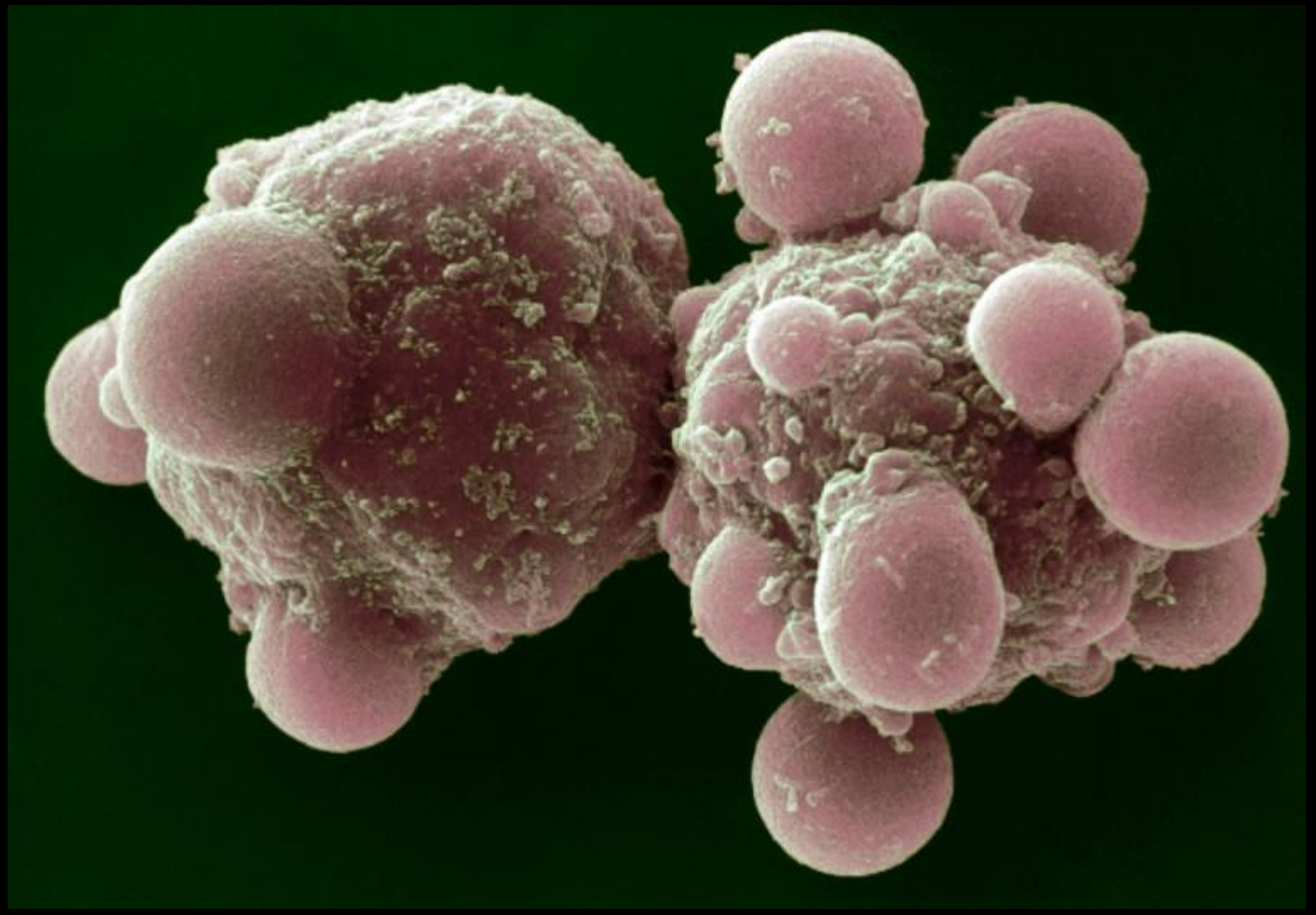
Muerte celular



Uriel Koziol
ukoziol@fcien.edu.uy

¿Por qué mueren las células?

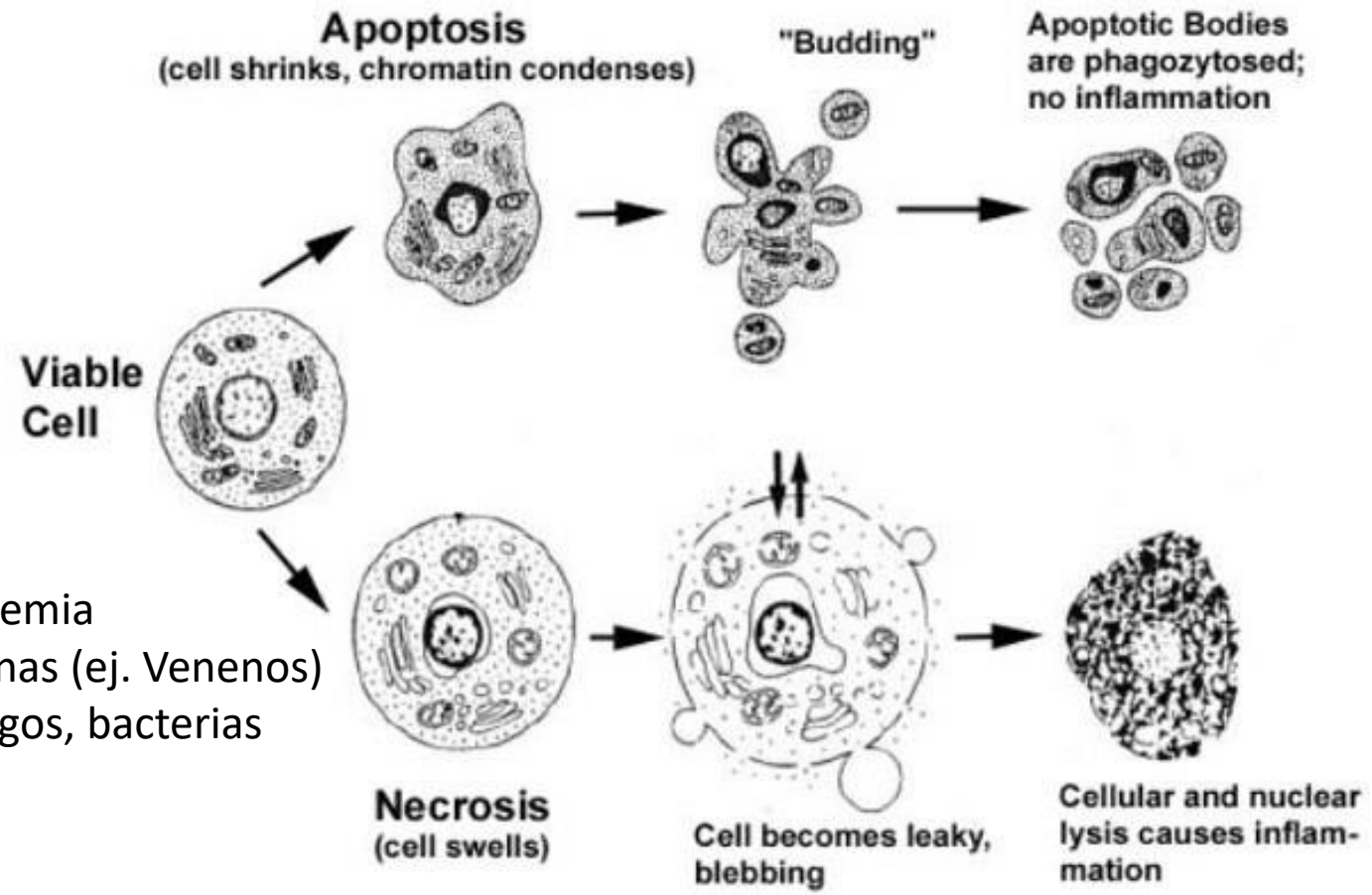
Muerte celular

- **Necrosis**

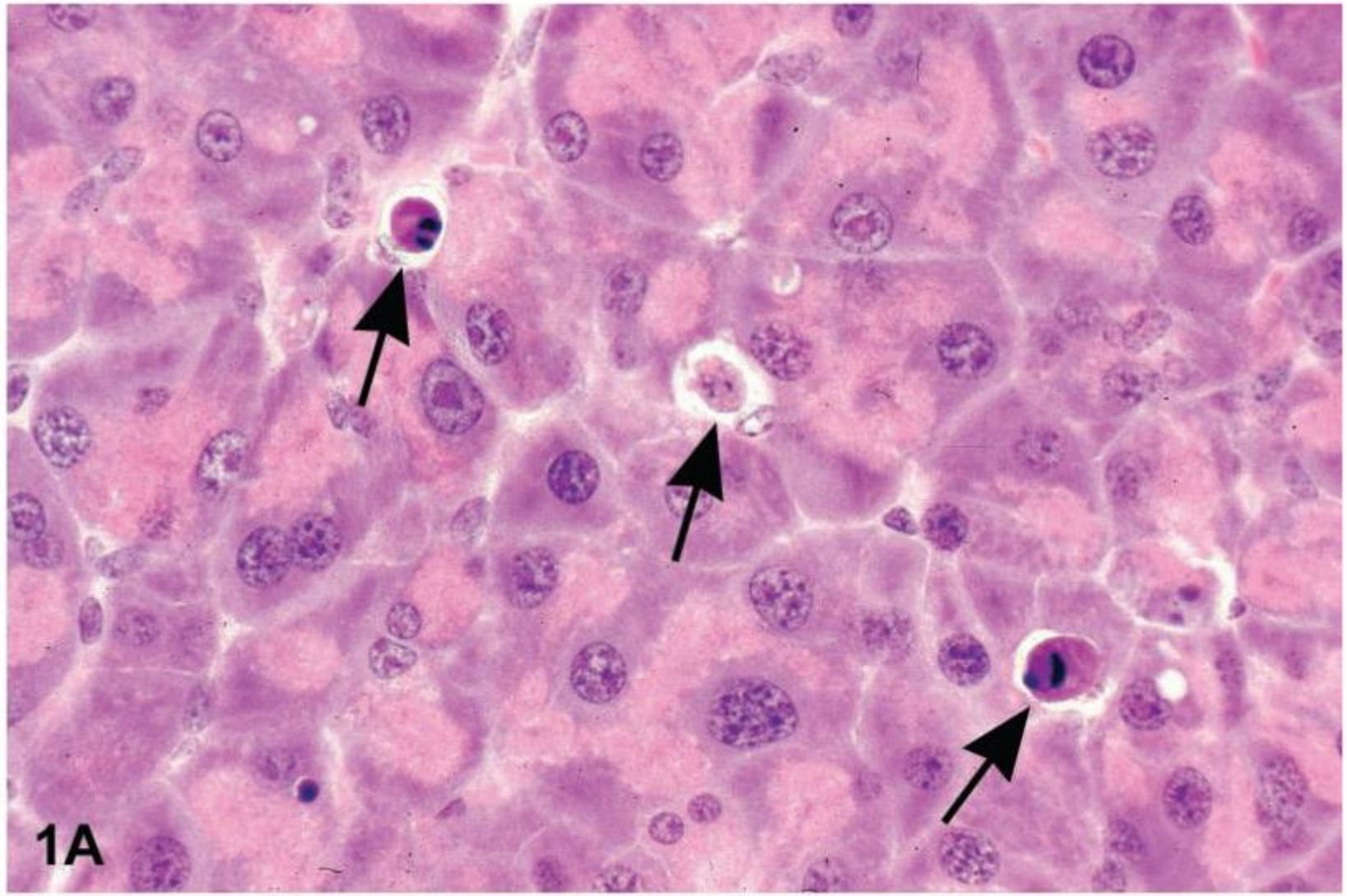
- **Muerte celular programada**
  (Programmed Cell Death, PCD)

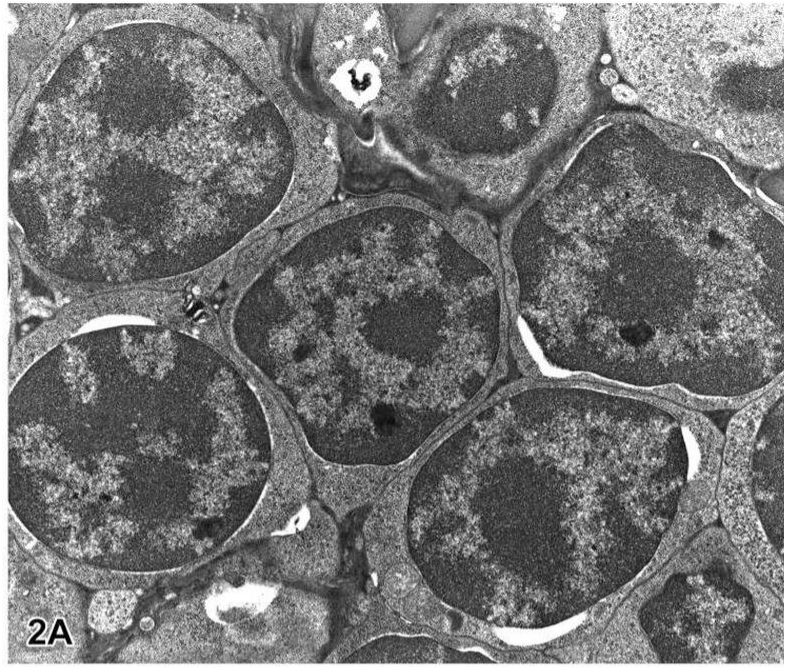
- **Apoptosis**
- **Otras formas de PCD** (Piroptosis, Necroptosis, etc.)

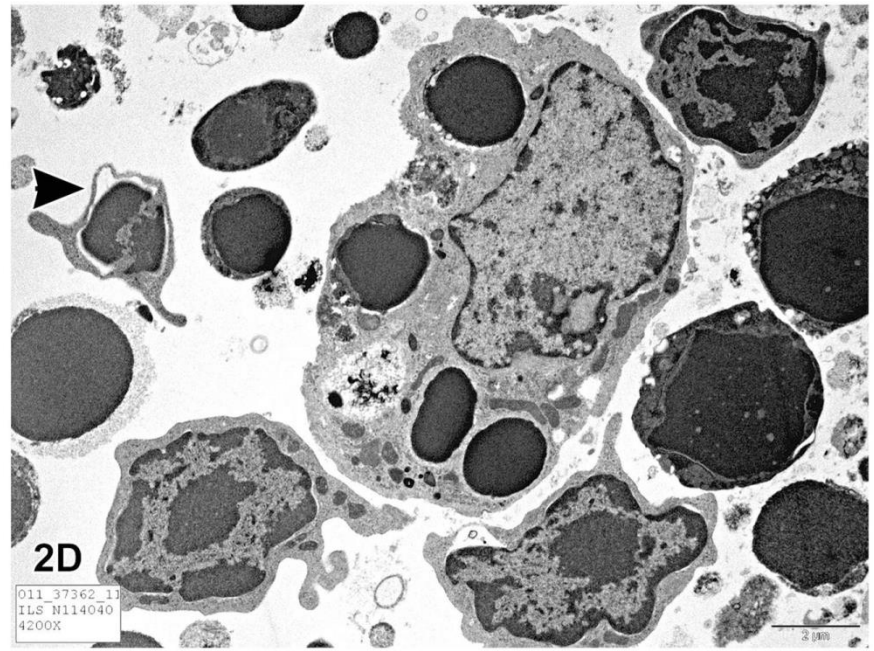
Apoptosis vs. Necrosis

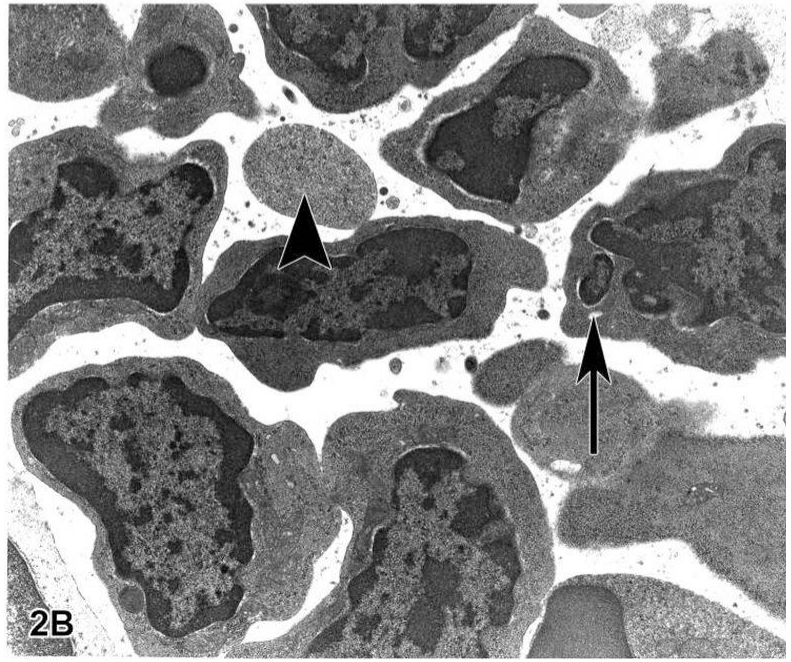


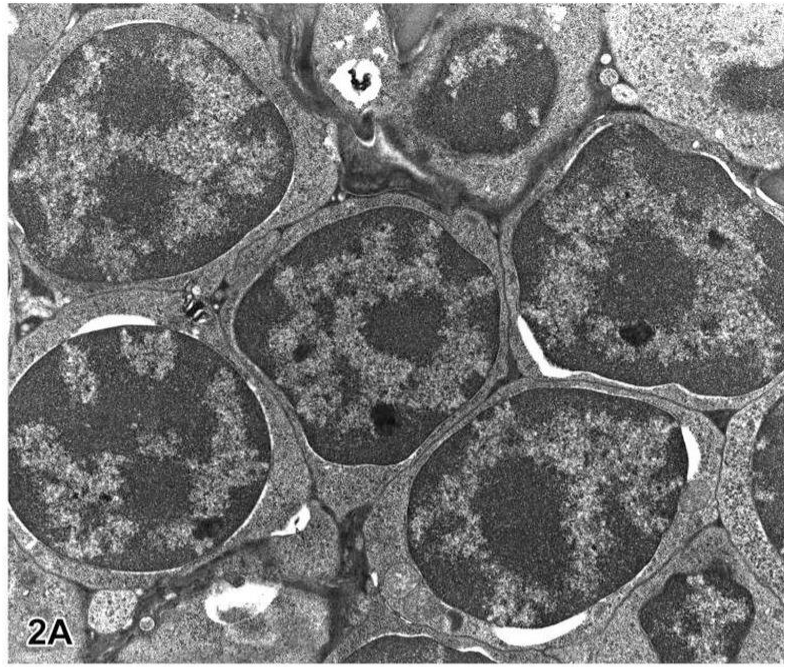
Isquemia
Toxinas (ej. Venenos)
Hongos, bacterias

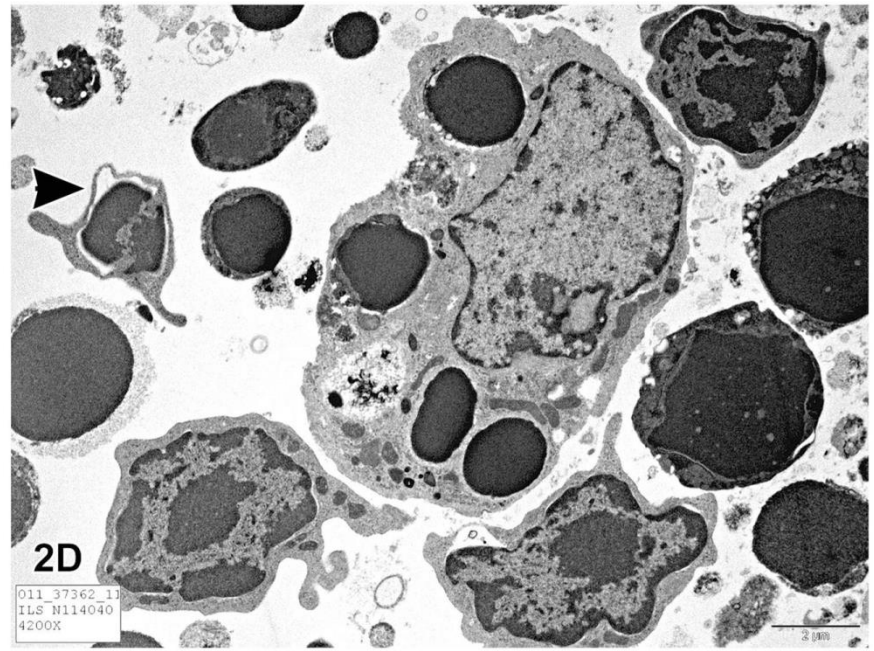

1A

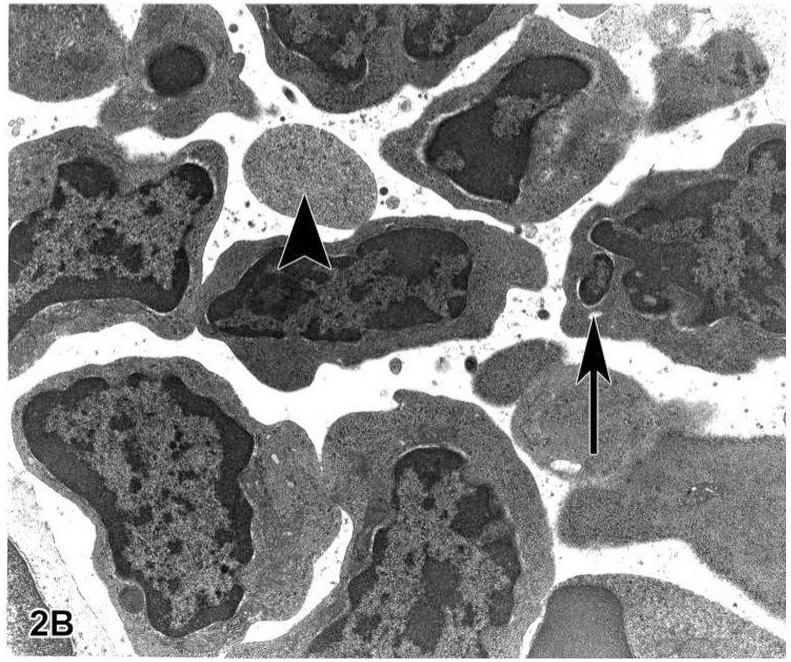

2A

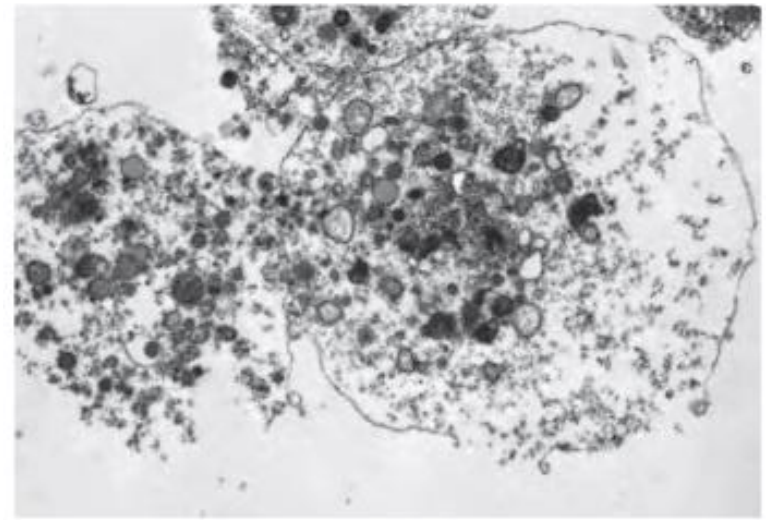

2D
011_37362_11
ILS N114040
4200X


2B


2A

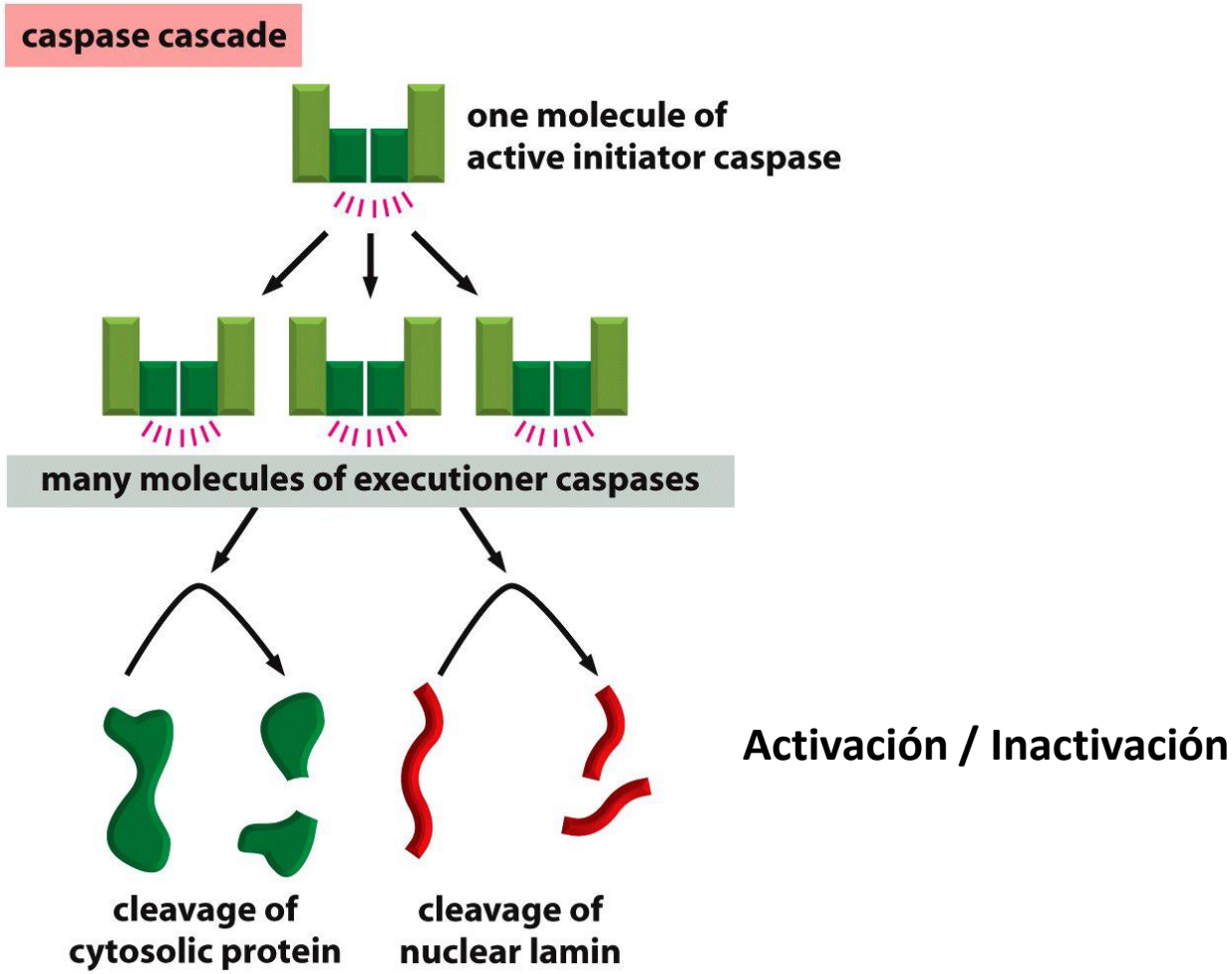

2D
011_37362_11
ILS N114040
4200X


2B



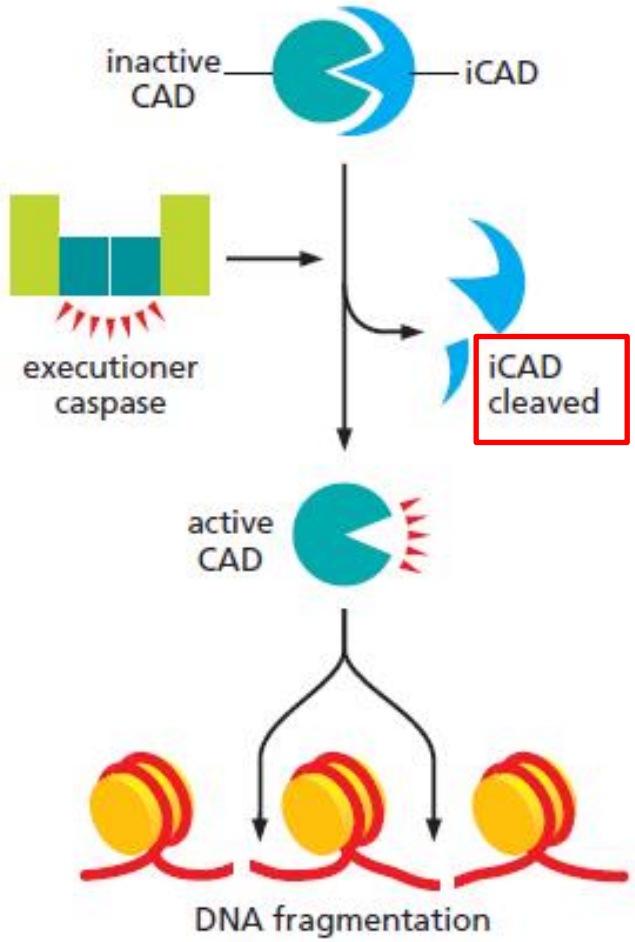
CASPASAS: PROTEASAS ENCARGADAS DE EJECUTAR EL PROGRAMA APOPTÓTICO

(= Cisteín-Proteasas que cortan blancos tras residuos de Aspartato)

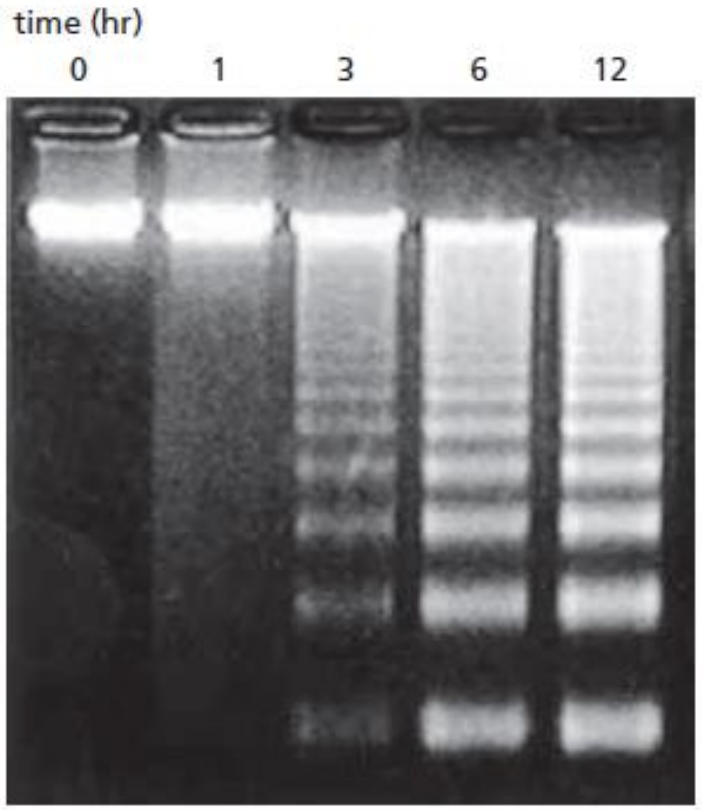


Activación / Inactivación

Caspase-Activated DNAse (CAD) degrada el ADN



(A)

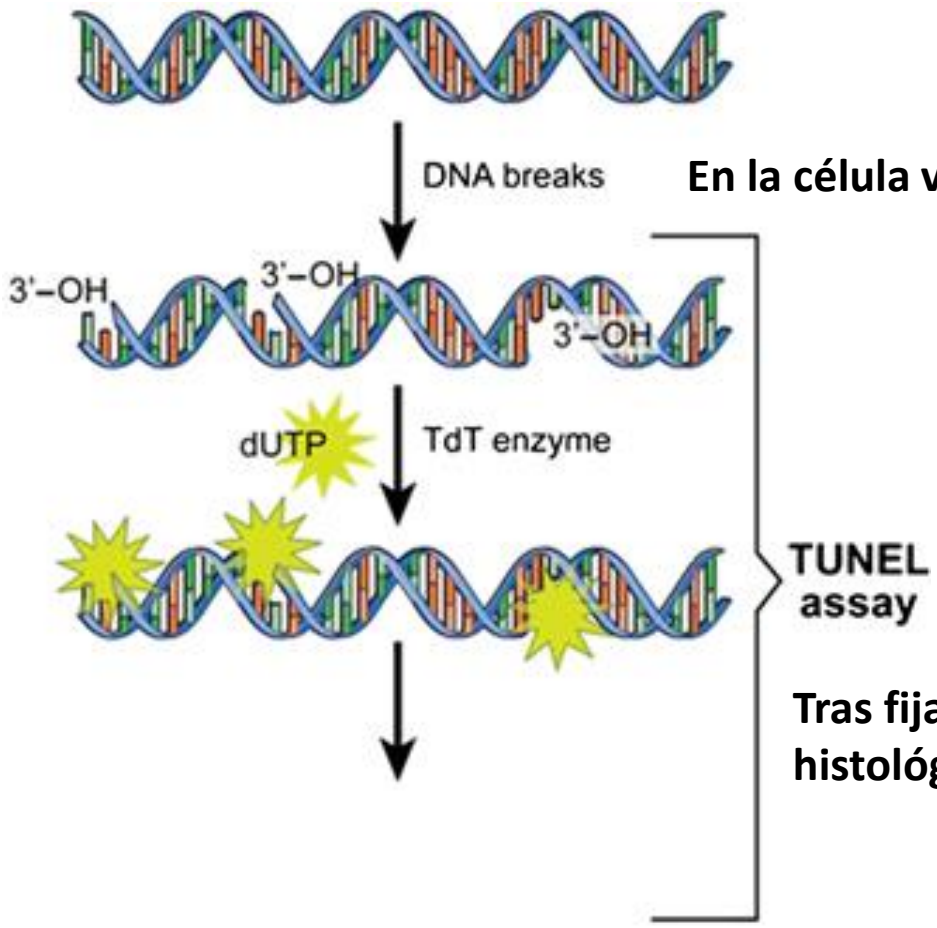


(B)

Técnica de TUNEL

(Terminal deoxynucleotidyl transferase dUTP nick end labeling)

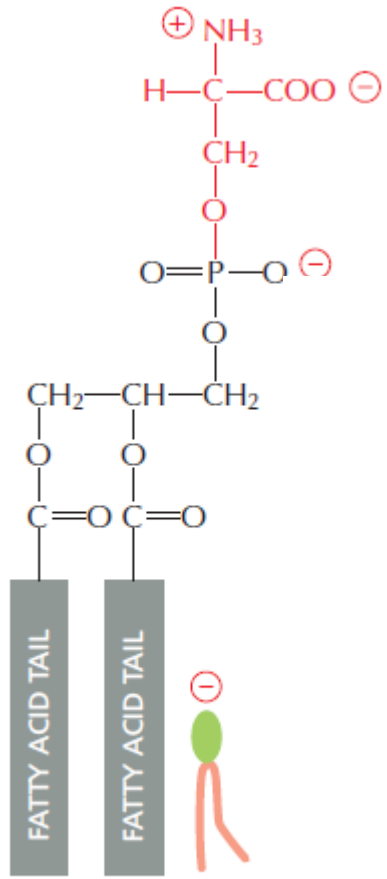
Permite observar experimentalmente la fragmentación del ADN



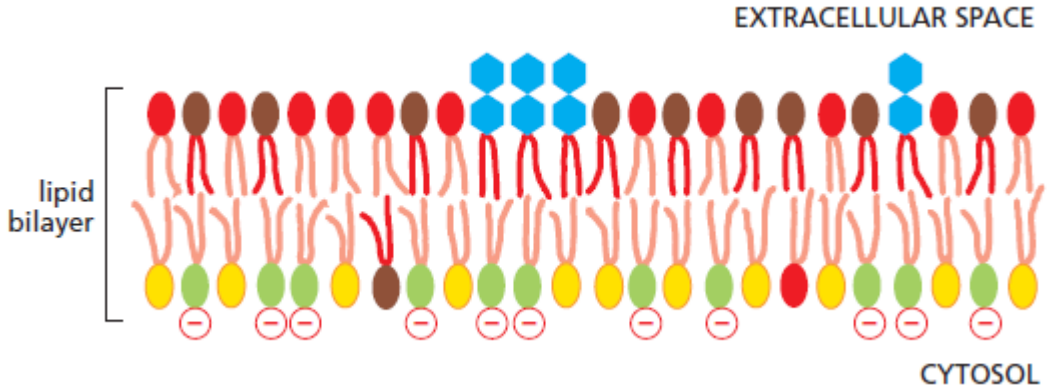
En la célula viva durante la apoptosis

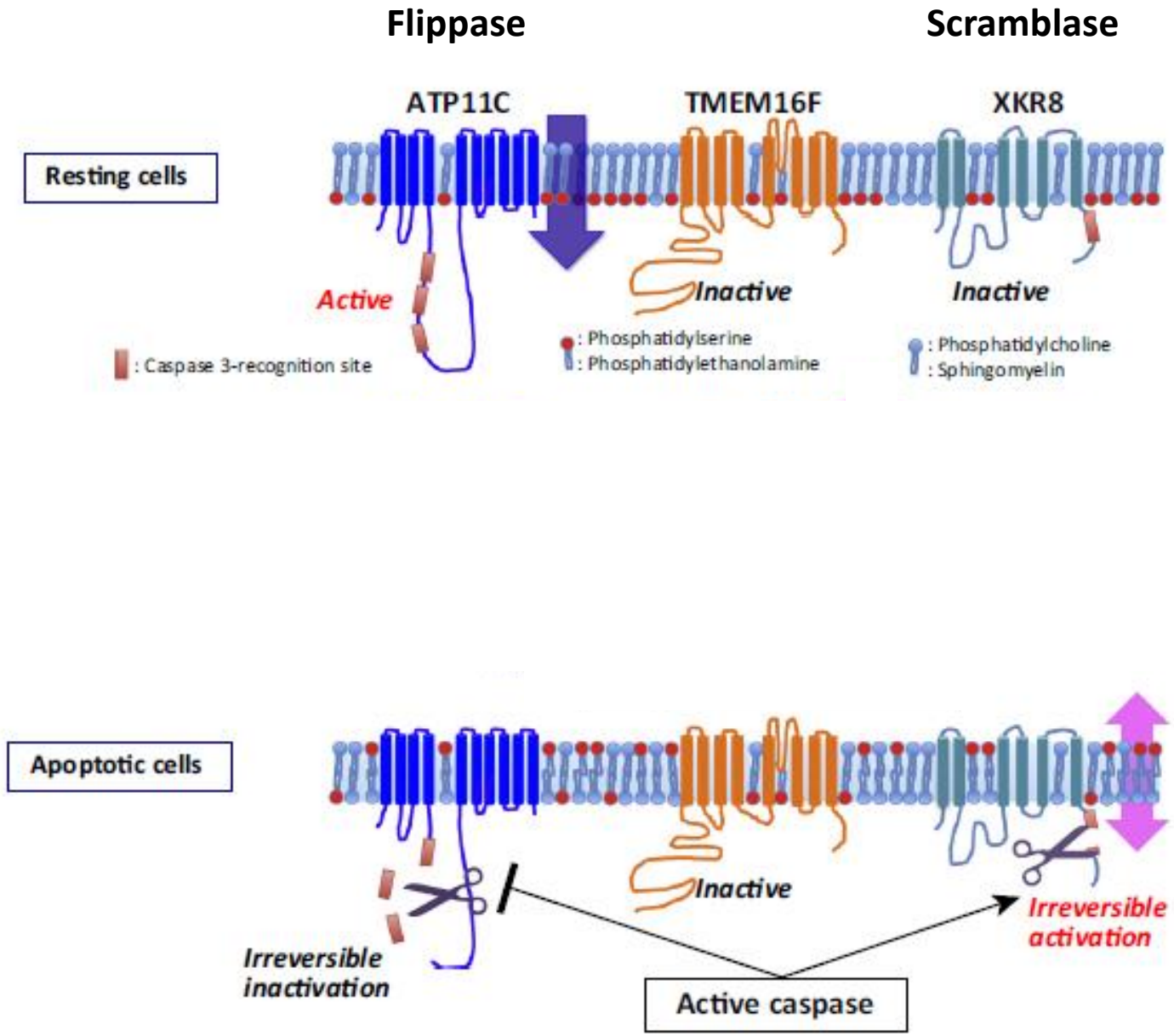
Tras fijar la célula, en cortes histológicos, placas, etc.

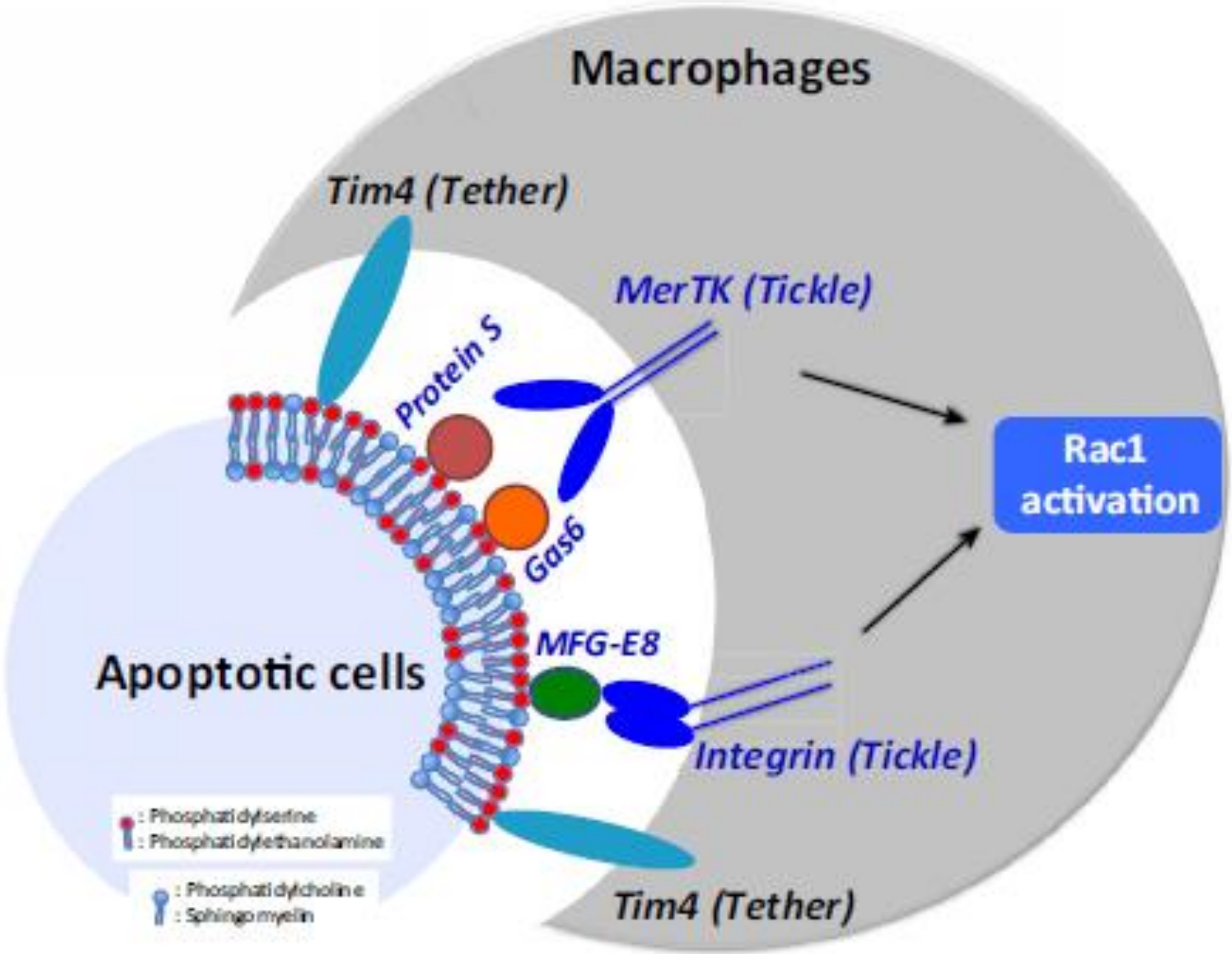
La Fosfatidilserina es expuesta en la superficie de la membrana plasmática



phosphatidylserine




Flippase
Scramblase
ATP11C
TMEM16F
XKR8
Resting cells
Active
Inactive
Inactive
: Caspase 3-recognition site
: Phosphatidylserine
: Phosphatidylethanolamine
: Phosphatidylcholine
: Sphingomyelin
Apoptotic cells
Inactive
Irreversible activation
Irreversible inactivation
Active caspase


Macrophages
Tim4 (Tether)
MerTK (Tickle)
Protein S
Gas6
Rac1
activation
MFG-E8
Apoptotic cells
Integrin (Tickle)
: Phosphatidylserine
: Phosphatidylethanolamine
: Phosphatidylcholine
: Sphingomyelin
Tim4 (Tether)

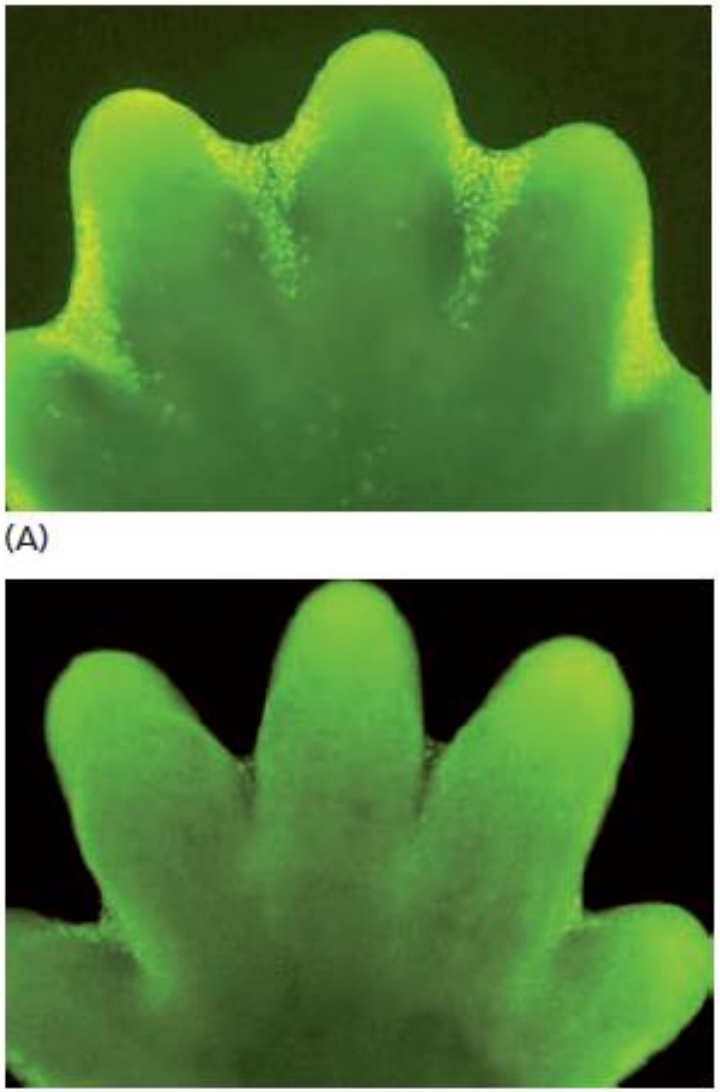
(Algunos) otros blancos de caspasas

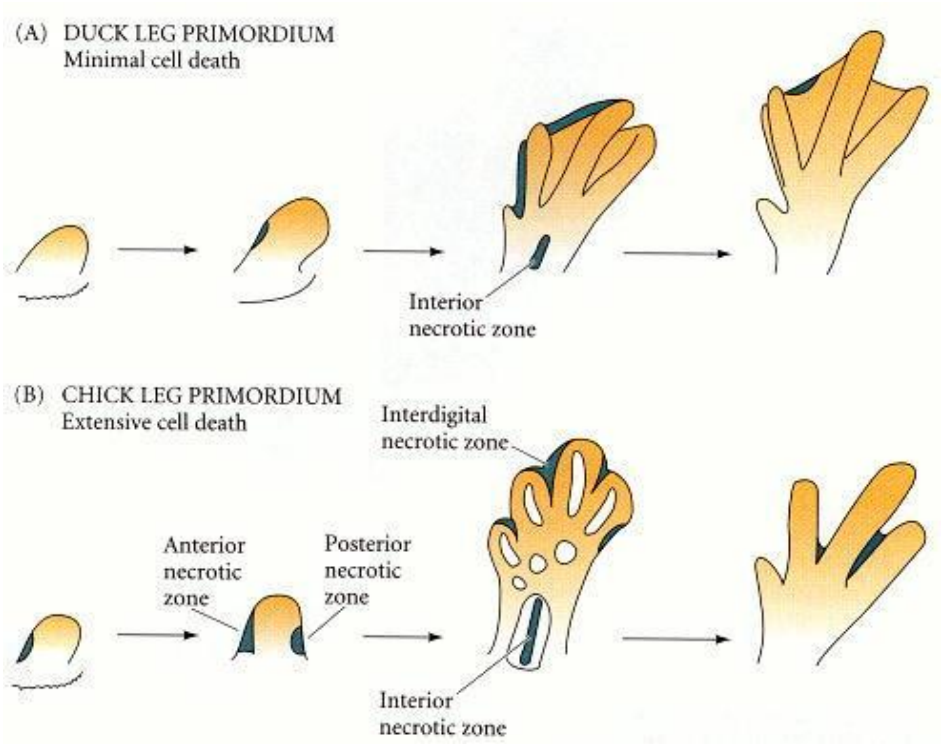
- **Laminas**
- **Espectrinas**
- **Citoqueratinas**

¿Por qué apoptosis?

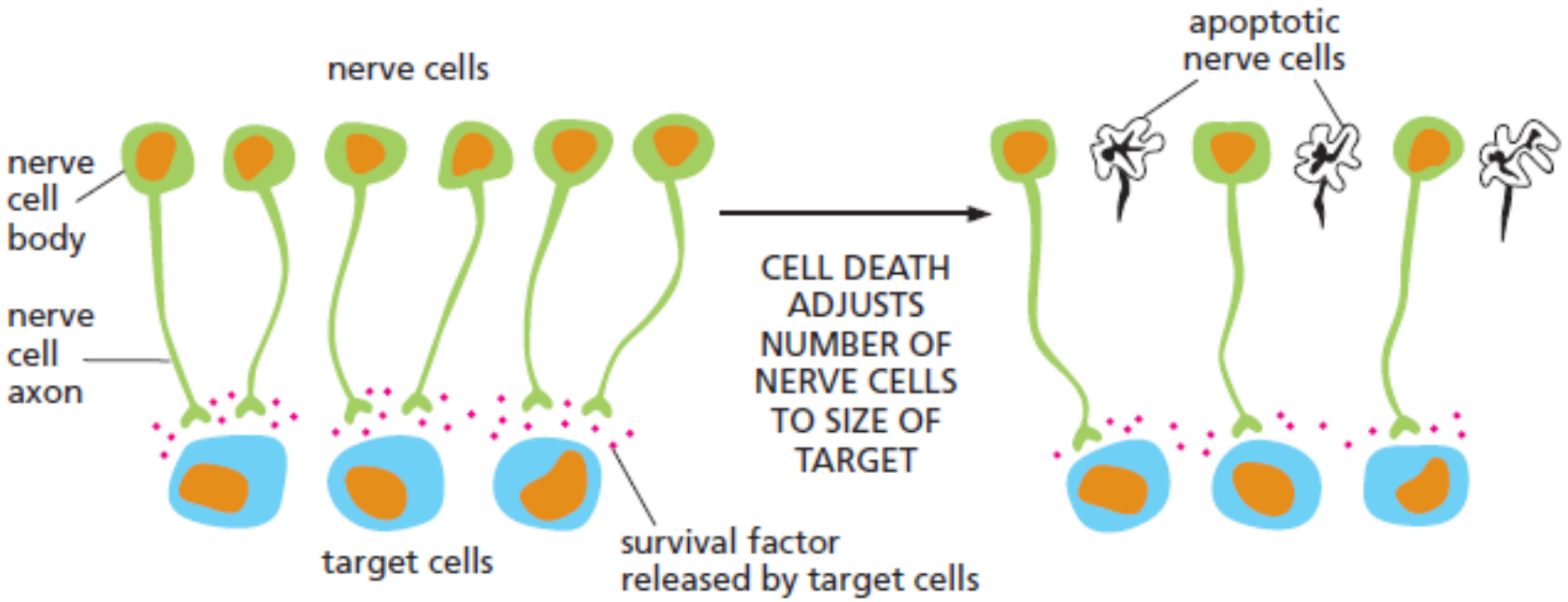
Desarrollo embrionario

TUNEL

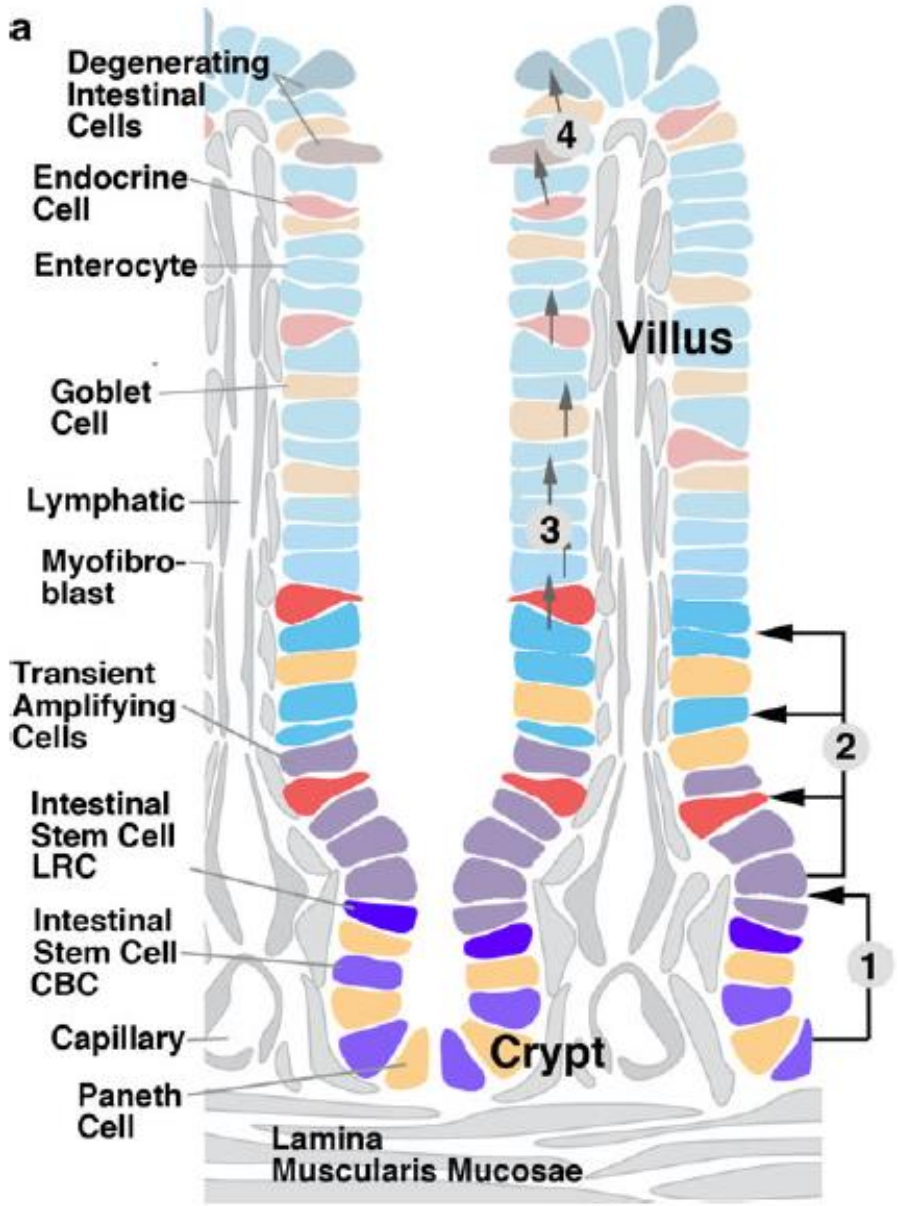




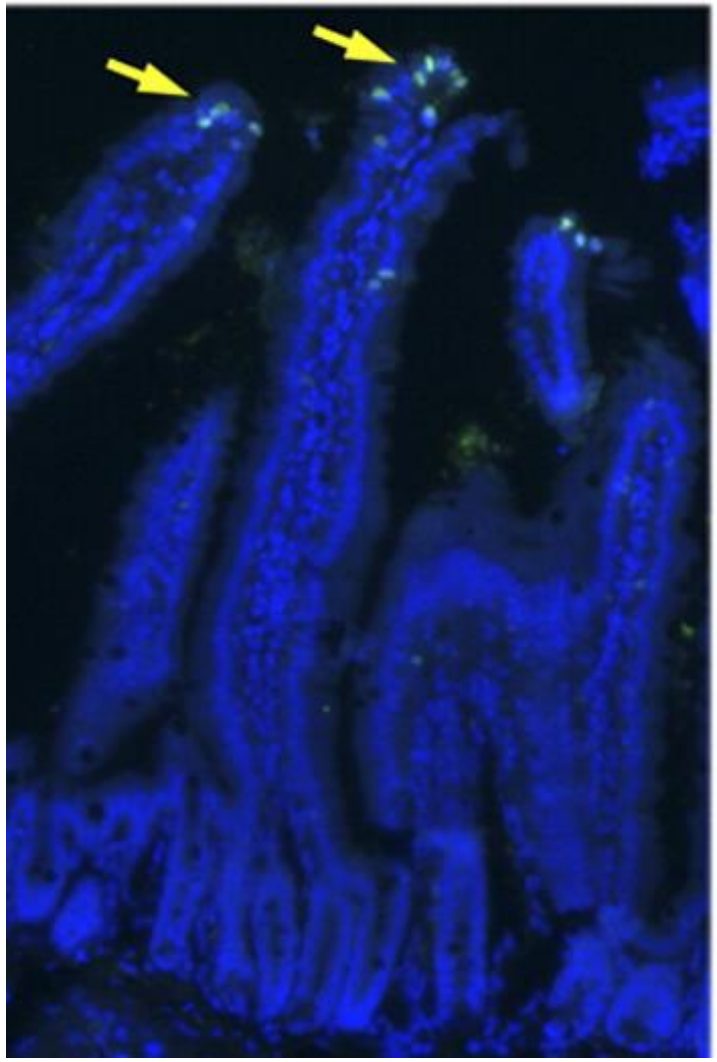
Regulación de la apoptosis por factores de supervivencia



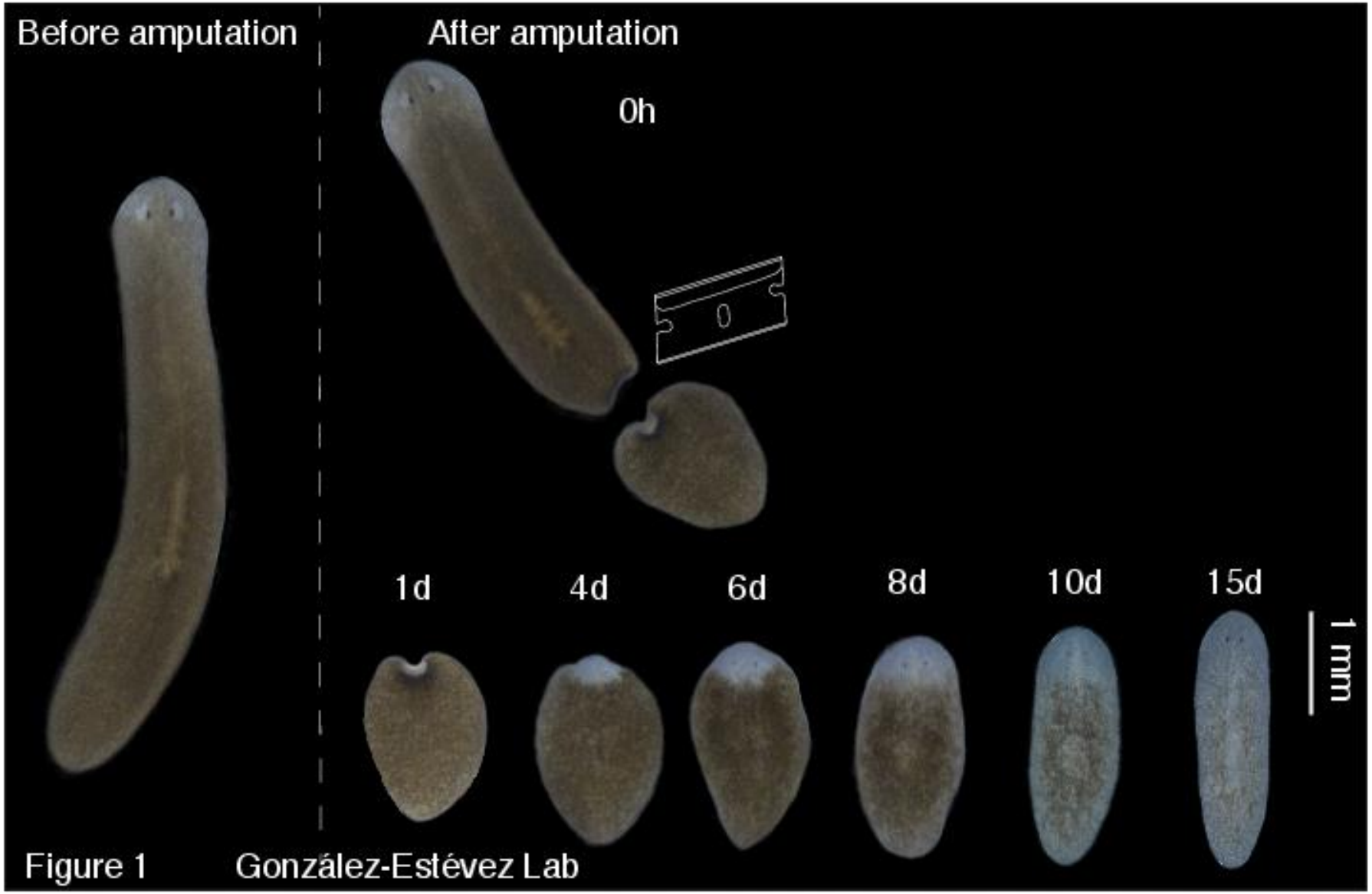
Renovación celular y Homeostasis de tejidos



Anoikis

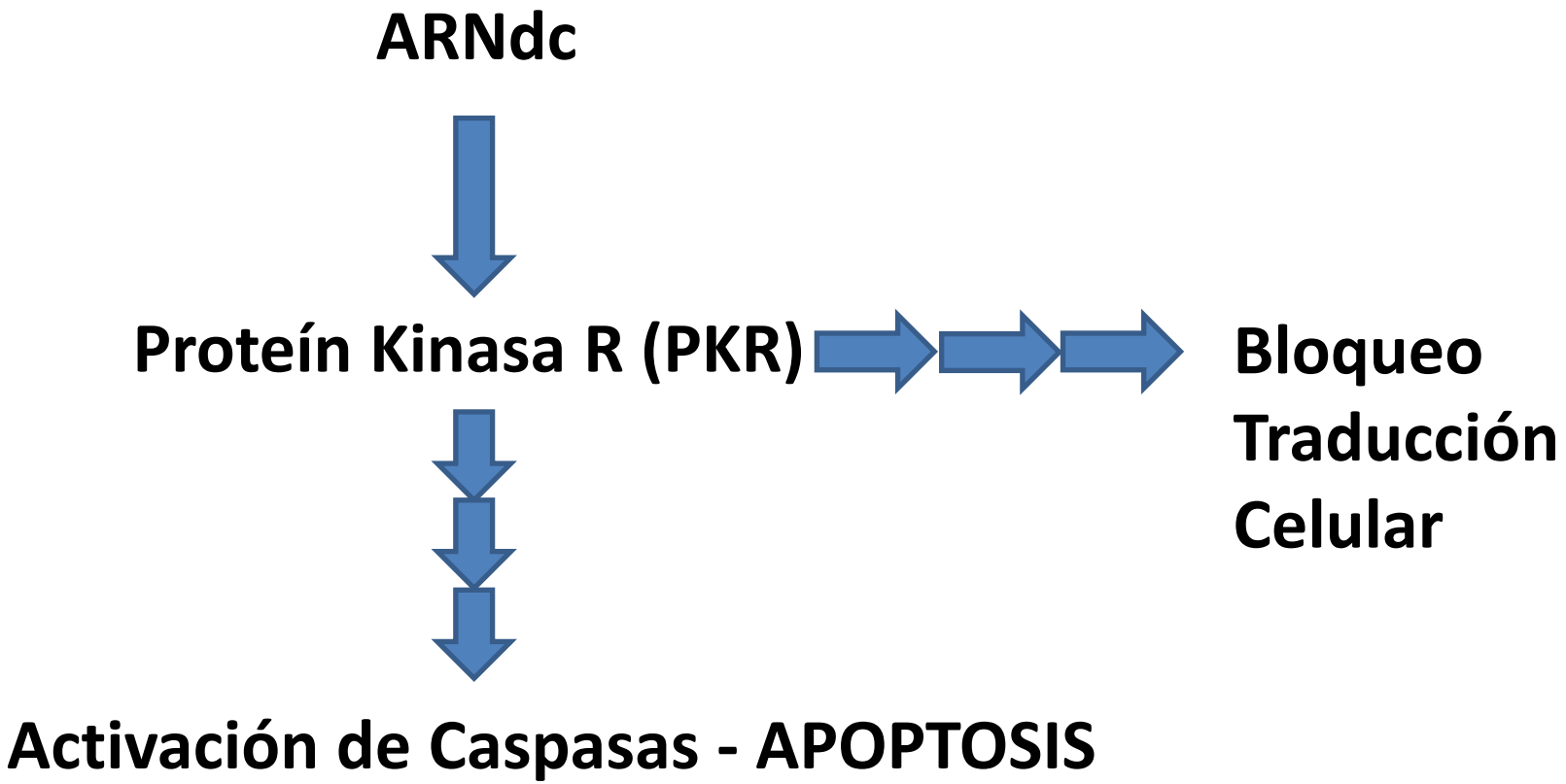


Regeneración



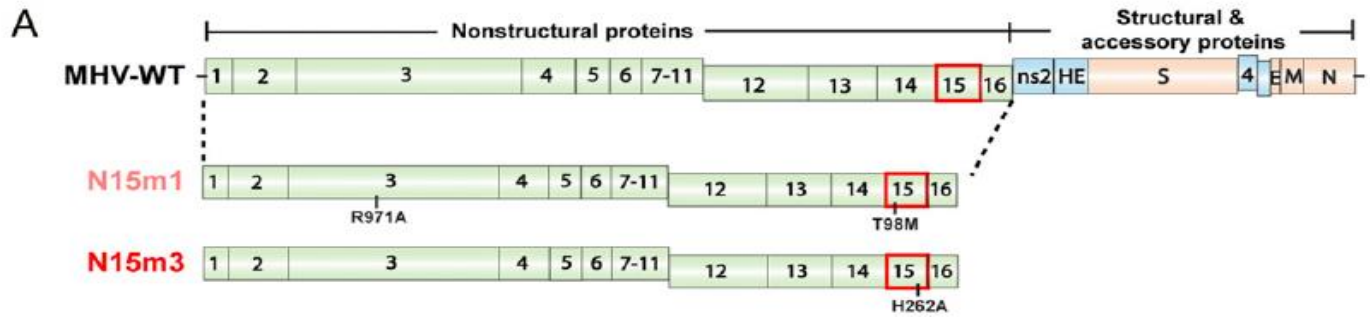

A
Baseline
4 Hours
3 Days
14 Days
Prepharyngeal amputation

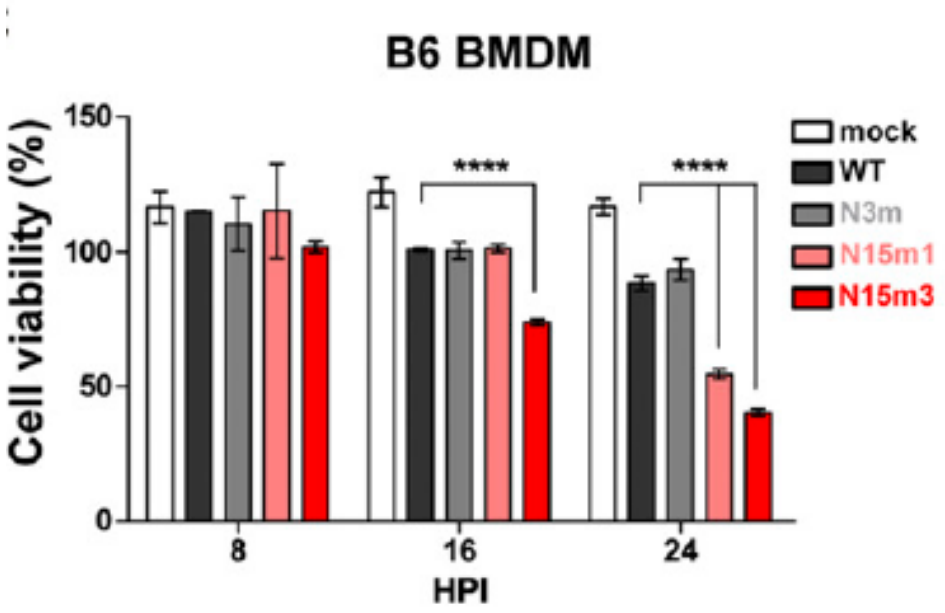
Respuesta contra virus y otros patógenos intracelulares

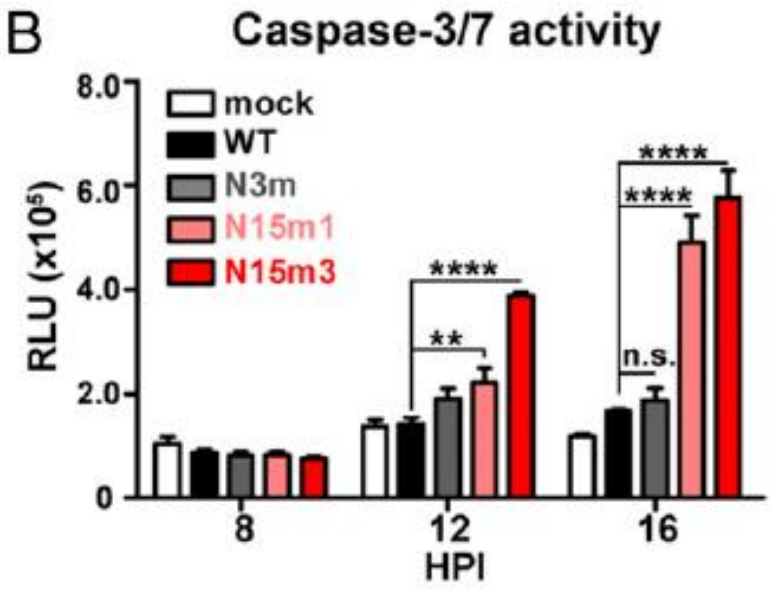


Respuesta contra virus y otros patógenos intracelulares

La proteína nsp15 de los coronavirus bloquea la apoptosis

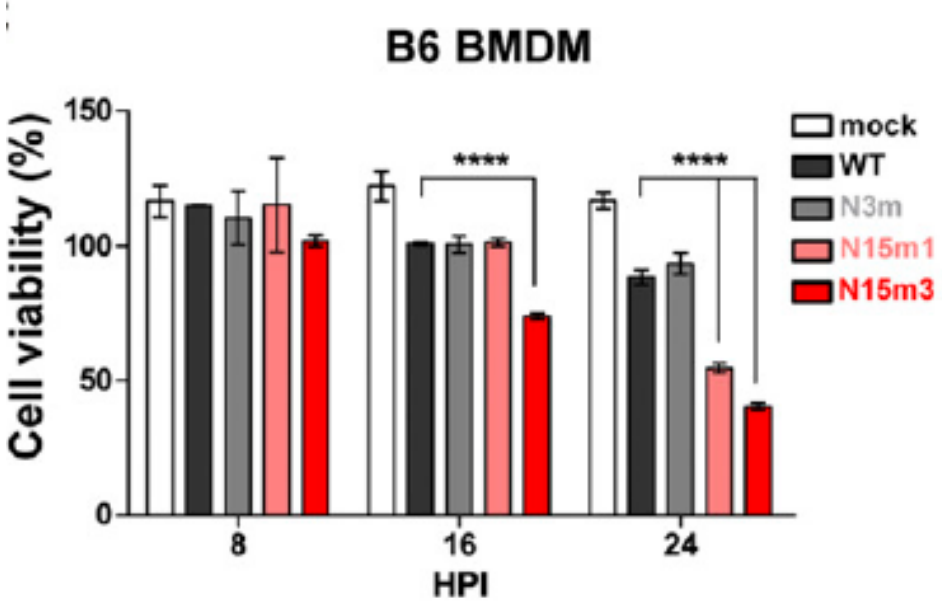


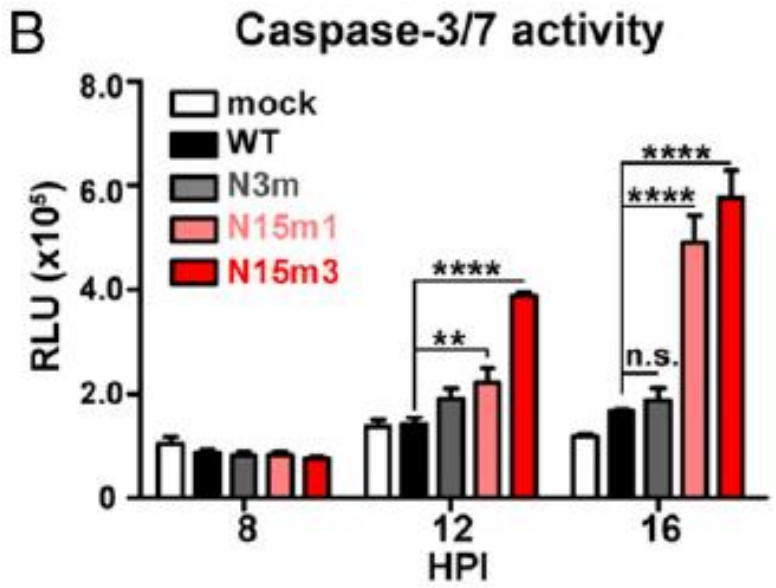




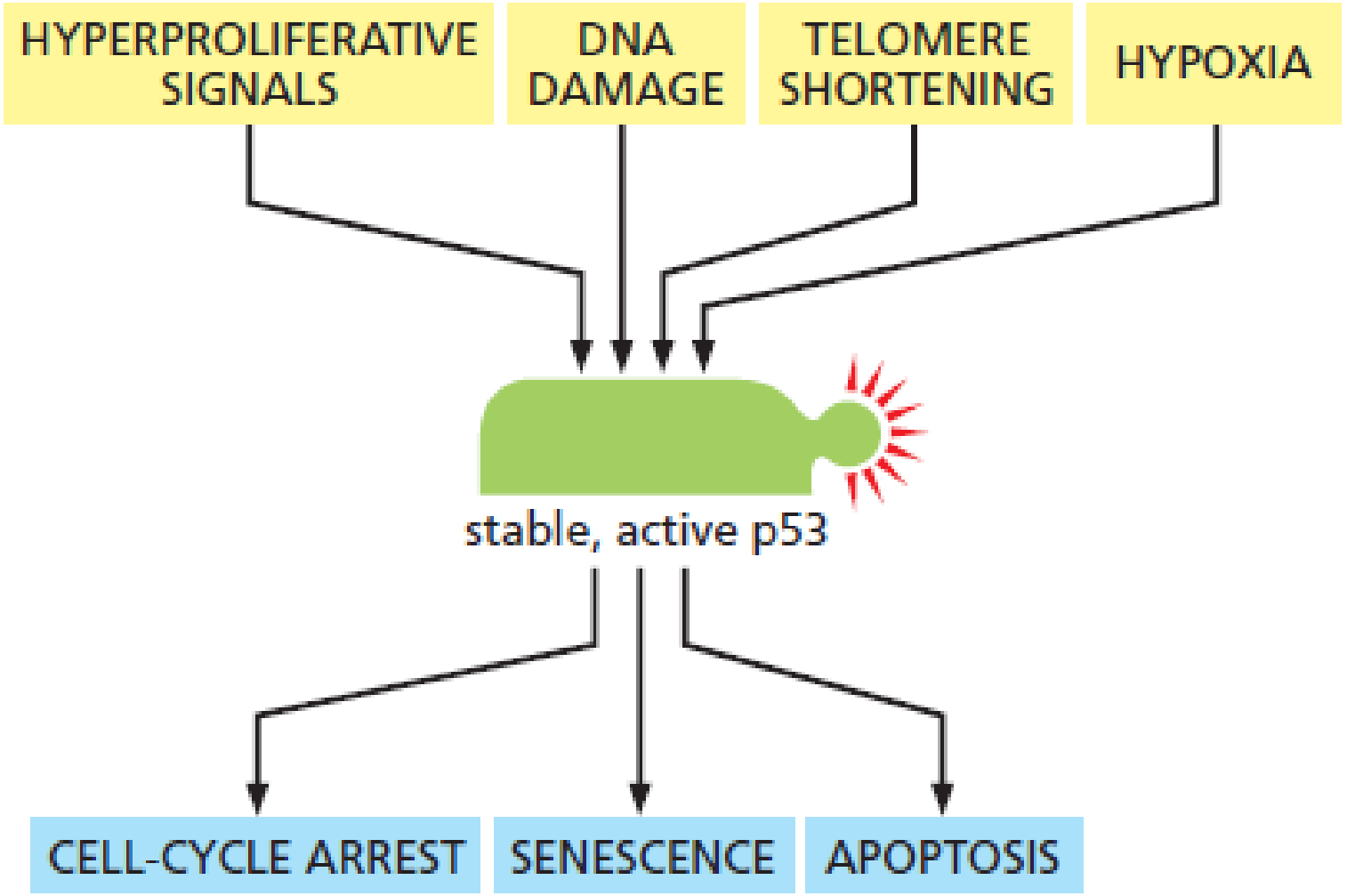
PNAS 2017 114 (21) E4251-E4260

¿PLAN B?


B6 BMDM
Cell viability (%)
150
100
50
0
8
16
24
HPI
****
****
mock
WT
N3m
N15m1
N15m3


B
Caspase-3/7 activity
RLU (x10⁵)
8.0
6.0
4.0
2.0
0
8
12
16
HPI
mock
WT
N3m
N15m1
N15m3
****
**
n.s.
****
****

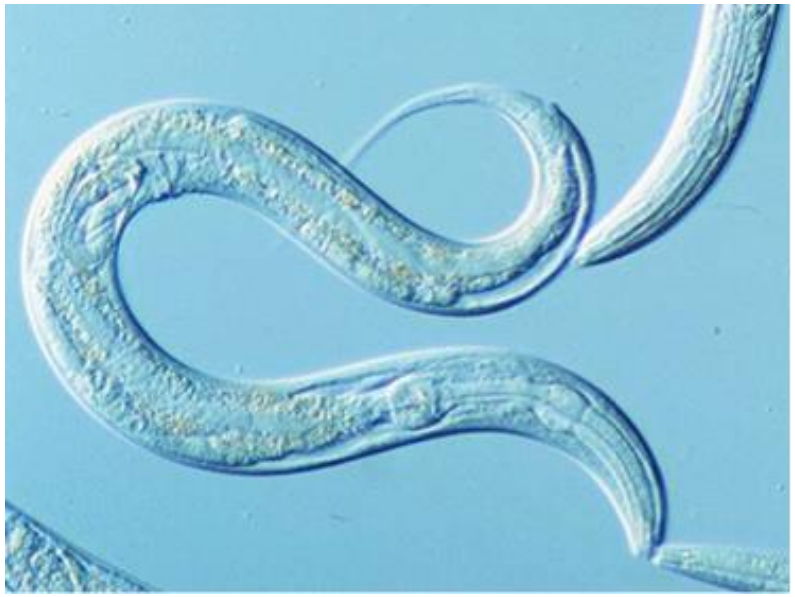
Eliminación de células con daño al ADN



¿Cómo se regula la apoptosis?

¿Cómo se descubrieron estos mecanismos?

Caenorhabditis elegans y las bases genéticas de la apoptosis



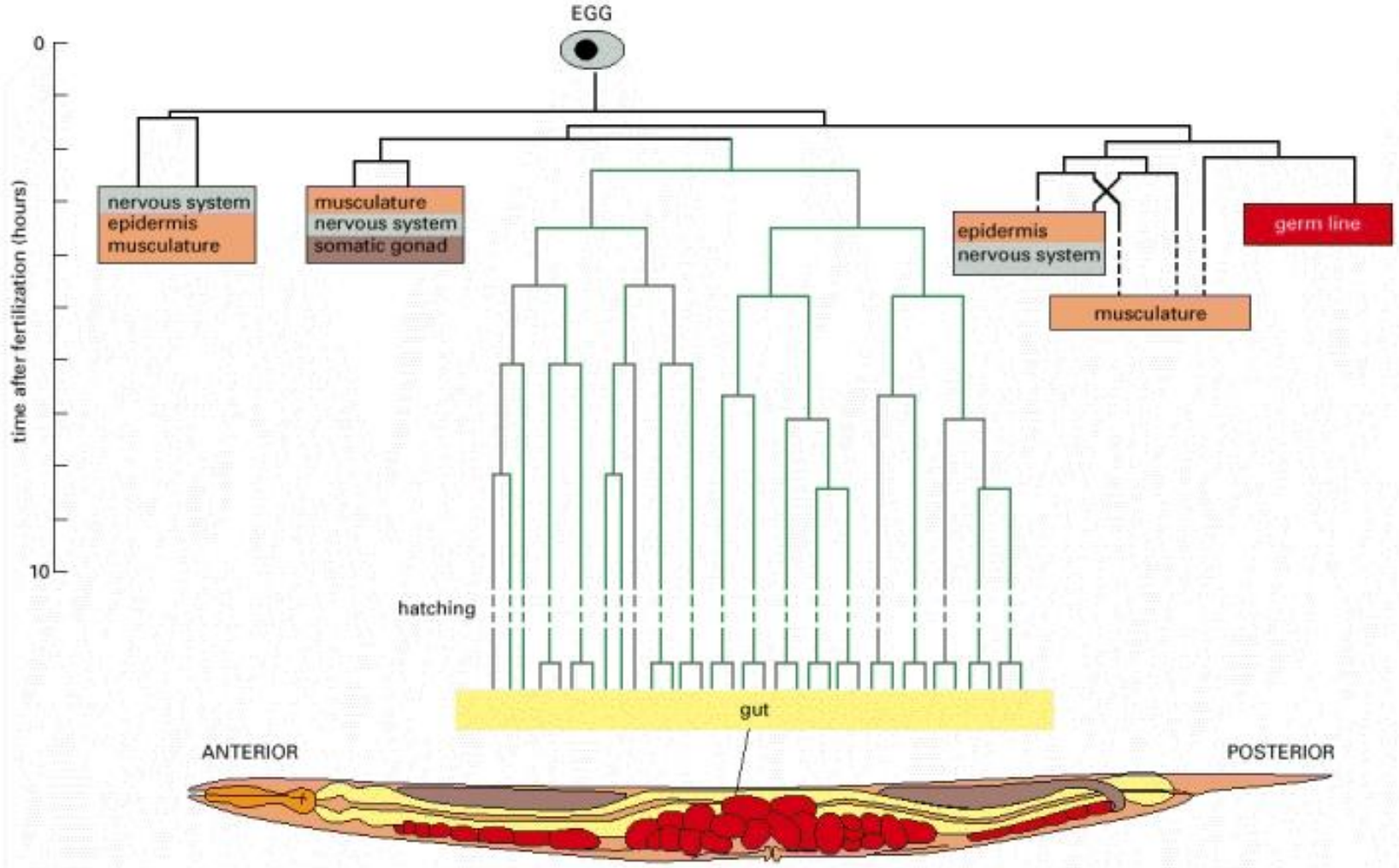
The Nobel Prize in Physiology or Medicine 2002

Sydney Brenner
Prize share: 1/3

H. Robert Horvitz
Prize share: 1/3

John E. Sulston
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2002 was awarded jointly to Sydney Brenner, H. Robert Horvitz and John E. Sulston *"for their discoveries concerning genetic regulation of organ development and programmed cell death'".*



Linaje invariable durante el desarrollo:

959 células somáticas
131 células mueren por apoptosis

Cell, Vol. 44, 817–829, March 28, 1986, Copyright © 1986 by Cell Press

Genetic Control of Programmed Cell Death in the Nematode C. elegans

Hilary M. Ellis,* and H. Robert Horvitz
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

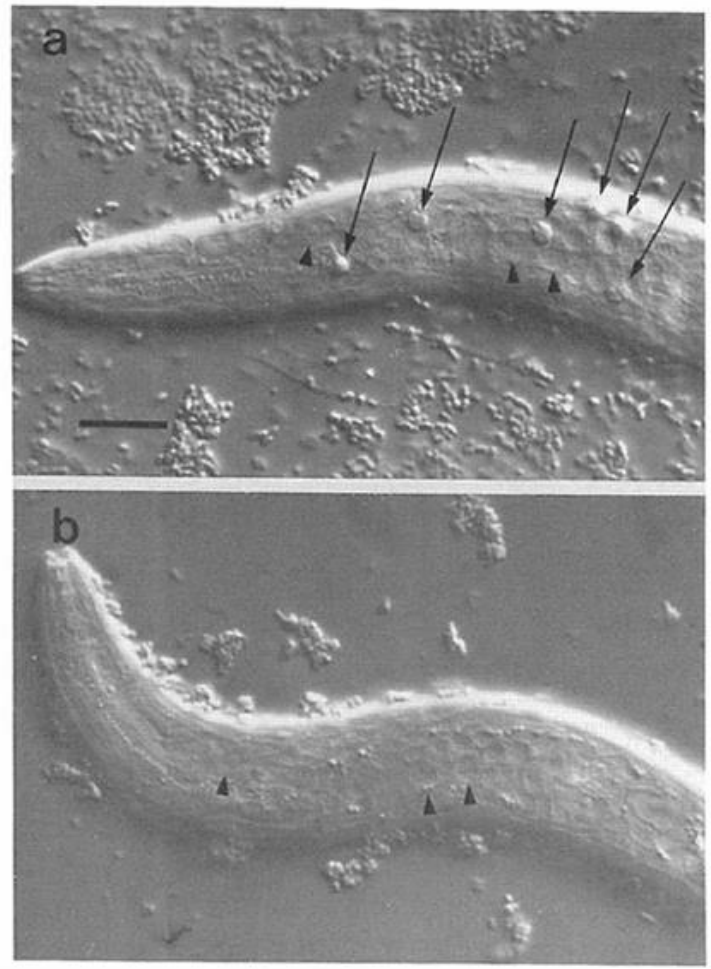
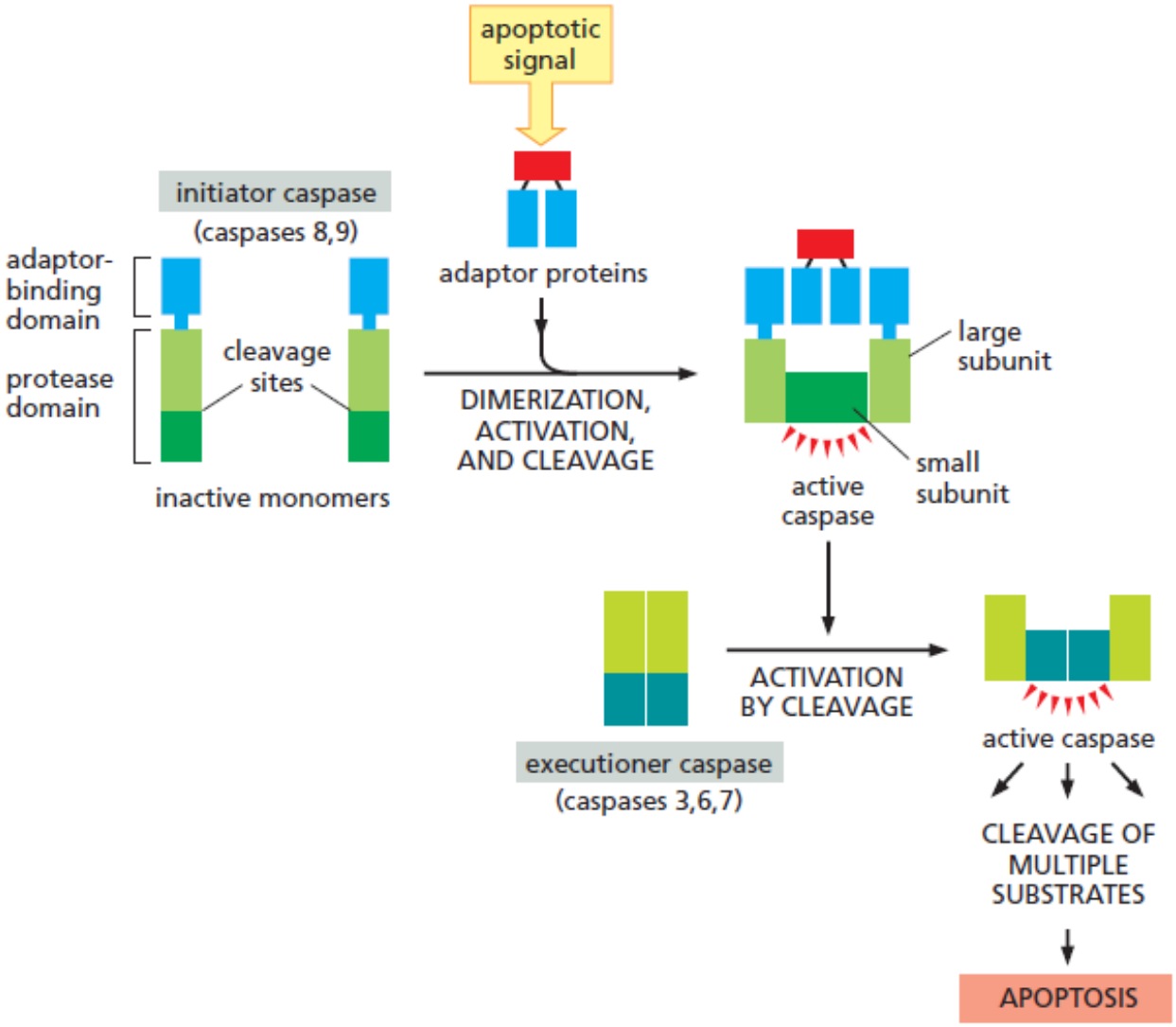


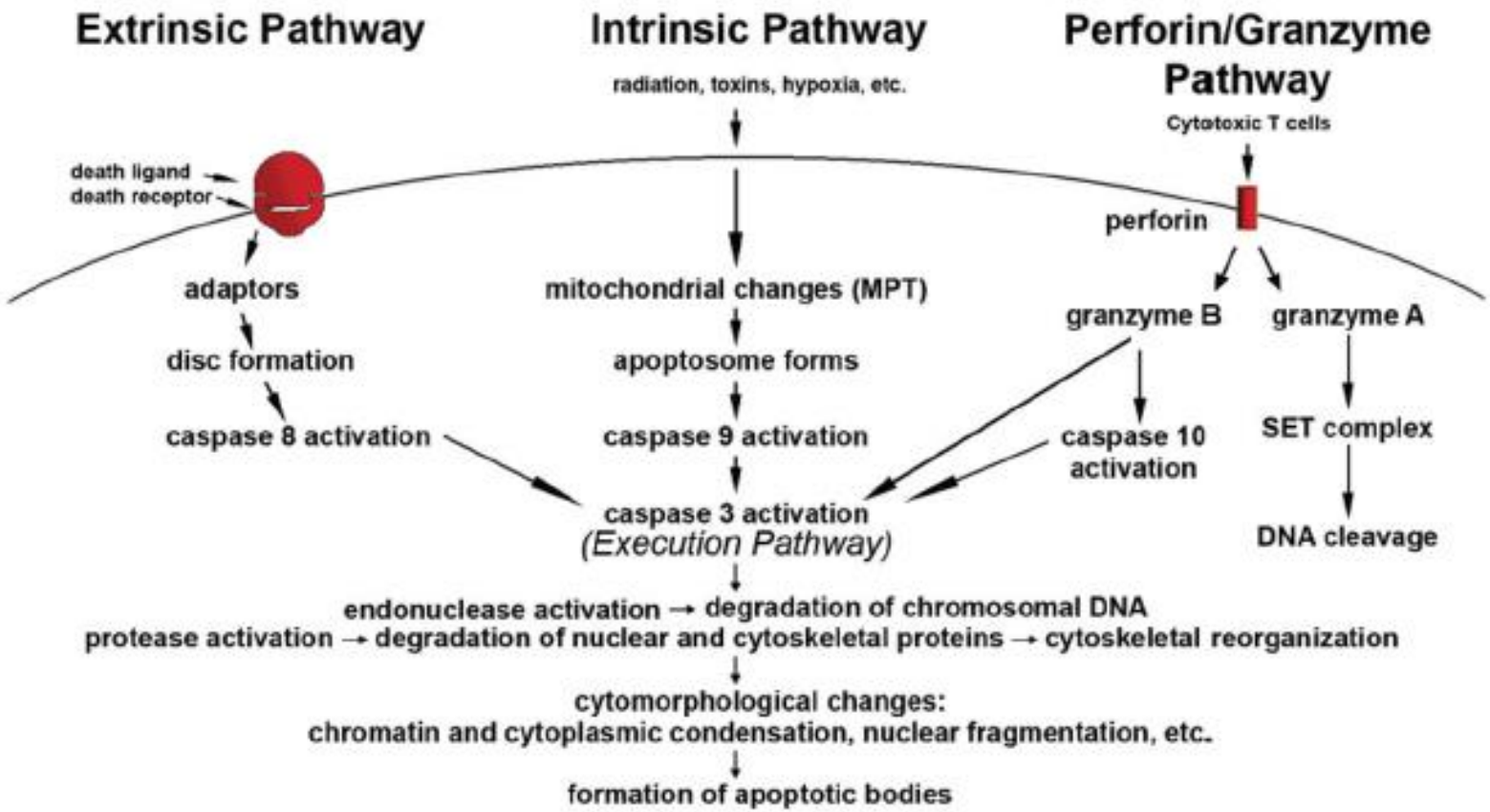
Figure 1. Absence of Cell Deaths in *ced-3* Animals

(a) Nomarski photomicrograph of a newly hatched *ced-1* larva. Arrows indicate dying cells. (b) Nomarski photomicrograph of a newly hatched *ced-1; ced-3* larva. Plane of focus is approximately that shown in (a). Arrowheads indicate several of the nuclei that can be seen in both (a) and (b). No cell deaths are seen in the *ced-1; ced-3* larva. Bar = 10 μ.

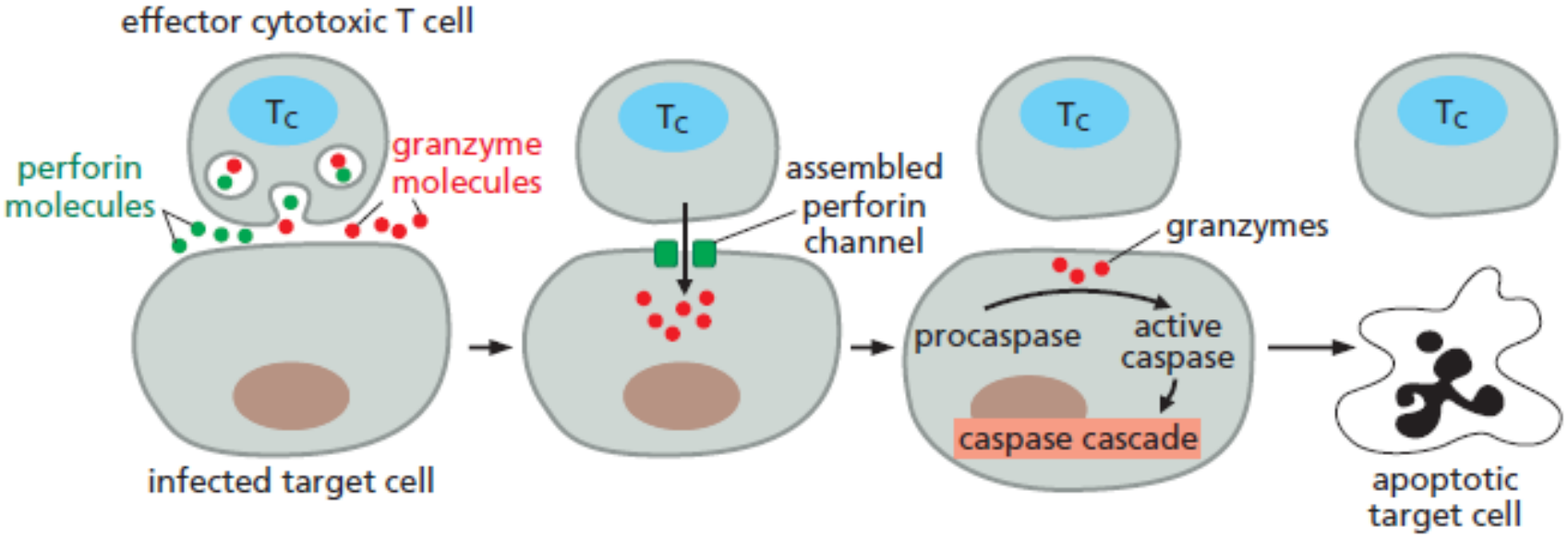
Mecanismos reguladores de la apoptosis: Caspasas



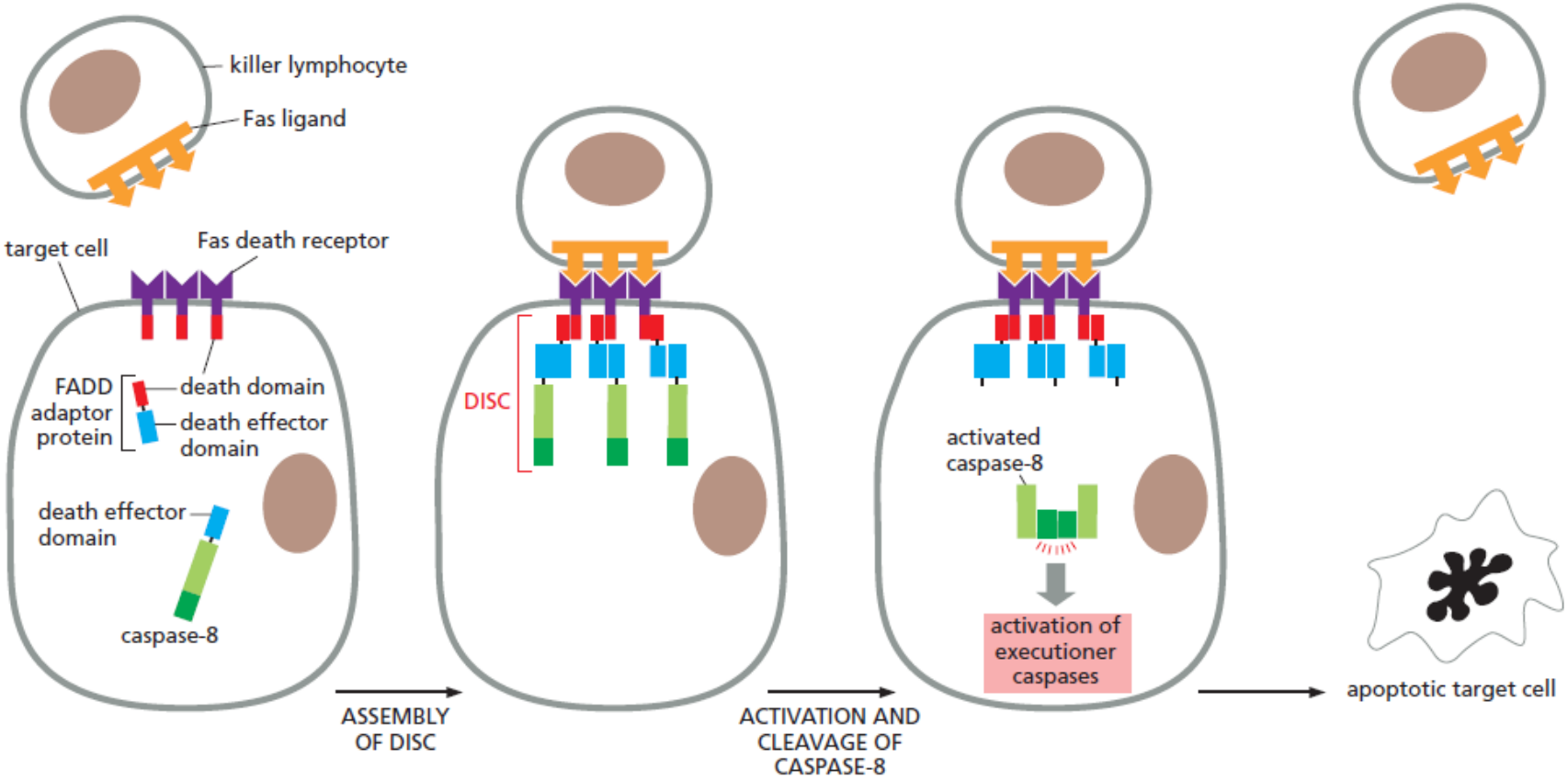
Mecanismos reguladores de la apoptosis: 3 vías principales que convergen



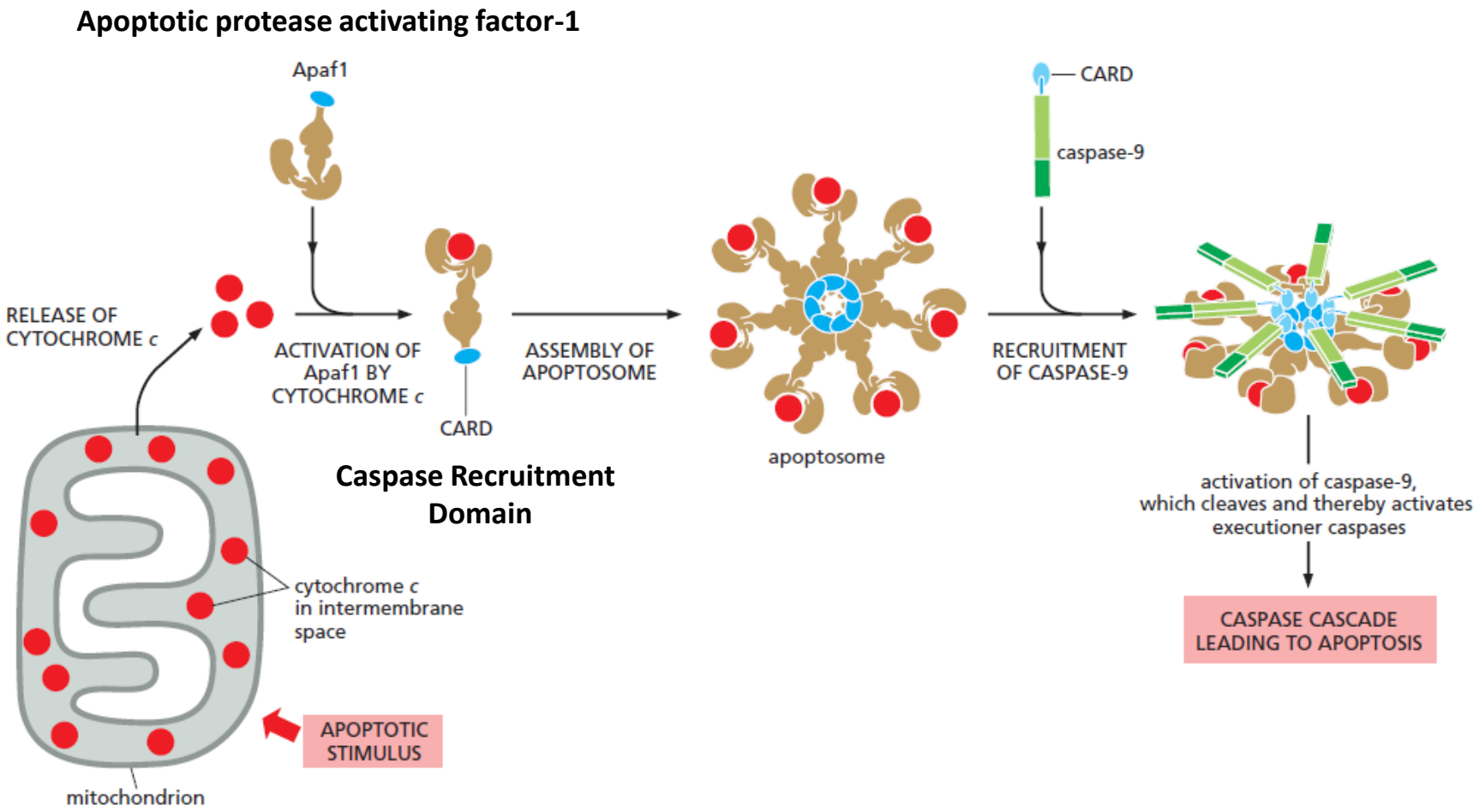
Apoptosis: Vía de perforina/granzima



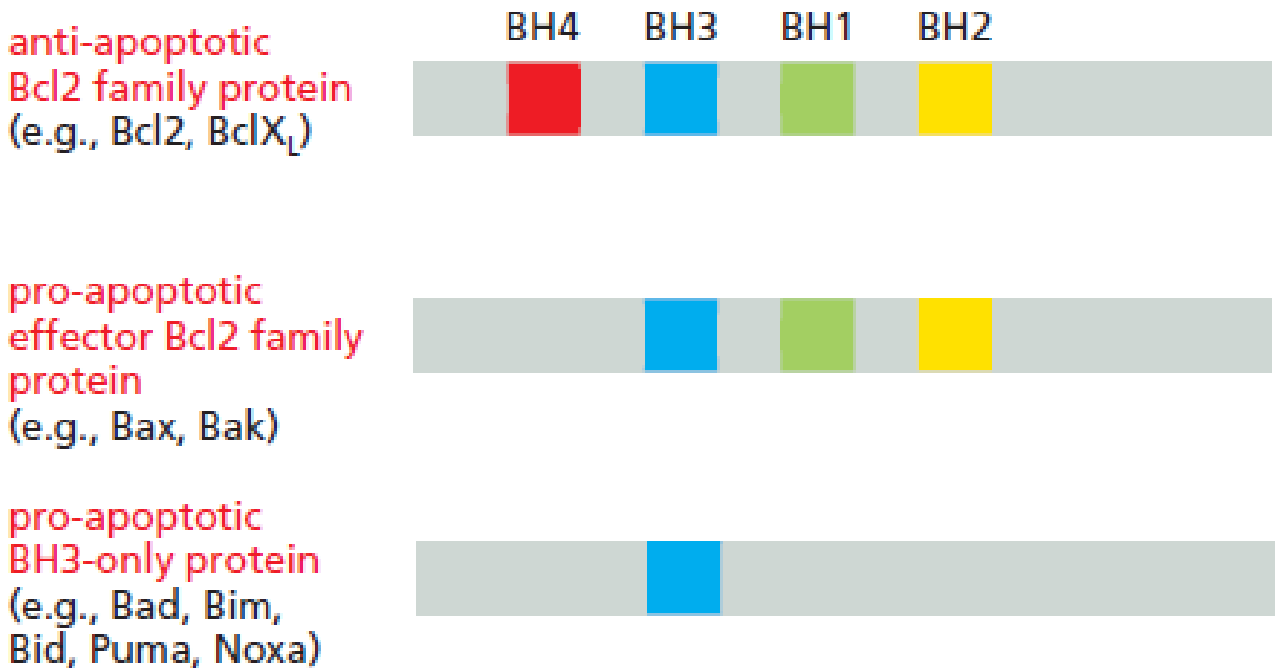
Apoptosis: Vía Extrínseca

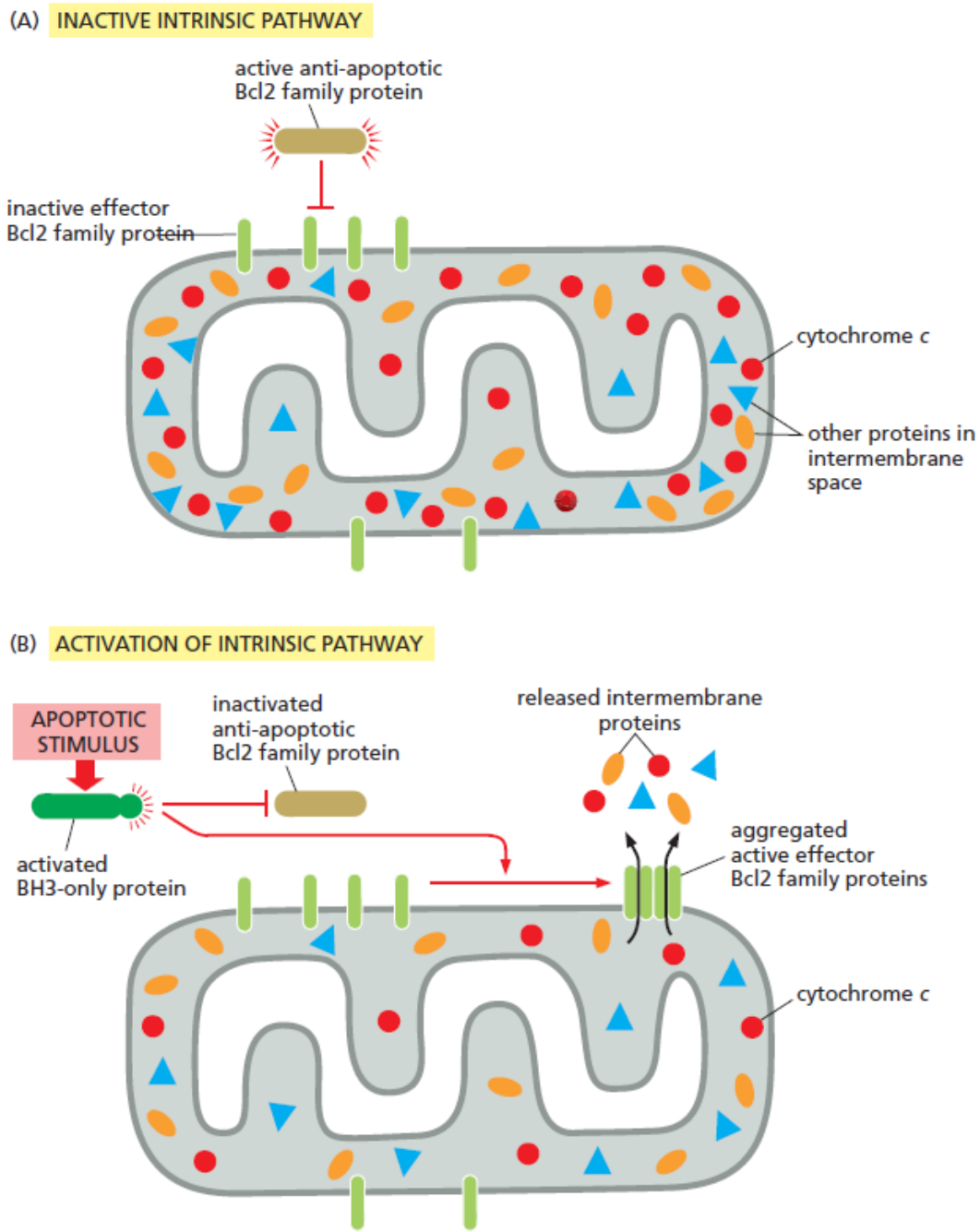


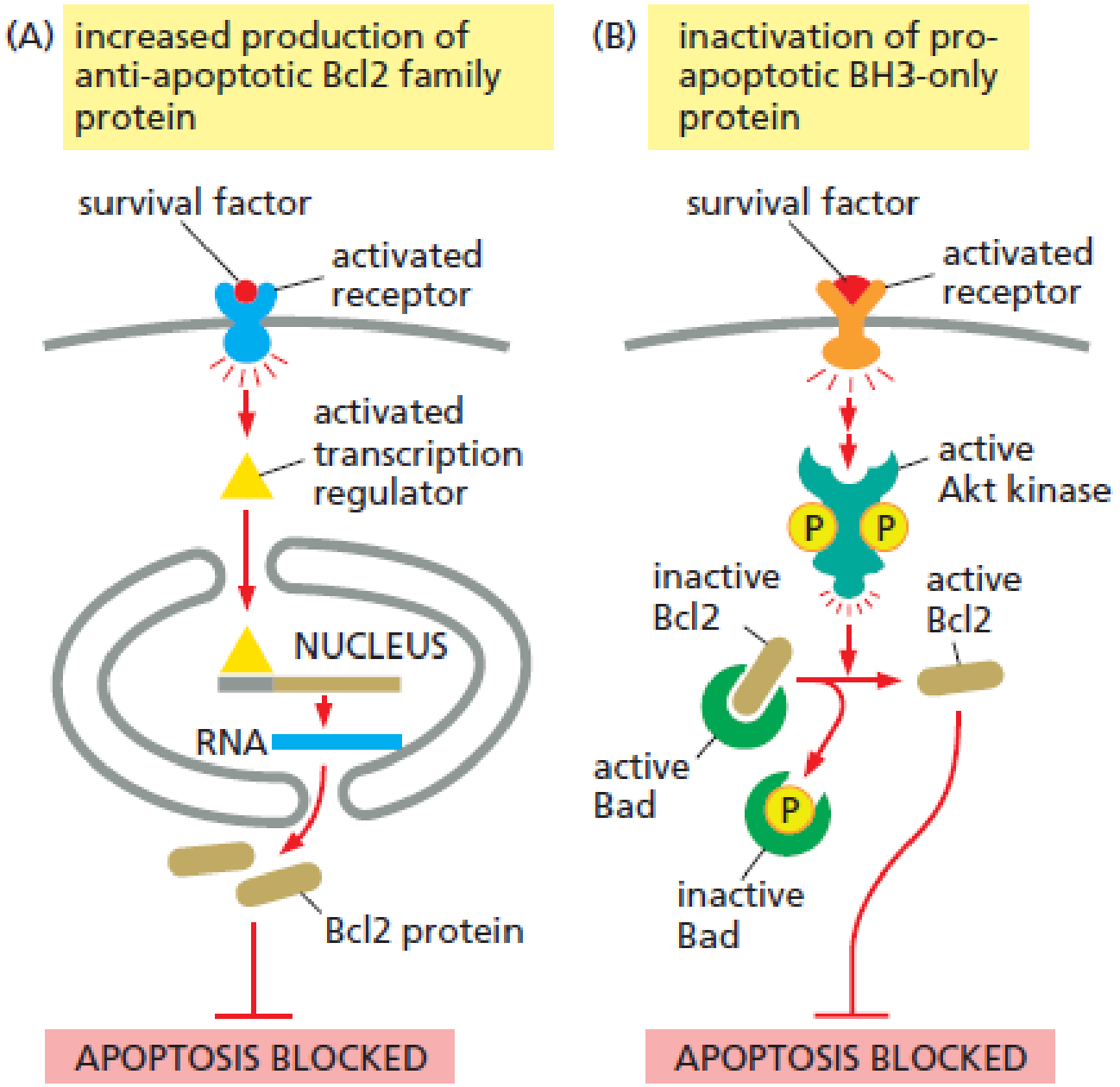
Apoptosis: Vía Intrínseca



Vía intrínseca: regulación

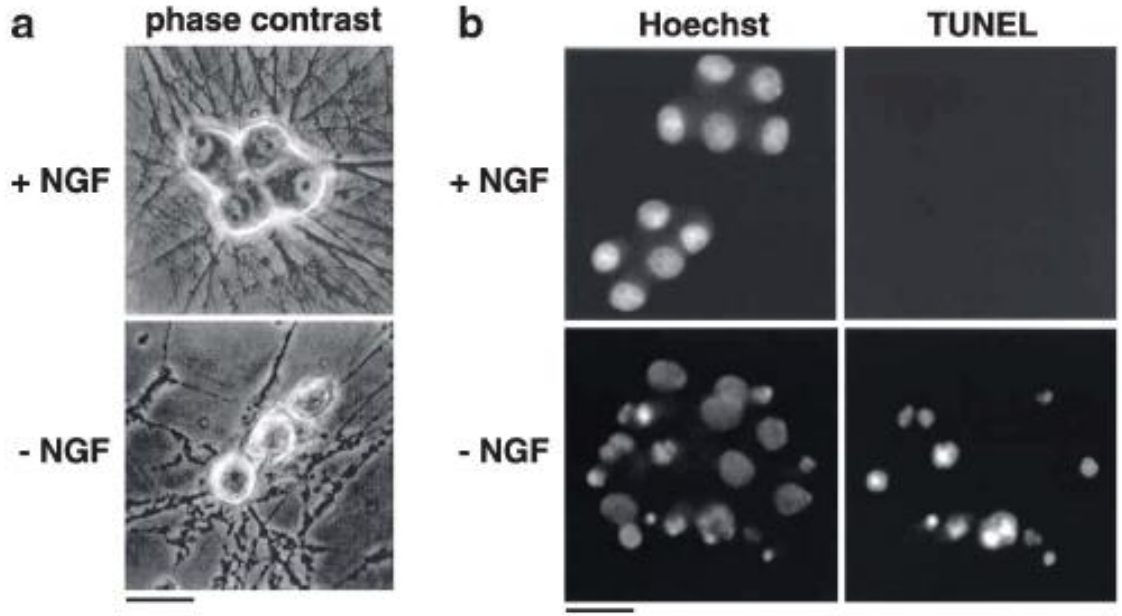



(A) INACTIVE INTRINSIC PATHWAY
active anti-apoptotic Bcl2 family protein
inactive effector Bcl2 family protein
cytochrome c
other proteins in intermembrane space
(B) ACTIVATION OF INTRINSIC PATHWAY
APOPTOTIC STIMULUS
inactivated anti-apoptotic Bcl2 family protein
released intermembrane proteins
activated BH3-only protein
aggregated active effector Bcl2 family proteins
cytochrome c

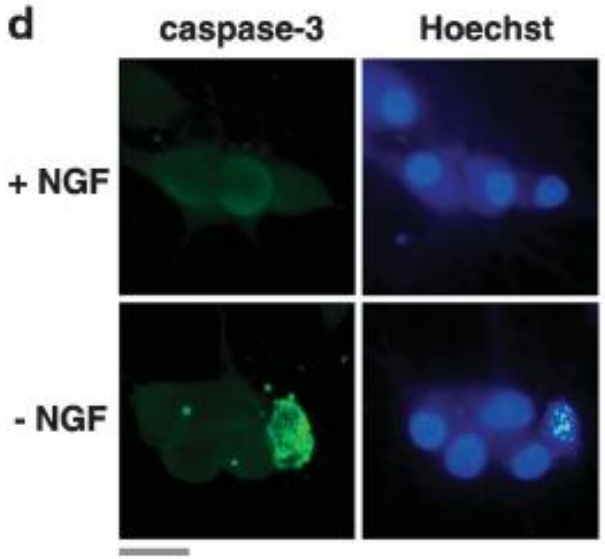

(A) increased production of anti-apoptotic Bcl2 family protein
survival factor
activated receptor
activated transcription regulator
NUCLEUS
RNA
Bcl2 protein
APOPTOSIS BLOCKED
(B) inactivation of pro-apoptotic BH3-only protein
survival factor
activated receptor
active Akt kinase
P
P
inactive Bcl2
active Bcl2
active Bad
P
inactive Bad
APOPTOSIS BLOCKED

Ejercicio

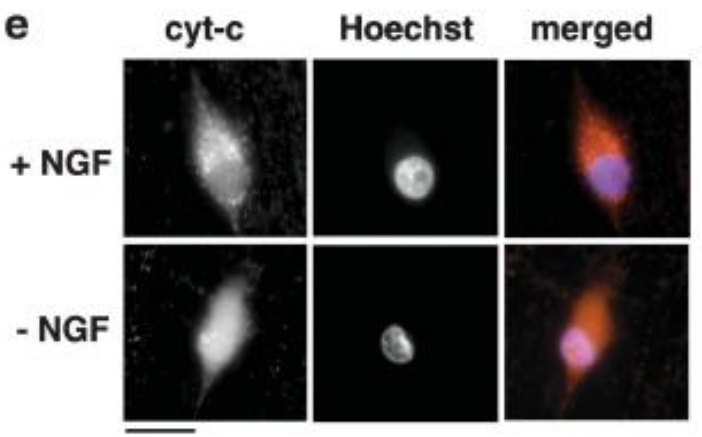
Cultivo de neuronas de ganglio simpático *in vitro* con Nerve Growth Factor (NGF) o sin NGF
Qué tipo de muerte celular ocurre en ausencia de NGF? Cuál podría ser el rol del NGF in vivo?



Hoechst: tinción de ADN
TUNEL: tiñe ADN fragmentado



Anticuerpo detecta caspasa 3 activada

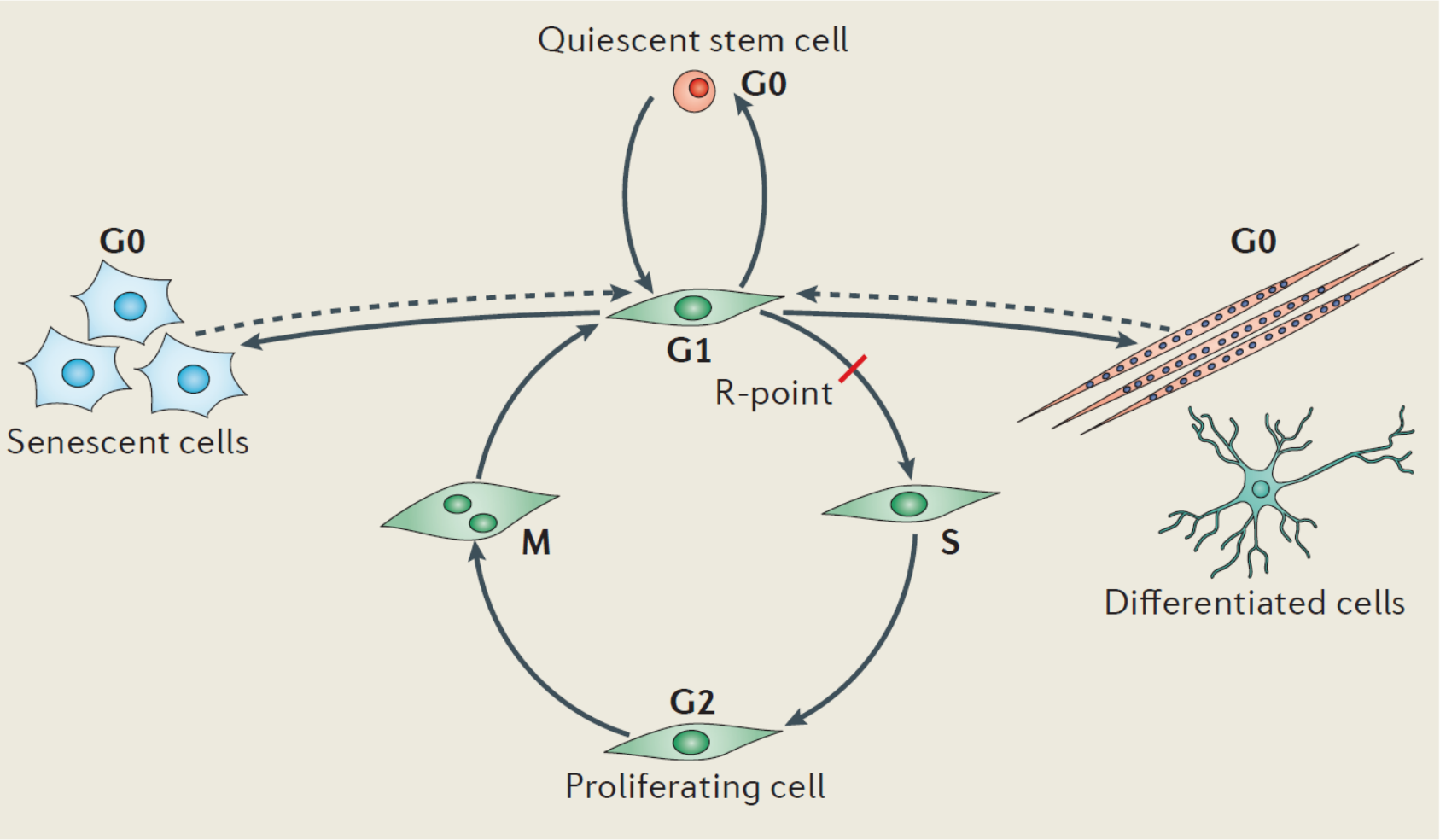


Anticuerpo detecta citocromo C

Inmunofluorescencias

Cell Death and Differentiation (2014) 21, 1025–1035

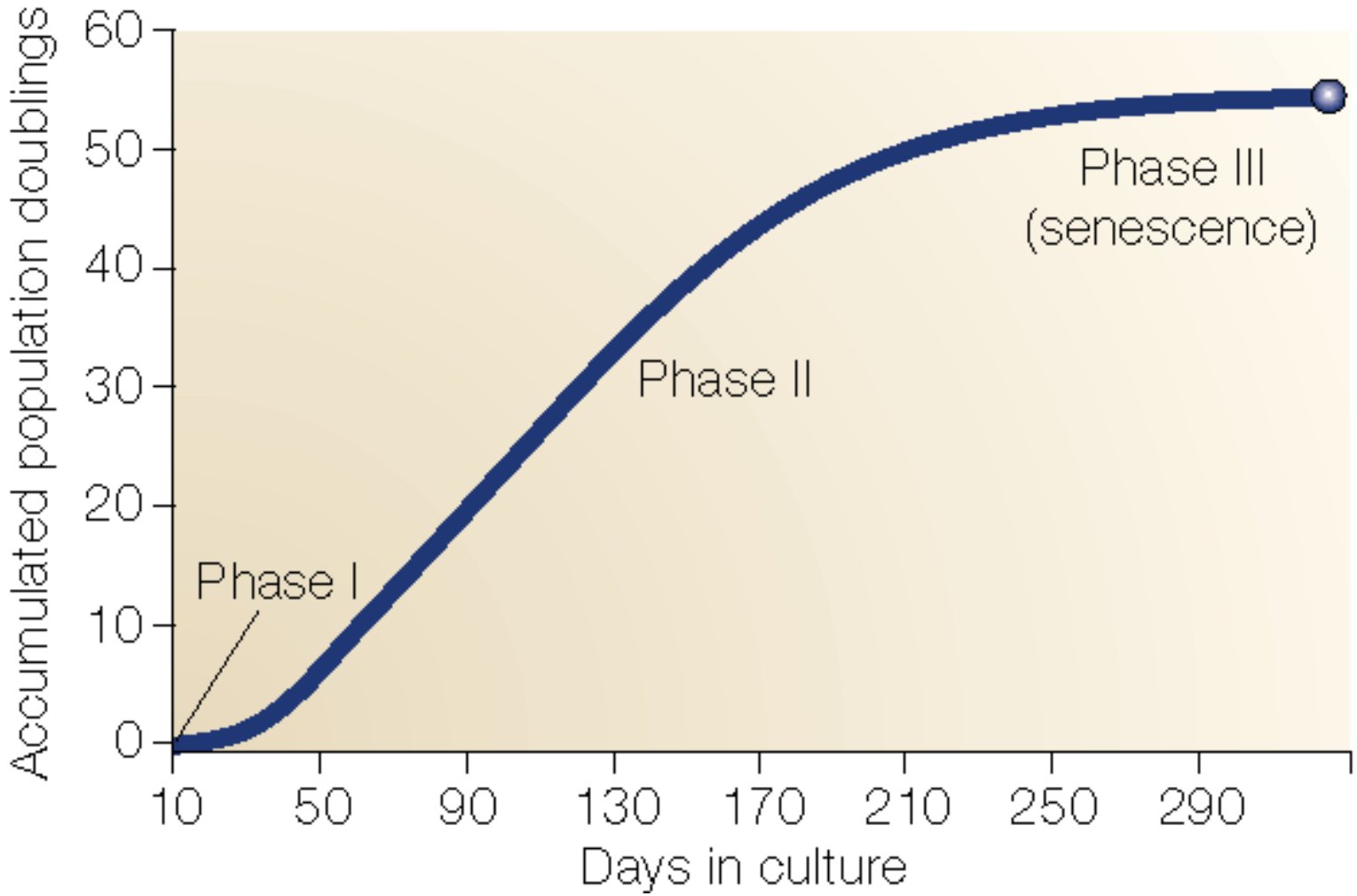
Senescencia celular



Senescencia celular

- Alternativa a la apoptosis
- Célula competente de proliferar entra en bloqueo permanente del ciclo ante algún tipo de stress.
- Incapaces de volver al ciclo celular tras estímulo mitogénico.
- “Fenotipo secretorio” – Inflamación y senescencia de células vecinas

Límite de Hayflick – senescencia replicativa



(40 pasajes)

Telomerasa y límite de Hayflick

Los Telómeros de los cromosomas están compuestos de secuencias sencillas repetitivas, que forman un “capuchón” que protege los extremos de cada cromosoma.

No se replican como el resto del genoma, si no que los repetidos son añadidos directamente por una enzima, la TELOMERASA

En ausencia de telomerasa, los telómeros se van acortando en cada división, y eventualmente los extremos sin capuchón son detectados como daño al ADN

En fibroblastos humanos, que casi no expresan telomerasa, su expresión mediante transgénesis evita la senescencia replicativa

La expresión anormal de telomerasa es también común en muchos tipos de cancer

Senescencia *in vivo*

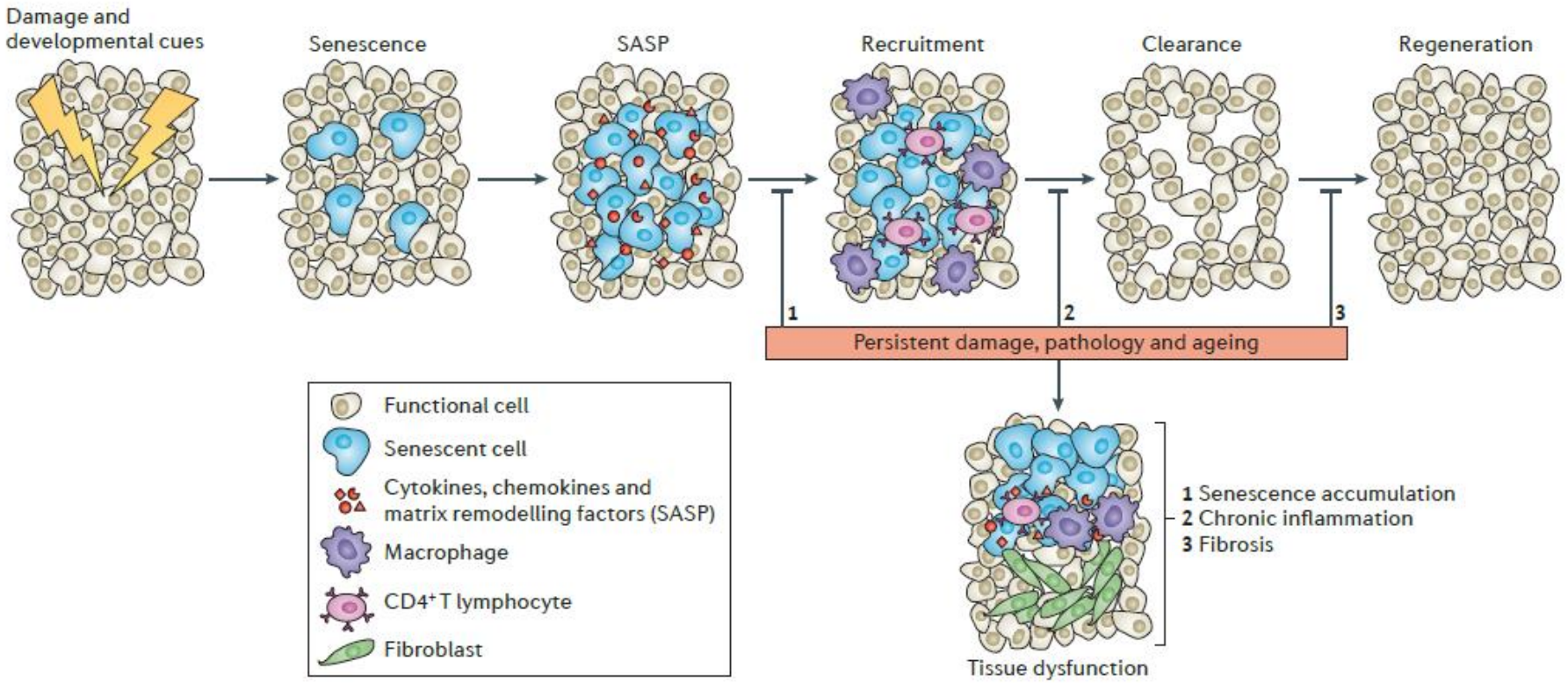


Figure 3 | **Unified model of senescence.** Senescence initiates a tissue remodelling process by recruiting immune cells through the senescence-associated secretory phenotype (SASP). Macrophages clear the senescent cells, and progenitor cells repopulate and regenerate the damaged tissue. This sequence of senescence–clearance–regeneration may be impaired upon persistent damage, pathological states or ageing. In these cases, senescent cells are not efficiently cleared and the tissue is not fully regenerated. Resolution of the damage in these cases involves a fibrotic scar with senescent cells, inflammatory cells and fibrotic tissue.

Envejecimiento - Hipótesis

- Deterioro con el tiempo de funciones necesarias para supervivencia y fertilidad

- Muchas hipótesis! No mutuamente excluyentes

Daño al ADN y senescencia

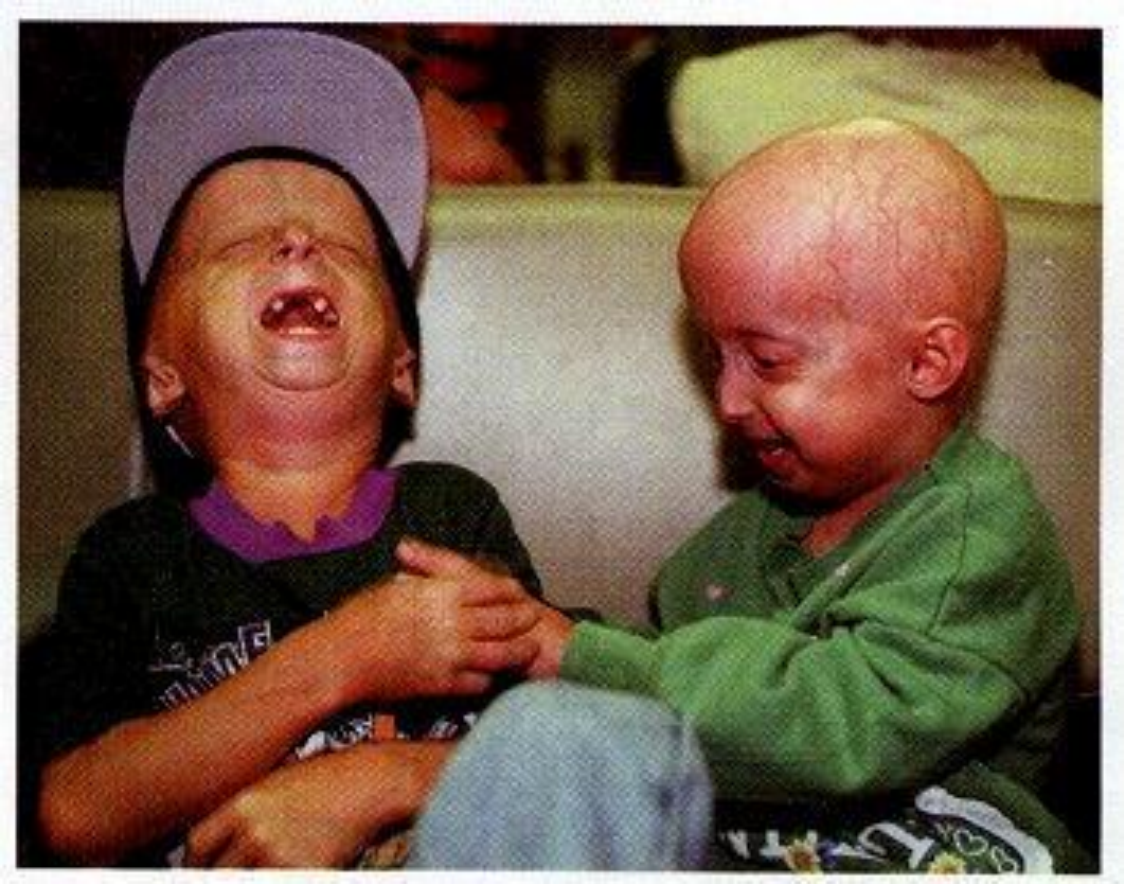
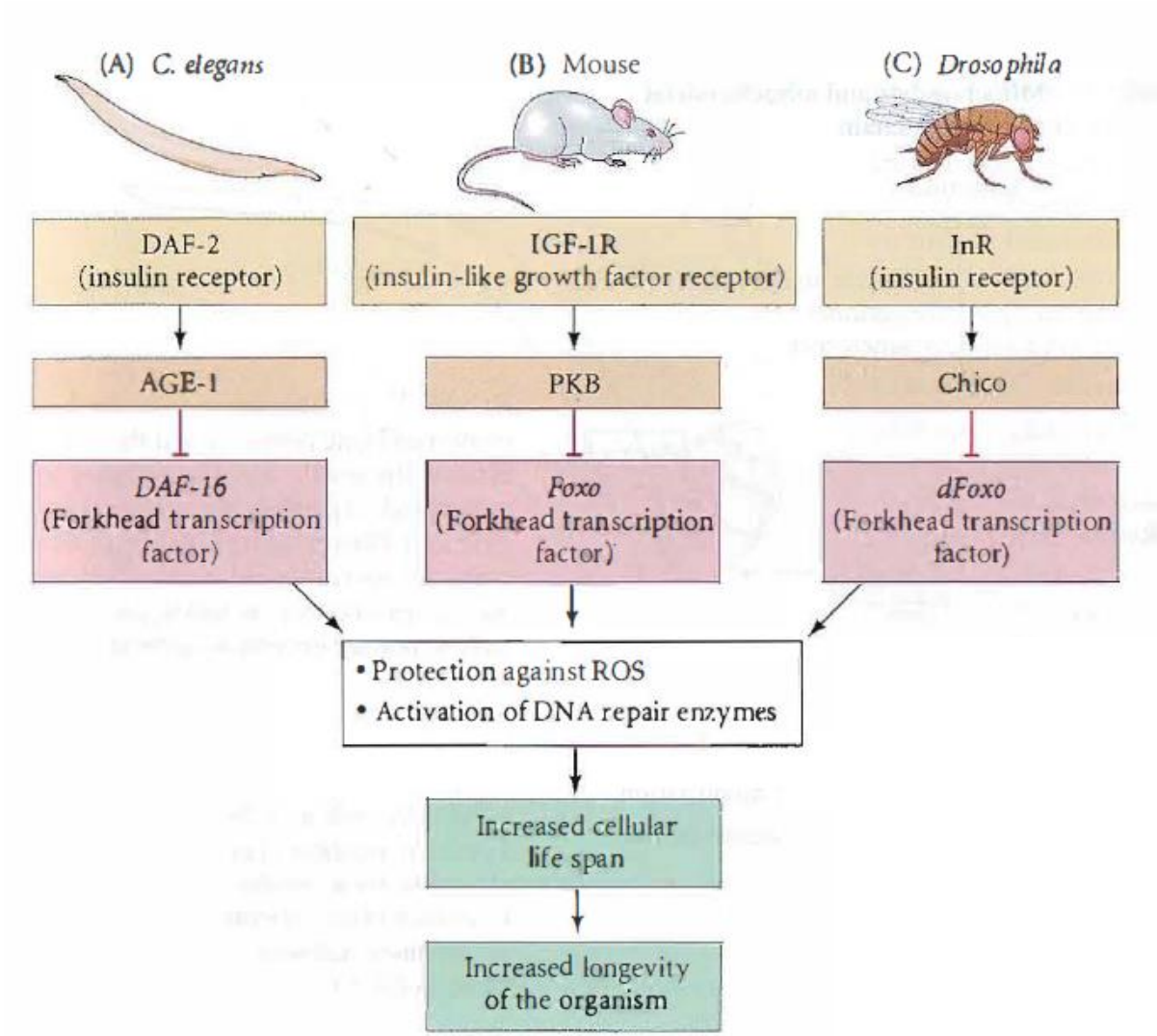


TABLE 5–2 Some Inherited Human Syndromes with Defects in DNA Repair

Name	Phenotype	Enzyme or process affected
MSH2, 3, 6, MLH1, PMS2	Colon cancer	Mismatch repair
Xeroderma pigmentosum (XP) groups A–G	Skin cancer, UV sensitivity, neurological abnormalities	Nucleotide excision repair
Cockayne syndrome	UV sensitivity; developmental abnormalities	Coupling of nucleotide excision repair to transcription
XP variant	UV sensitivity, skin cancer	Translesion synthesis by DNA polymerase ν
Ataxia telangiectasia (AT)	Leukemia, lymphoma, γ-ray sensitivity, genome instability	ATM protein, a protein kinase activated by double-strand breaks
BRCA1	Breast and ovarian cancer	Repair by homologous recombination
BRCA2	Breast, ovarian, and prostate cancer	Repair by homologous recombination
Werner syndrome	Premature aging, cancer at several sites, genome instability	Accessory 3′-exonuclease and DNA helicase used in repair
Bloom syndrome	Cancer at several sites, stunted growth, genome instability	DNA helicase needed for recombination
Fanconi anemia groups A–G	Congenital abnormalities, leukemia, genome instability	DNA interstrand cross-link repair
46 BR patient	Hypersensitivity to DNA-damaging agents, genome instability	DNA ligase I

Regulación genética – longevidad vs. reproducción



Pregunta de examen

¿Qué son las caspasas? ¿Cuáles son sus roles en la apoptosis?