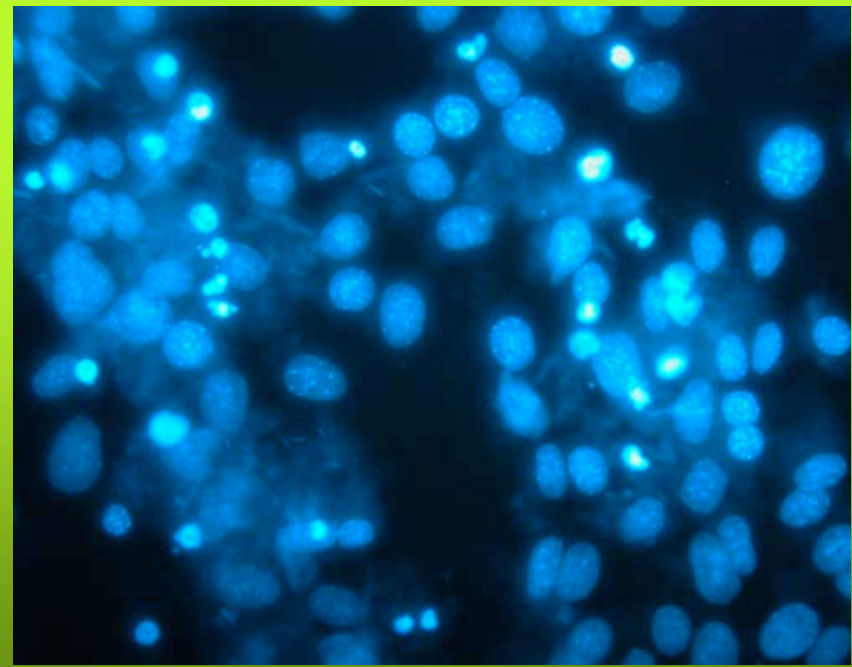


Nuclei

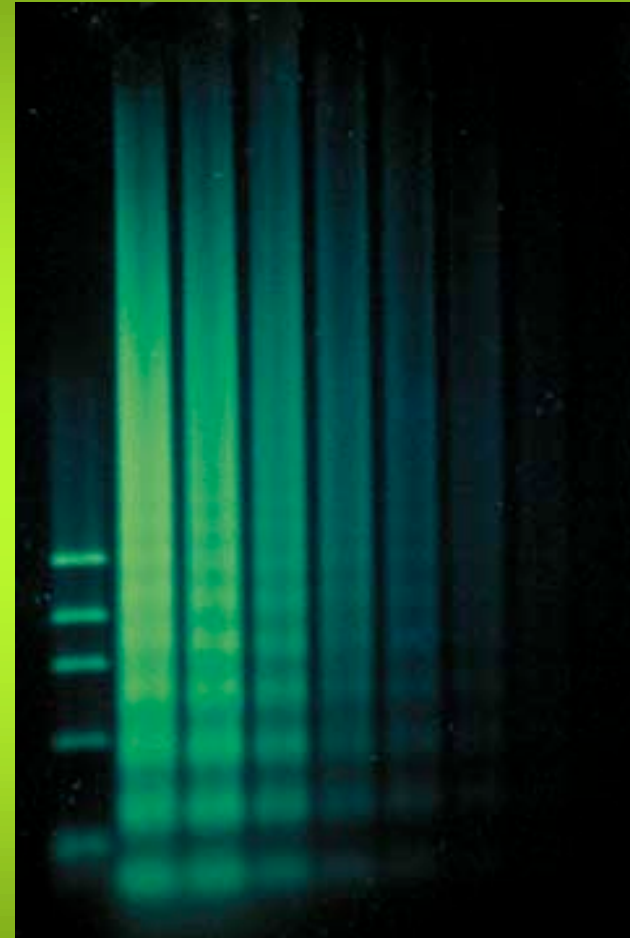
Controllo



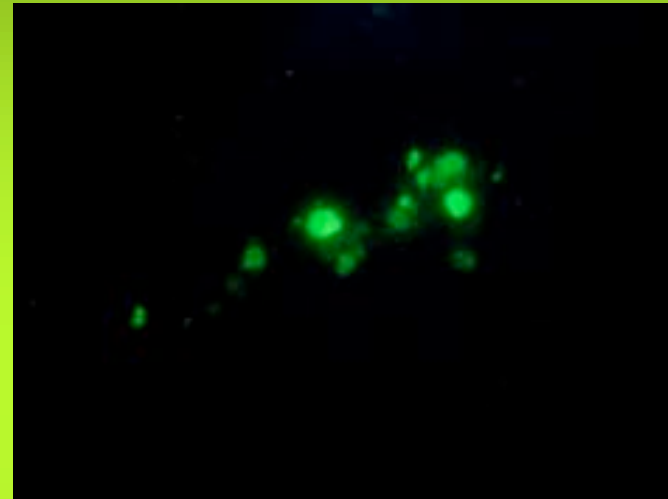
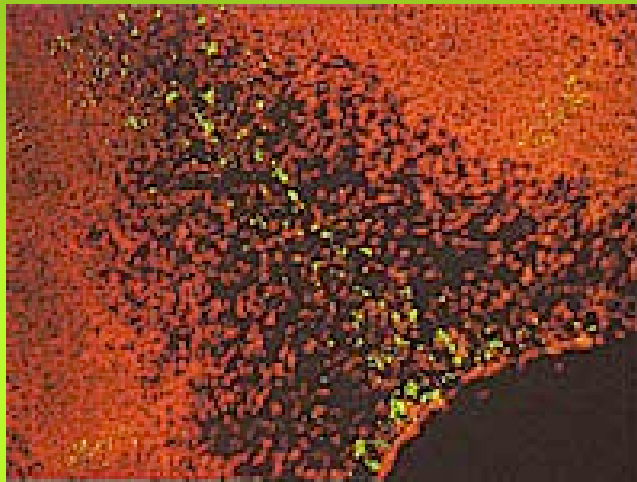
Apoptosi



La cromatina si frammenta
con un pattern tipico



Questo processo di frammentazione ci consente di individuare le cellule apoptotiche con una colorazione tipica (TUNEL; Terminal-transferase-mediated dUTP Nick End Labelling)



Tunel



Dapi

I segnali che provocano l'apoptosi possono essere endogeni o esogeni

Esempi

- Ligando-recettore
- Assenza di fattori di crescita
- Mutazioni DNA
- P53

STIMOLO
APOPTOTICO

CASPASI
ESECUTRICI

Lamine

CAD/ICAD

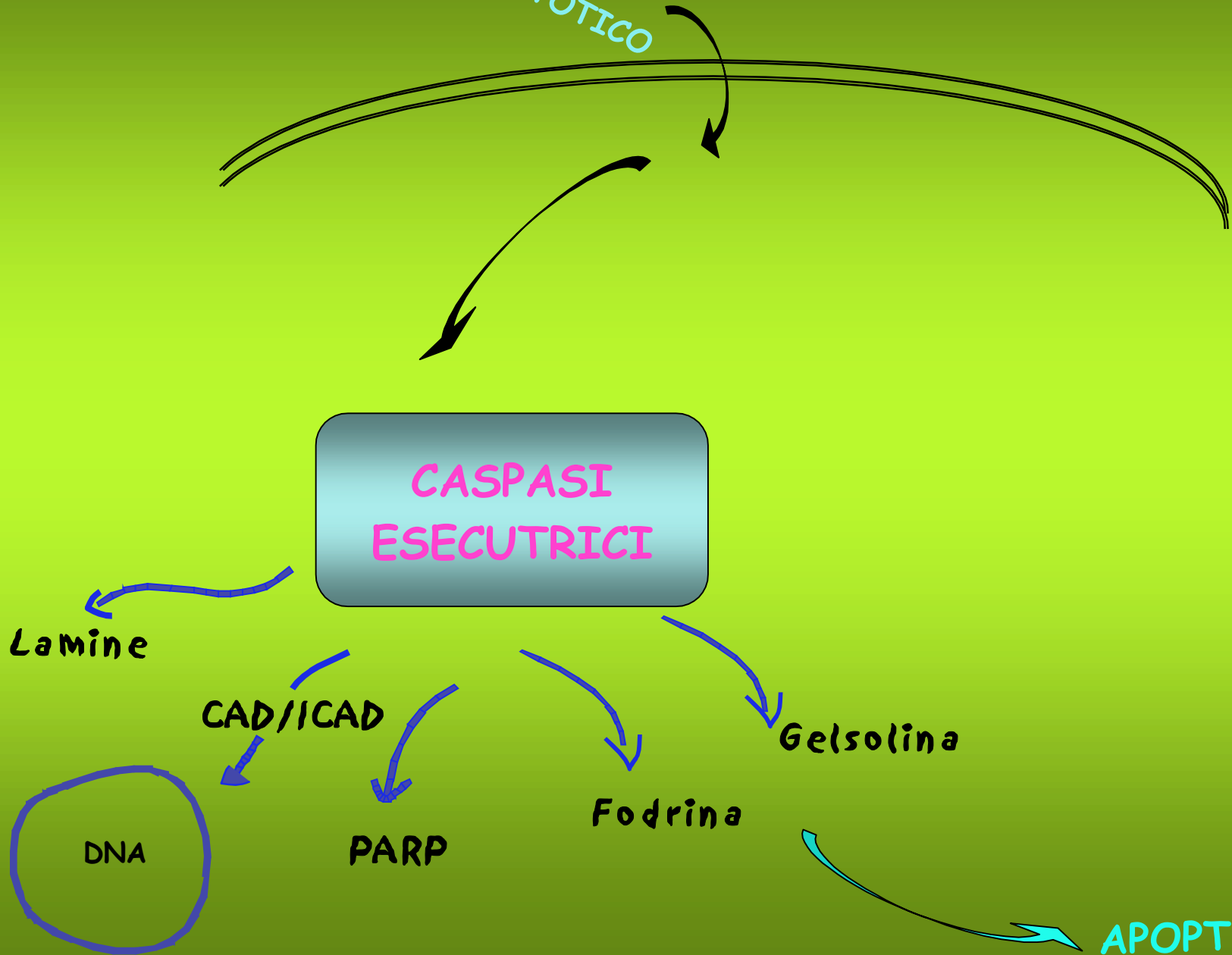
DNA

PARP

Fodrina

Gelsolina

APOPTOSI



CASPASI

C: cisteina

asp: specificita' per l'acido aspartico

asi: proteasi

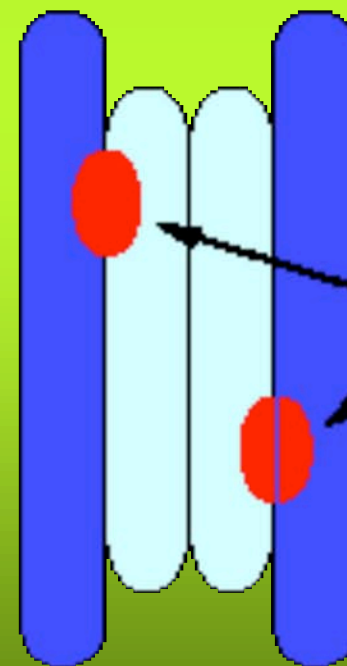
Enzimi molto selettivi

Esempio di sito bersaglio: **DEV D-X**

Inactive Proenzyme



Proenzyme is cleaved at caspase cleavage sequences (Asp-x)



2 large and 2 small subunits combine to form the active tetrameric enzyme

Active Caspase

14 CASPASI

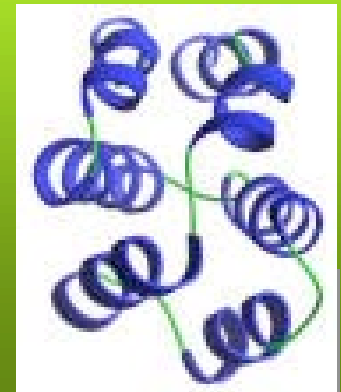
Caspasi EFFETTRICI: Prodominio piccolo

3, 6, 7

Caspasi INIZIATRICI: Prodominio grande

8, 9

CARD domain



Protéines Kin
PKC δ
PKC τ
PKC-related kinase2
PKN
Ca/Cam dependent protein kinase IV
Pak 2
PITSLRE kinase α 2-1
Mst1 kinase
Mst 2 kinase
FAK
Fyn
MEKK-1
Wee1 kinase

Polypeptides
Protéines cyto
Gelsoline
Gas-2
Fodrine
β -Caténine
Cytokératine 18
Protéines Nuc
SRF
Lamine A
Lamine B1
NuMA
Protéine 70kDa de U1 snRNP
mdm2
Protéines imp
PARP
DNA-PKcs
Topo-isomérase I

Polypeptides
Autres protéin
Pro-interleukine 1 β
Pro-interleukine 16
Pro-interleukine 18
Proteine phosphatase 2A sous-unité A α
Phospholipase A2 Cytosolique
Stat 1
NF κ Bp50, p65
I κ B
Calpastatine

Protéines imp
p21 ^{Waf1}
p27 ^{kip1}
Proteine Rb
Protéines imp
Huntington
Préséniline-1
Préséniline-2
Protéines de l
Bcl-2
Bcl-X _L
FLIP _L
Bid
Bax
ICAD

STIMOLO
APOPTOTICO

CASPASI
ESECUTRICI

Lamine

CAD/ICAD

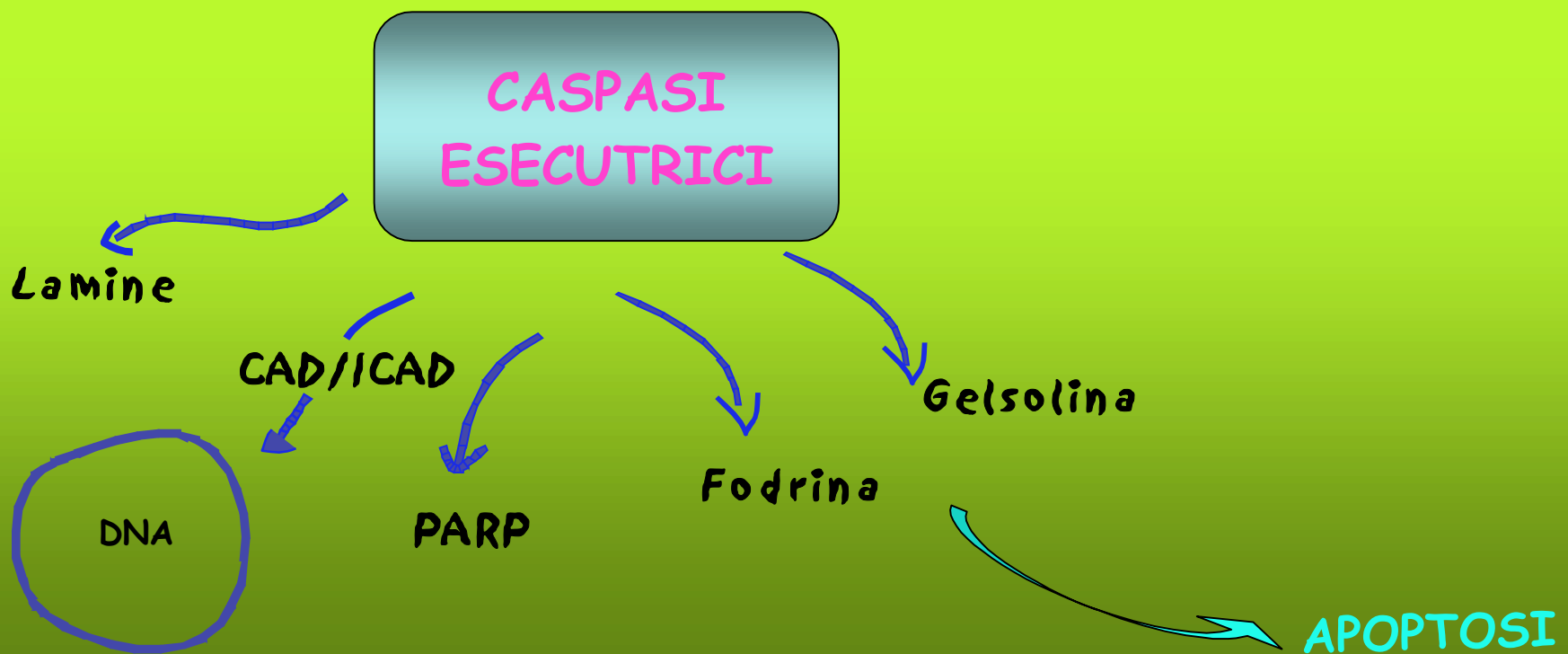
PARP

Fodrina

Gelsolina

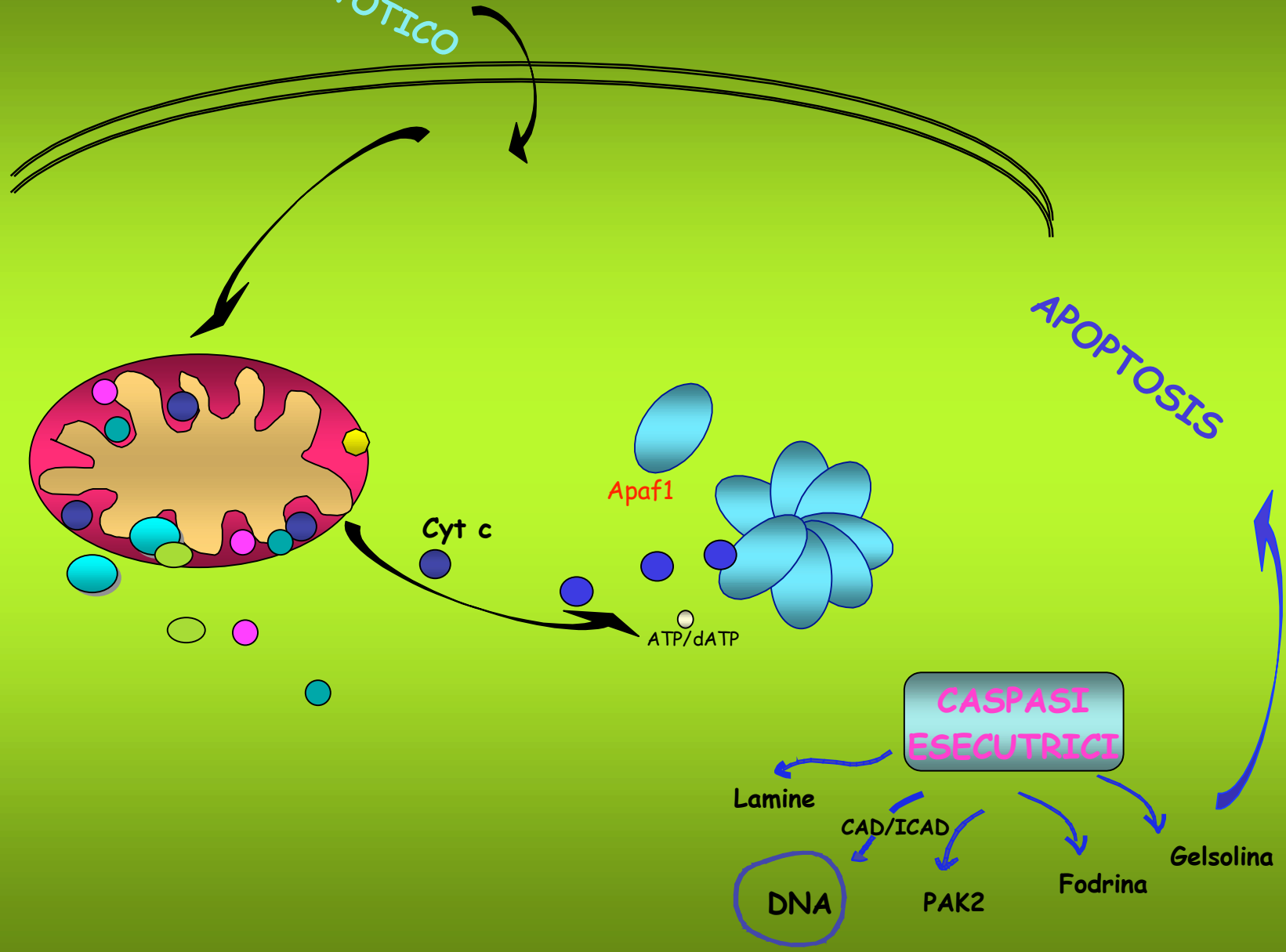
DNA

APOPTOSI



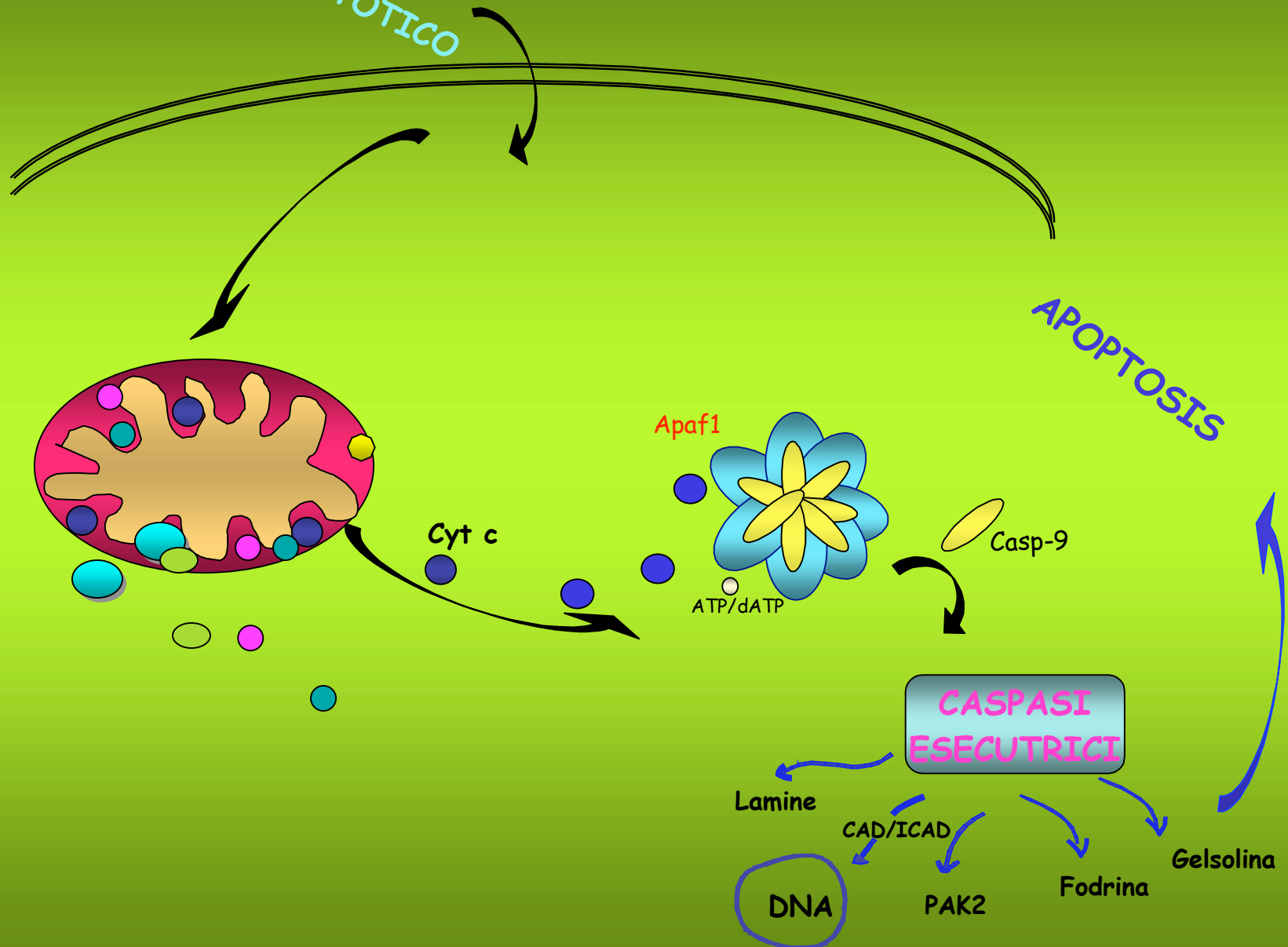
STIMOLO
APOPTOTICO

APOPTOSIS



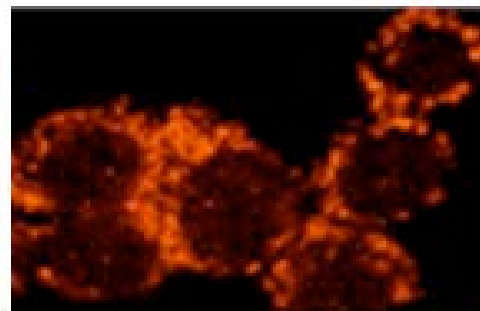
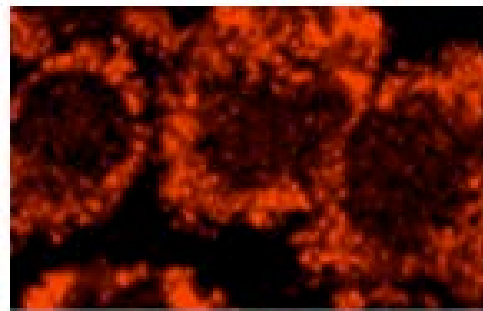
STIMOLO
APOPTOTICO

APOPTOSIS

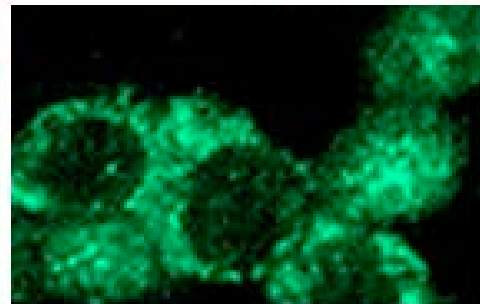
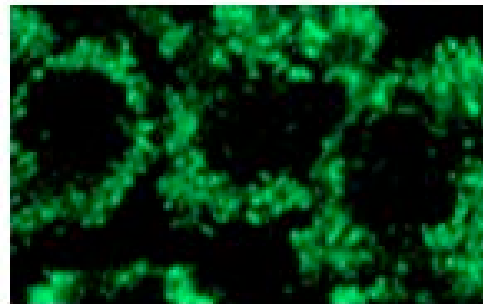


Control

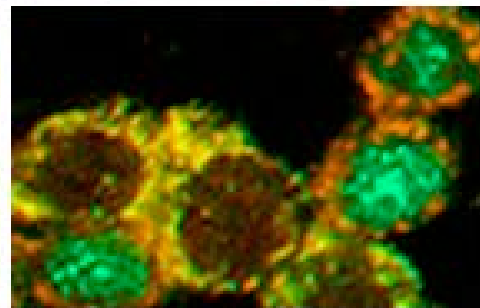
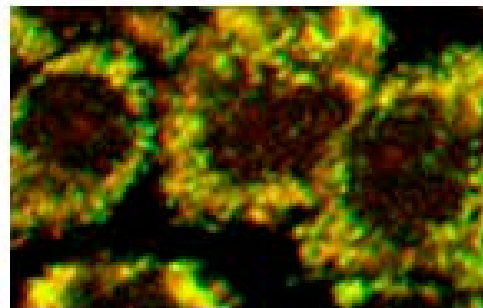
STS



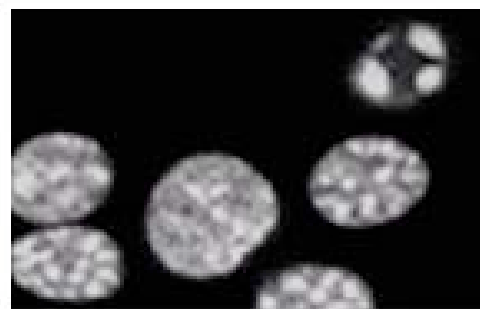
Hsp 60



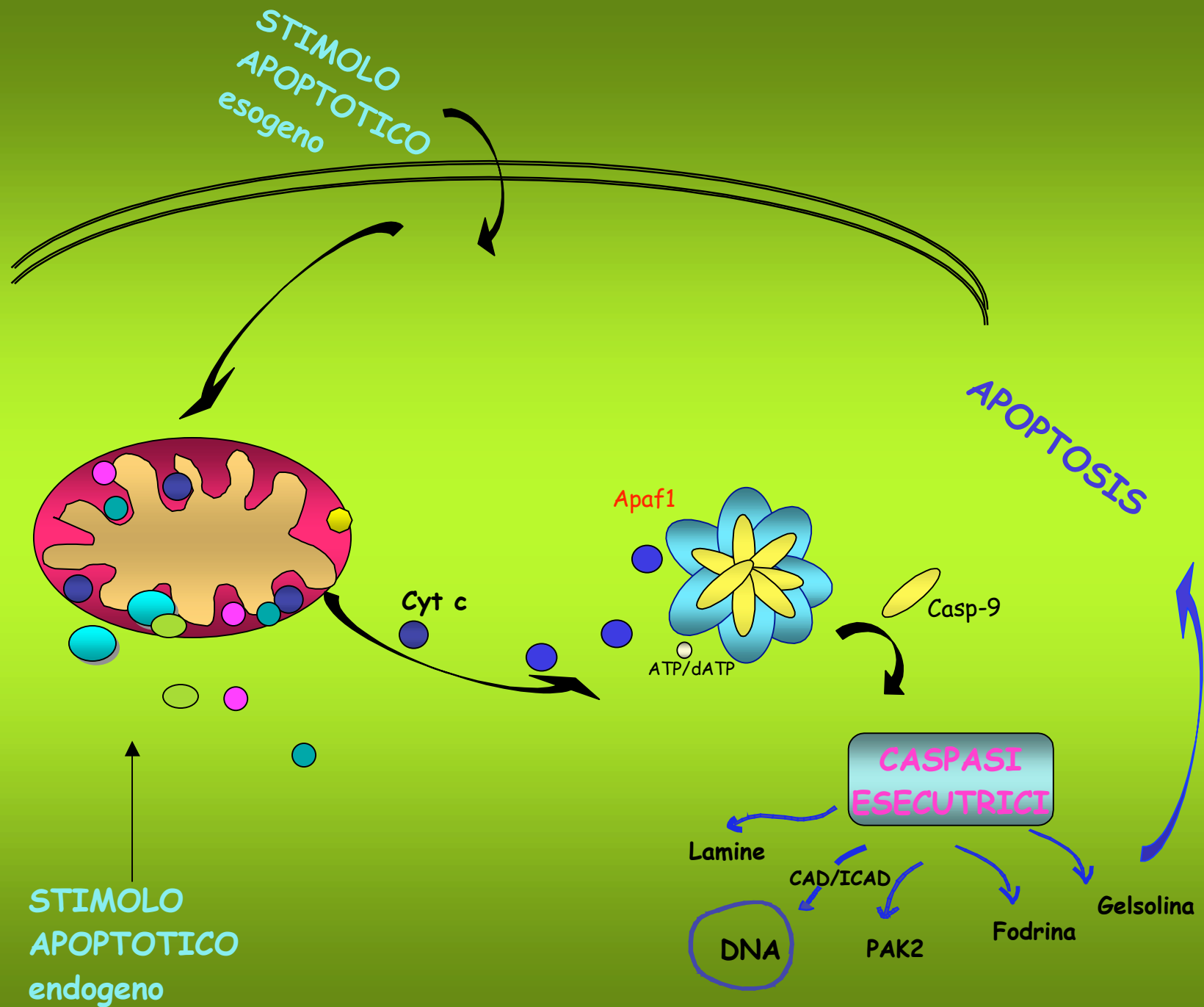
Cyt c



Merge



Nuclei



I segnali che provocano l'apoptosi possono essere endogeni o esogeni

Esempi

- Ligando-recettore
- Assenza di fattori di crescita
- Mutazioni DNA
- P53

Le proteine della famiglia di Bcl-2

Le proteine della famiglia di Bcl-2
controllano la permeabilità
mitocondriale e il rilascio di
citocromo c.

Si distinguono in:

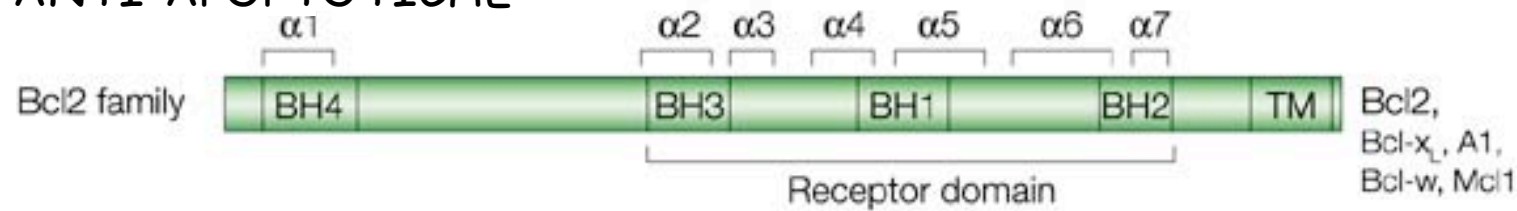
- proapoptotiche
- antiapoptotiche

Condividono dei domini di omologia detti **BH** (Bcl-2 homology).

Sono **BH1, BH2, BH3, BH4**

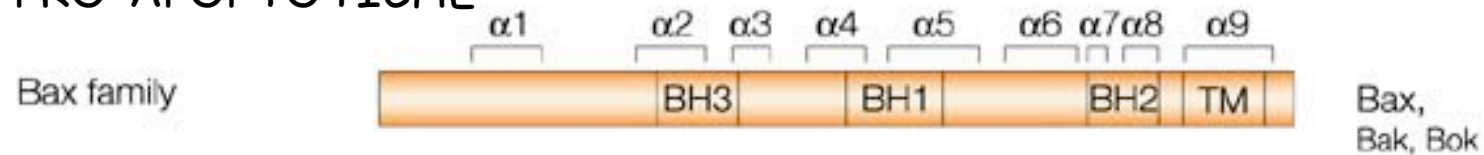
Le proteine Bcl-2: Classificazione

ANTI-APOPTOTICHE

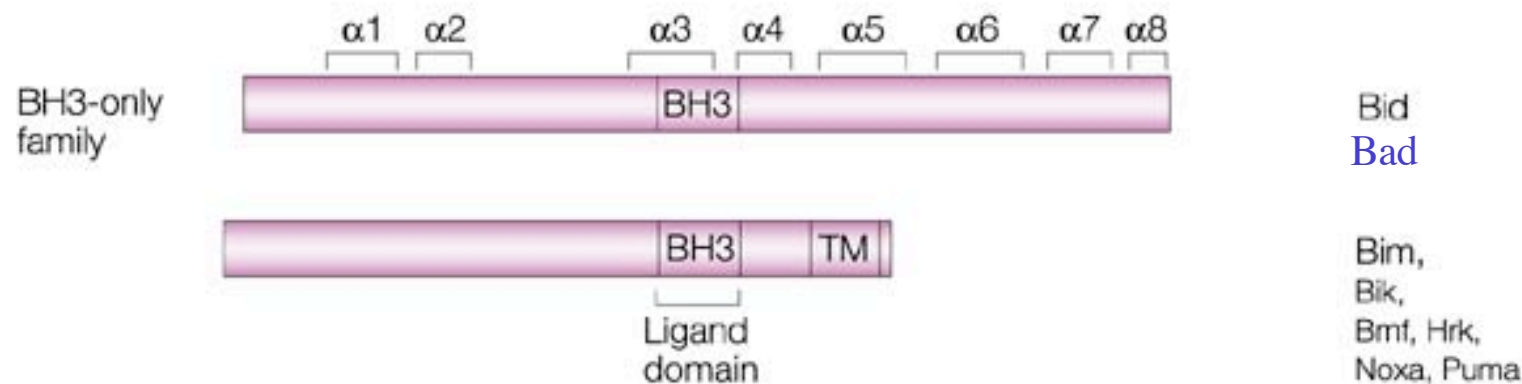


Bcl-2
Bcl-XL

PRO-APOPTOTICHE



Bax
Bak



Bid
Bad

Le molecole della famiglia Bcl-2 sono molto conservate

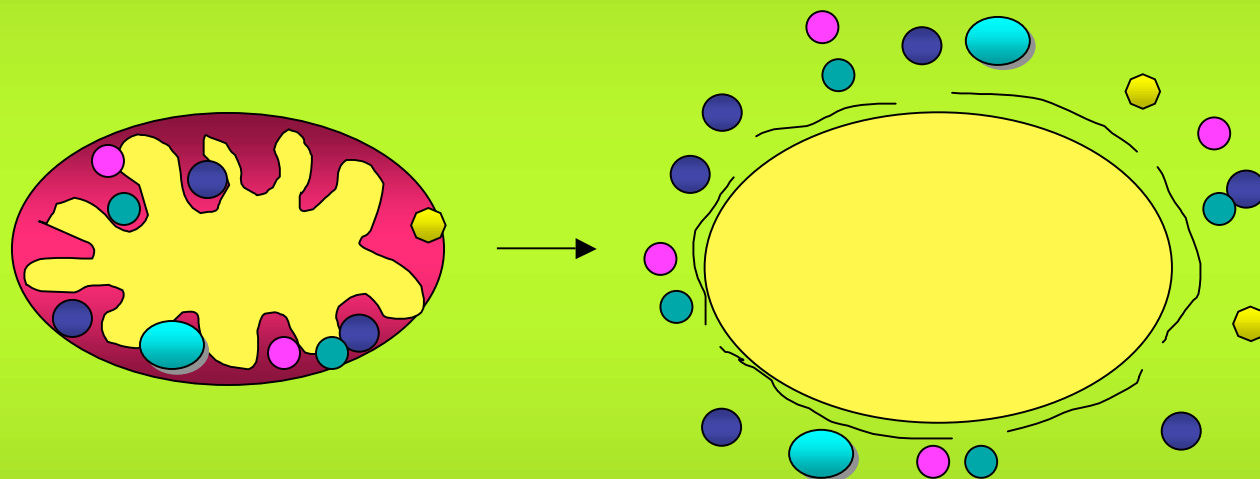
proapoptotiche (Bid, Bad, Bax, Bak)

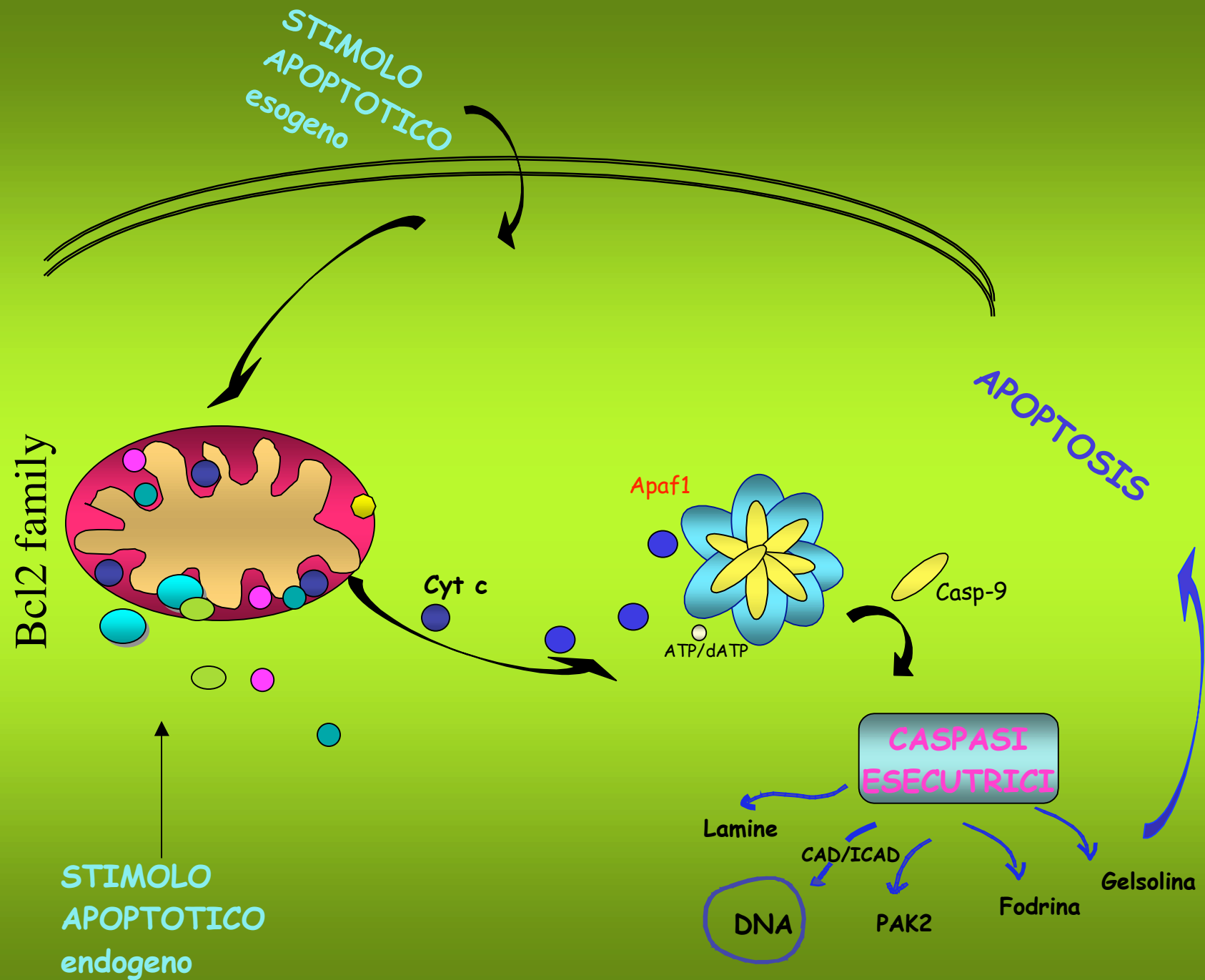
antiapoptotiche (Bcl-2; Bcl-XL)

Interagiscono tra loro
per formare
eterodimeri o omodimeri

Similitudine di Bcl-2, BclxL e Bax con alcune tossine batteriche (colicine, tossina difterica) che formano **pori in membrana**.

Potrebbero formare dei pori attraverso cui passano le proteine rilasciate oppure destabilizzare l'equilibrio ionico e indurre la rottura della membrana esterna





I segnali che provocano l'apoptosi possono essere endogeni o esogeni

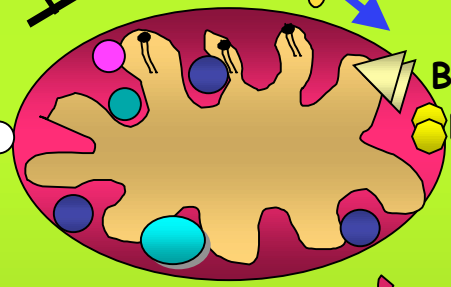
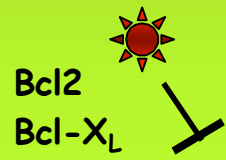
Esempi

- Ligando-recettore
- Assenza di fattori di crescita
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STIMOLO
APOPTOTICO
esogeno



Casp-8



Bak
Bax

Bad

Cyt c

Apaf1

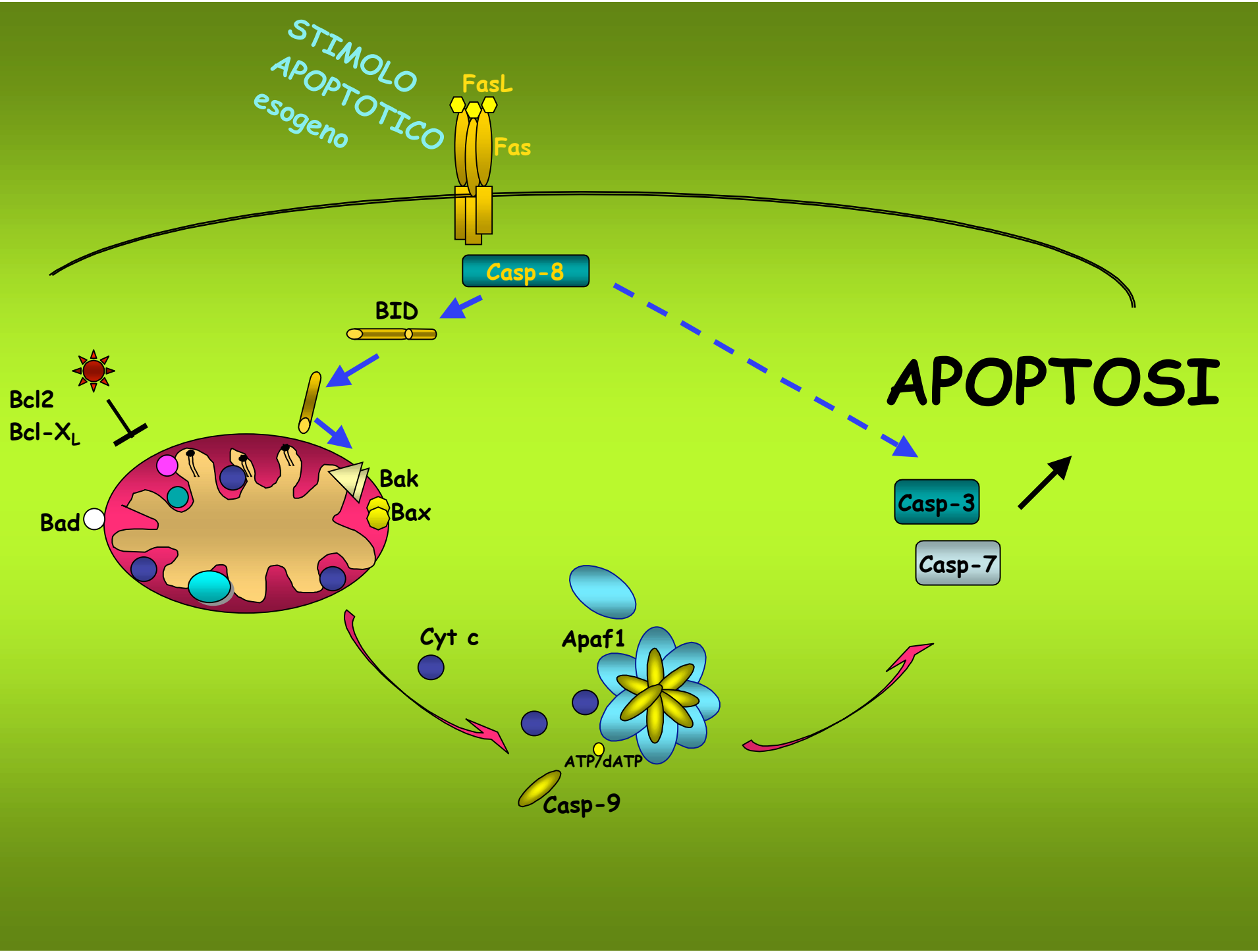
ATP/dATP

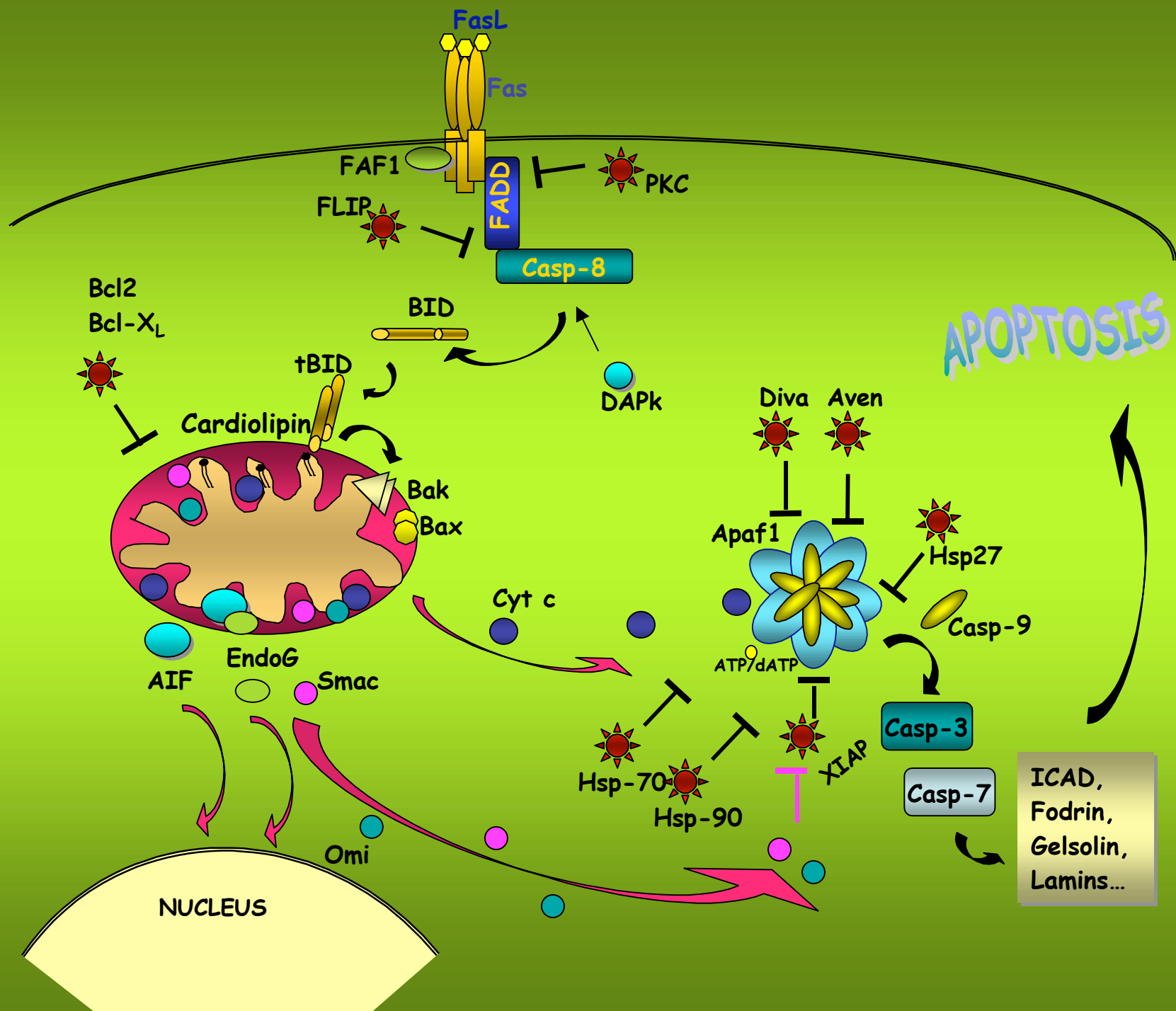
Casp-9

APOPTOSI

Casp-3

Casp-7





Evento

Esempio

Normale sviluppo embrionale/fetale

Eliminazione della membrana interdigitale

Normale turnover cellulare

Villi intestinali

Metamorfosi

Regressione della coda degli anfibi

Citotossicità cellulo-mediata

Uccisione T-cell mediata

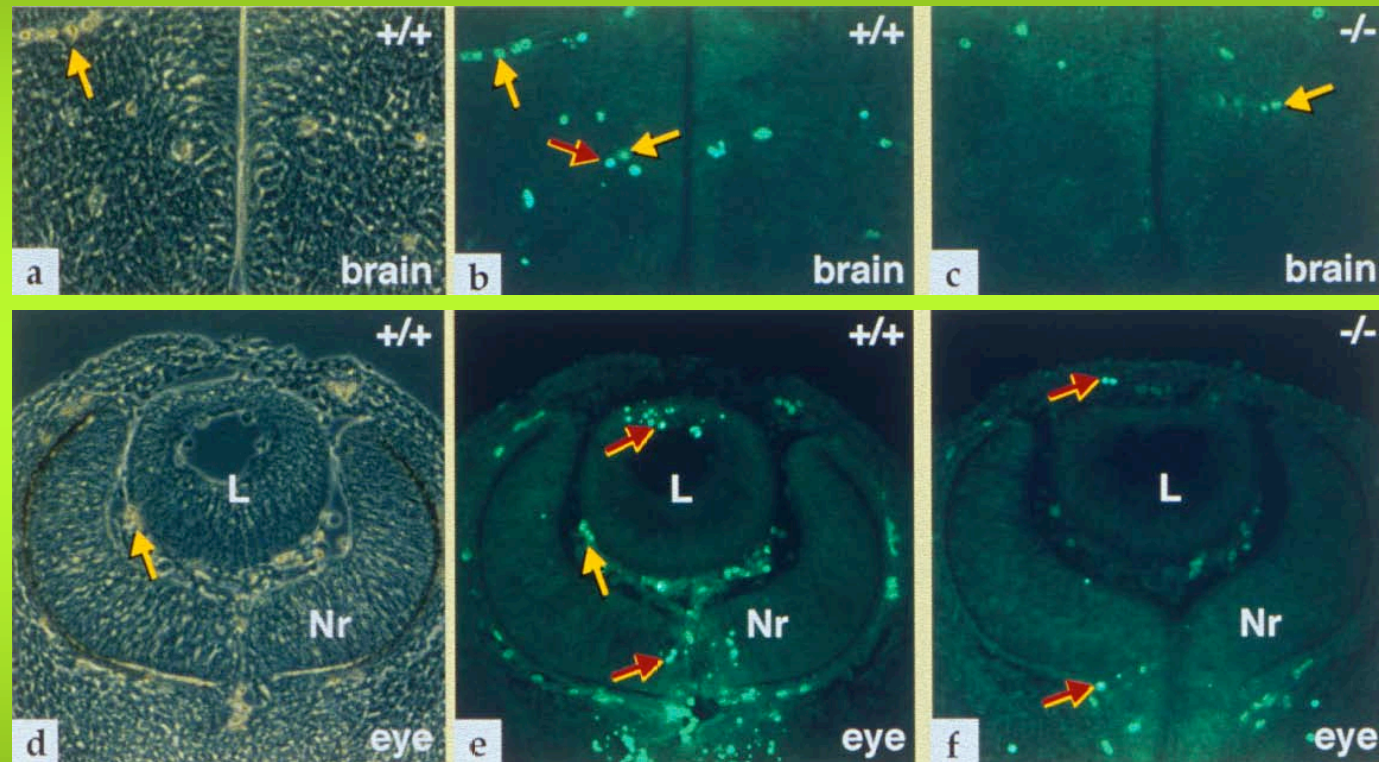
Condizioni di crescita
sfavorevoli/patologiche

Deprivazione di fattori di crescita
Irradiazione
Esposizione a tossine

Apoptosi nello sviluppo

*Knock-out e transgenici hanno aiutato a capire
in quali tessuti uno specifico gene apoptotico
funziona*

L'apoptosi e' ridotta negli embrioni Apaf1-/-

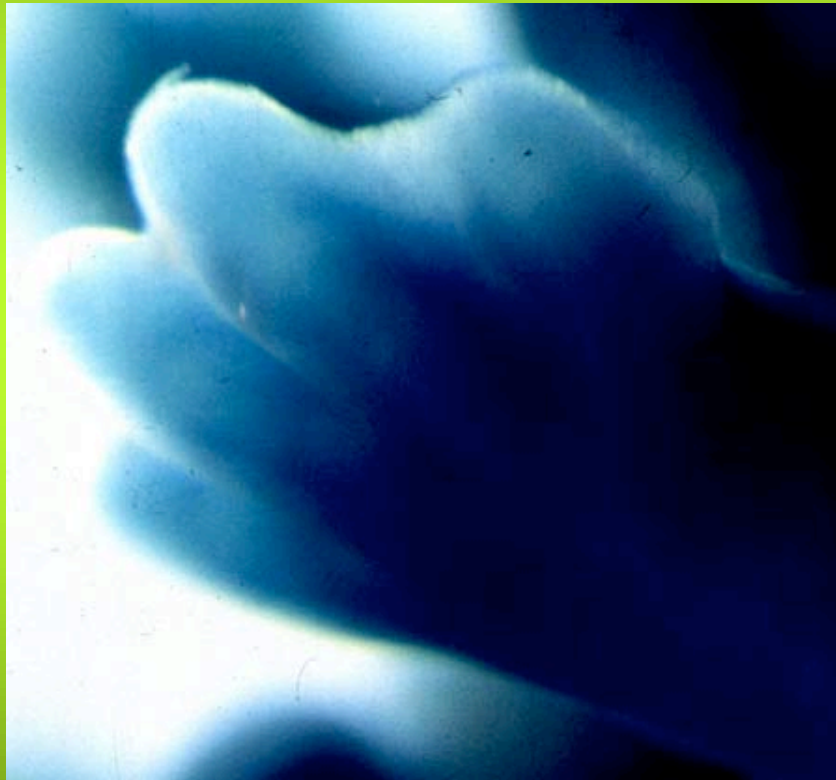


Il fenotipo del KO *Apaf1*^{-/-}

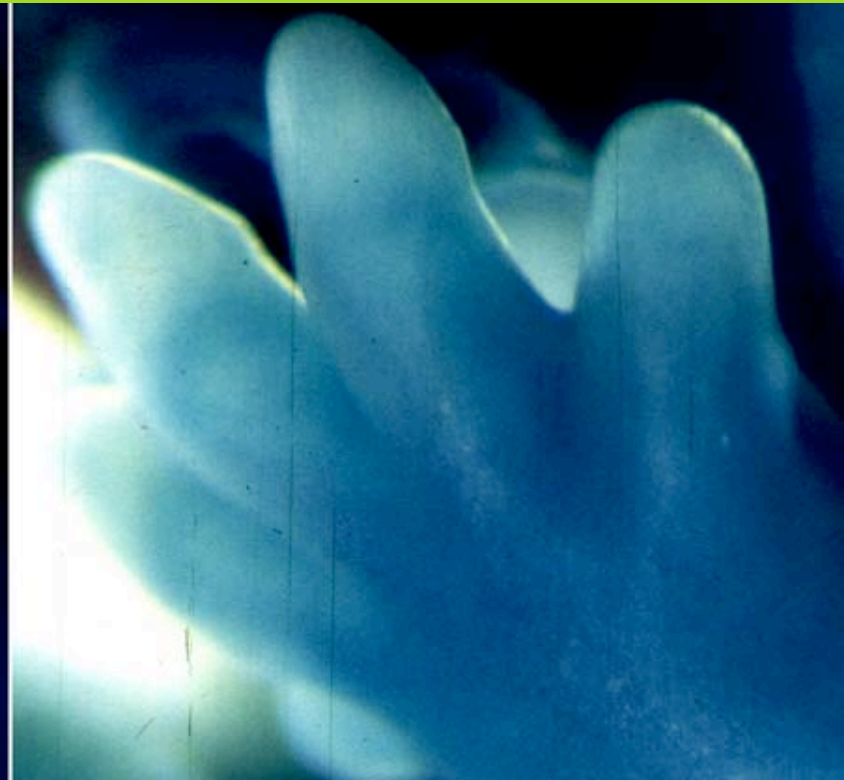
Arti	Persistenza degli spazi interdigitali
CNS	Mancata chiusura del tubo neurale, spina bifida. Iperplasia cerebrale dovuta ad una espansione dei progenitori neuronali; esencefalia.
Palato	Mancata fusione del palato
Cranio	Scorretta ossificazione.
Occhio	Iperproliferazione della retina e riduzione della dimensione della lente.
Orecchio interno	Malformazione della vescicola otica, scorretta morfogenesi .

Formazione degli spazi interdigitali

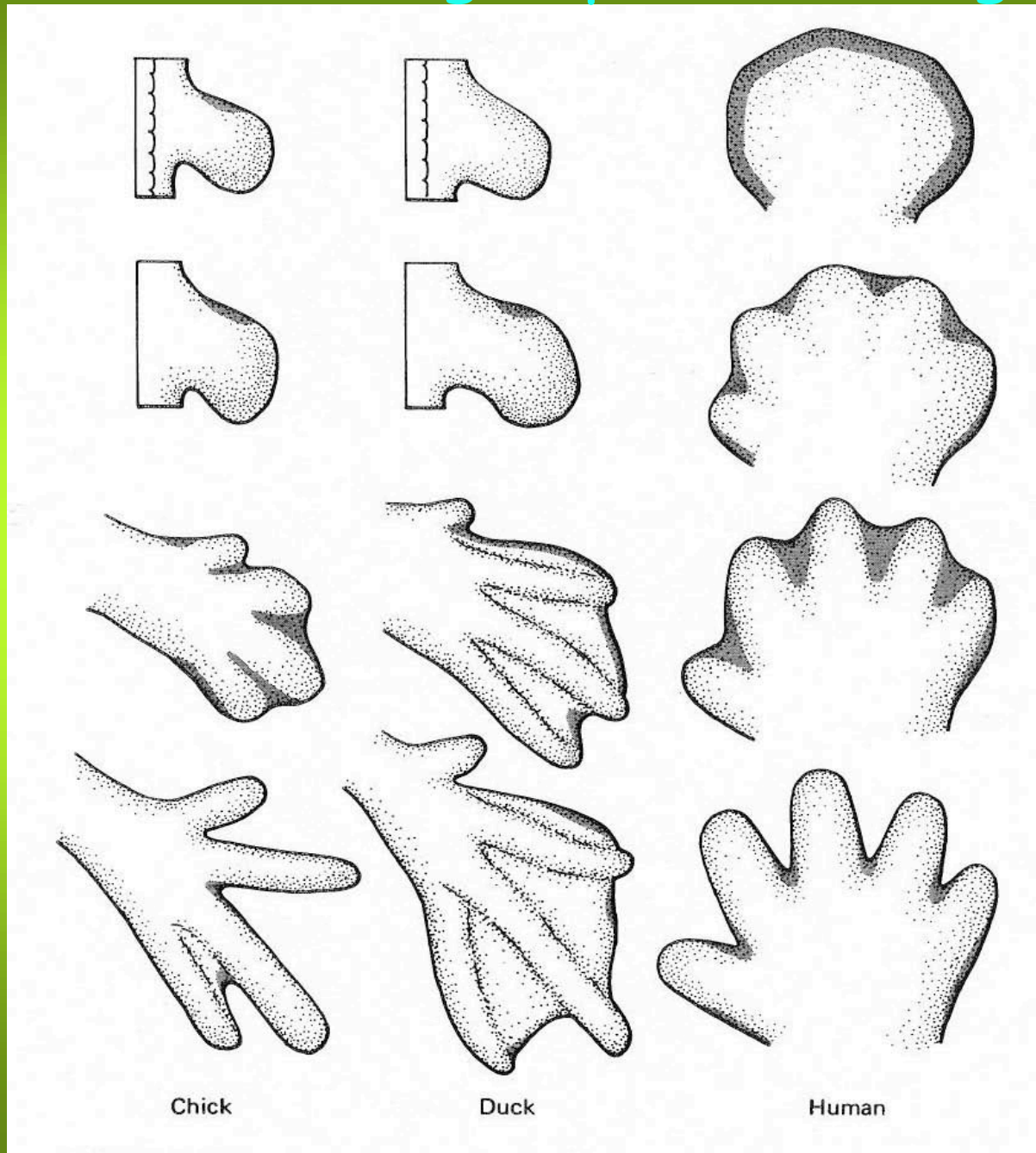
Assenza di apoptosi



Apoptosi regolare

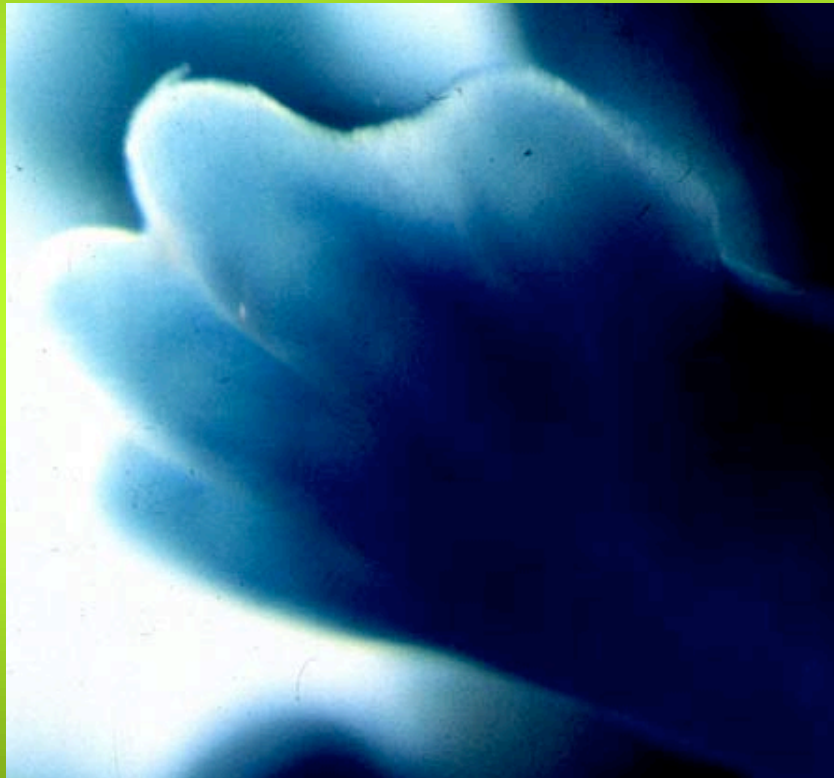


Formazione degli spazi interdigitali

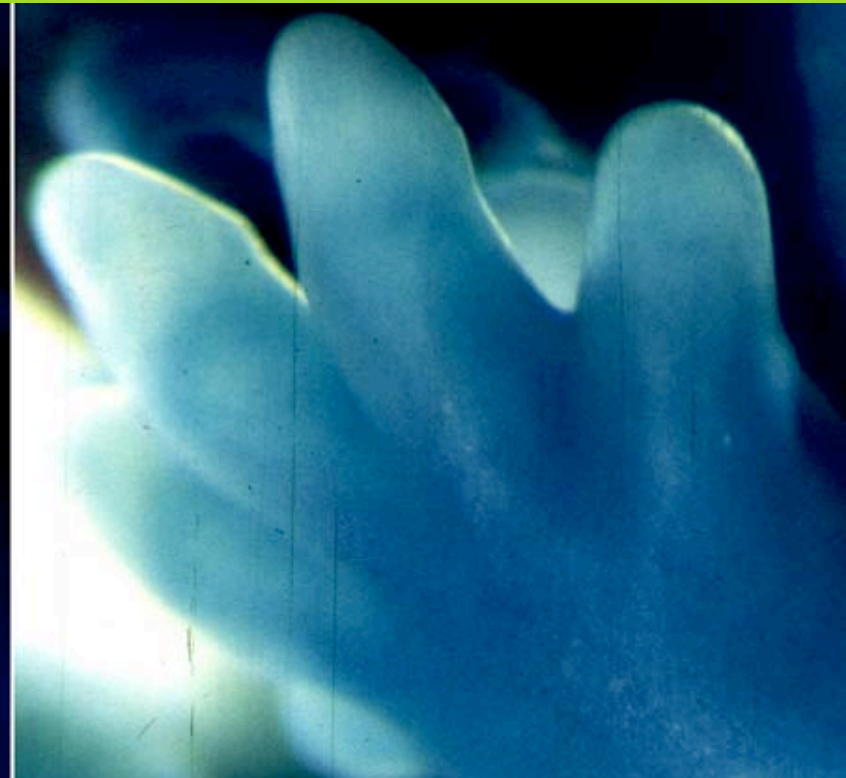


Formazione degli spazi interdigitali

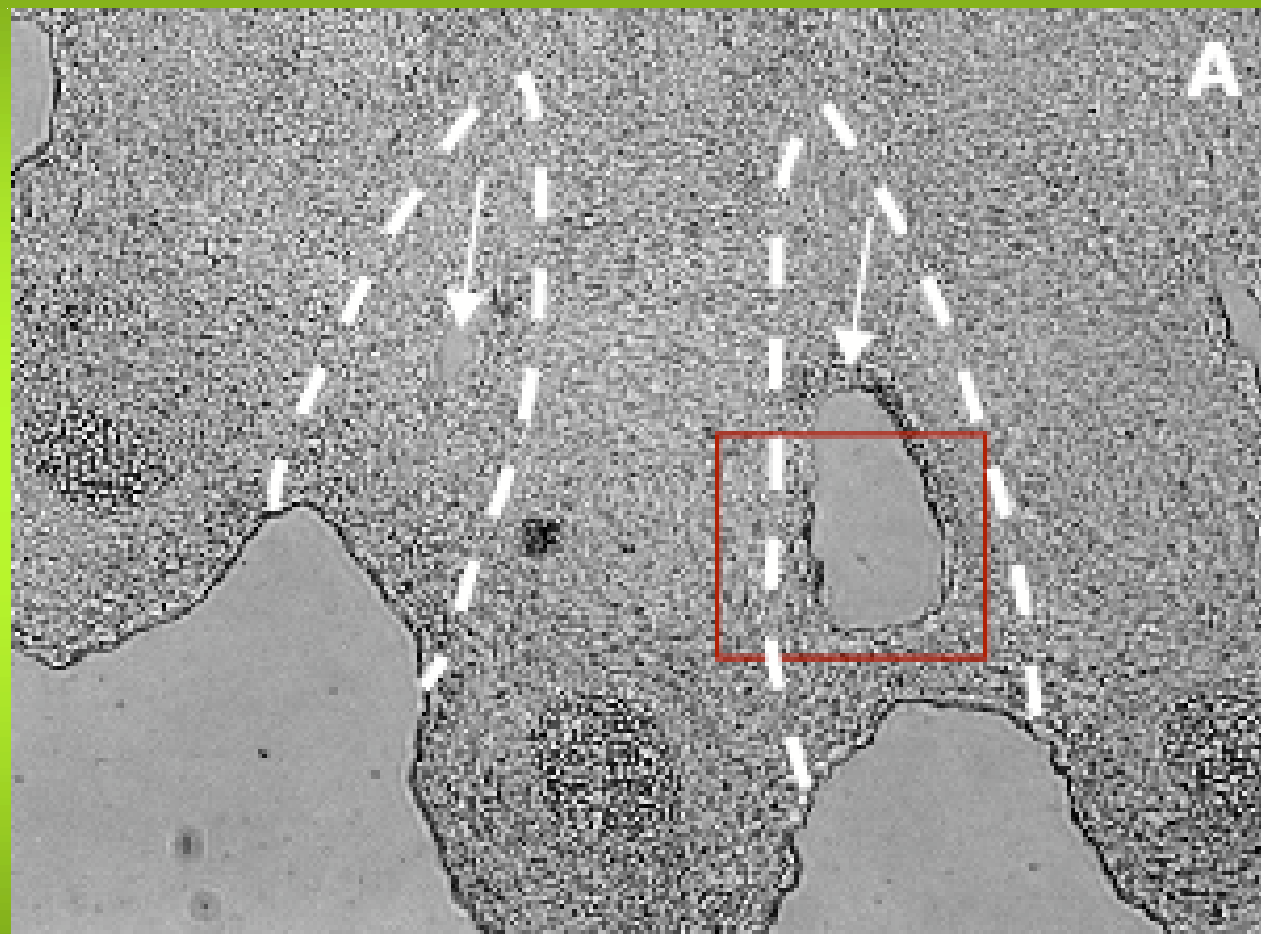
Assenza di apoptosi



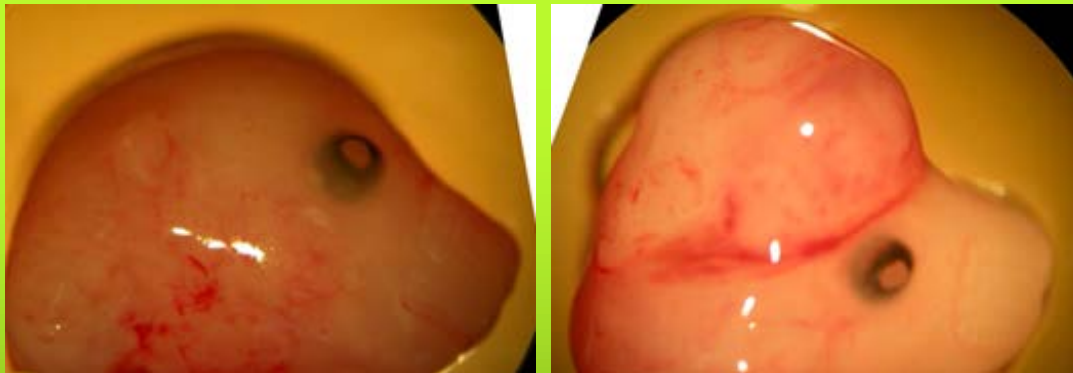
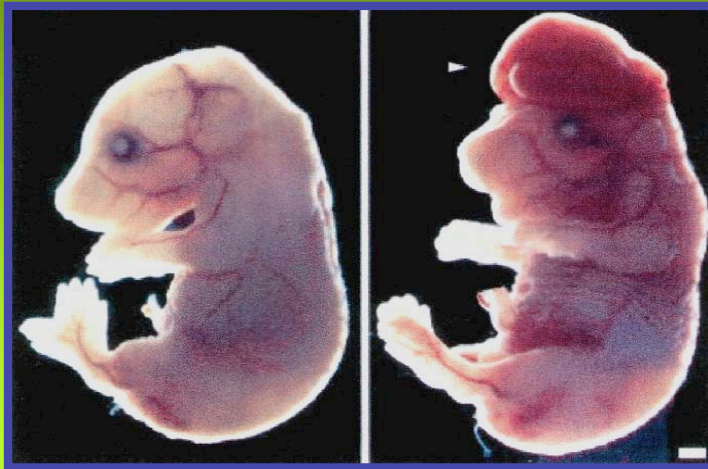
Apoptosi regolare



Formazione degli spazi interdigitali



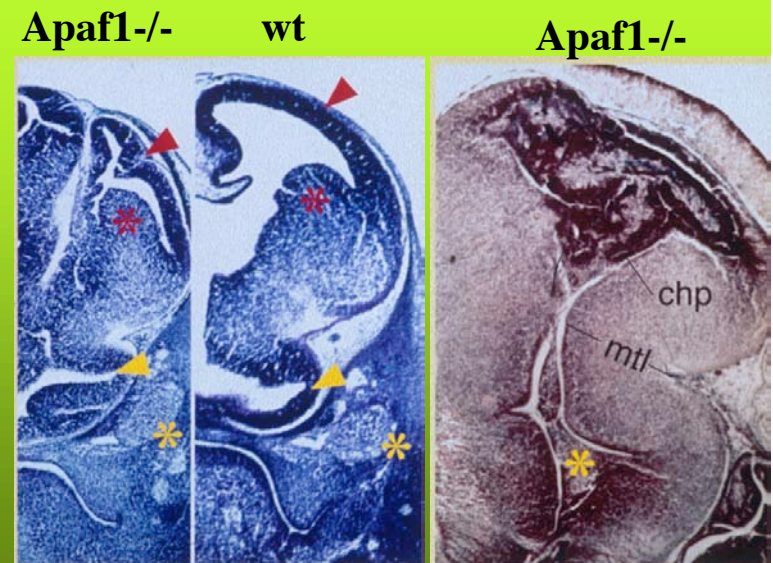
Iperplasia cerebrale ed esencefalia



wt



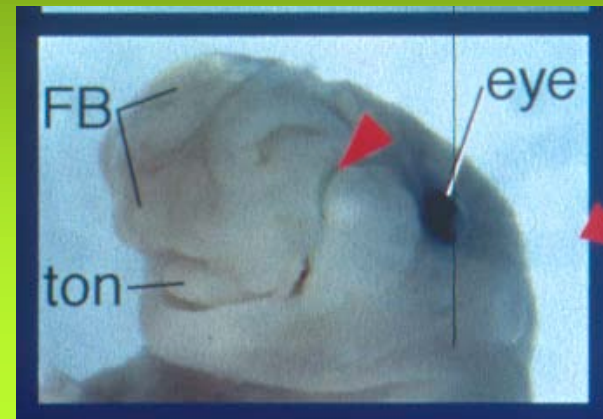
Apaf1-/-



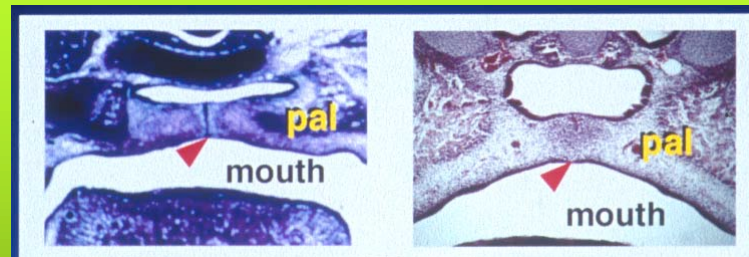
Malformazioni di tipo cranio-facciale



wt

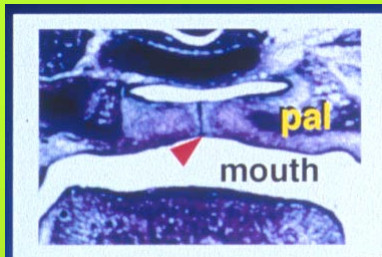


Apaf1-/-



Palato

Apaf1^{-/-}



wt

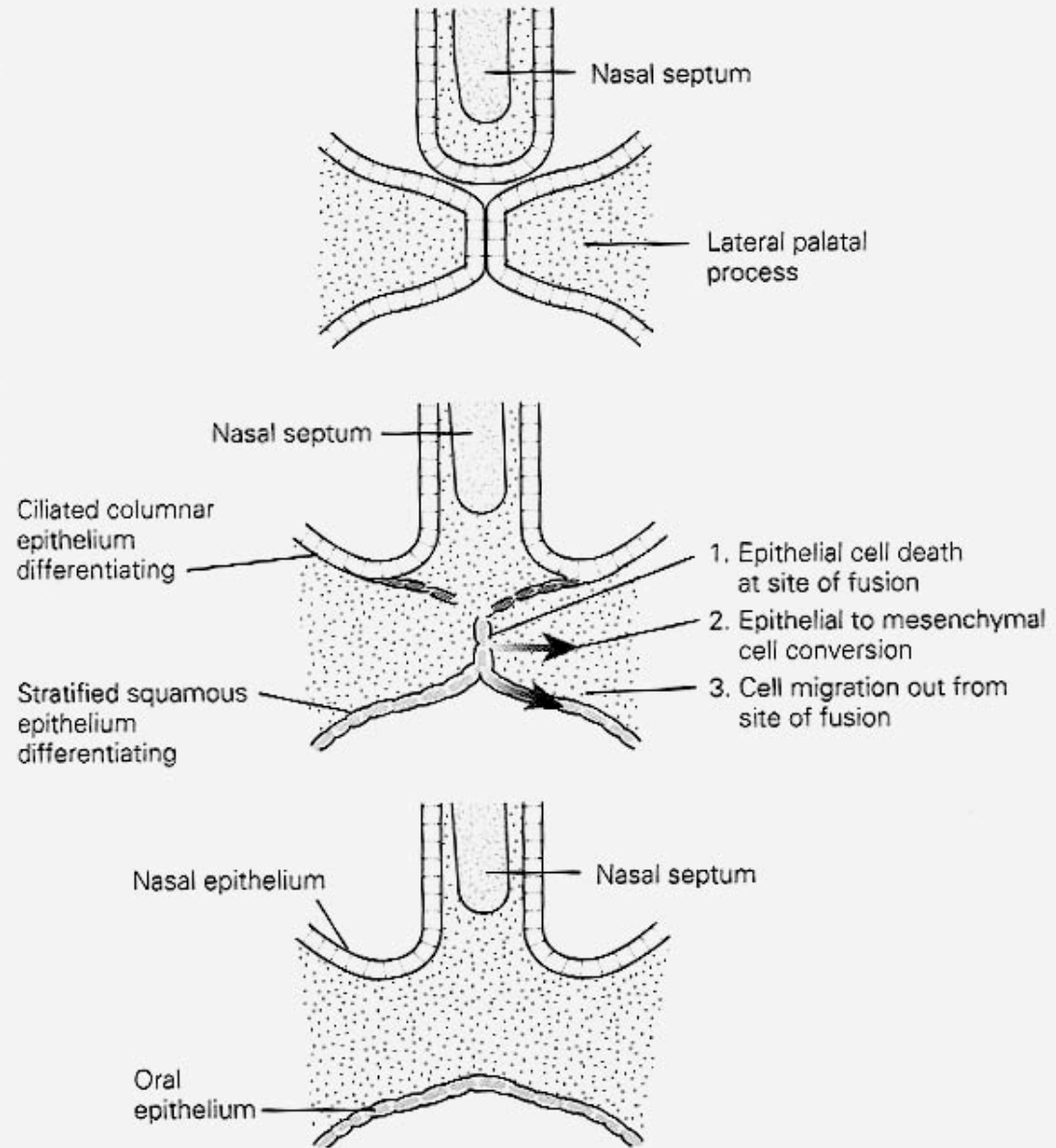
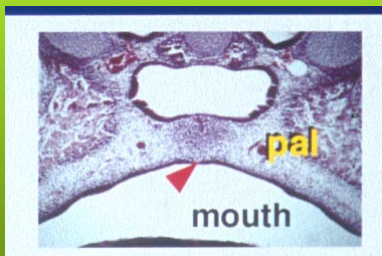
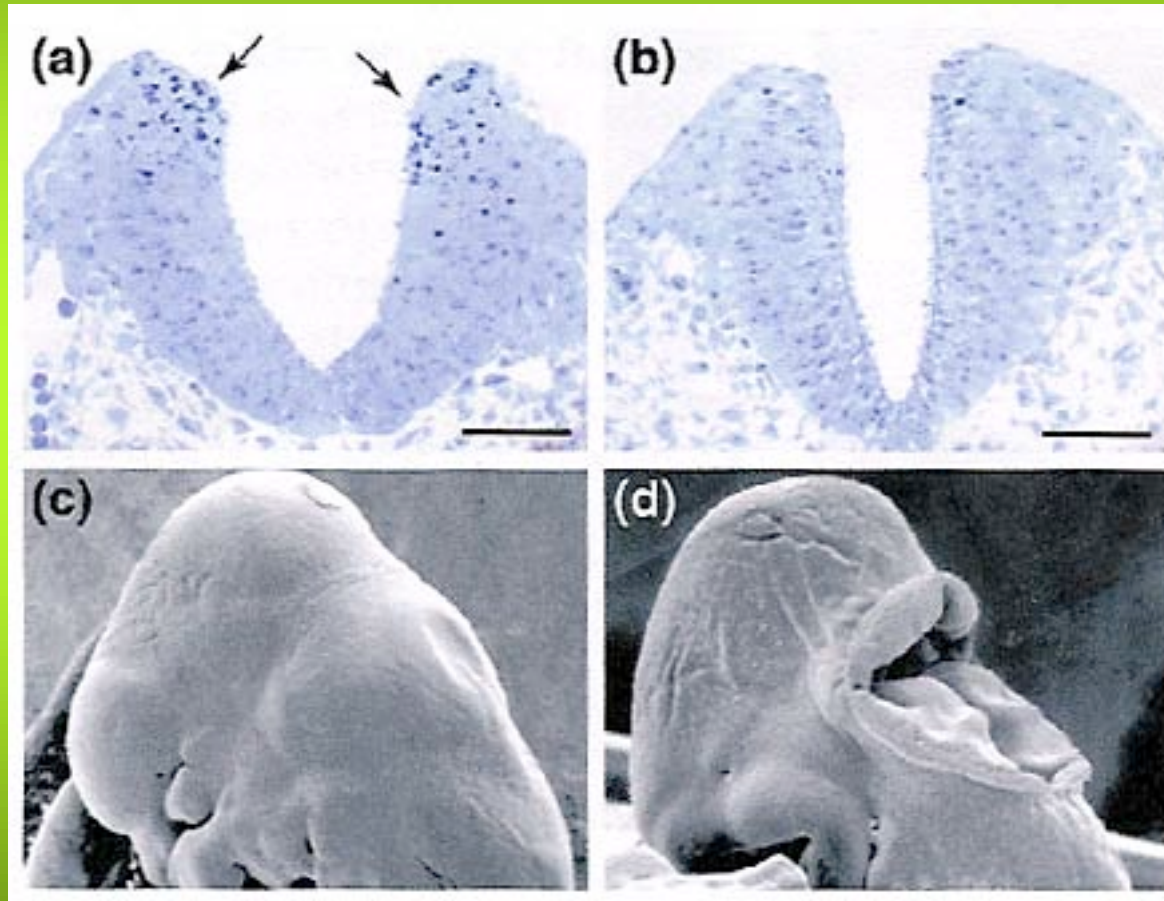


FIGURE 16-8
Developmental processes associated with fusion of the palatal shelves and nasal septum.

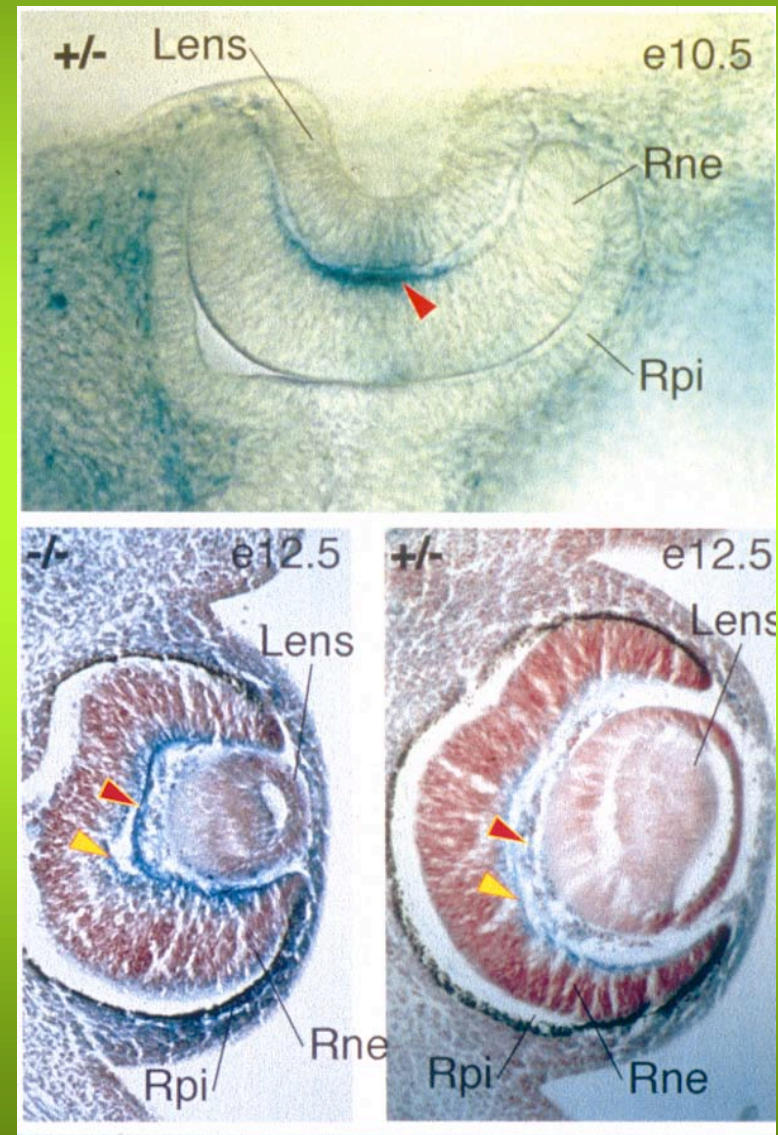
L'apoptosi e' richiesta per la corretta chiusura del tubo neurale



Wt

mutante

Retina iperplastica nei mutanti Apaf1 $-/-$



Apoptosi e Cancro

Se il meccanismo apoptotico e' non funzionante questo impedisce alla cellula di morire e il blocco dell'apoptosi e' un meccanismo che promuove la formazione di tumori.

Apoptosi e Neurodegenerazioni

Se, viceversa, il meccanismo apoptotico e' attivato oltre la norma allora si ha la distruzione non fisiologica di cellule. Questo puo' portare a malattie come le neurodegenerazioni in cui c'e' un'eccessiva morte neuronale.