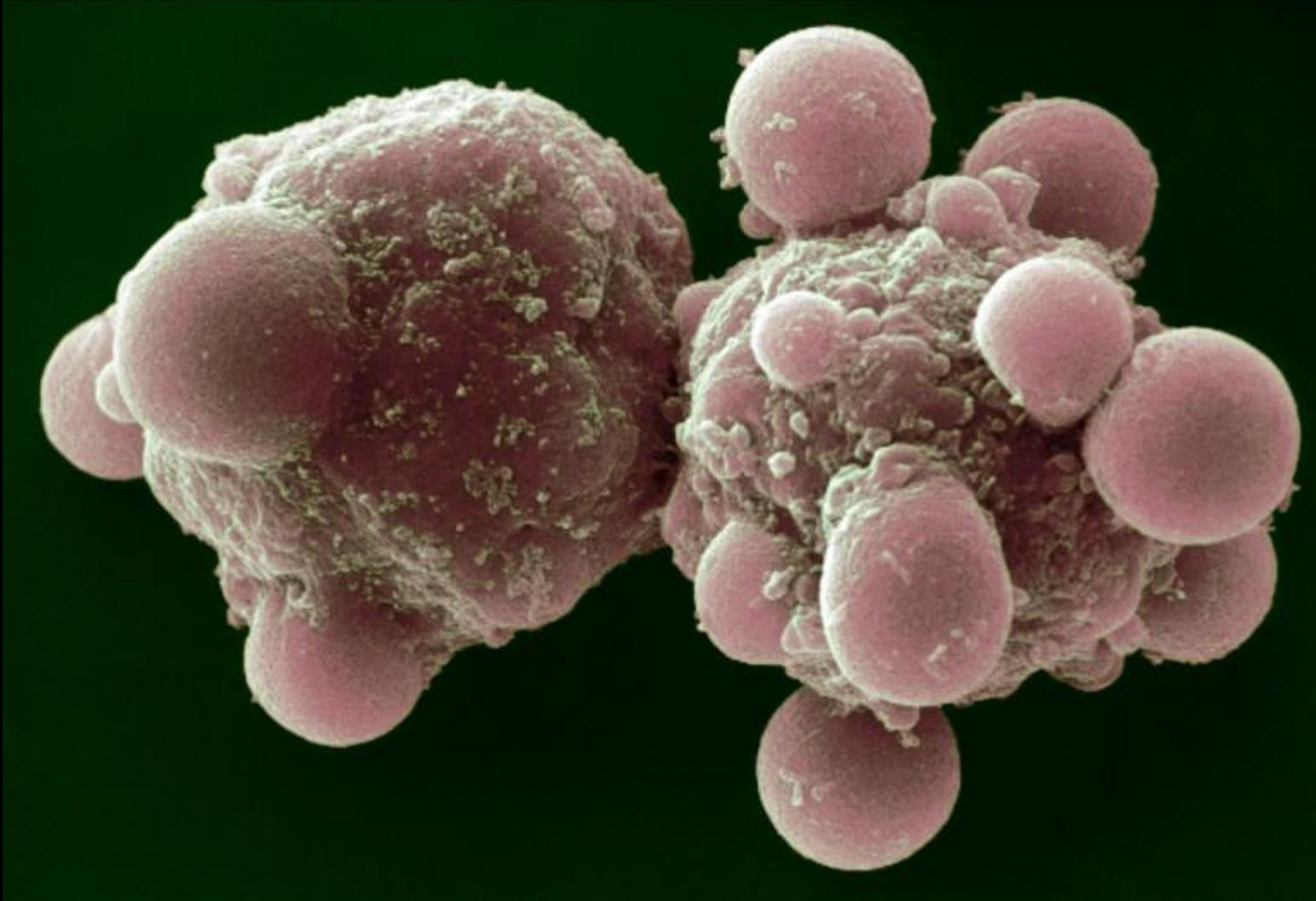


Muerte celular



Uriel Koziol
ukoziol@fcien.edu.uy

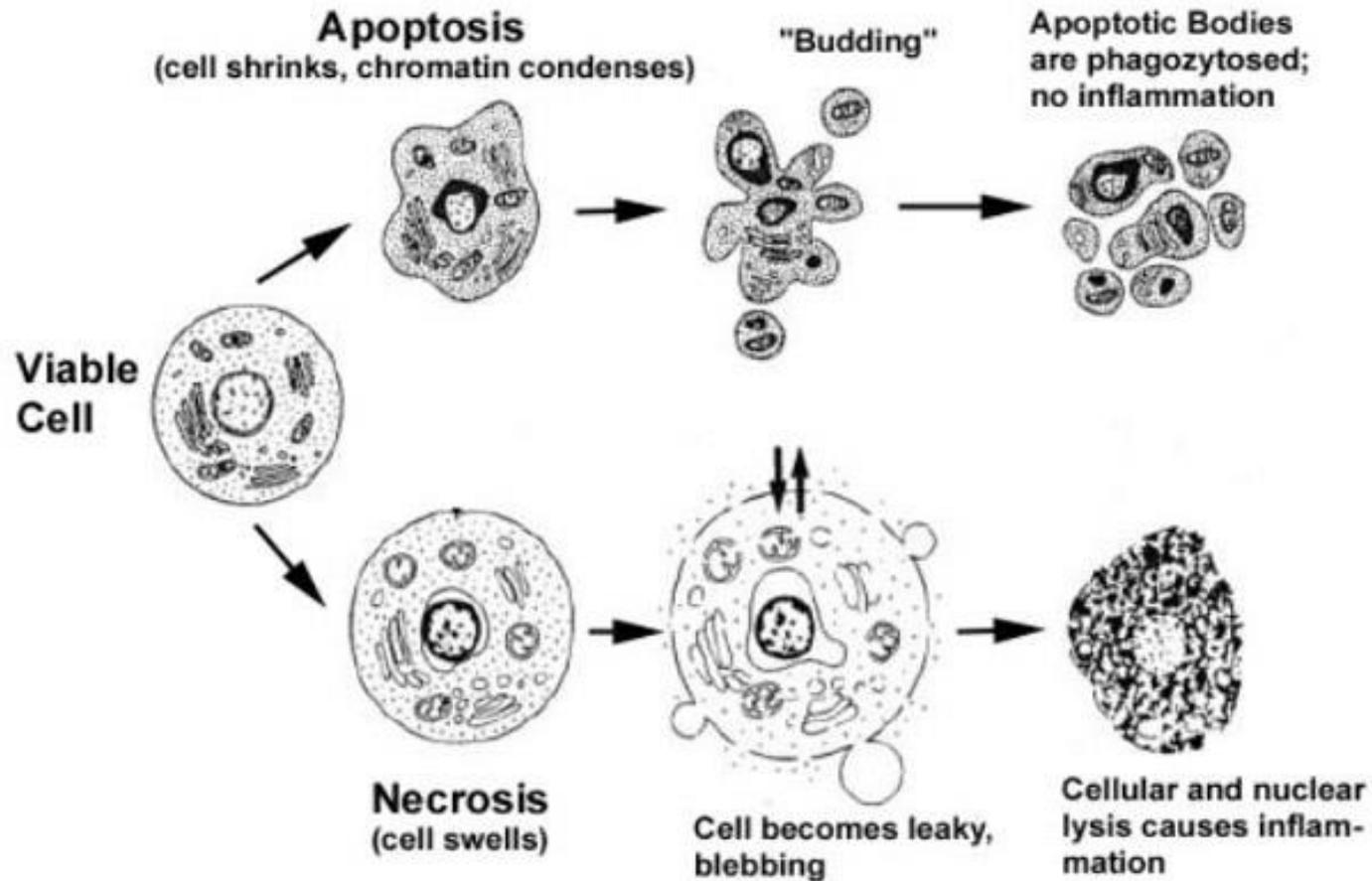


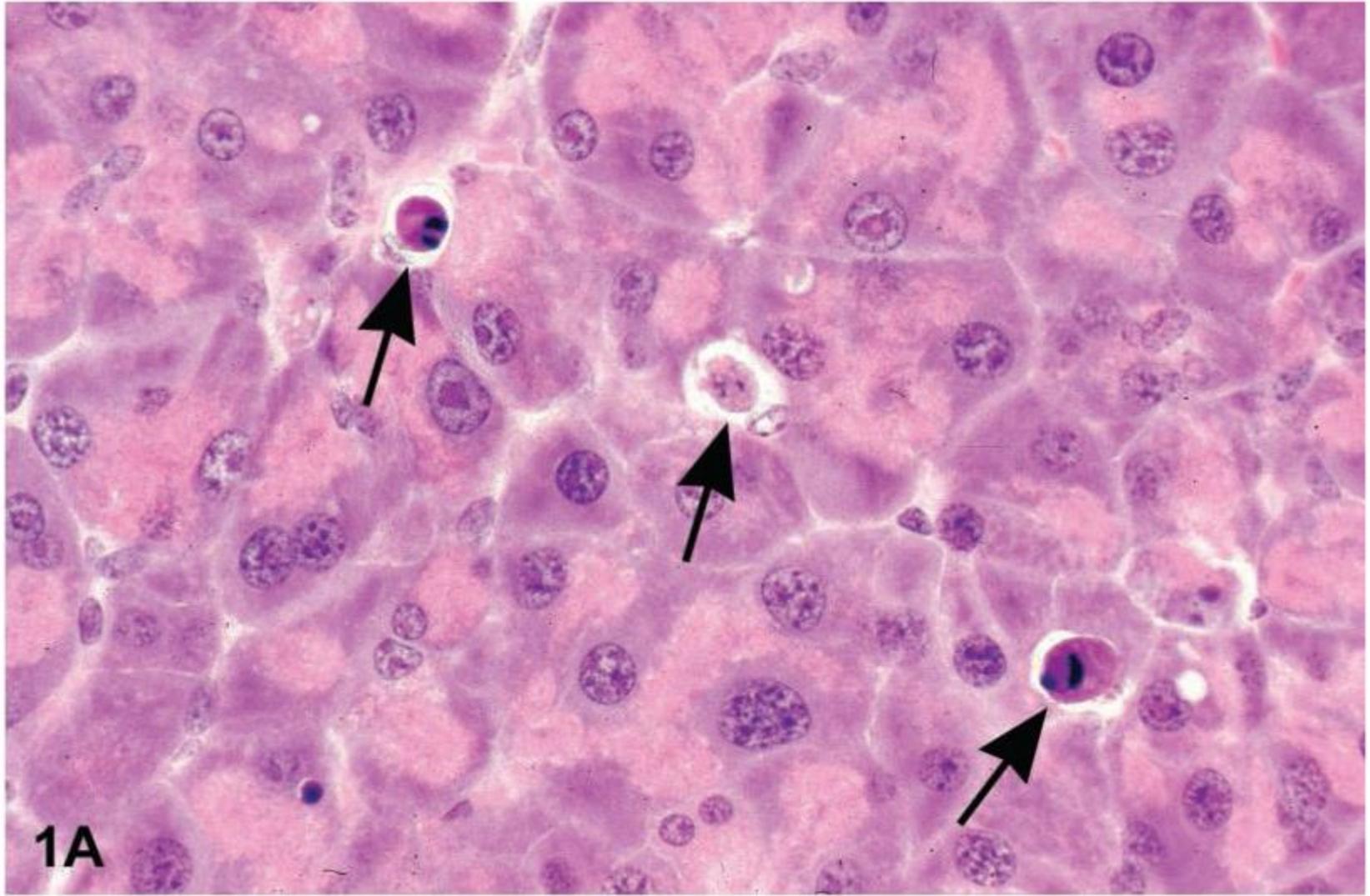
Muerte celular

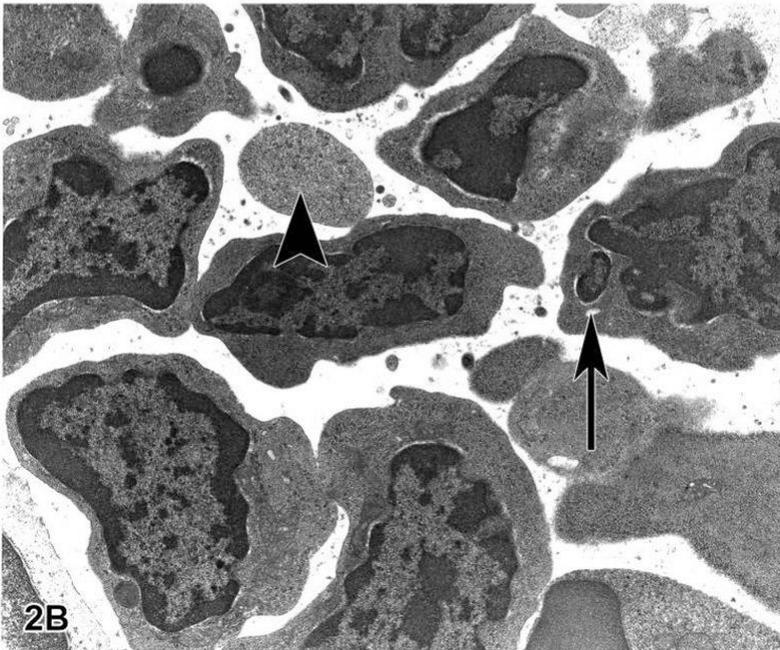
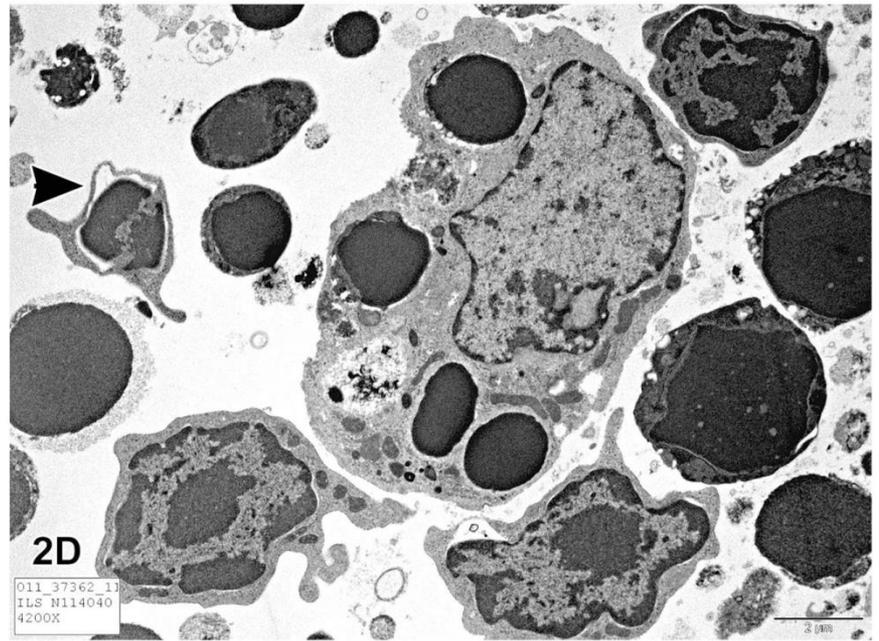
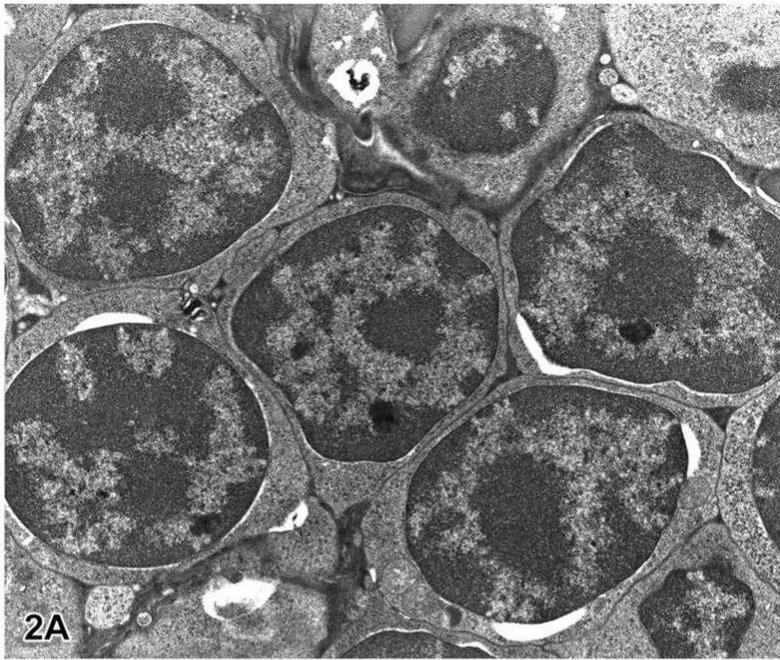
- **Necrosis**
- **Muerte celular programada**
(Programmed Cell Death, PCD)
 - **Apoptosis**
 - **Otras formas de PCD** (Piroptosis, Necroptosis, etc.)

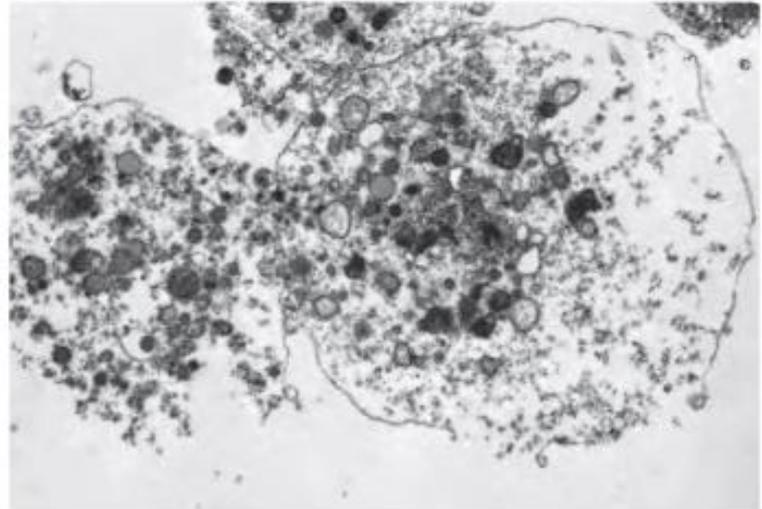
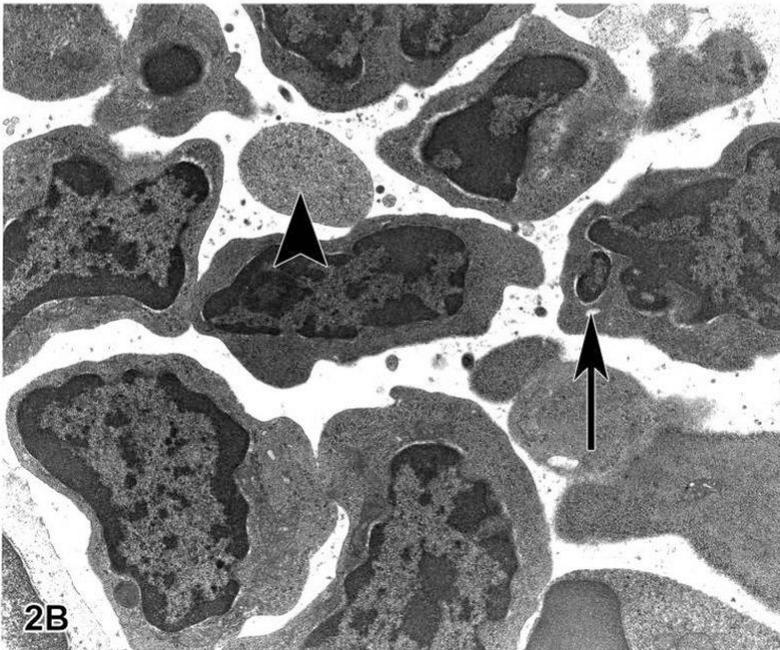
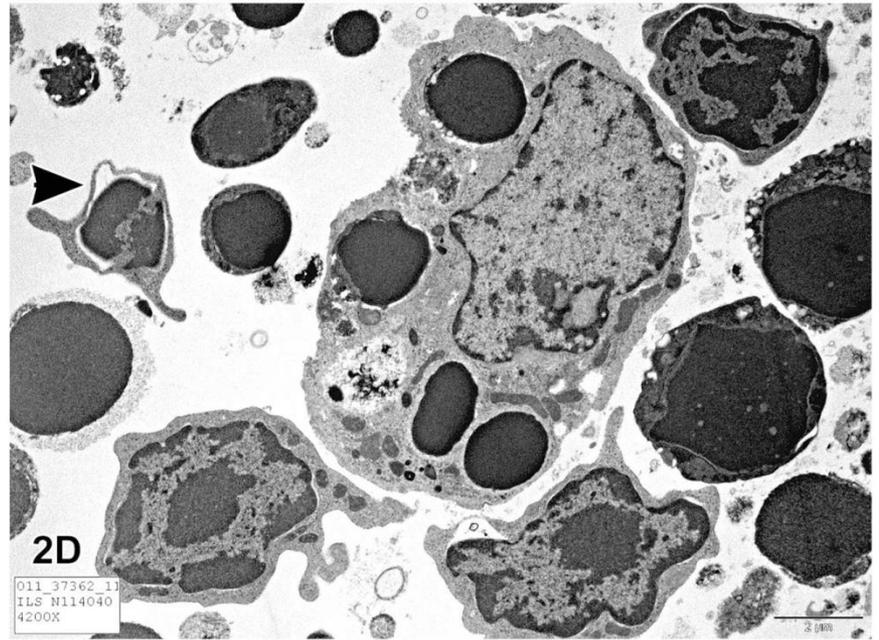
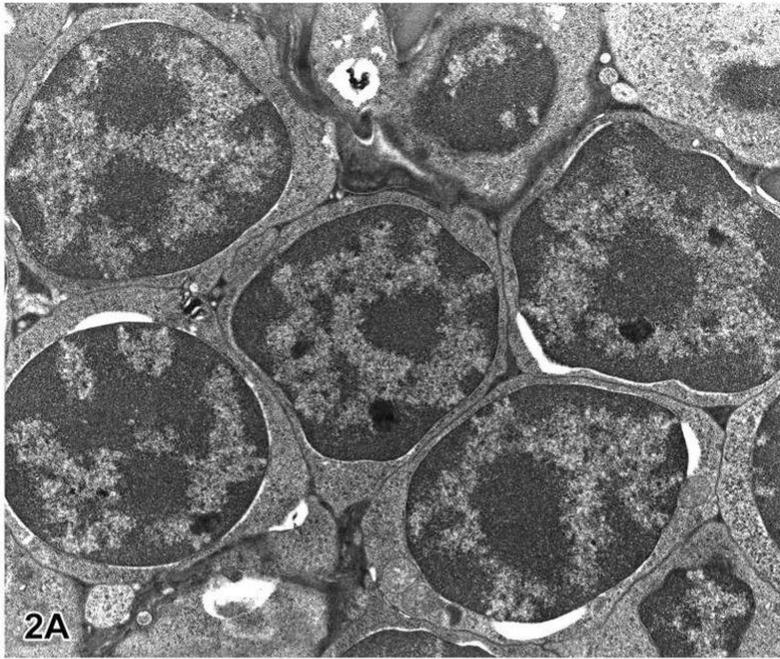


Apoptosis vs. Necrosis









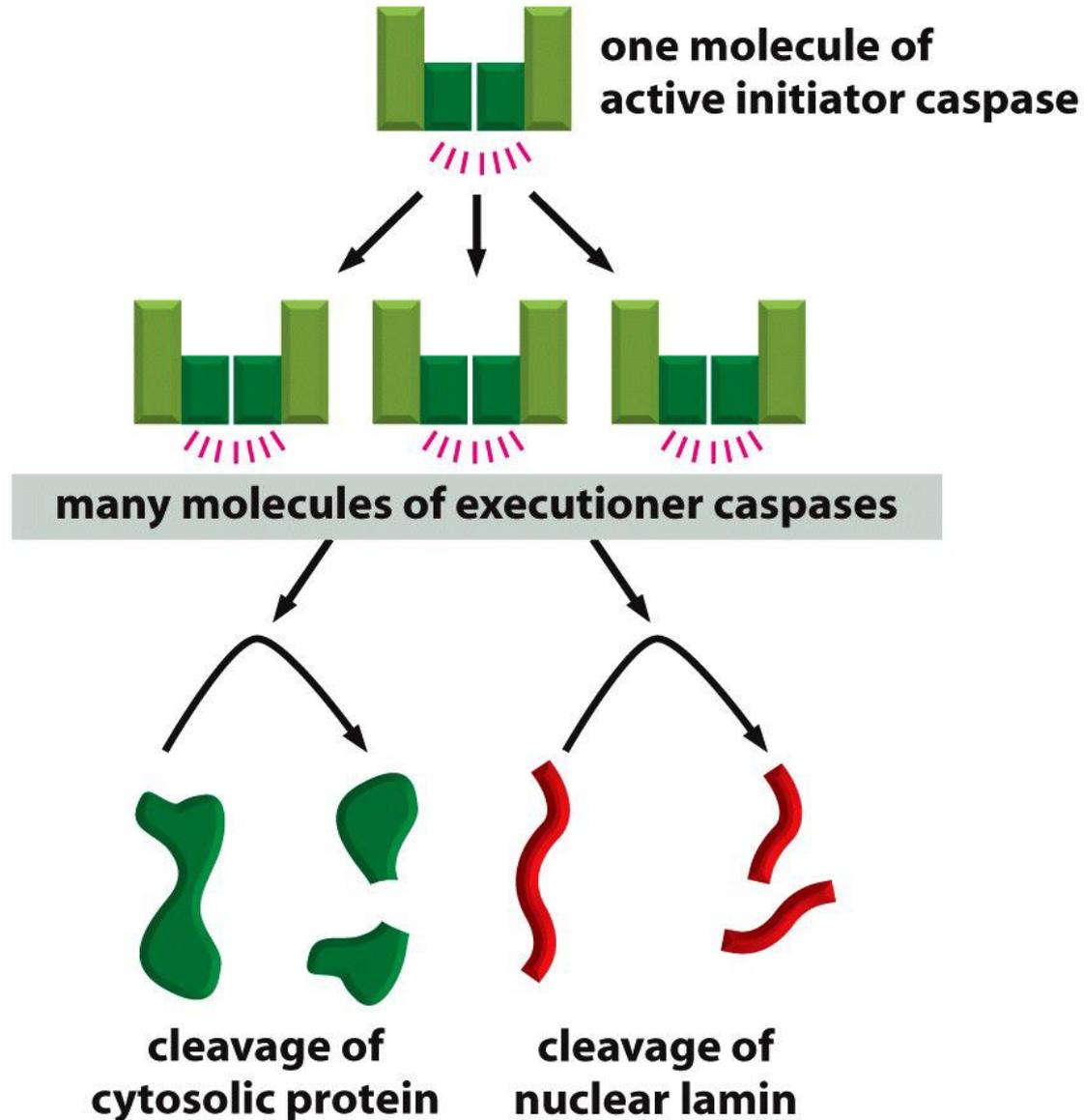
Apoptosis vs. Necrosis

Apoptosis	Necrosis
Activo	Pasivo
Condensación del citoplasma	Hinchazón del citoplasma
Condensación y fragmentación nuclear (picnosis)	Condensación y fragmentación nuclear (picnosis) / lisis nuclear (cariólisis)
Membrana intacta	Membrana comprometida
Citoplasma retenido en cuerpos apoptóticos	Citoplasma liberado a medio extracelular
No hay inflamación	Inflamación
Dependiente de caspasas	Independiente de caspasas

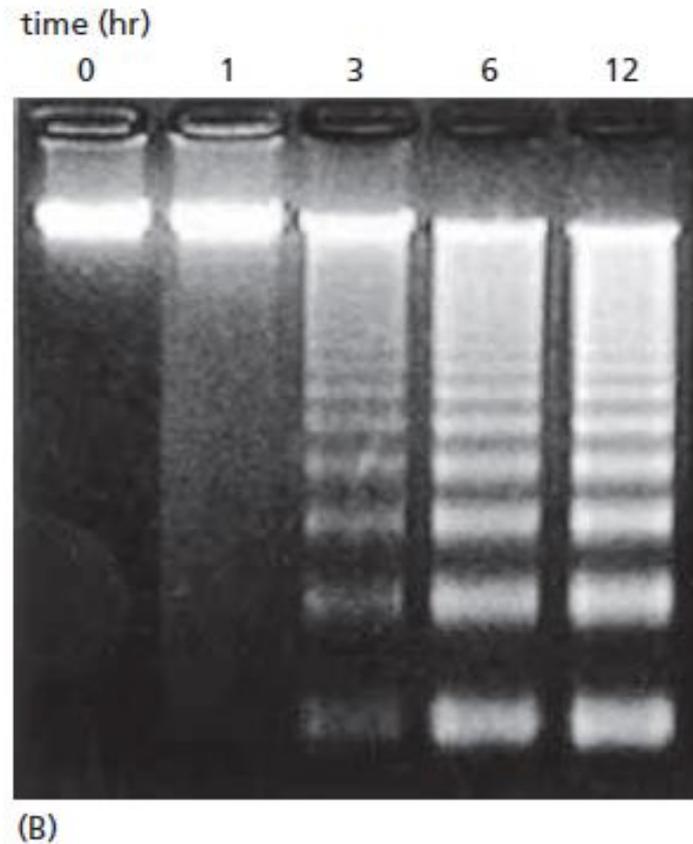
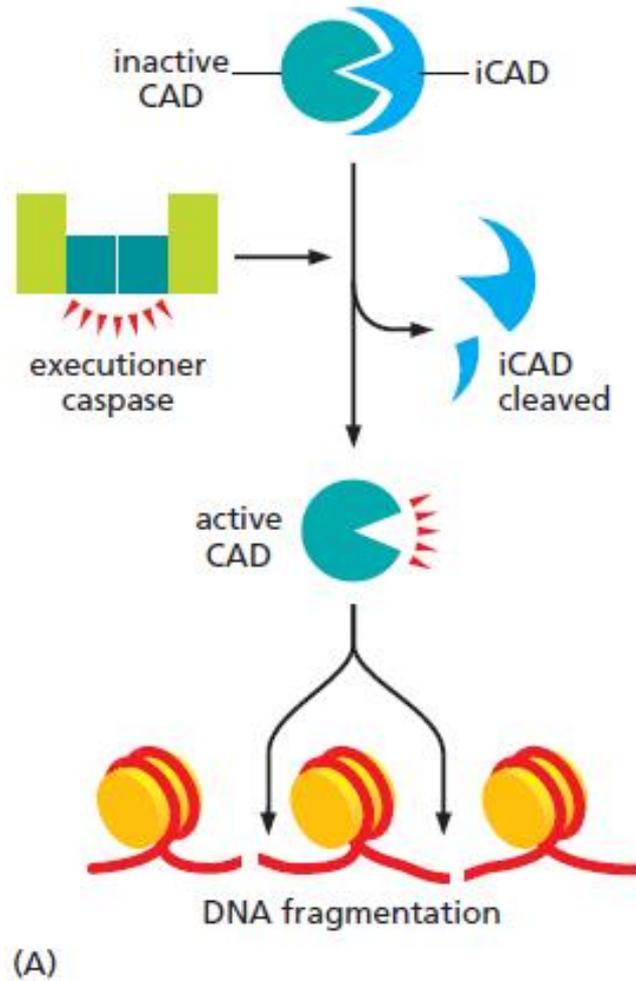
CASPASAS = Cisteín-Proteasas que cortan blancos tras residuos de Aspartato



caspase cascade



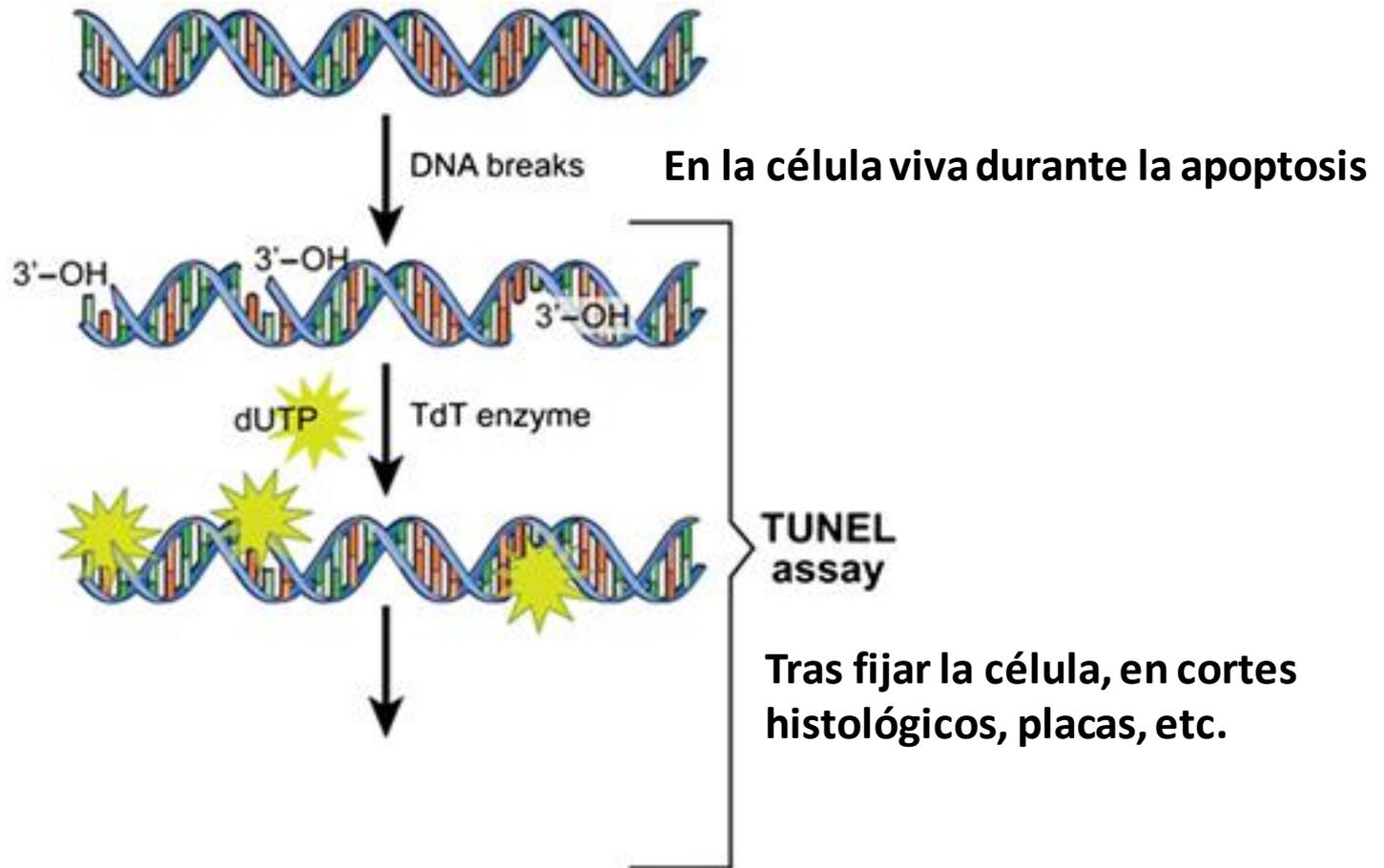
Caspase-Activated DNase (CAD) degrada el ADN

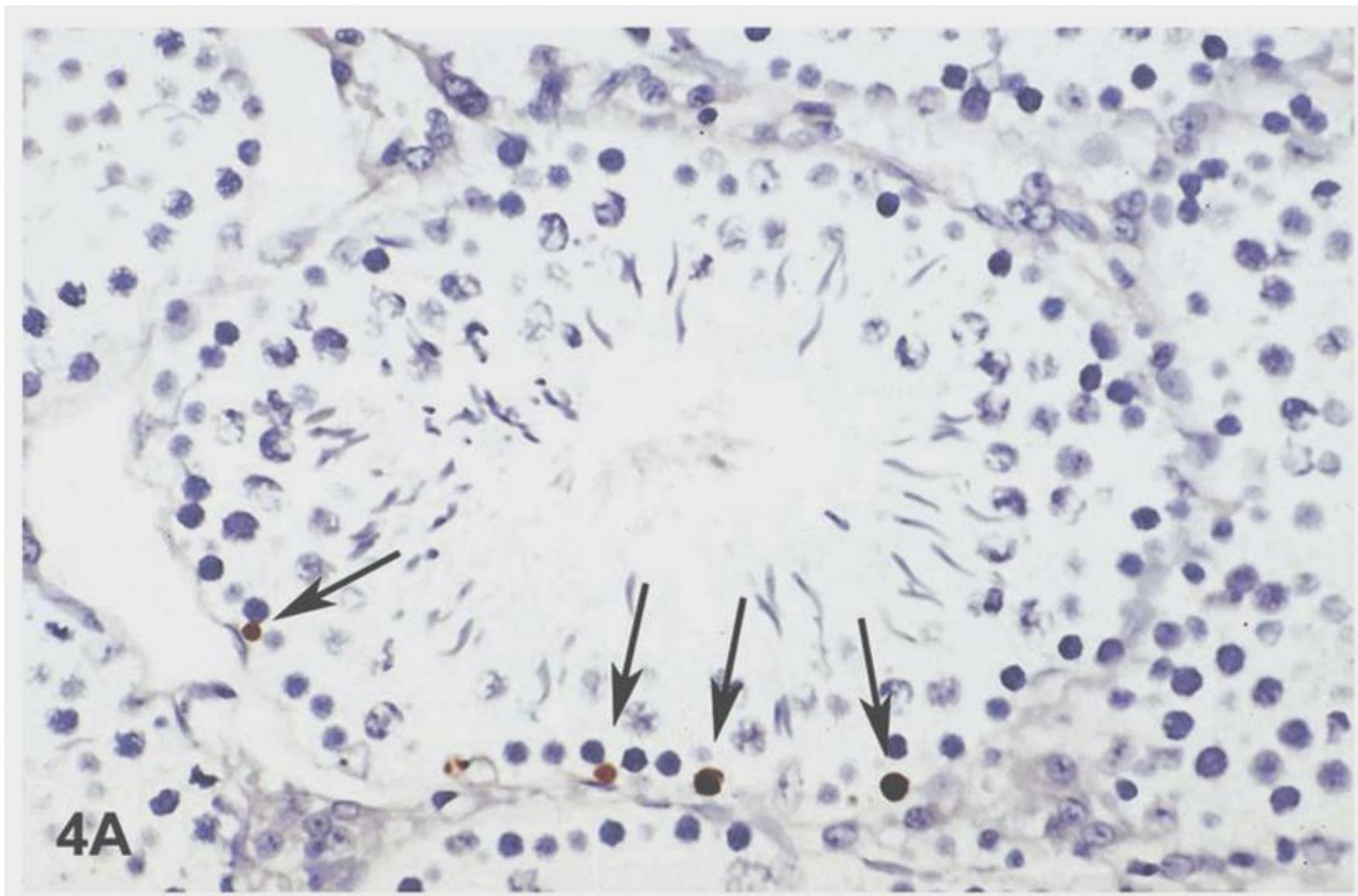


Técnica de TUNEL

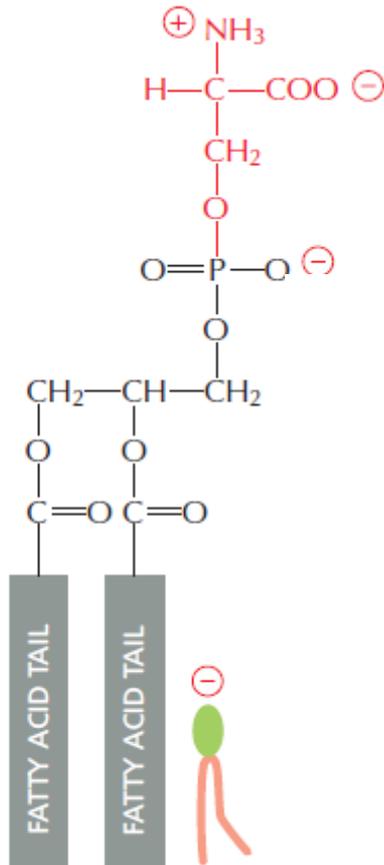
(Terminal deoxynucleotidyl transferase dUTP nick end labeling)

Permite observar experimentalmente la fragmentación del ADN

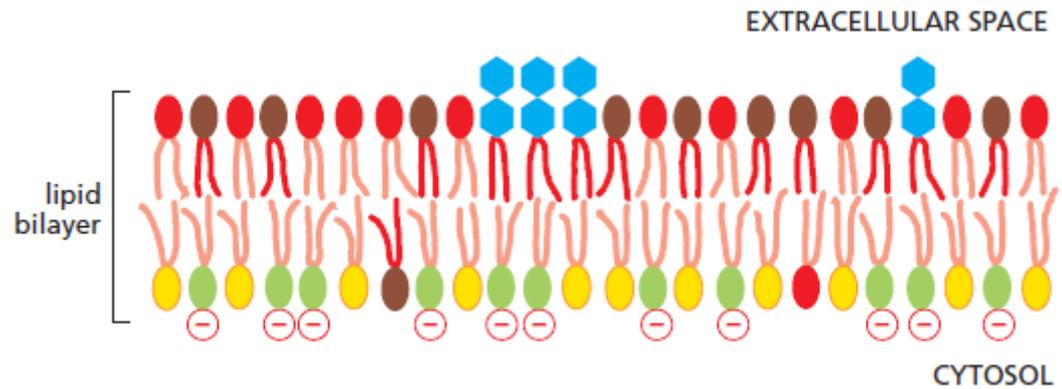




La Fosfatidilserina es expuesta en la superficie de la membrana plasmática

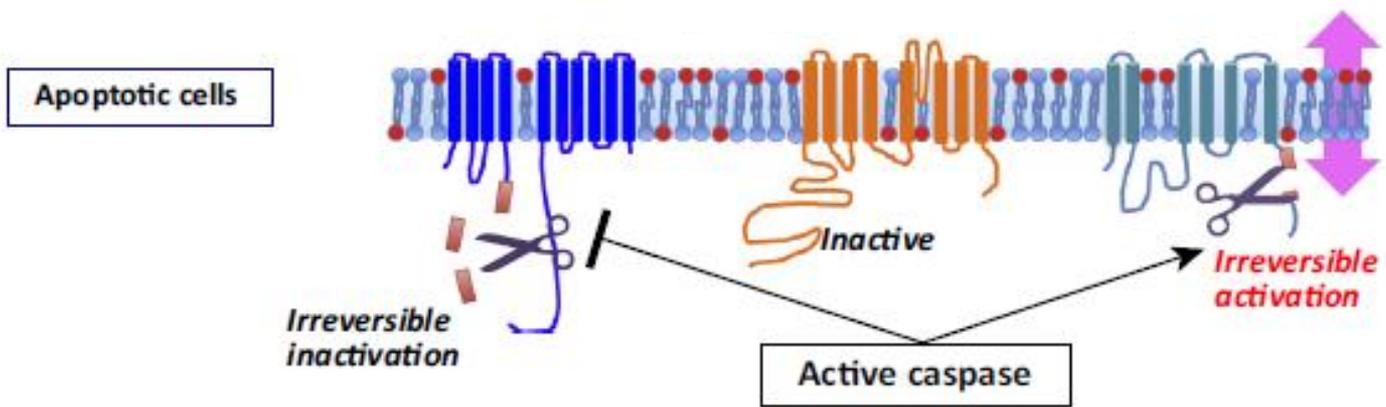
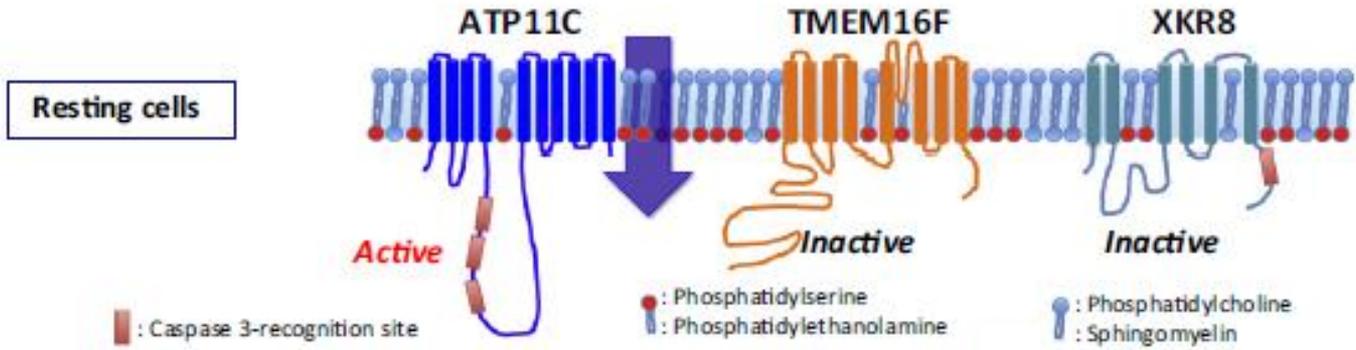


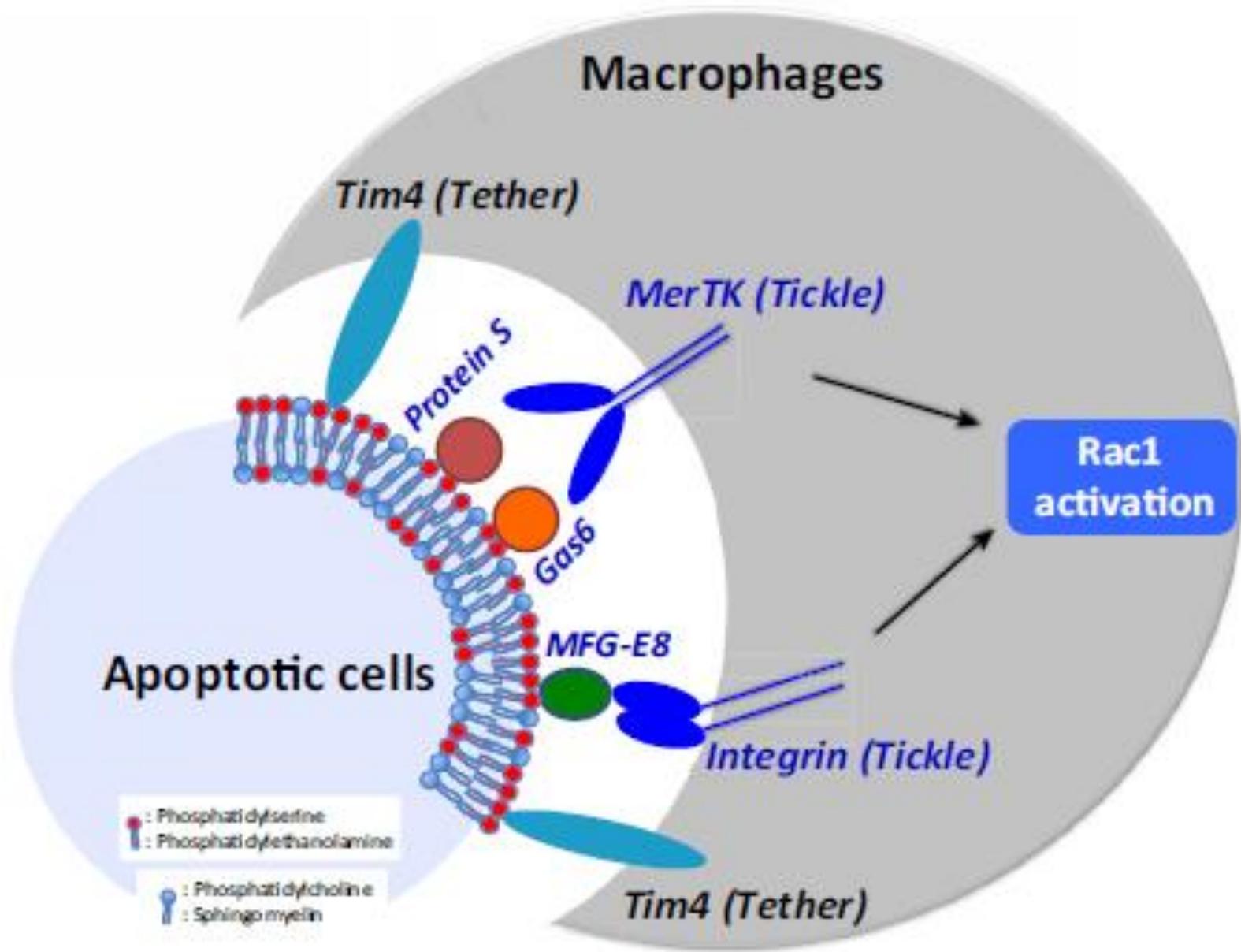
phosphatidylserine



Flippase

Scramblase





Otros blancos de caspasas

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



¿Por qué apoptosis?

¿Cuándo?

¿Cómo?



***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

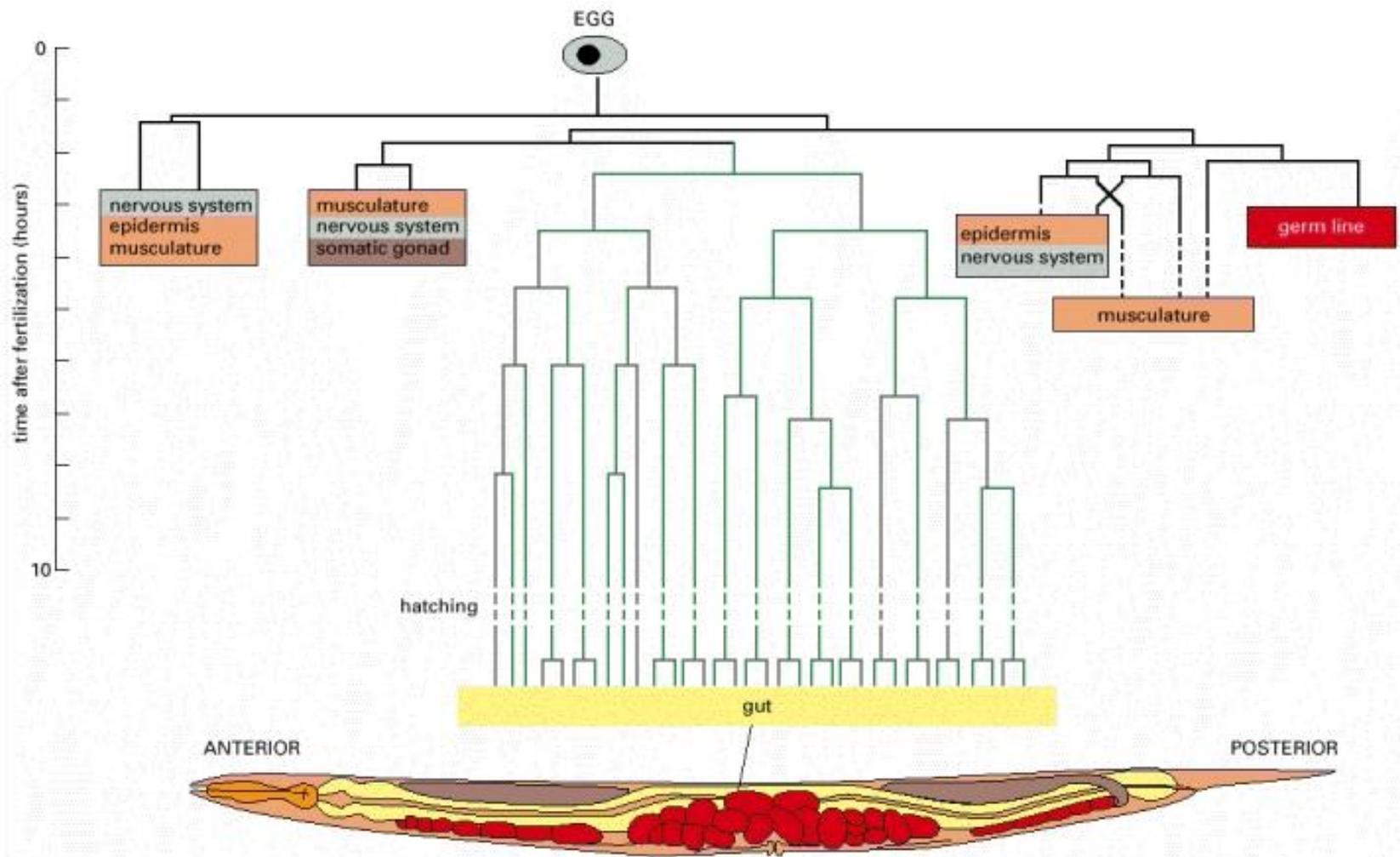


John E. Sulston

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



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

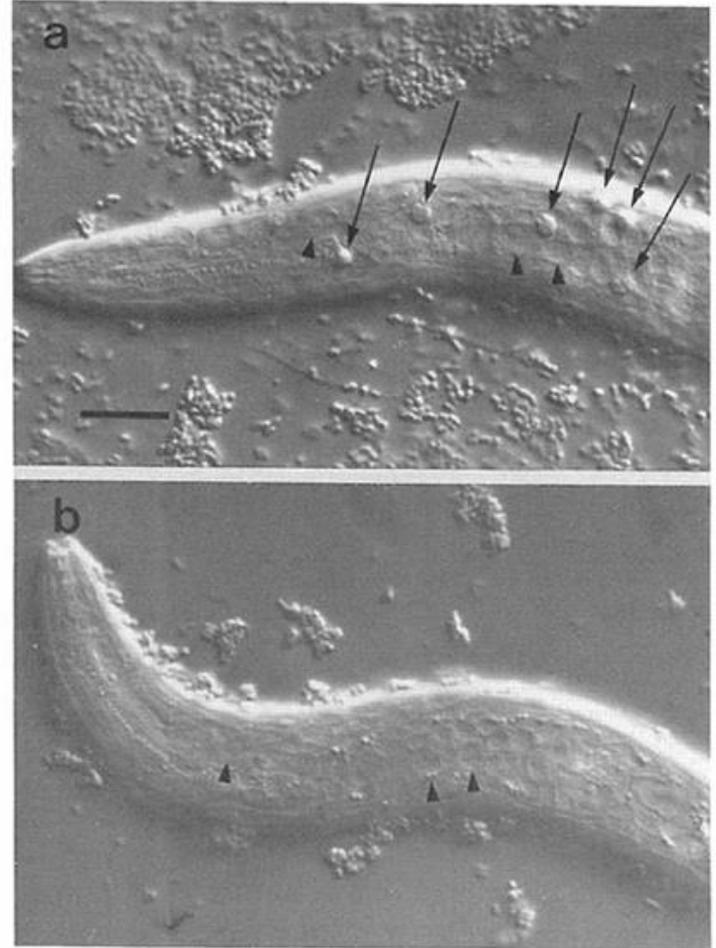
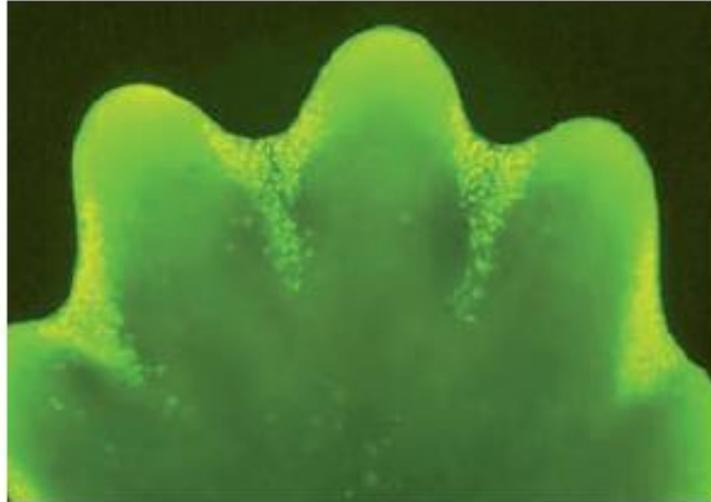


Figure 1. Absence of Cell Deaths in *ced-3* Animals

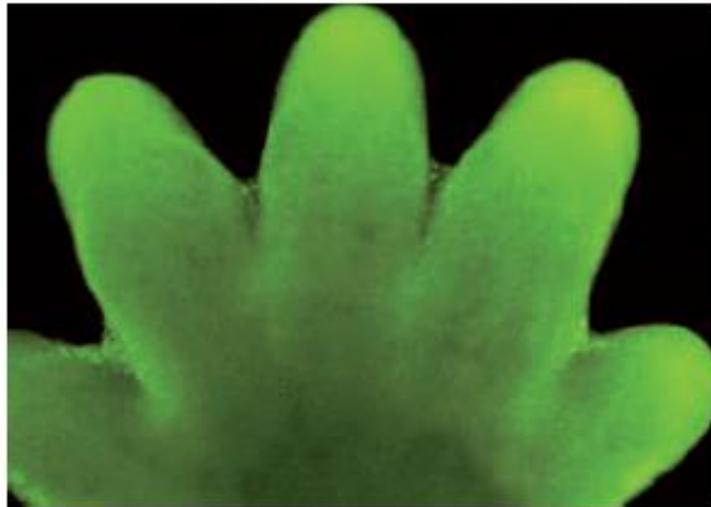
(a) Nomarski photomicrograph of a newly hatched *ced-1* larva. Arrows indicate dying cells. (b) Nomarski photomicrograph of a newly hatched *ced-1; ced-3* larva. Plane of focus is approximately that shown in (a). Arrowheads indicate several of the nuclei that can be seen in both (a) and (b). No cell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



Desarrollo embrionario



(A)

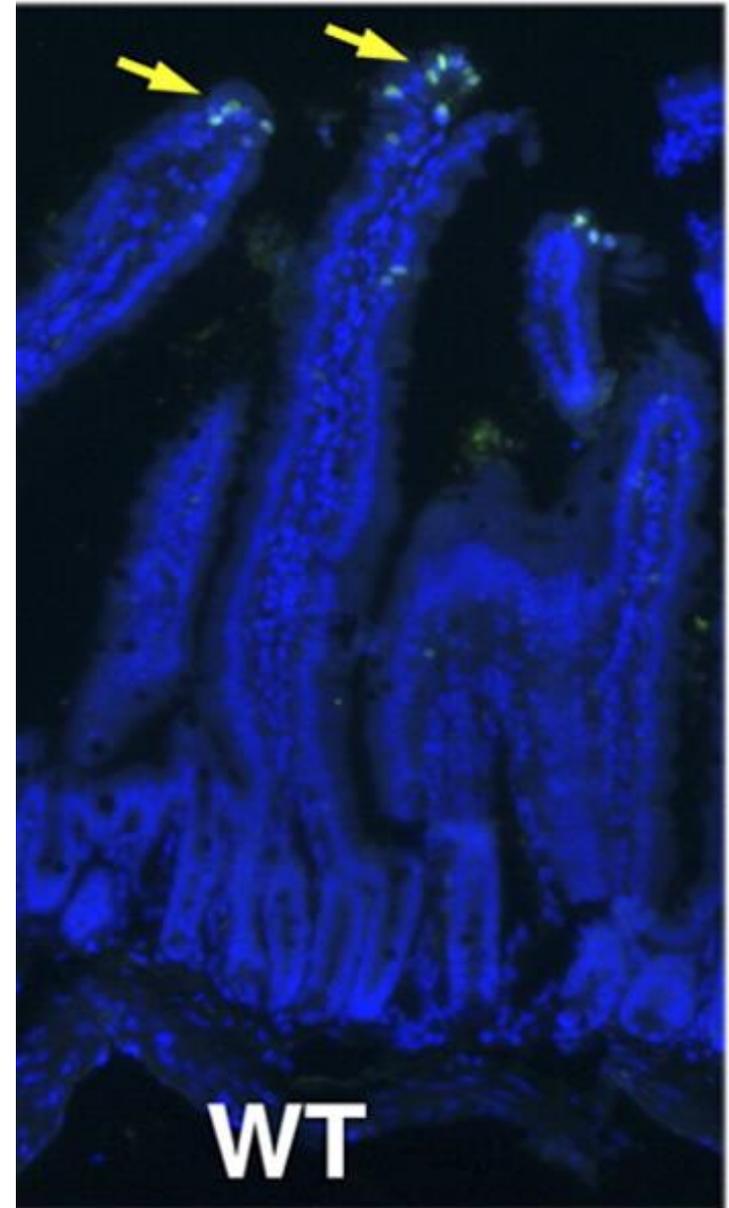
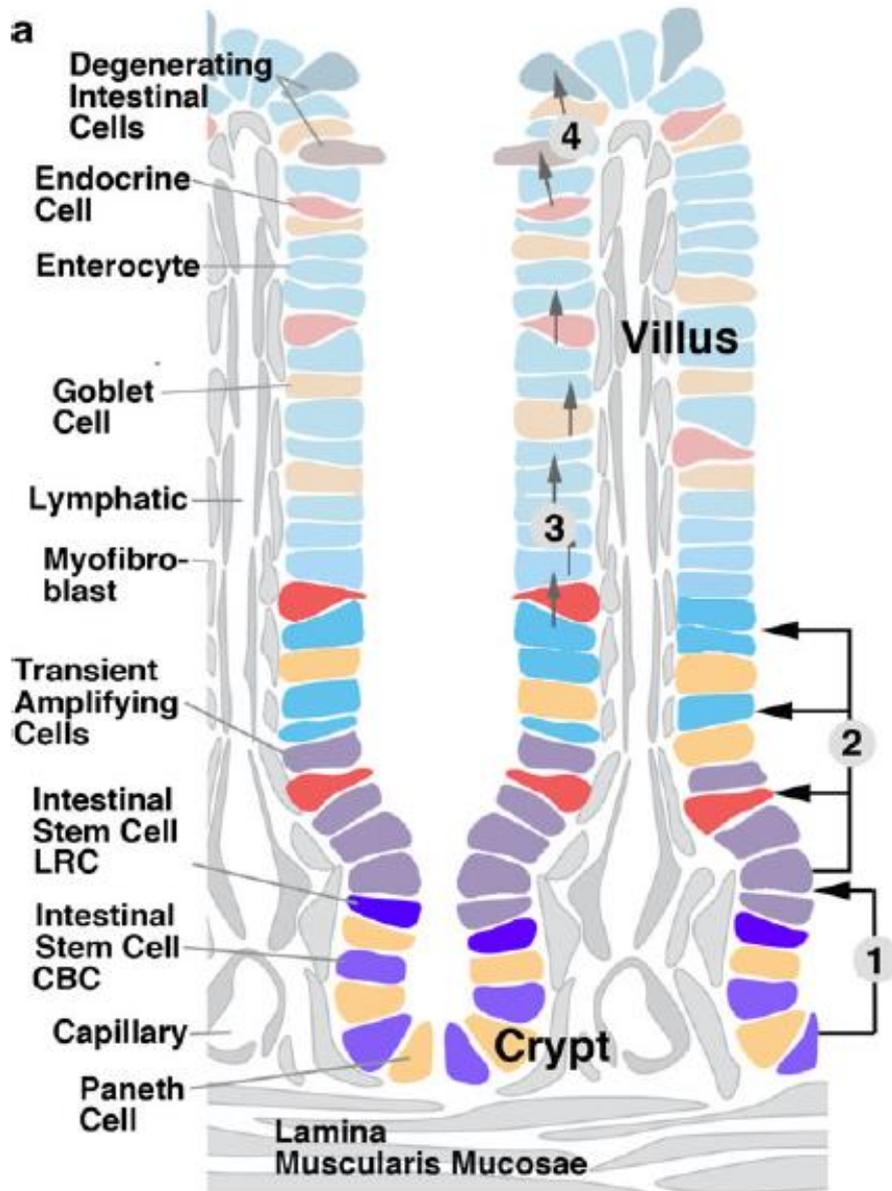


(B)

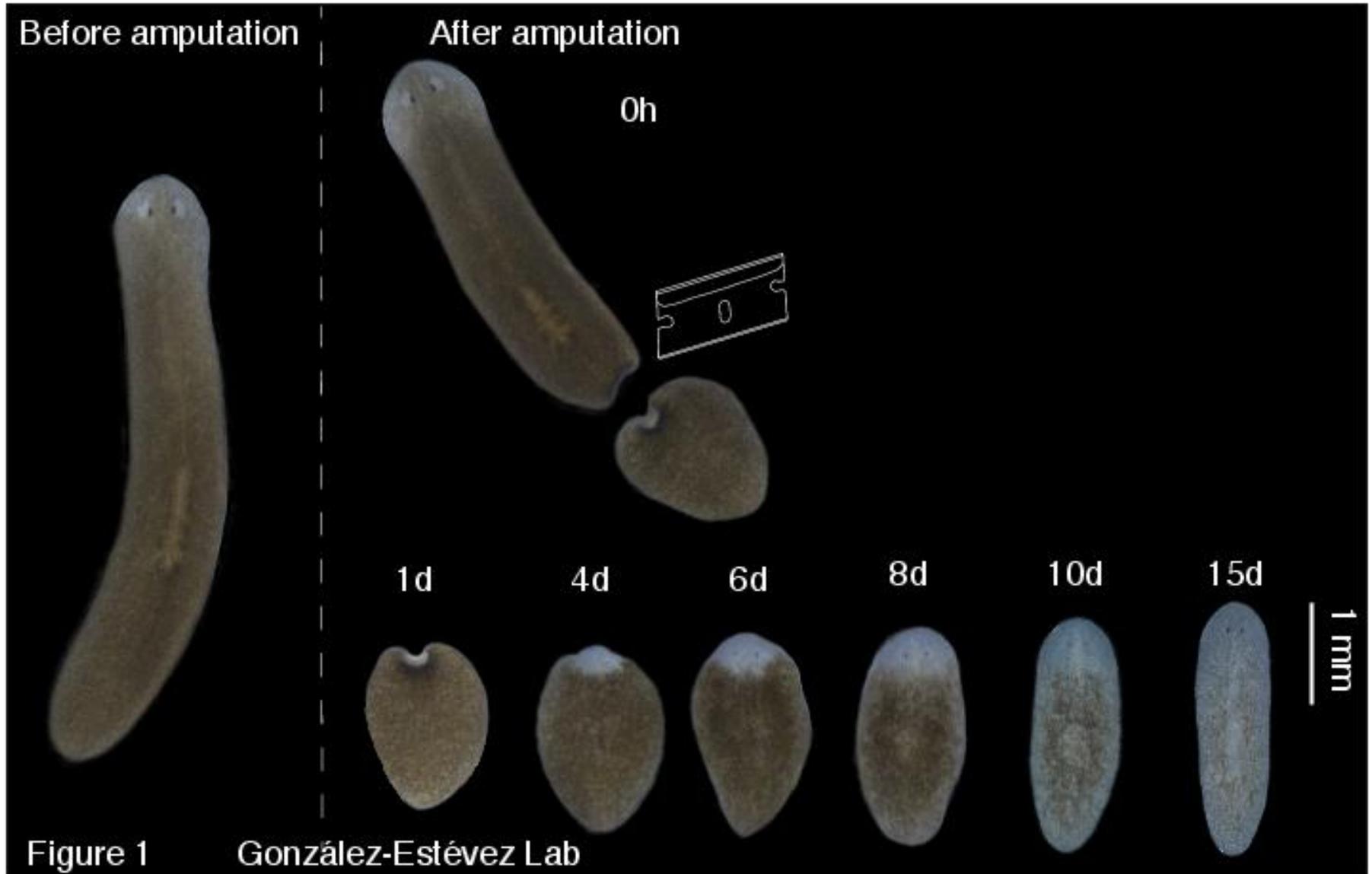
1 mm



Renovación celular y Homeostasis de tejidos



Regeneración



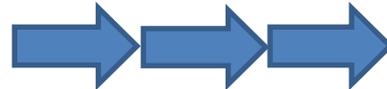


Respuesta contra virus y otros patógenos intracelulares

ARNdc



Proteín Kinasa R (PKR)



**Bloqueo
Traducción
Celular**

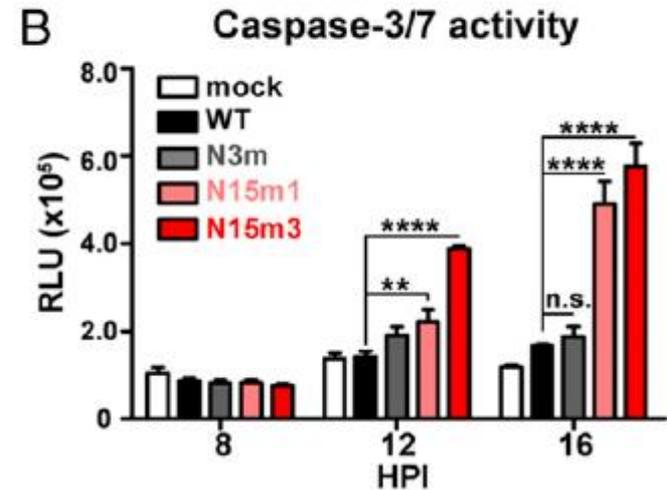
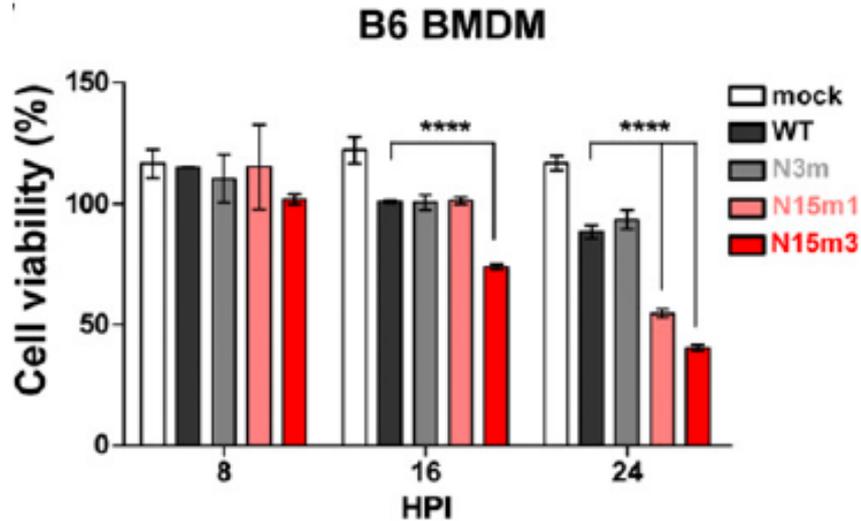
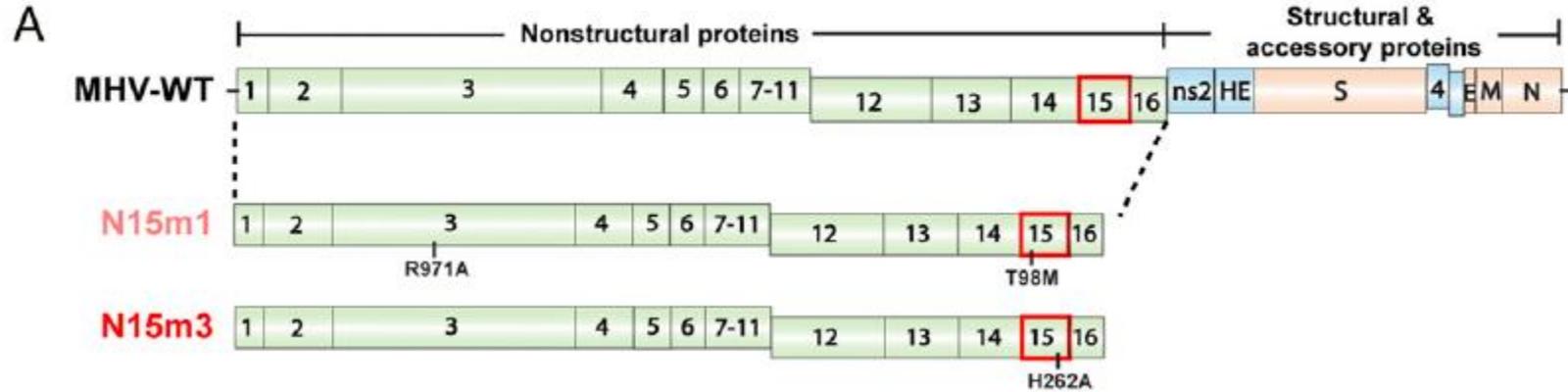


Activación de Caspasas - APOPTOSIS

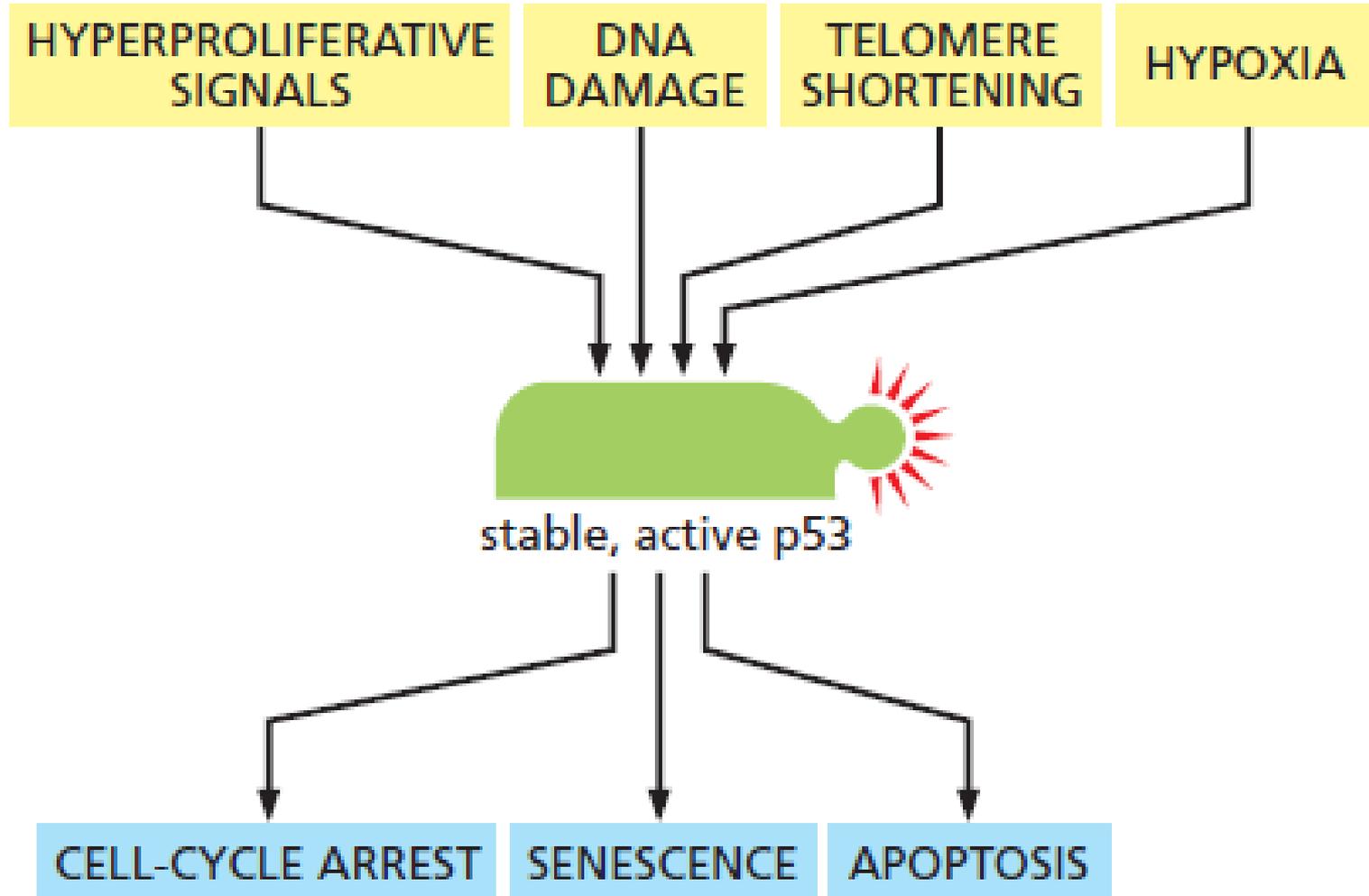


Respuesta contra virus y otros patógenos intracelulares

La proteína nsp15 de los coronavirus bloquea la apoptosis



Eliminación de células con daño al ADN

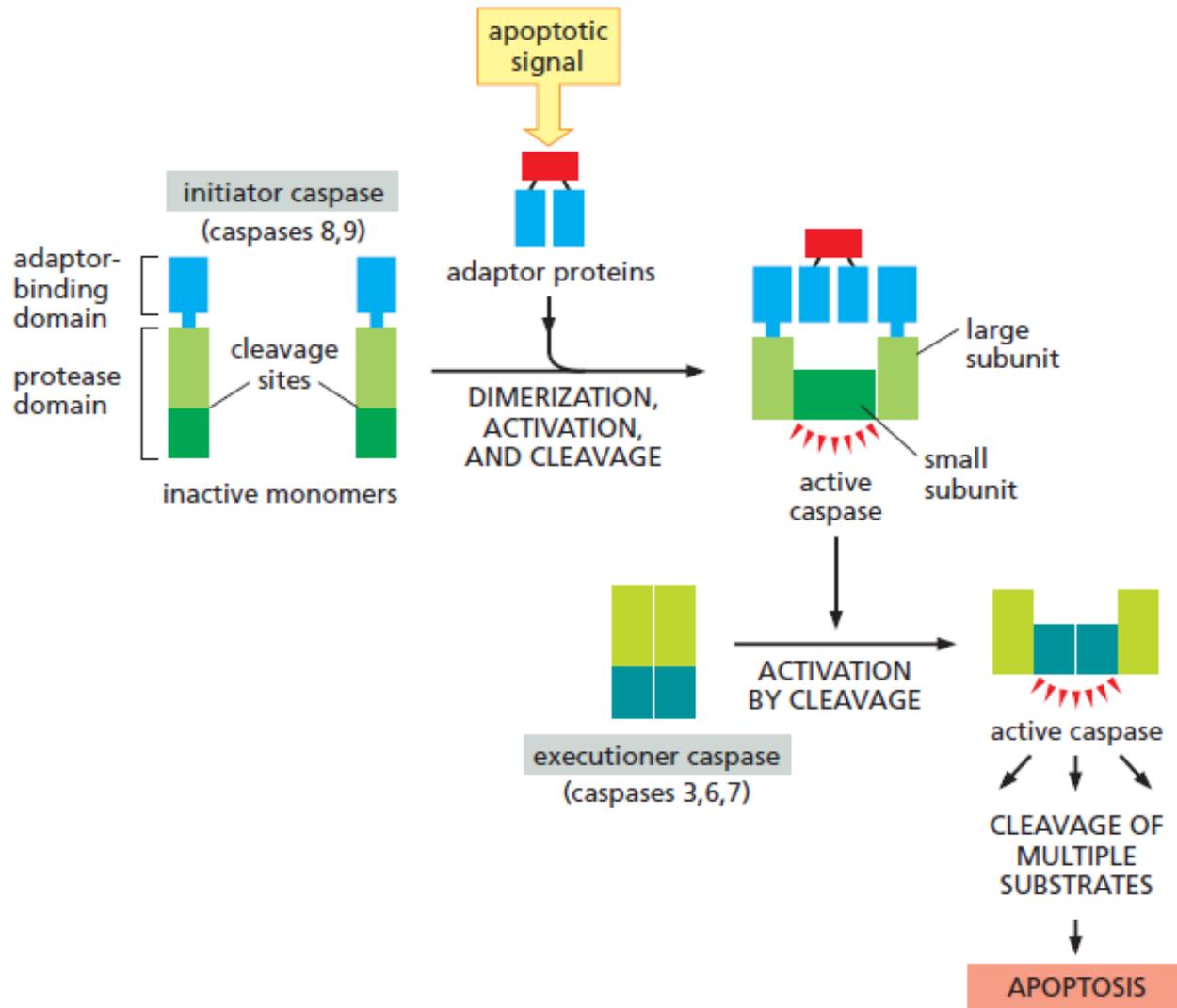


Cómo se regula la apoptosis?

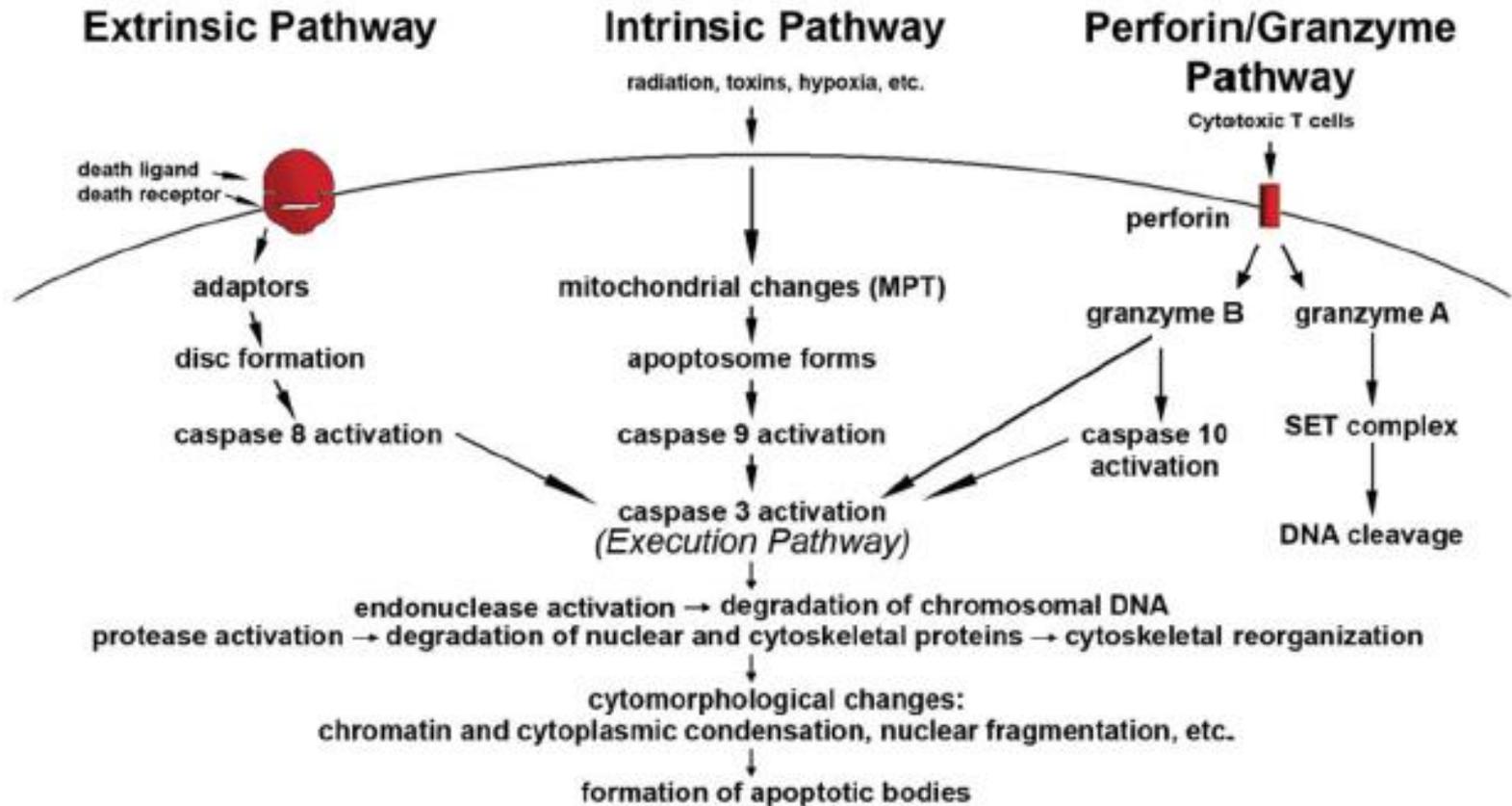


Mecanismos reguladores de la apoptosis:

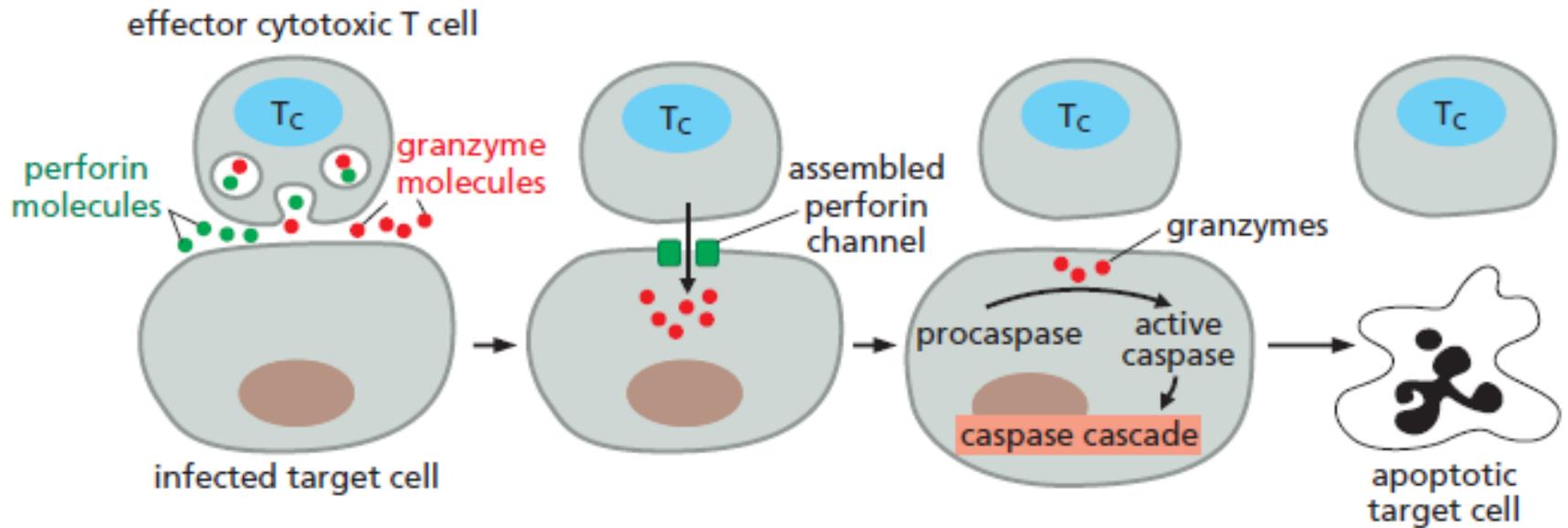
Caspasas



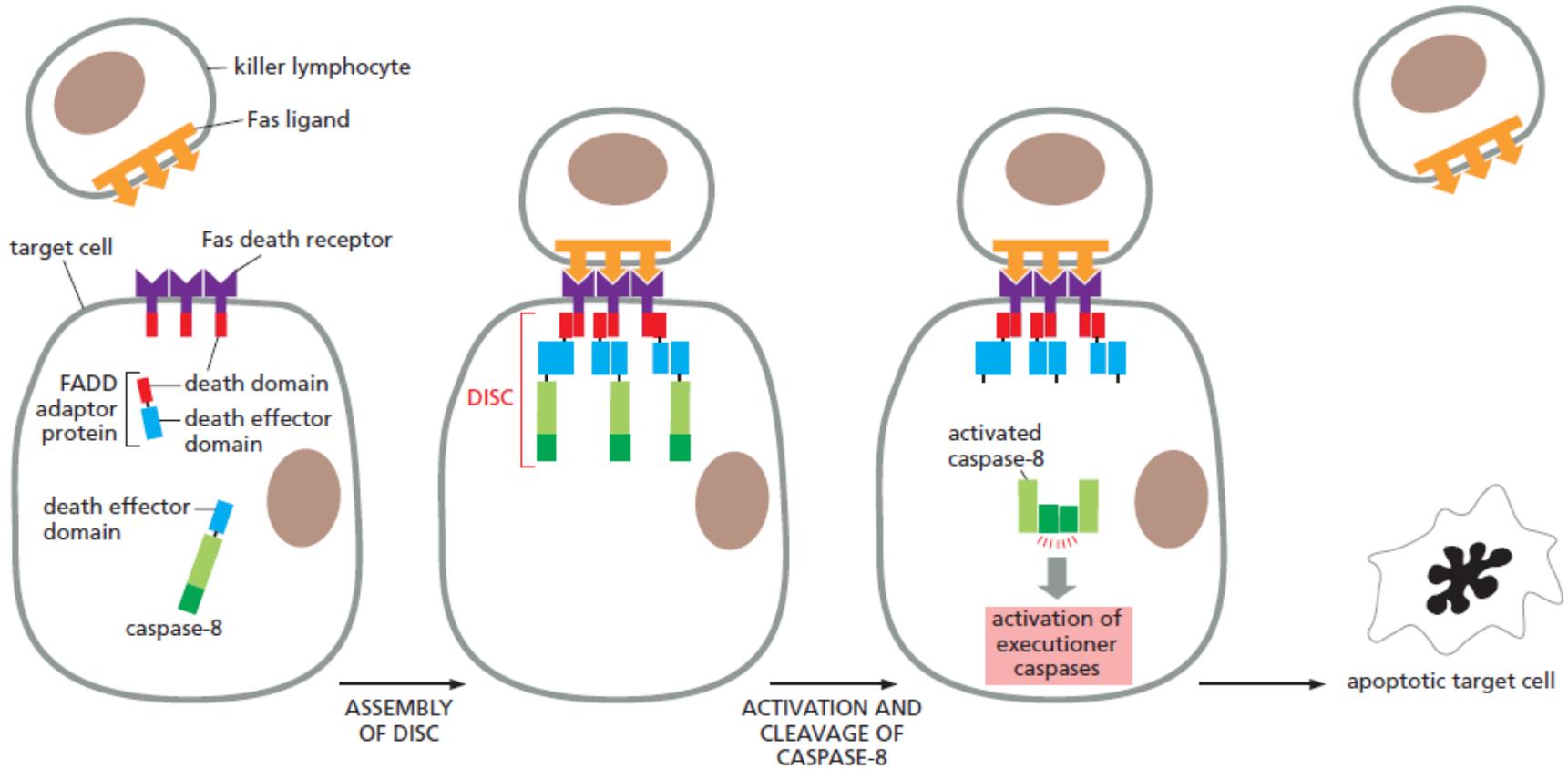
Mecanismos reguladores de la apoptosis: 3 vías principales que convergen



Apoptosis: Vía de perforina/granzima

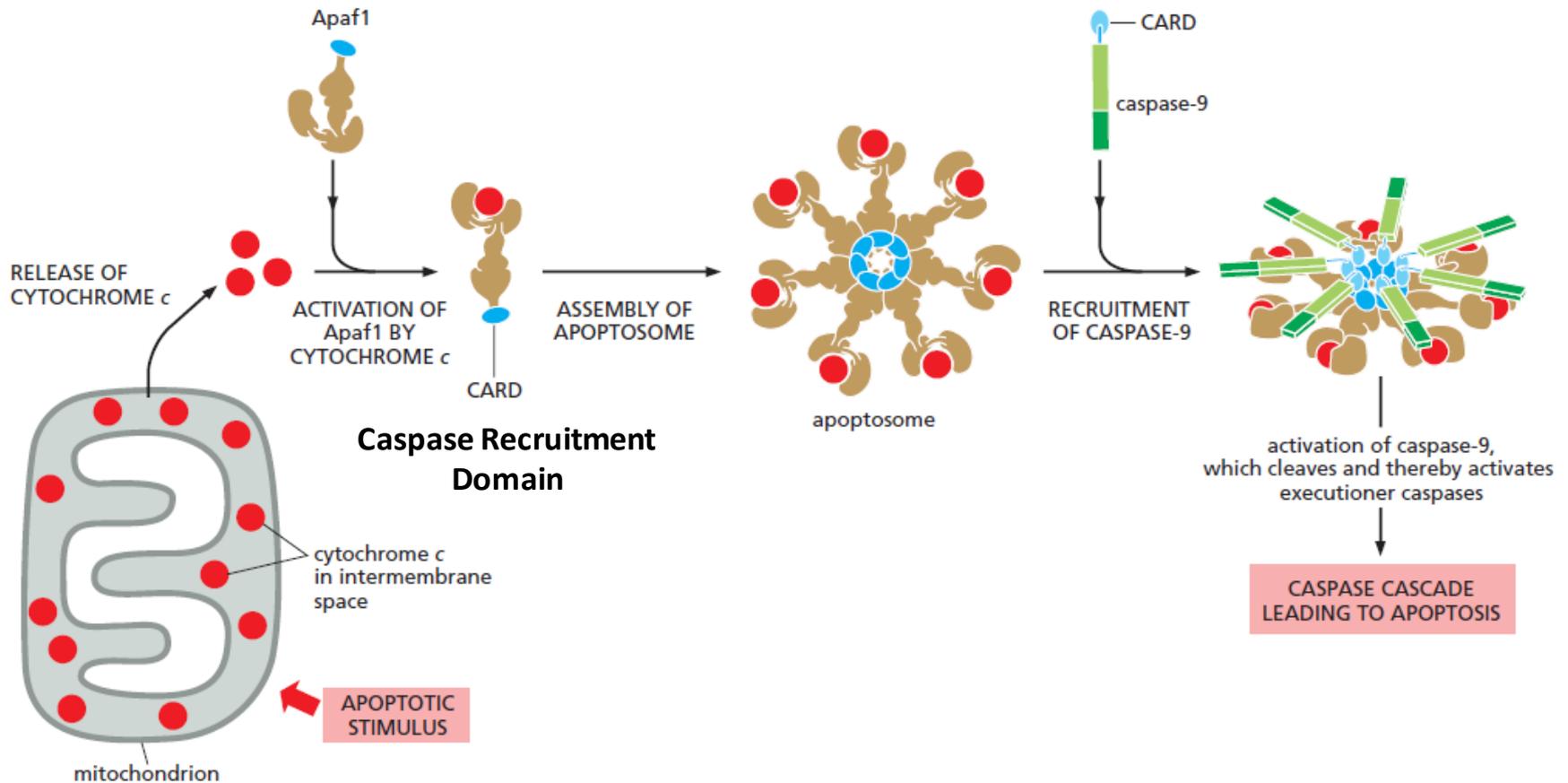


Apoptosis: Vía Extrínseca



Apoptosis: Vía Intrínseca

Apoptotic protease activating factor-1



Vía intrínseca: regulación

anti-apoptotic
Bcl2 family protein
(e.g., Bcl2, BclX_L)



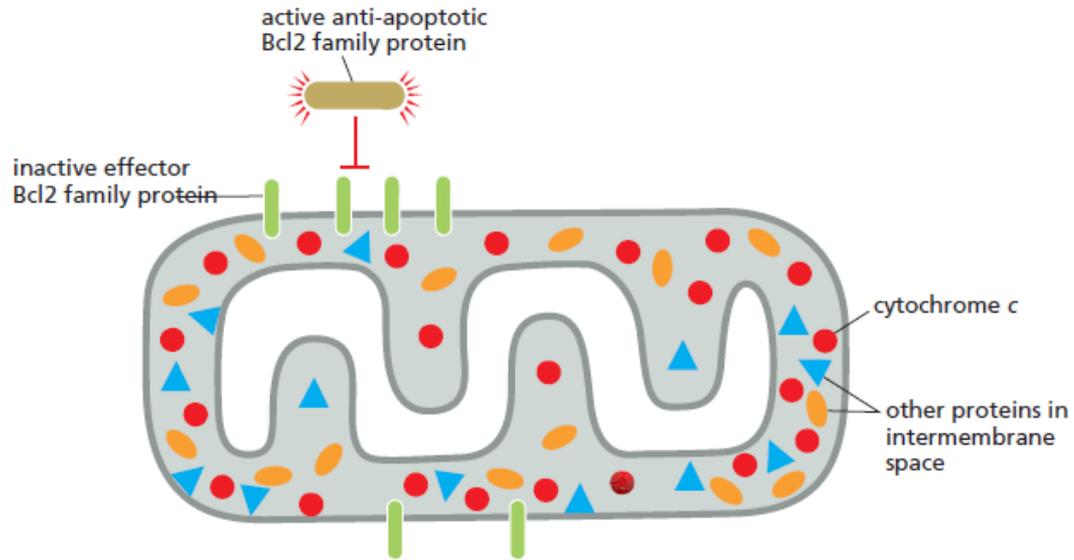
pro-apoptotic
effector Bcl2 family
protein
(e.g., Bax, Bak)



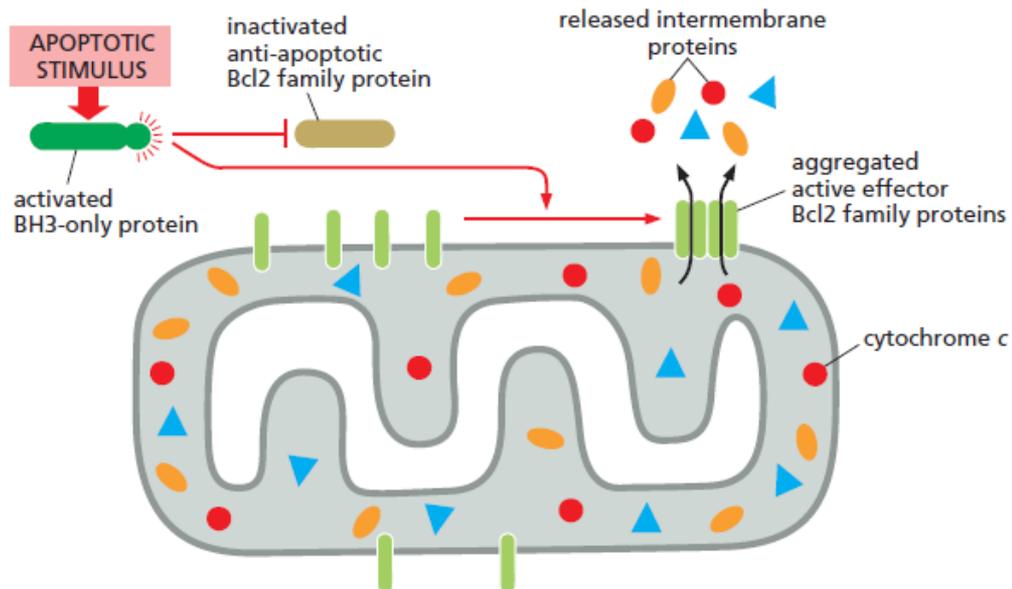
pro-apoptotic
BH3-only protein
(e.g., Bad, Bim,
Bid, Puma, Noxa)



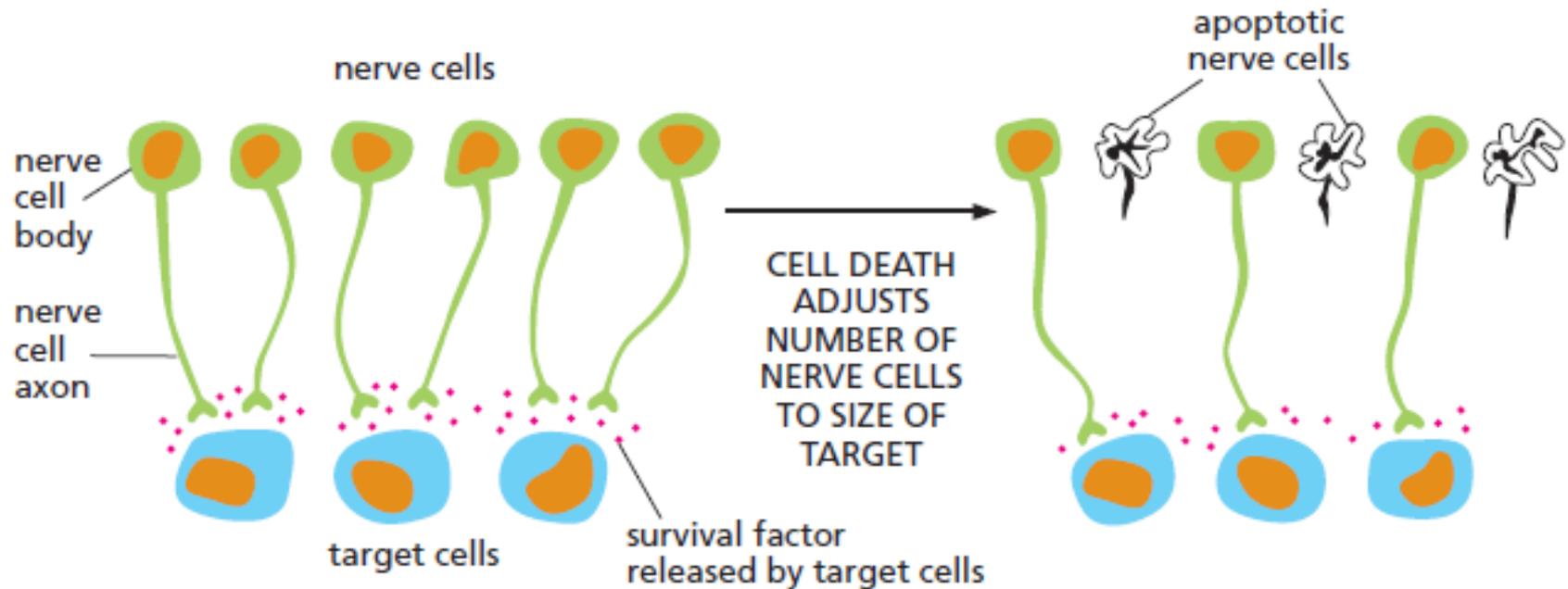
(A) INACTIVE INTRINSIC PATHWAY



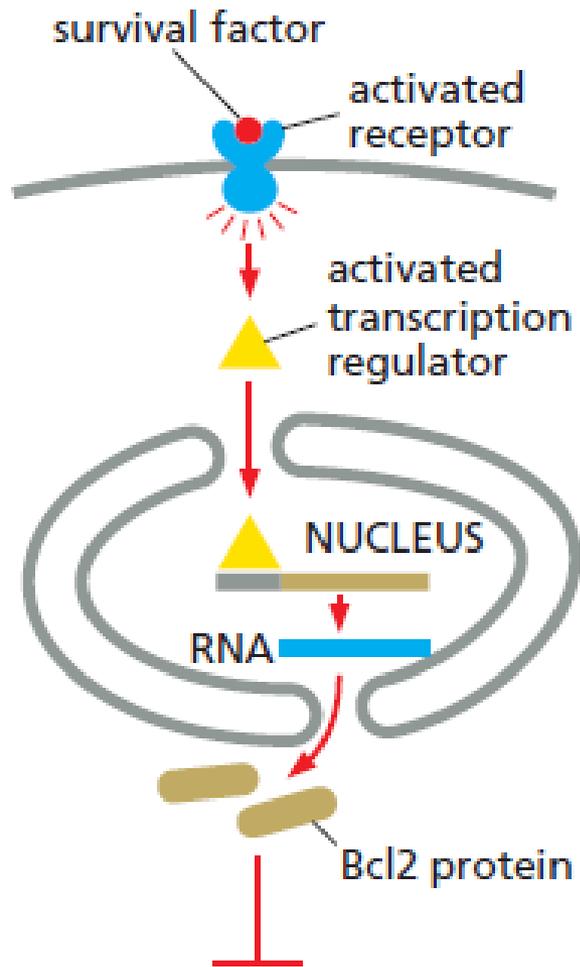
(B) ACTIVATION OF INTRINSIC PATHWAY



Regulación de la apoptosis por factores de supervivencia

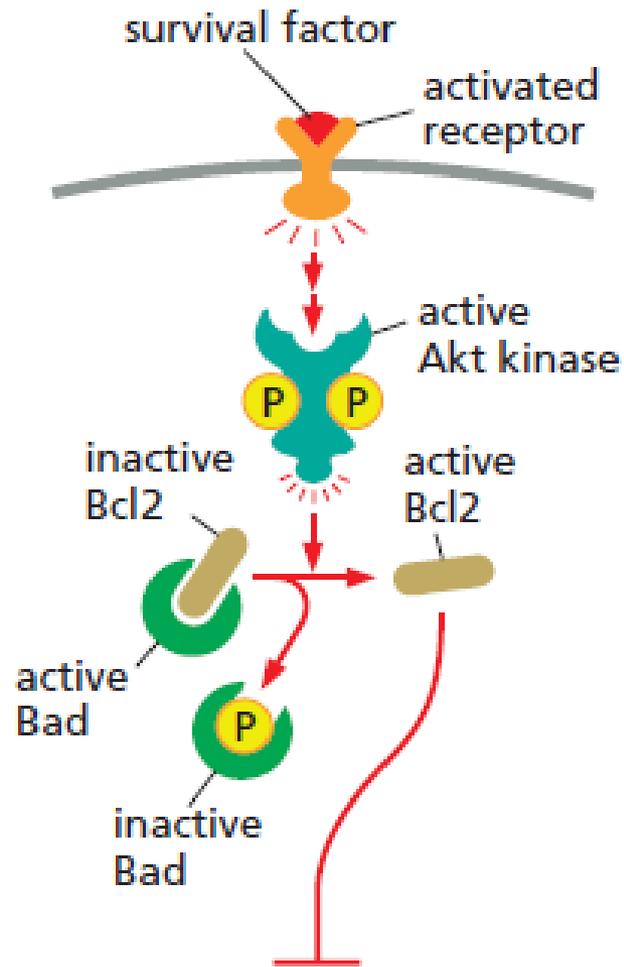


(A) increased production of anti-apoptotic Bcl2 family protein



APOPTOSIS BLOCKED

(B) inactivation of pro-apoptotic BH3-only protein



APOPTOSIS BLOCKED

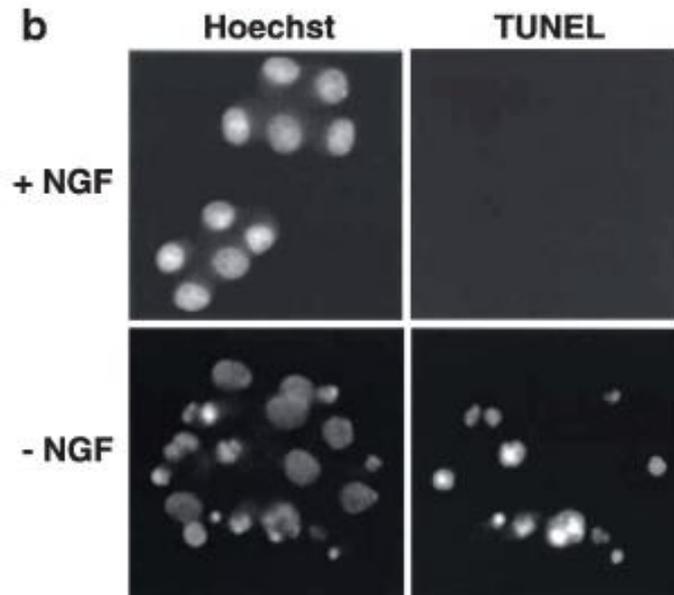
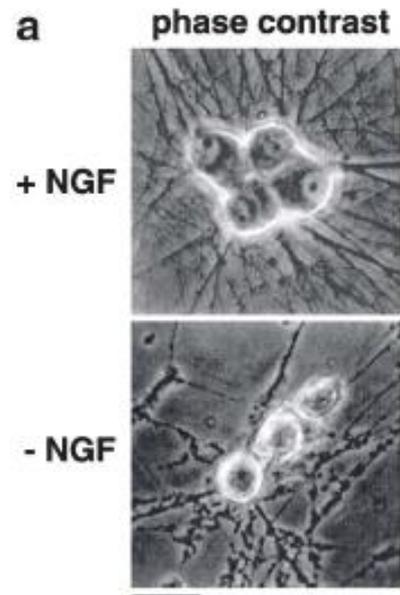


Ejercicio (queda en EVA)

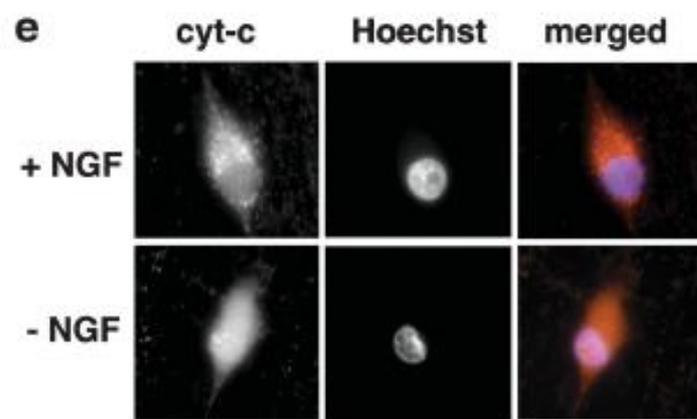
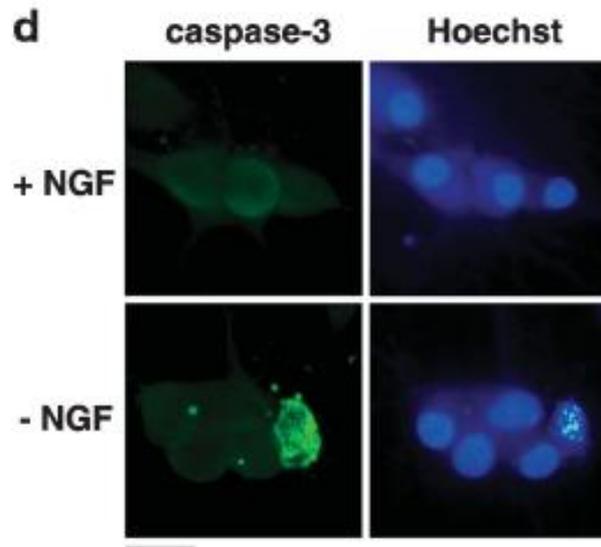


Cultivo de neuronas de ganglio simpático *in vitro* con Nerve Growth Factor (NGF) o sin NGF

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



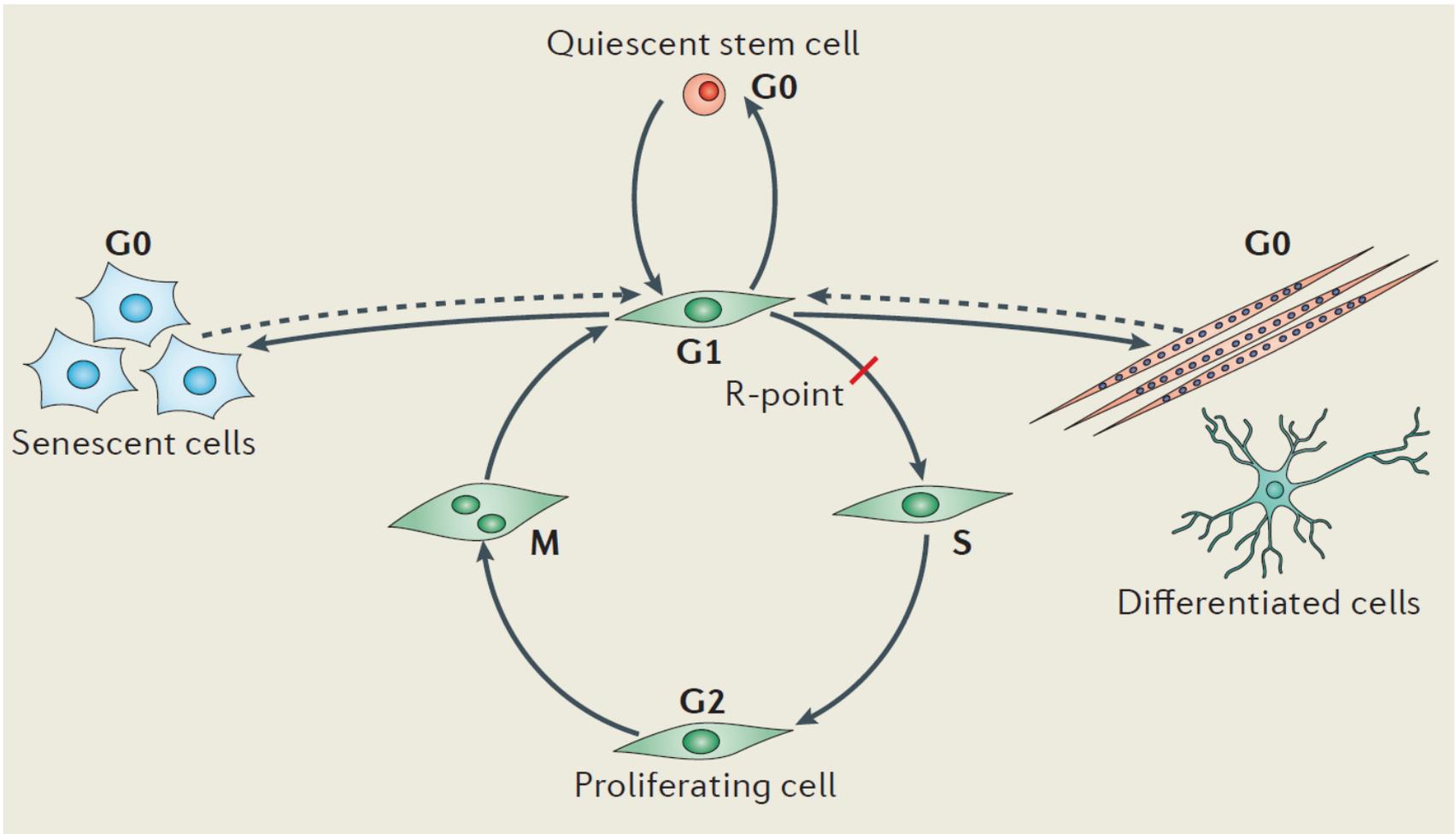
Immunofluorescencias

Anticuerpo detecta citocromo C

Anticuerpo detecta caspasa 3 activada



Senescencia celular

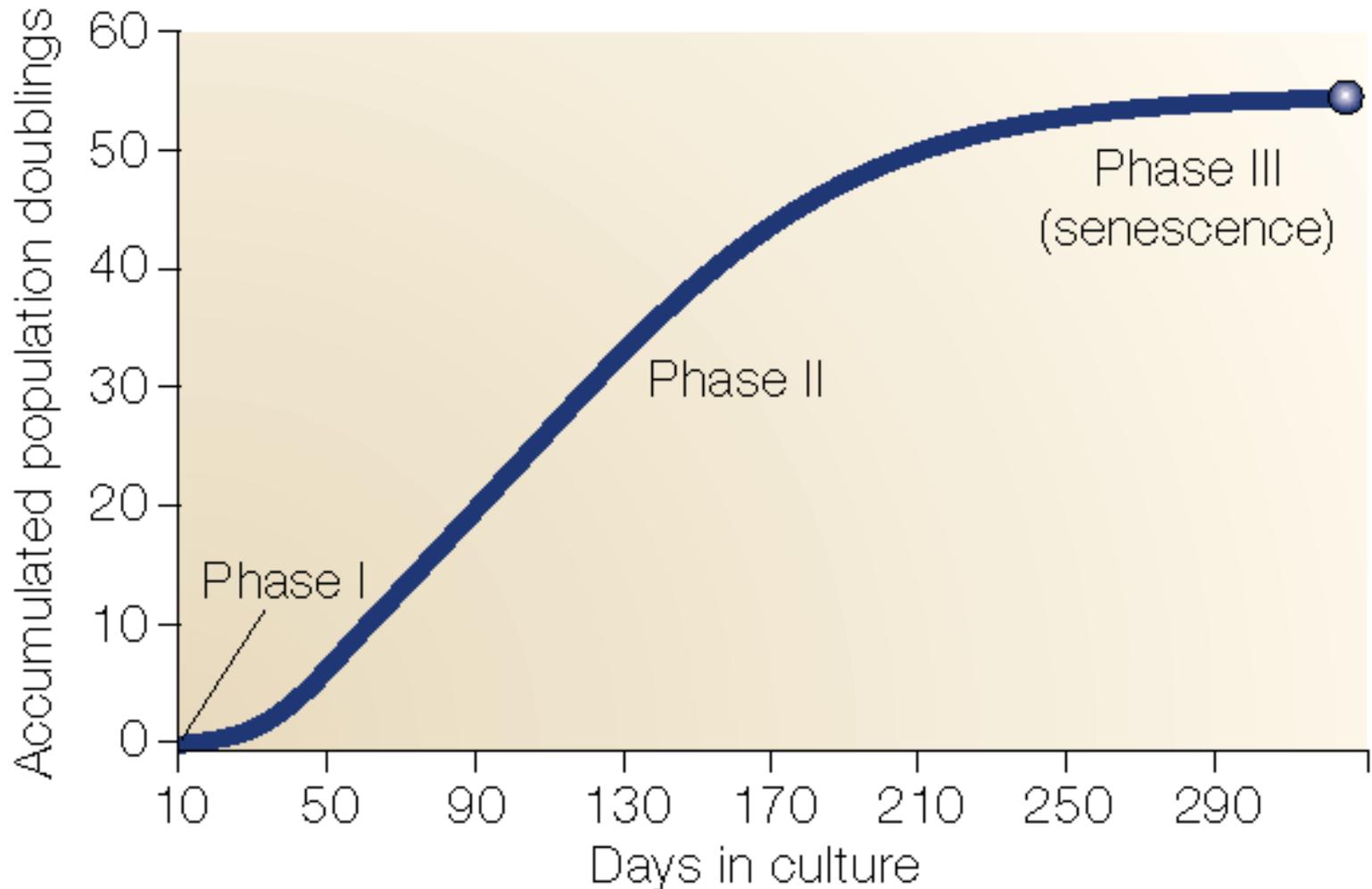


Senescencia celular

- 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*

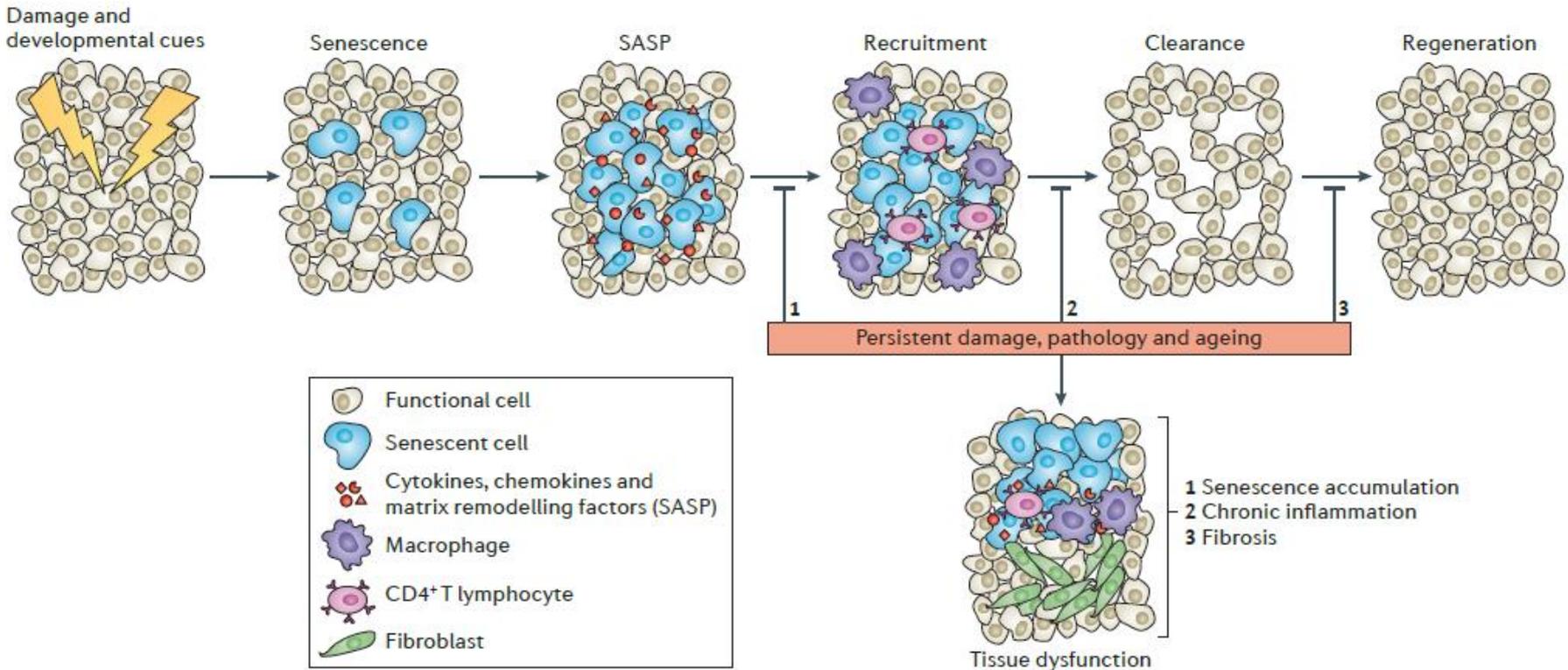


Figure 3 | Unified model of senescence. Senescence initiates a tissue remodelling process by recruiting immune cells through the senescence-associated secretory phenotype (SASP). Macrophages clear the senescent cells, and progenitor cells repopulate and regenerate the damaged tissue. This sequence of 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

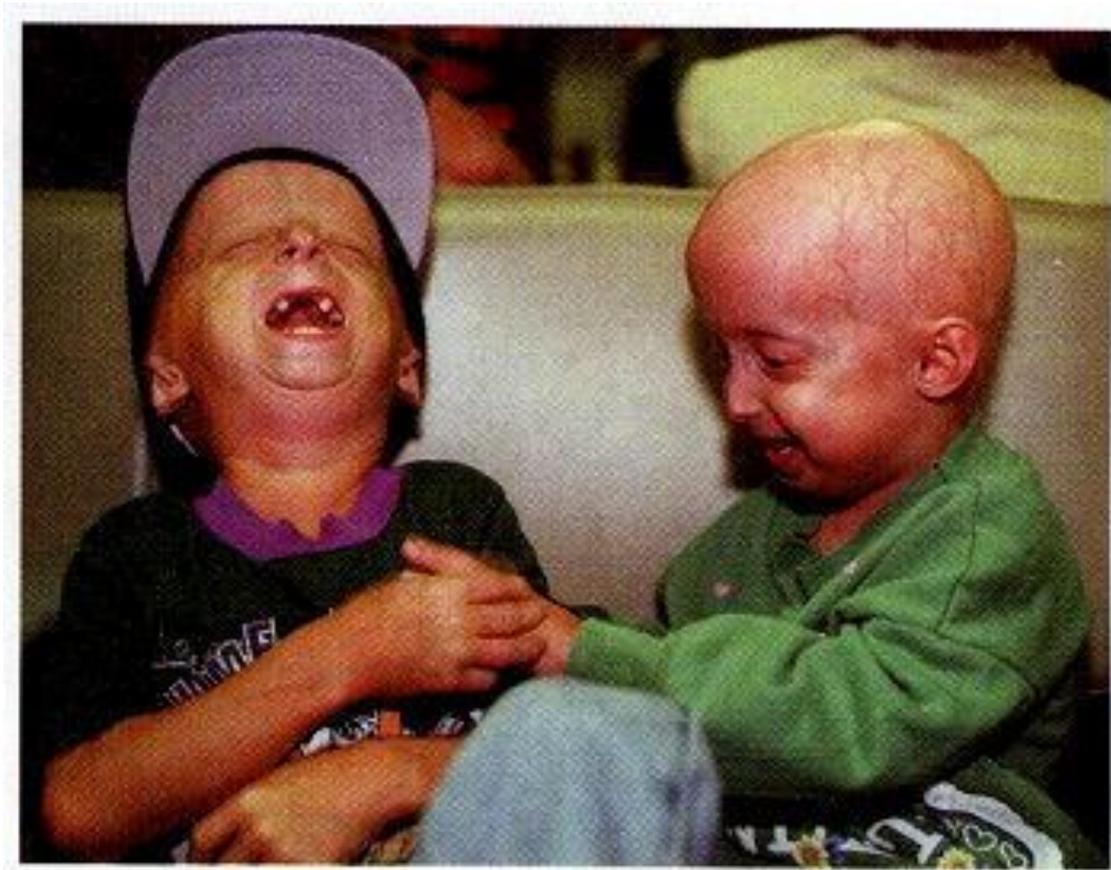
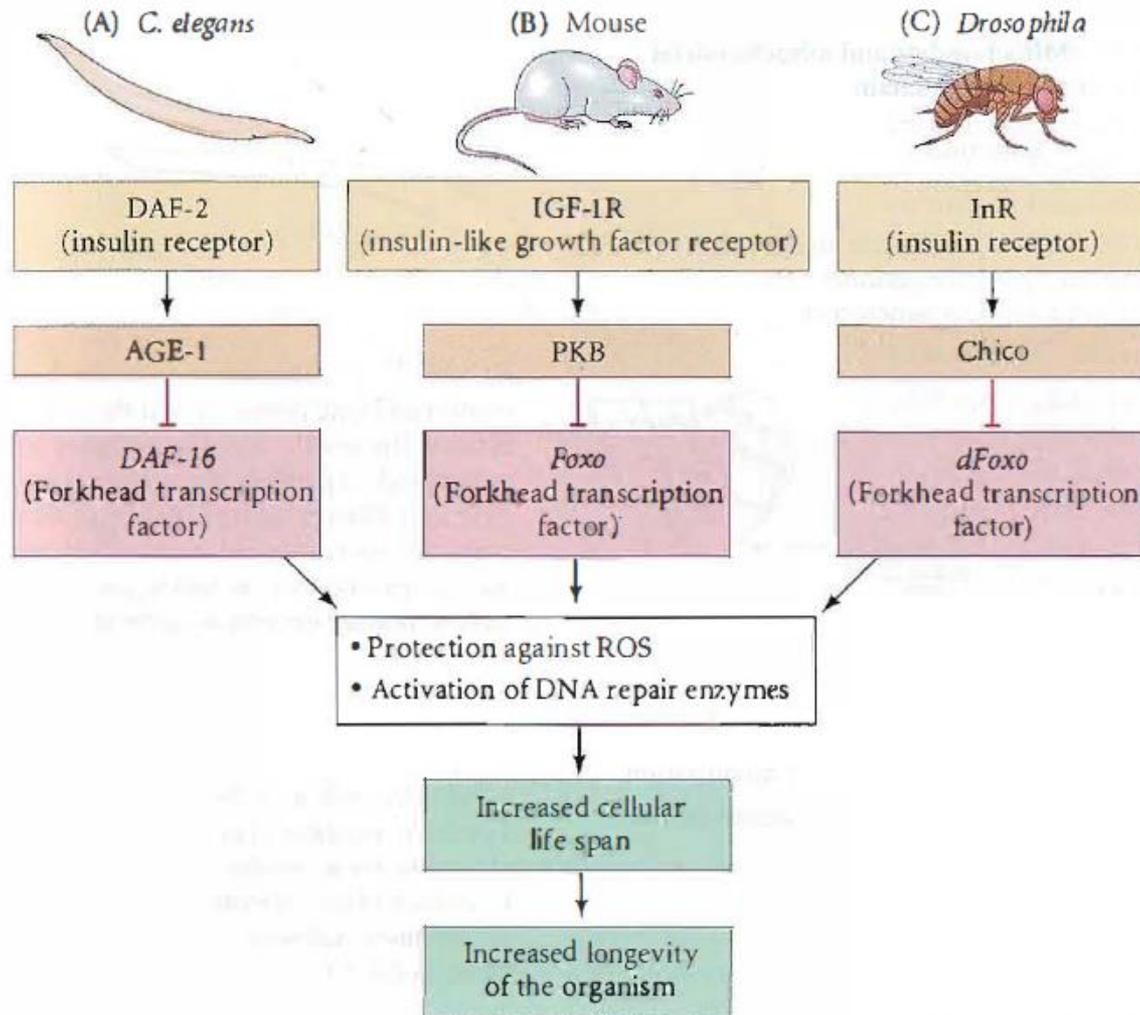


TABLE 5–2 Some Inherited Human Syndromes with Defects in DNA Repair

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?

