Immortalisation et cancers

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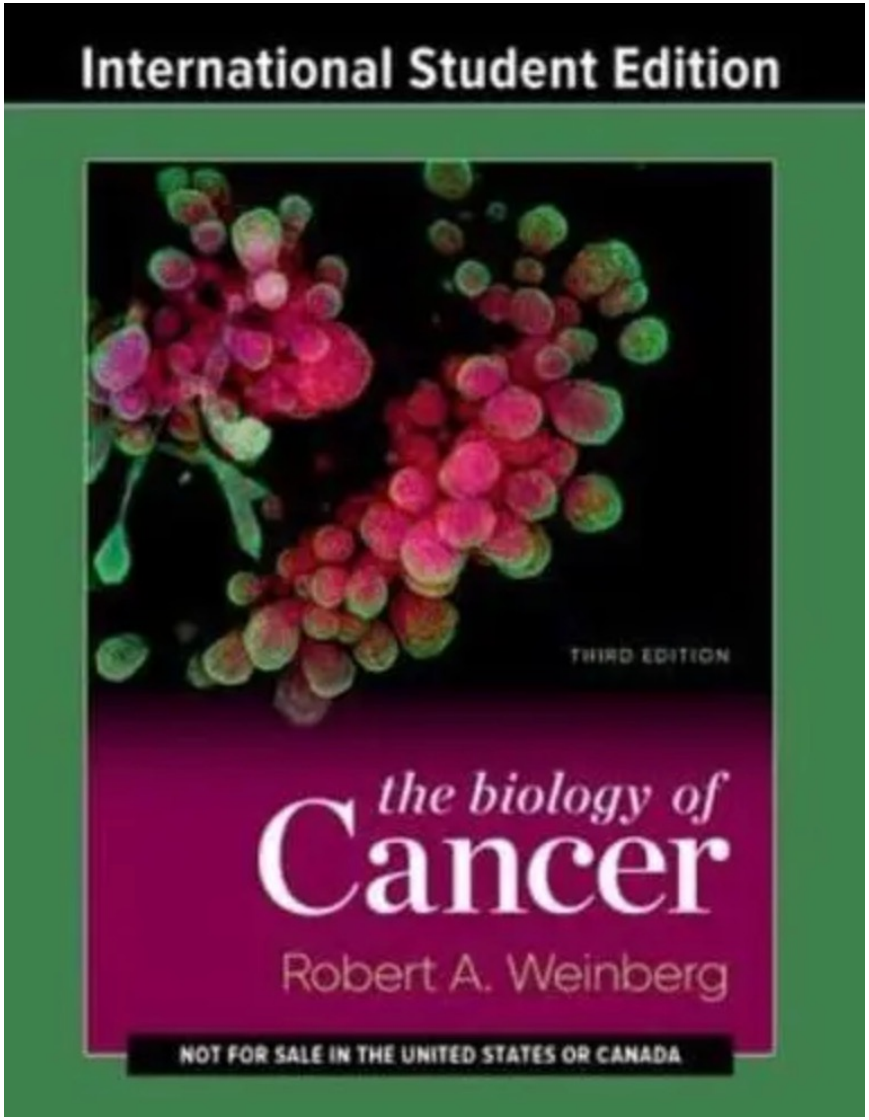
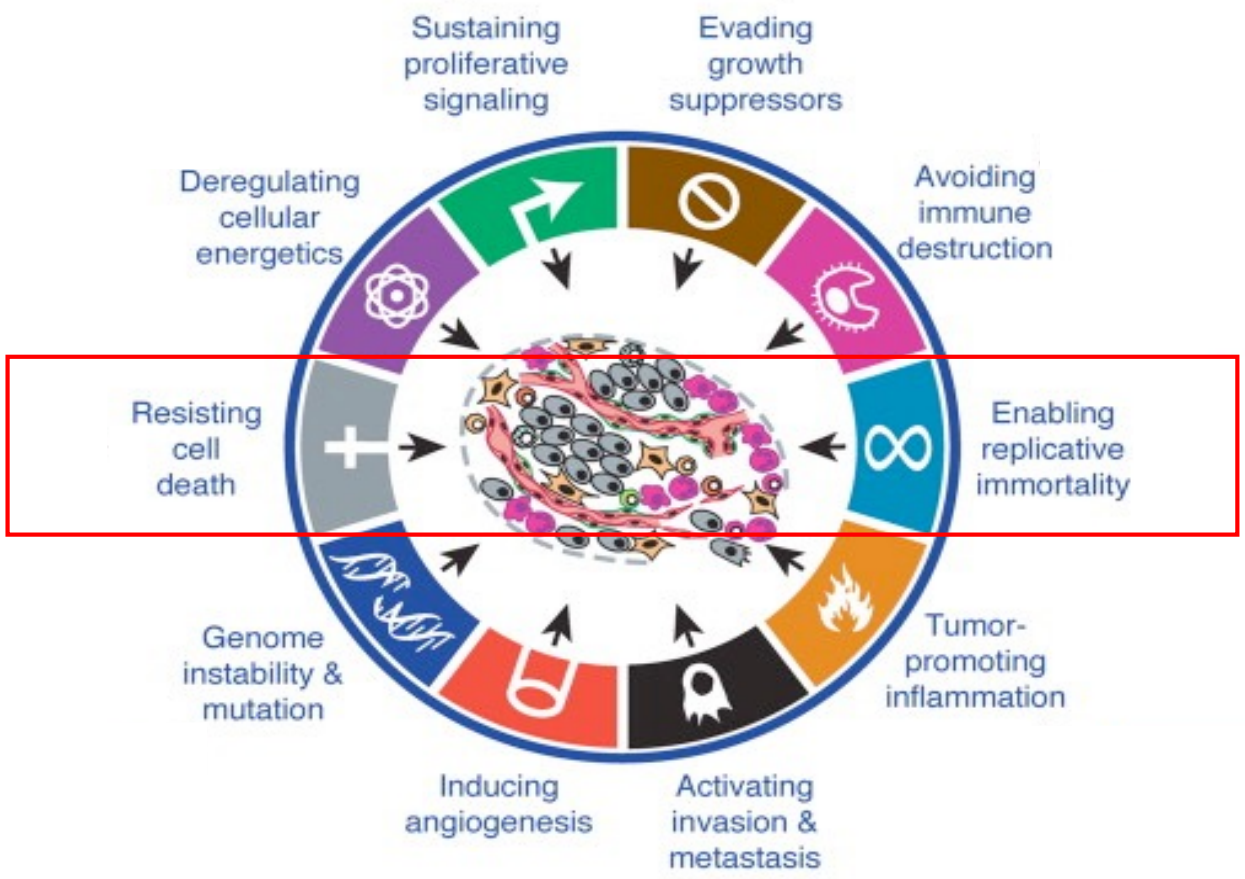
Lecture recommandée



The Hallmarks of Cancer



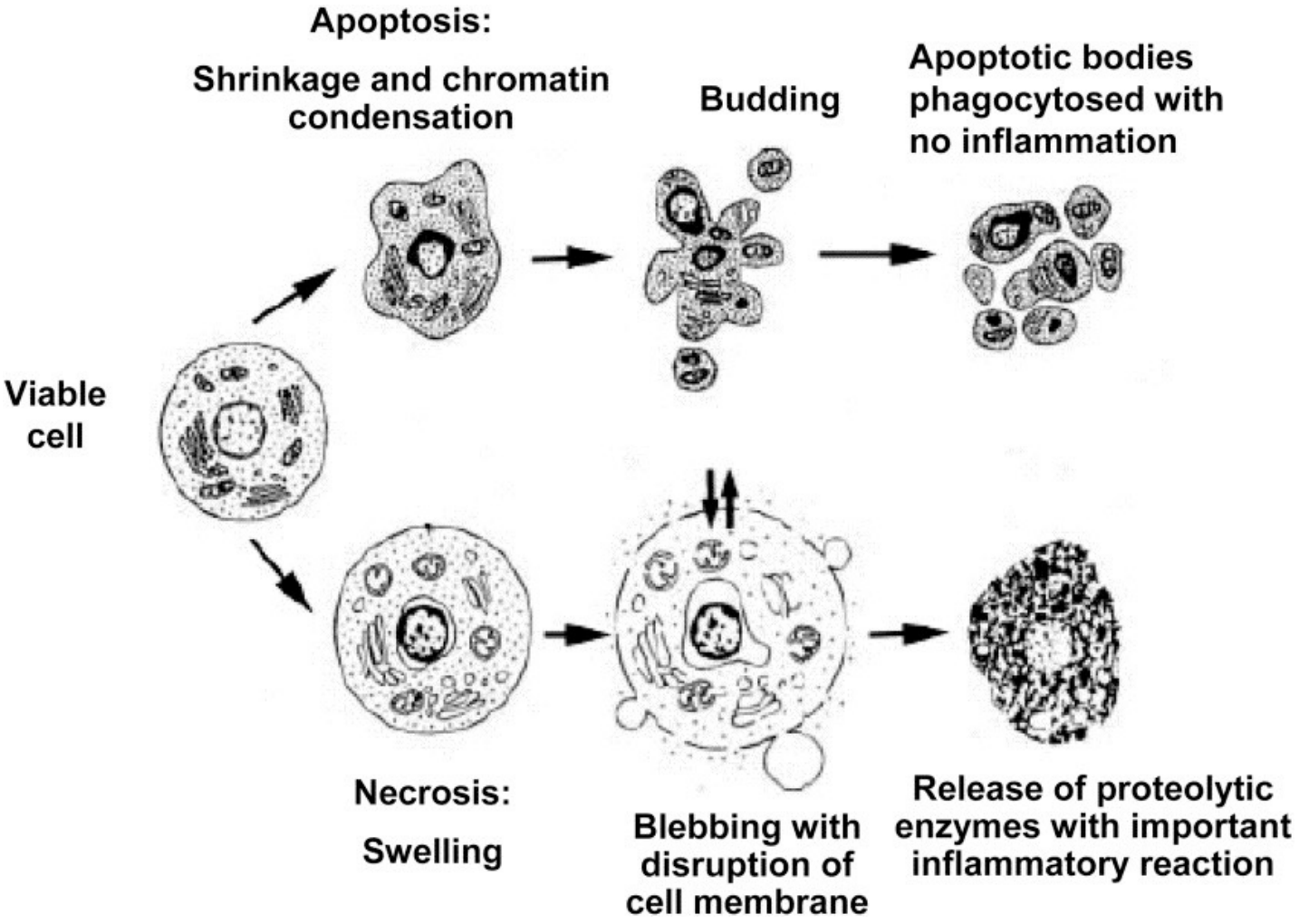
Hanahan & Weinberg, Cell, 2011

I/ Echapper à la mort cellulaire

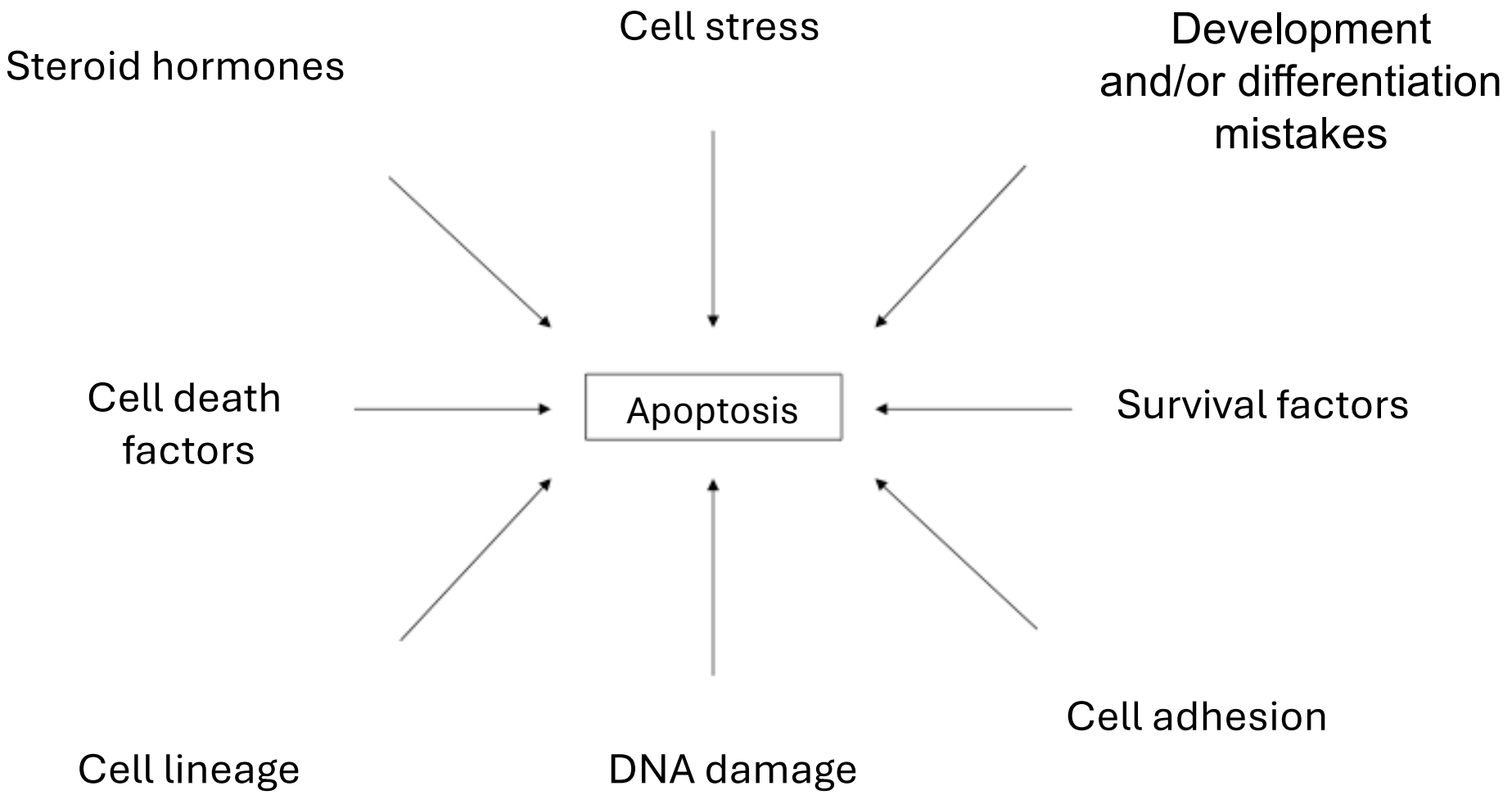
Il existe différentes formes de morts cellulaires:

- Nécrose et Nécroptose
- Apoptose
- Mort cellulaire autophagique
- Pyroptose
- Ferroptose

Apoptosis vs Necrosis



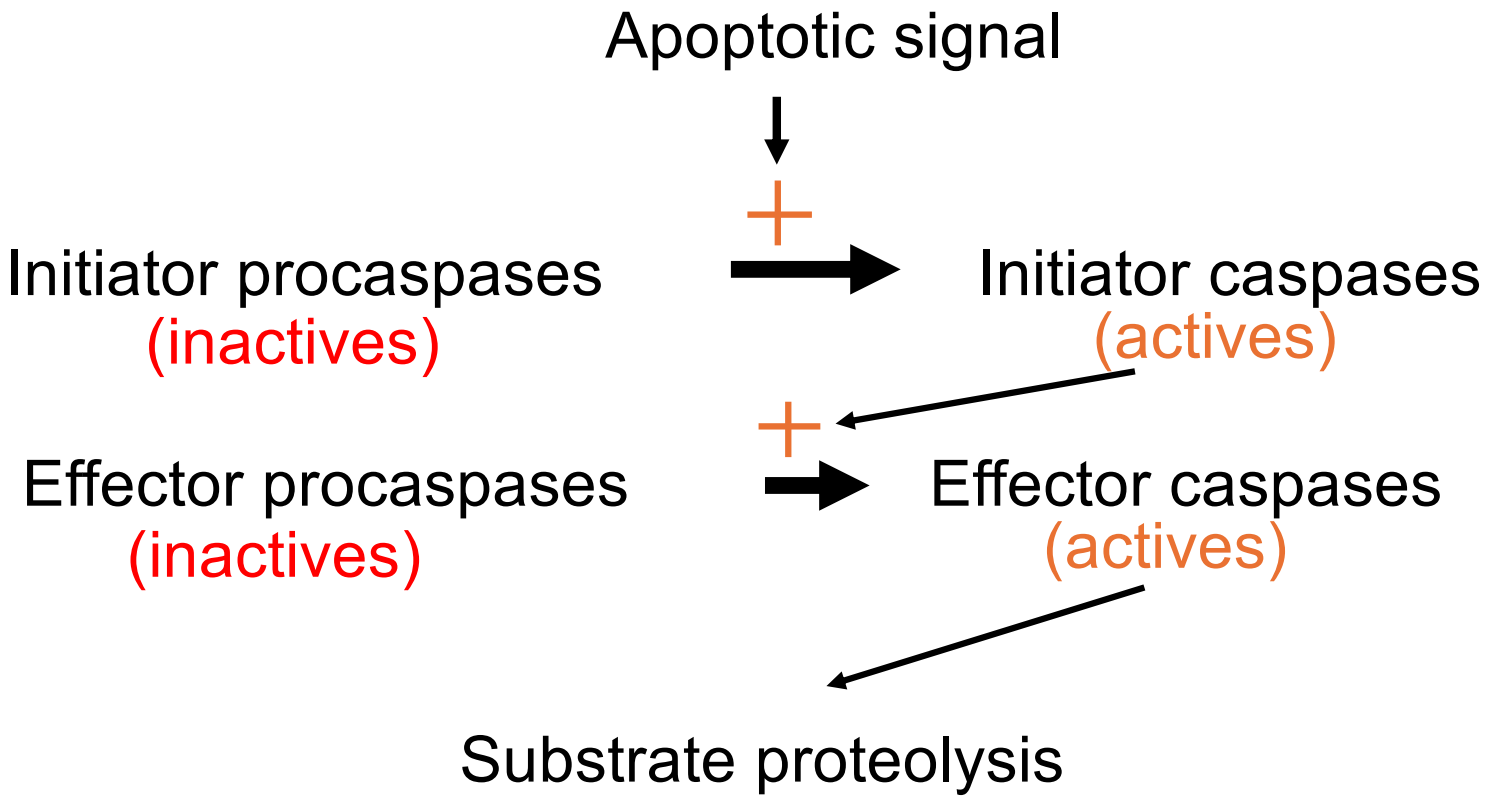
Causes of Apoptosis



Key actors of apoptosis

Caspases are the executioners of the canonical apoptosis!

- Initiator caspases autoactivate in response to apoptotic signals
- activated initiators activate other procaspases: effector caspases (amplification in cascade)
- Effector caspases cleave many cytoplasmic or nuclear target proteins
- Different levels of control exist



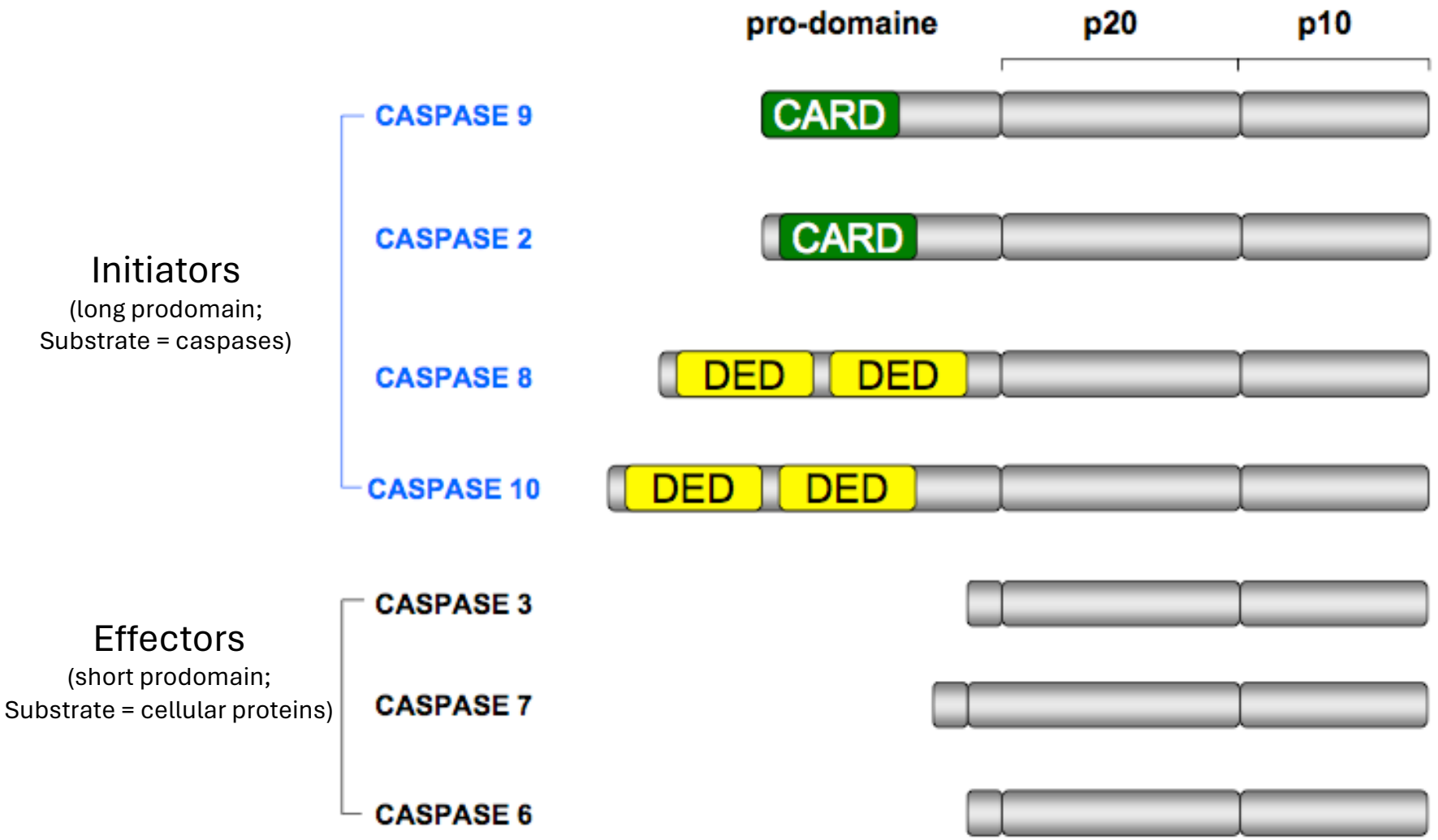
CASPASES (Cystine ASPartyl-specific proteASES)

Caspases = cystine proteases

Catalytic site : a well conserved pentapeptide **QAC<u>X</u>G**
Cleavage site: Cterminus of an Aspartate (D) residue

Specificity group[a]	P_4–P_1 specificity[b]	Consensus	Proposed role
Group I			
Caspase-1 (ICE)	WEHD	WEHD	Maturation of multiple pro-inflammatory cytokine
Caspase-4 (ICE_{rel}-II, TX, ICH-2)	(W/L)EHD		
Caspase-5 (ICE_{rel}-III, TY)	(W/L)EHD		
Group II			
Caenorhabditis elegans CED-3	DETD	DExD	Cleavage of DxxD apoptotic substrates
Caspase-3 (CPP32, apopain, Yama)	DEVD		
Caspase-7 (Mch3, ICE-LAP3, CMH-1)	DEVD		
Caspase-2 (ICH-1)	DEHD		
Group III			
Caspase-6 (Mch2)	VEHD	(IVL)ExD	Activation of group-II caspases, activation of other group-III caspases, cleavage of non-DxxD apoptotic structures
Caspase-8 (MACH, FLICE, Mch5)	LETD		
Caspase-9 (ICE-LAP6, Mch6)	LEHD		
Granzyme B	IEPD		

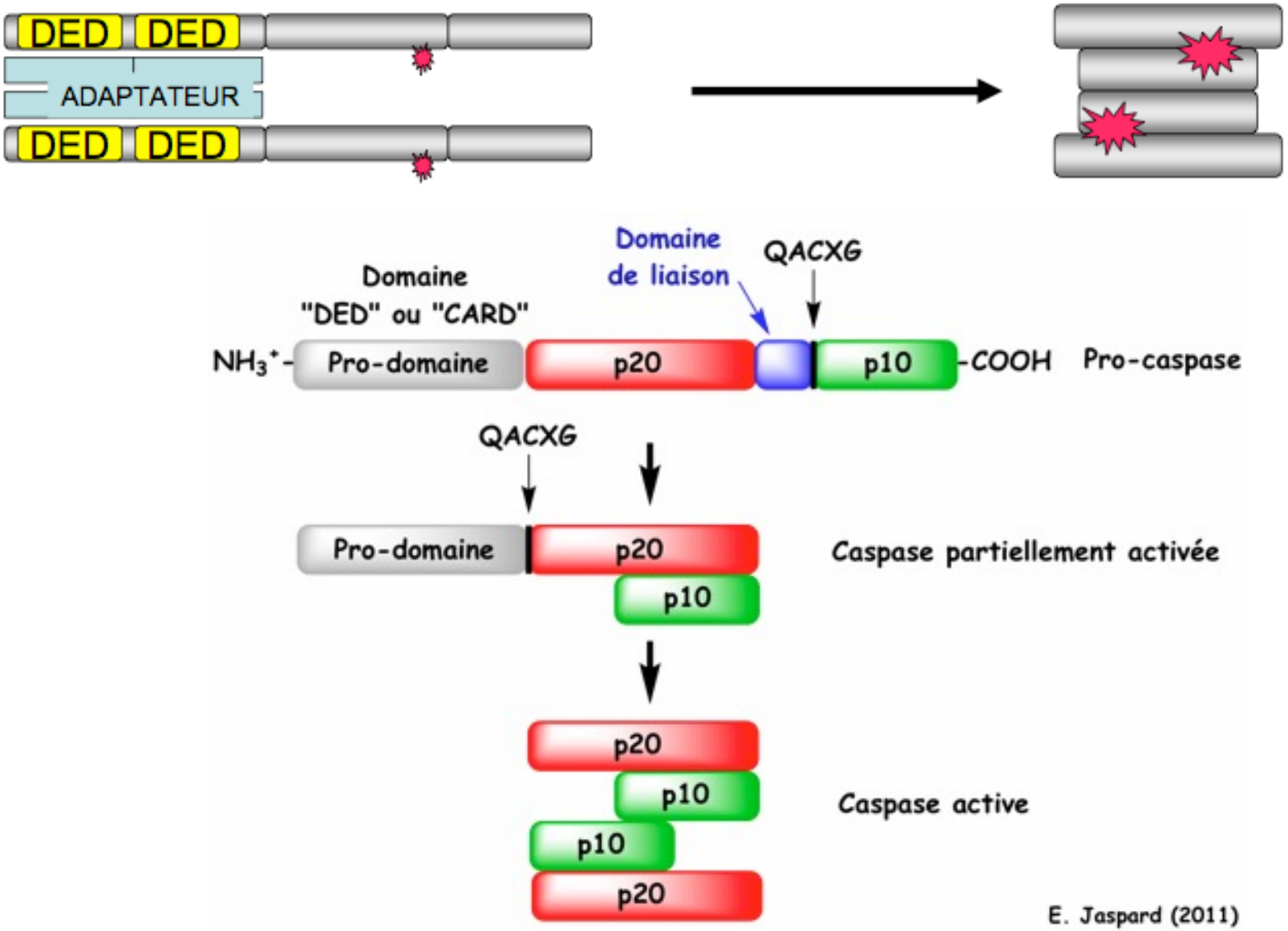
P4

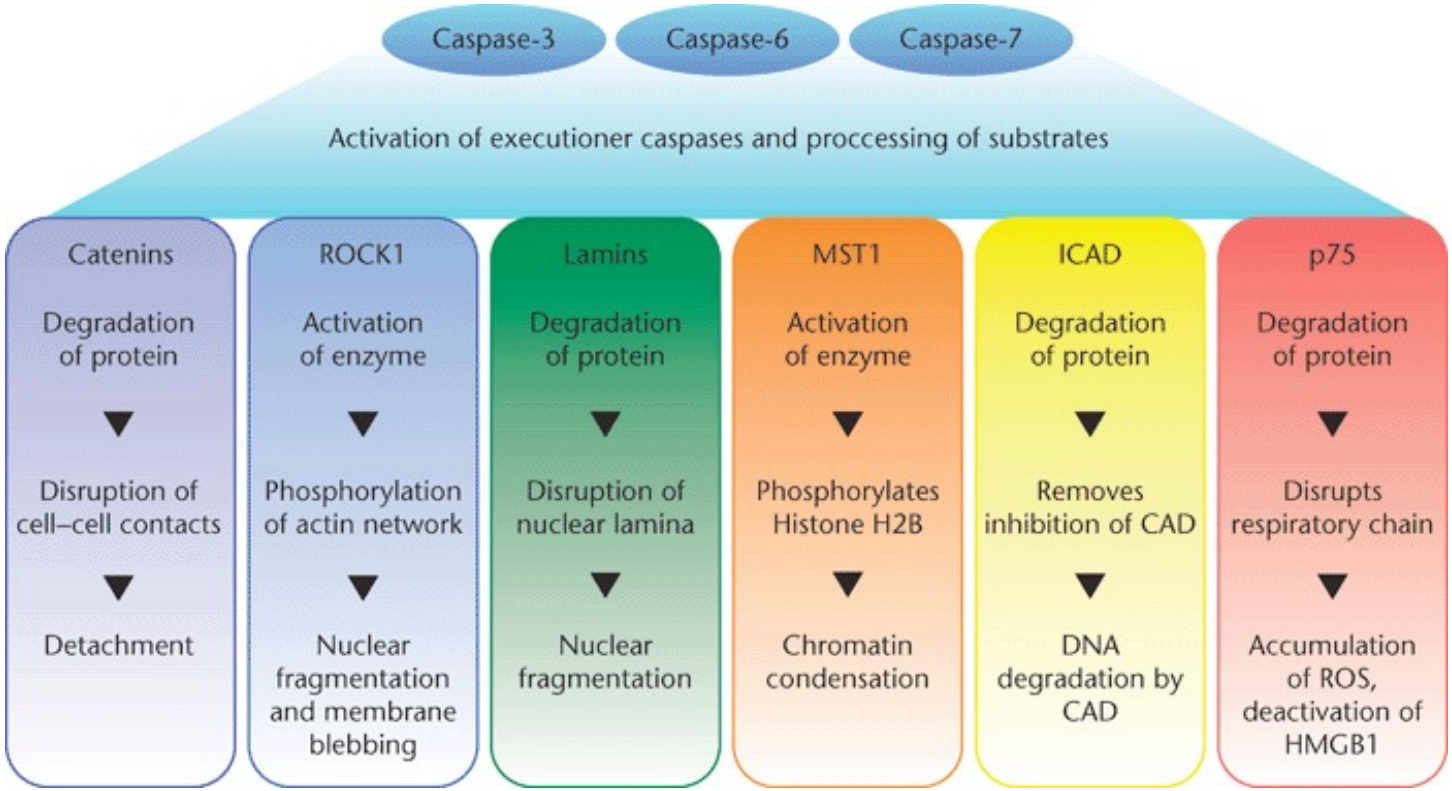


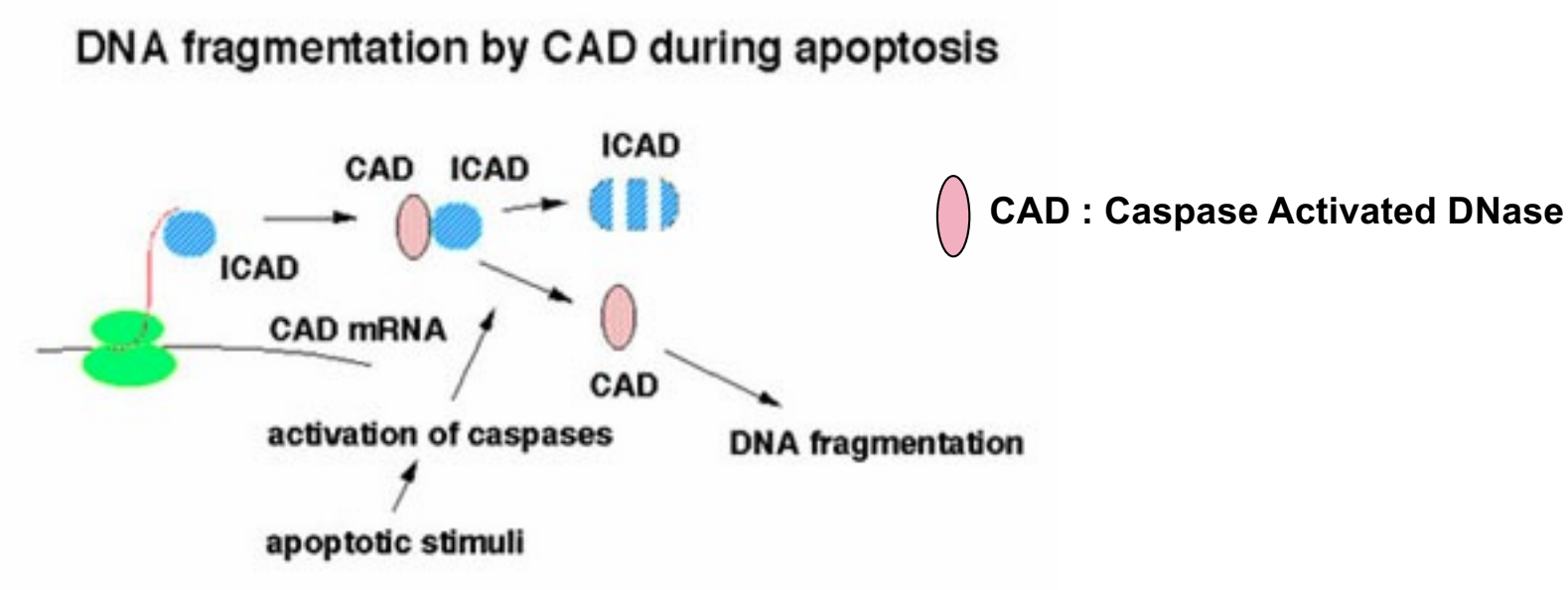
Two distinct, but structurally related propeptides have been identified:
The caspase recruitement domain (CARD) and the death effector domain (DED)

Both domains typically facilitate interaction with proteins that contain the same motifs.

Activation of initiators by dimerization

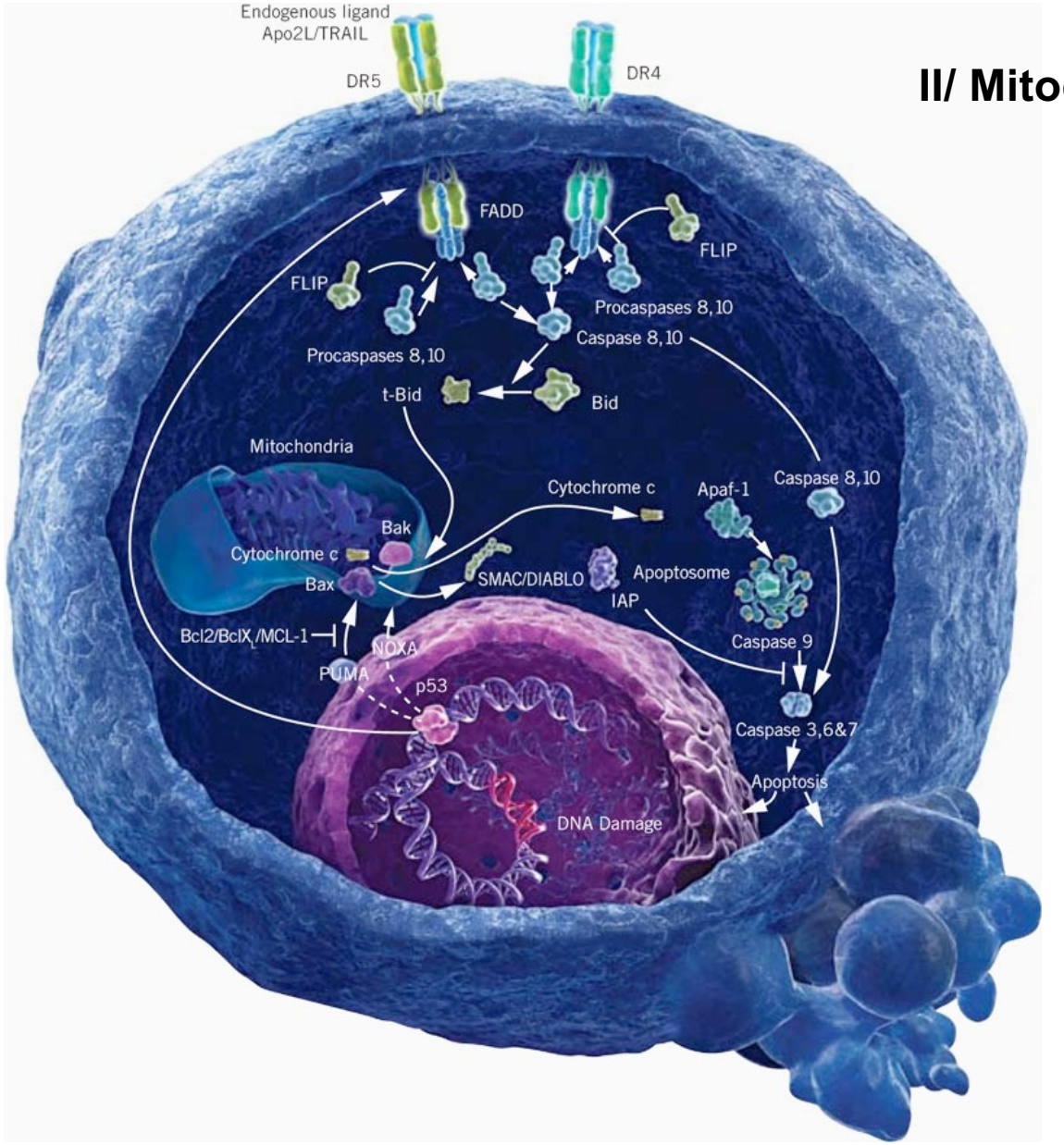



Caspase-3
Caspase-6
Caspase-7
Activation of executioner caspases and proccessing of substrates
Catenins
Degradation of protein
Disruption of cell–cell contacts
Detachment
ROCK1
Activation of enzyme
Phosphorylation of actin network
Nuclear fragmentation and membrane blebbing
Lamins
Degradation of protein
Disruption of nuclear lamina
Nuclear fragmentation
MST1
Activation of enzyme
Phosphorylates Histone H2B
Chromatin condensation
ICAD
Degradation of protein
Removes inhibition of CAD
DNA degradation by CAD
p75
Degradation of protein
Disrupts respiratory chain
Accumulation of ROS, deactivation of HMGB1


DNA fragmentation by CAD during apoptosis
CAD
ICAD
ICAD
ICAD
CAD mRNA
CAD
activation of caspases
DNA fragmentation
apoptotic stimuli
CAD : Caspase Activated DNase

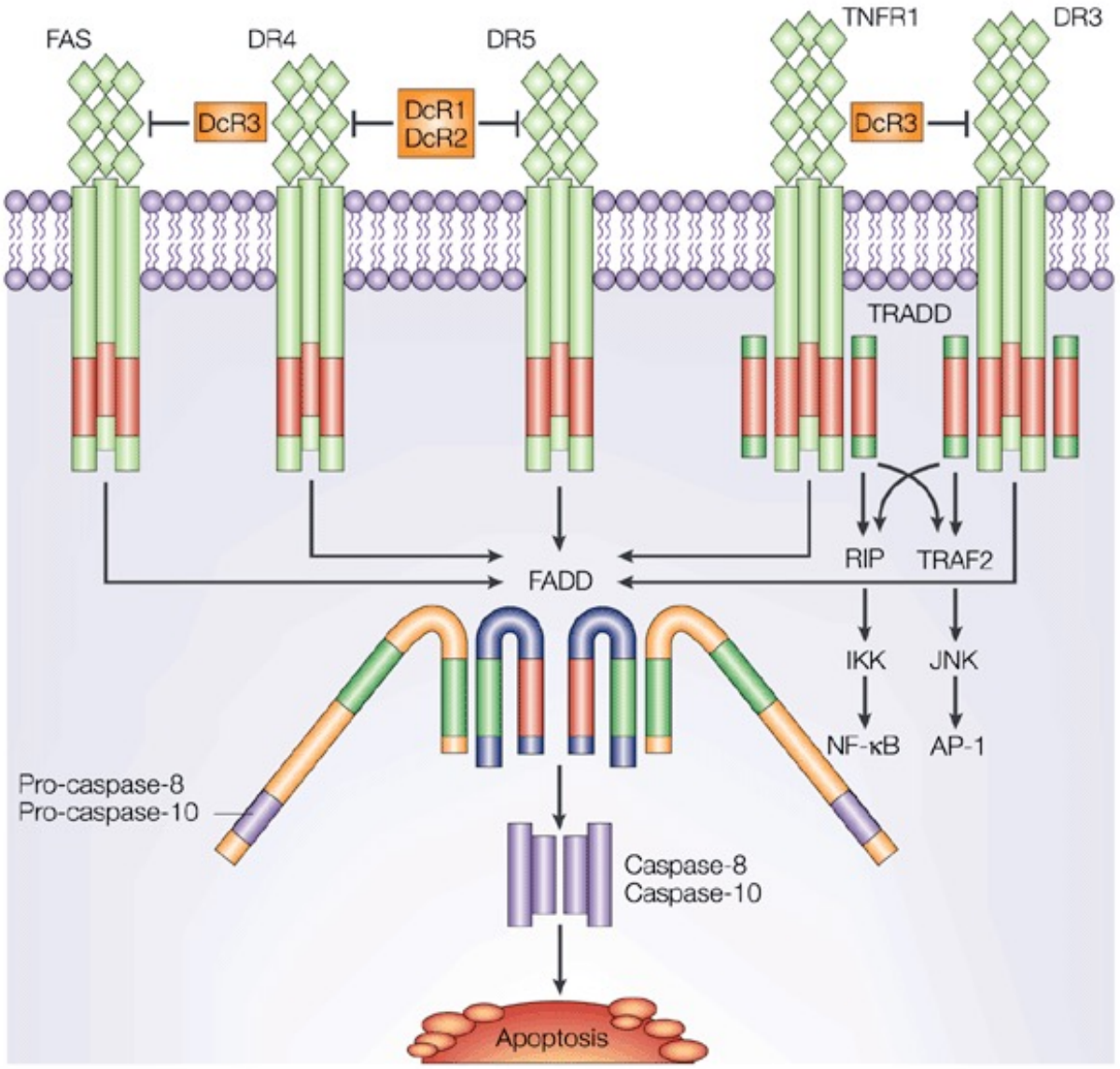
The two apoptotic pathways :

I/ Cell death receptor pathway.



II/ Mitochondrial pathway.

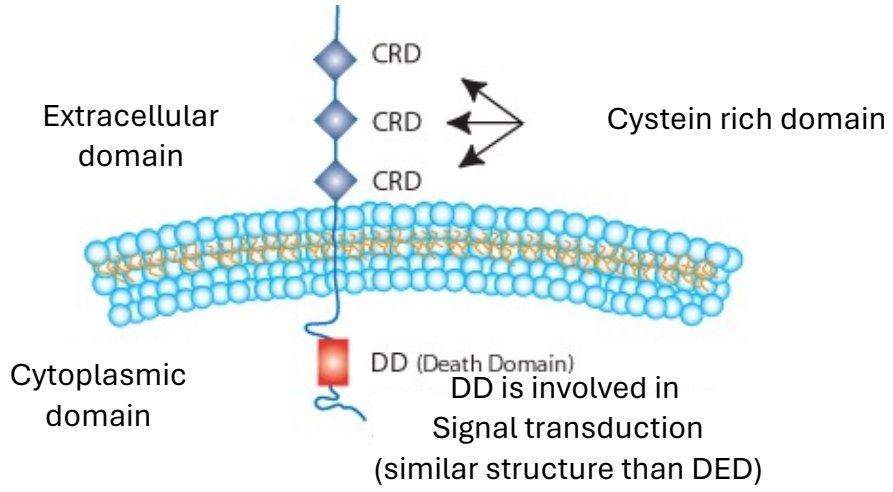
Cell death receptor pathway



Nature Reviews | Cancer

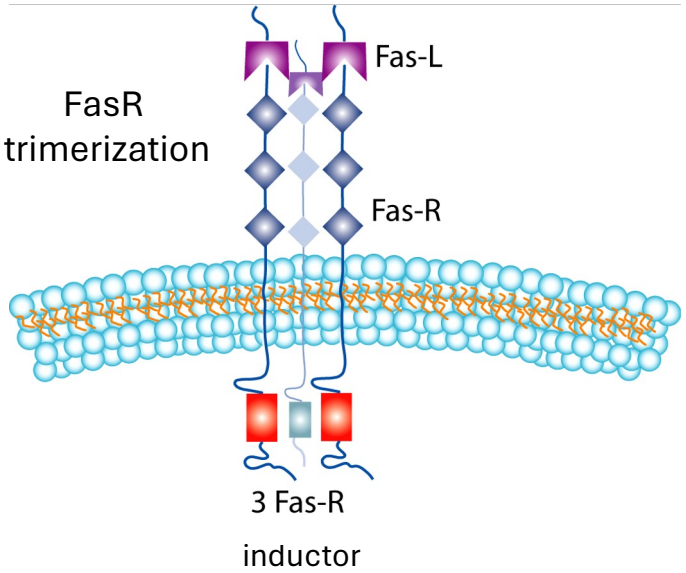
Cell death receptor pathway

1- Death receptors



Ex: Fas receptor (TNF receptor family)

2- Receptor trimerization



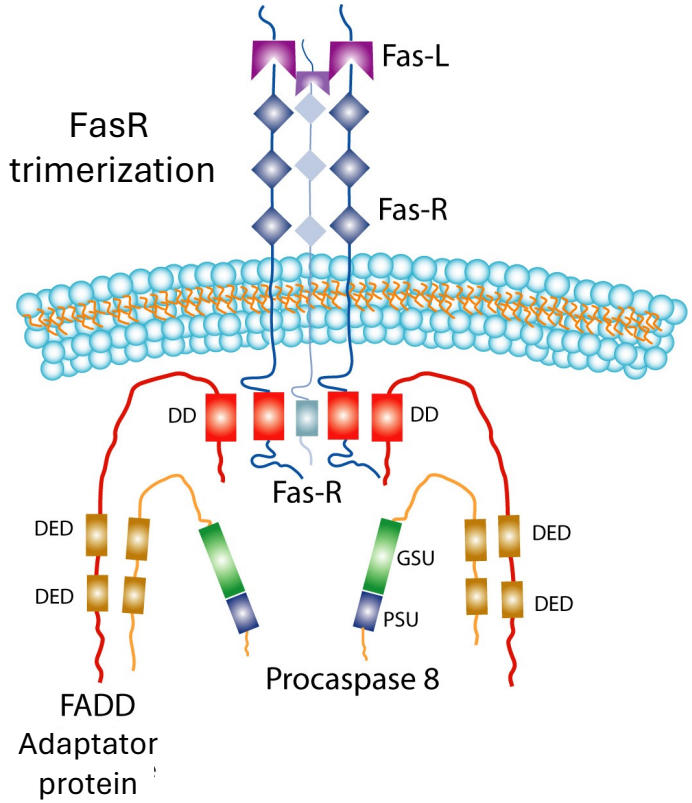
Death receptor pathway

Fas-R trimerization induce the formation of the Death Inducing signaling Complex (DISC)

•Interaction between FADD (an adaptator) and Fas-R through their respective DD

•Interaction between FADD and Procaspase 8 through their respective DED

> Procaspase 8 aggregation and activation

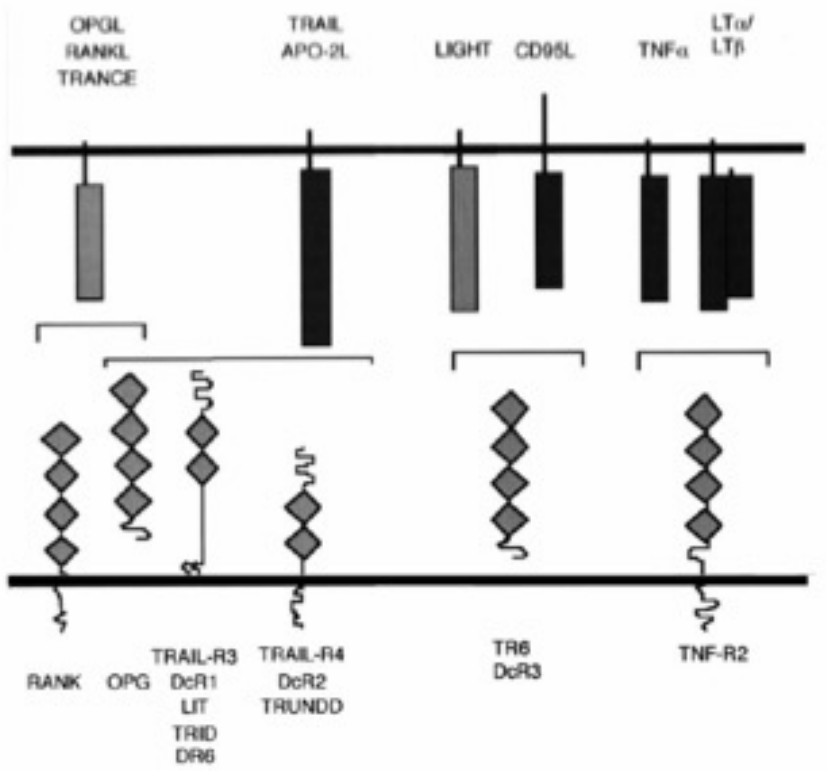


Formation of the Death Inducing Signaling Complex (DISC)

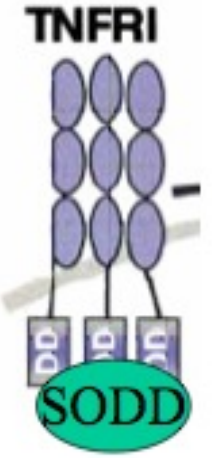
Modulation of Death Receptor Pathway

1/ At the receptor level:

-Glycosylation status (Fas, TNF-R1)
- p53-induced transcription after DNA damage (Fas, DR5)
- Decoy receptors (prevent ligand binding to the functional receptors)



Modulation of Death Receptor Pathway



2/ Inside the cell:

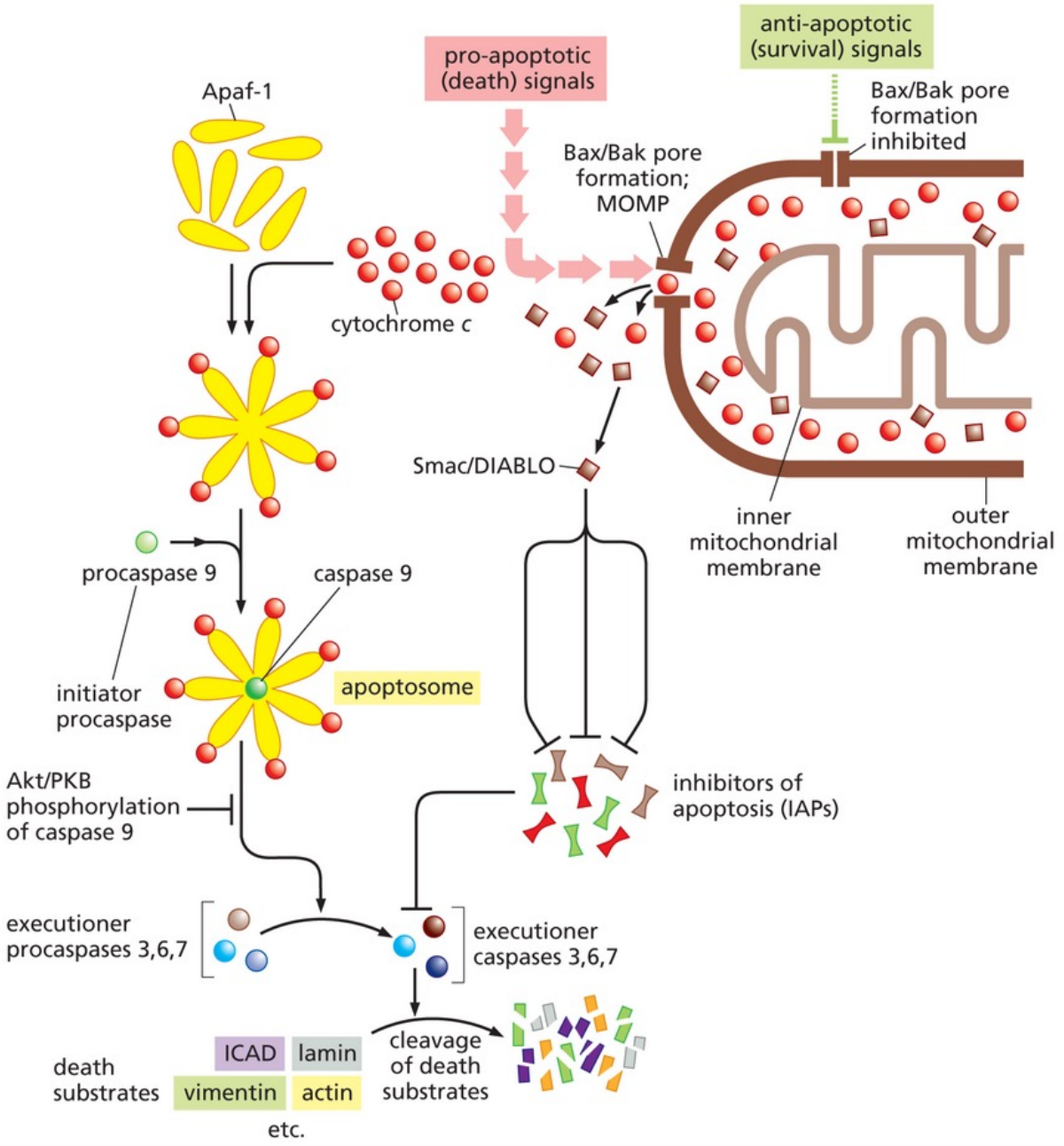
-Targeting the DD domain (TNF-R1 & DR3)
Ex : SODD (Silencer Of Death Domain protein)

- Targeting the DED domain (procaspases 8/10 or FADD)

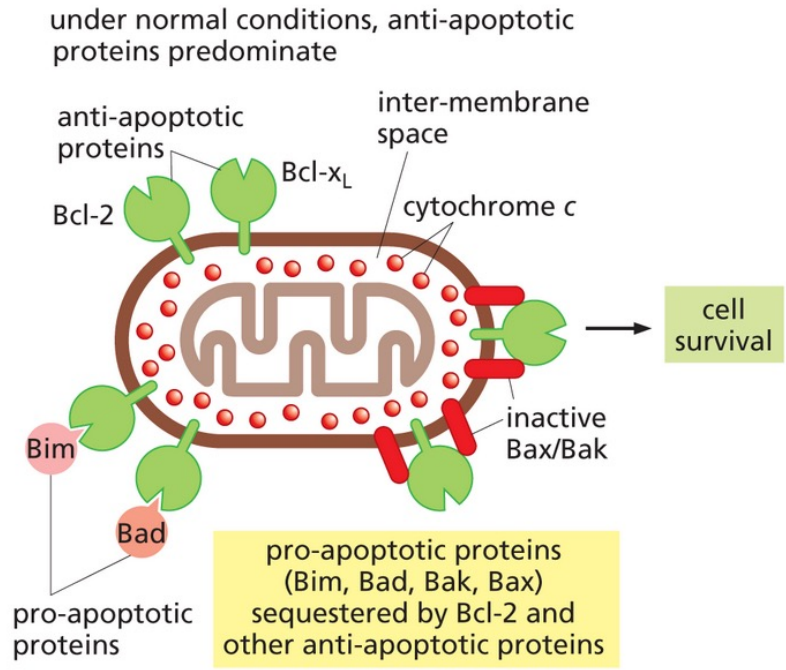
Flash & DEDD proteins ⟶ Stimulation of Apoptosis

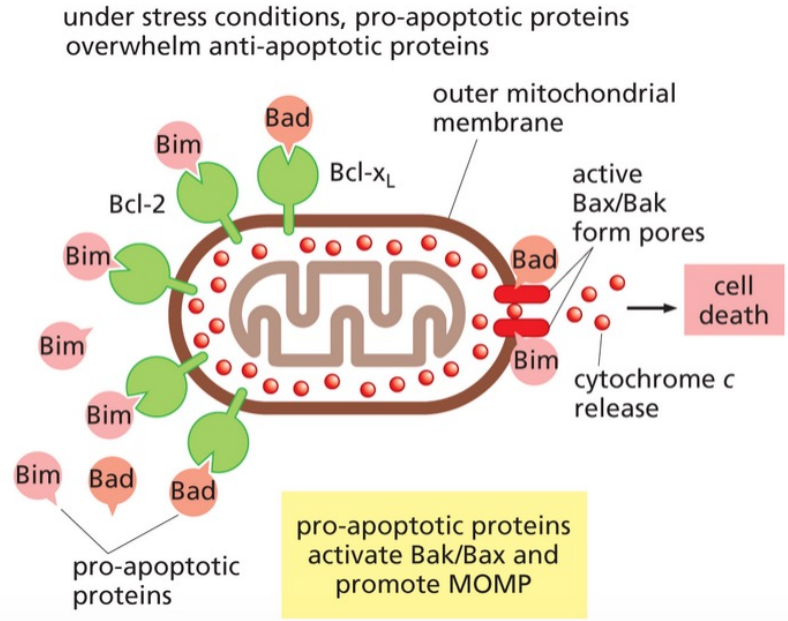
c-Flip ⟶ Inhibition of Apoptosis

The mitochondrial death pathway



Control of cytochrome c release by members of the Bcl2 family

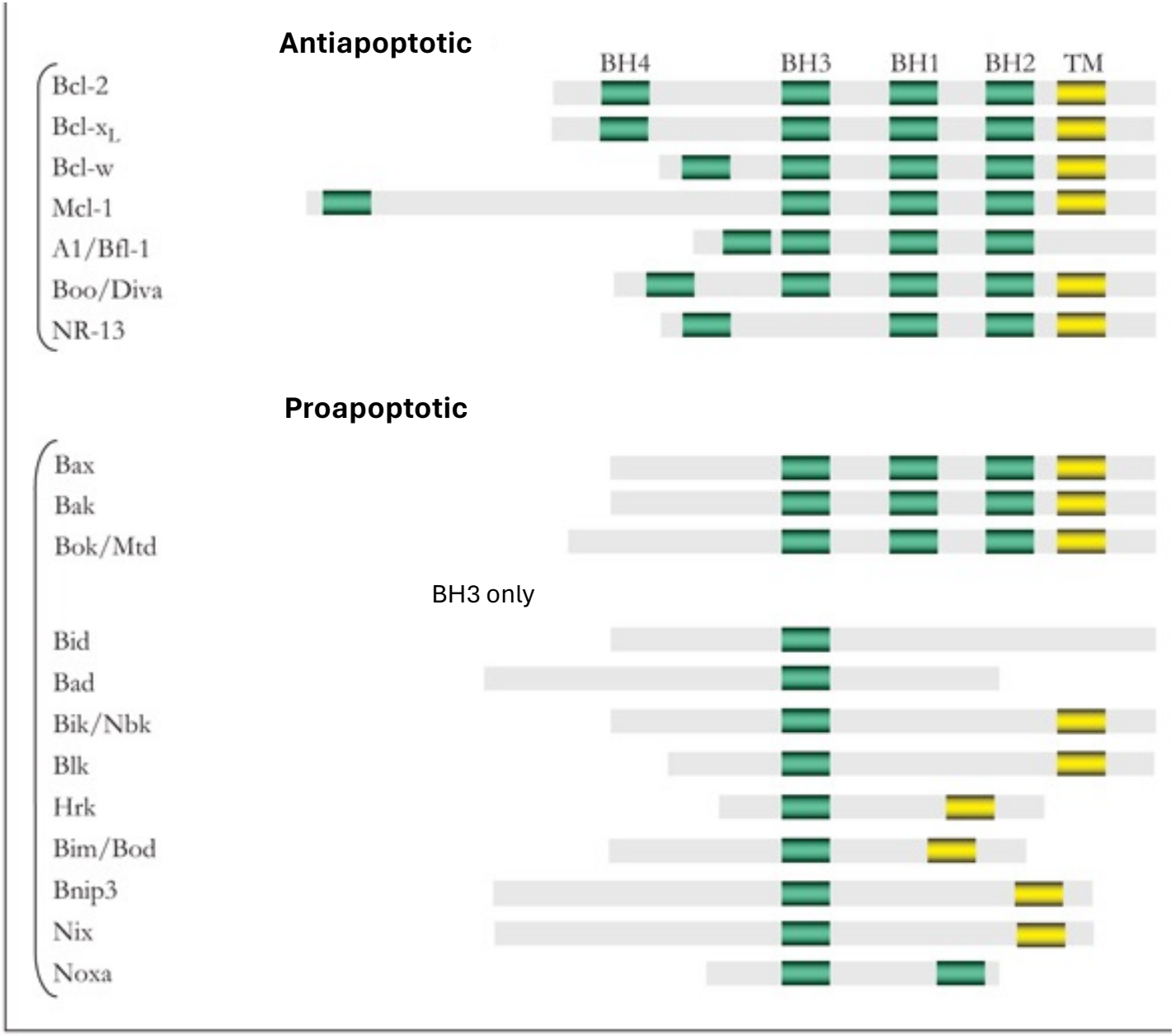




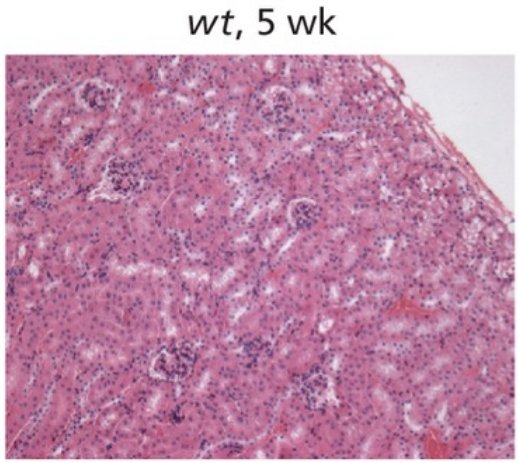
Members of the Bcl-2 family

Antiapoptotic:
Members with the BH4 domain

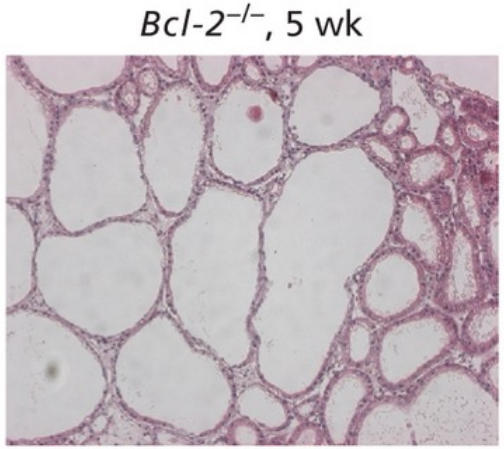
Proapoptotic:
Members lacking the BH4 domain



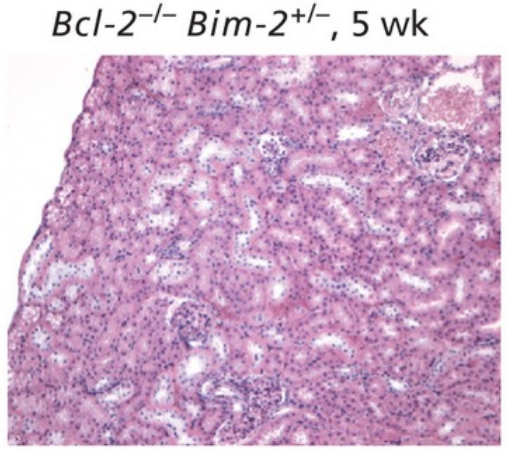
The delicate balance between pro- and anti-apoptotic proteins



(A)



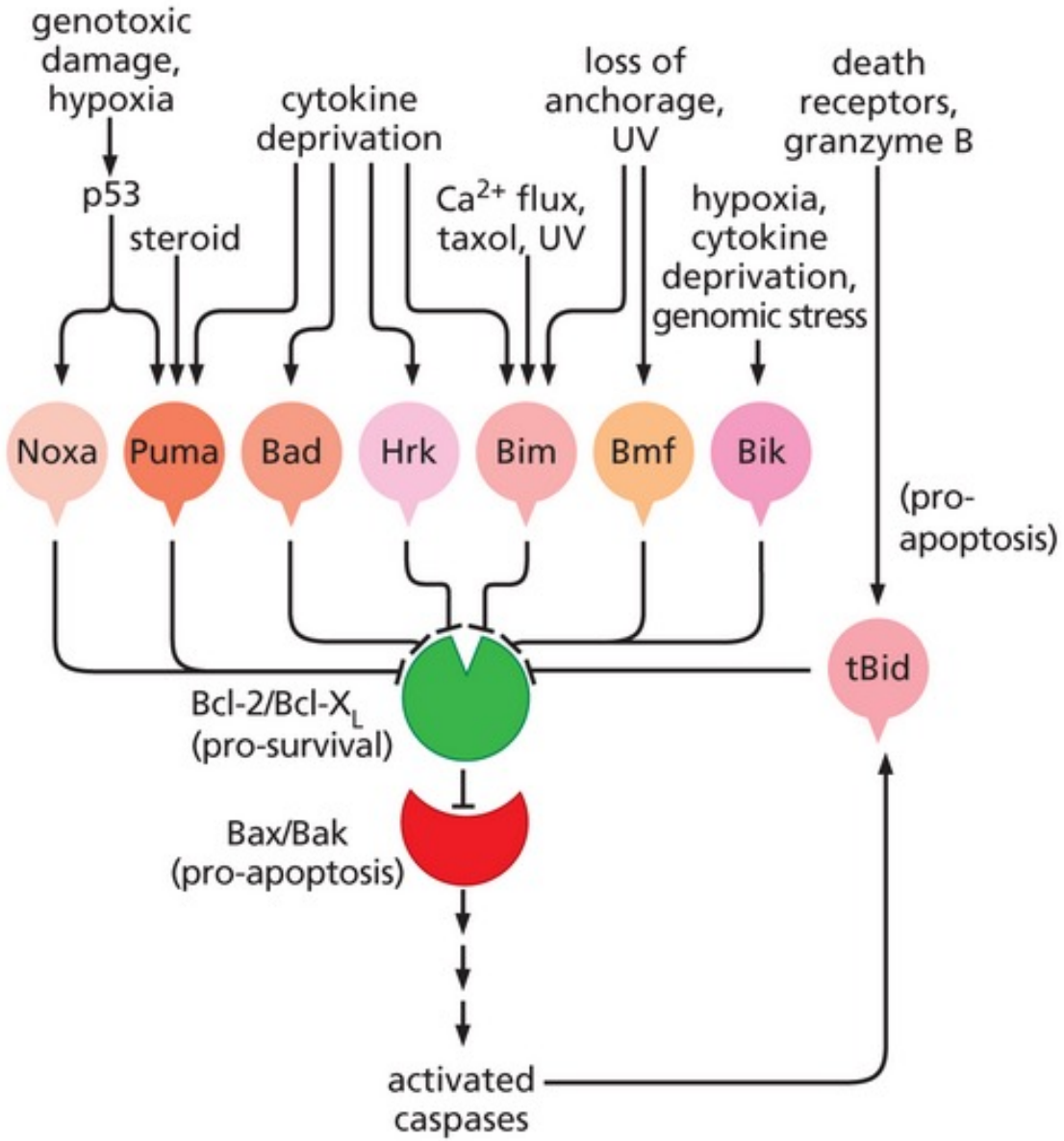
(B)



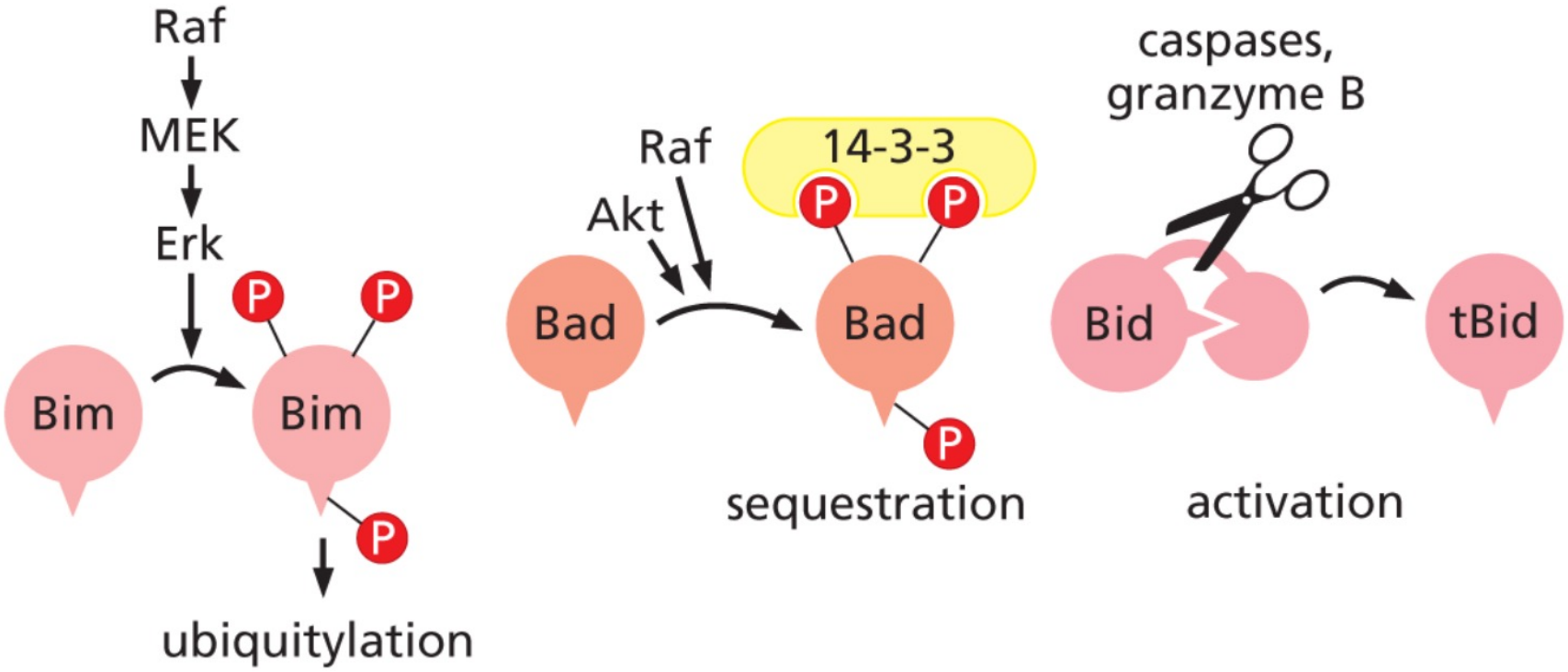
(C)

Bcl2 KO mice exhibit widespread apoptosis and polycystic kidney disease!
This phenotype can be rescued on a Bim2+/- background

Various cell physiological stresses operate through different pro-apoptotic proteins to antagonize anti-apoptotic proteins

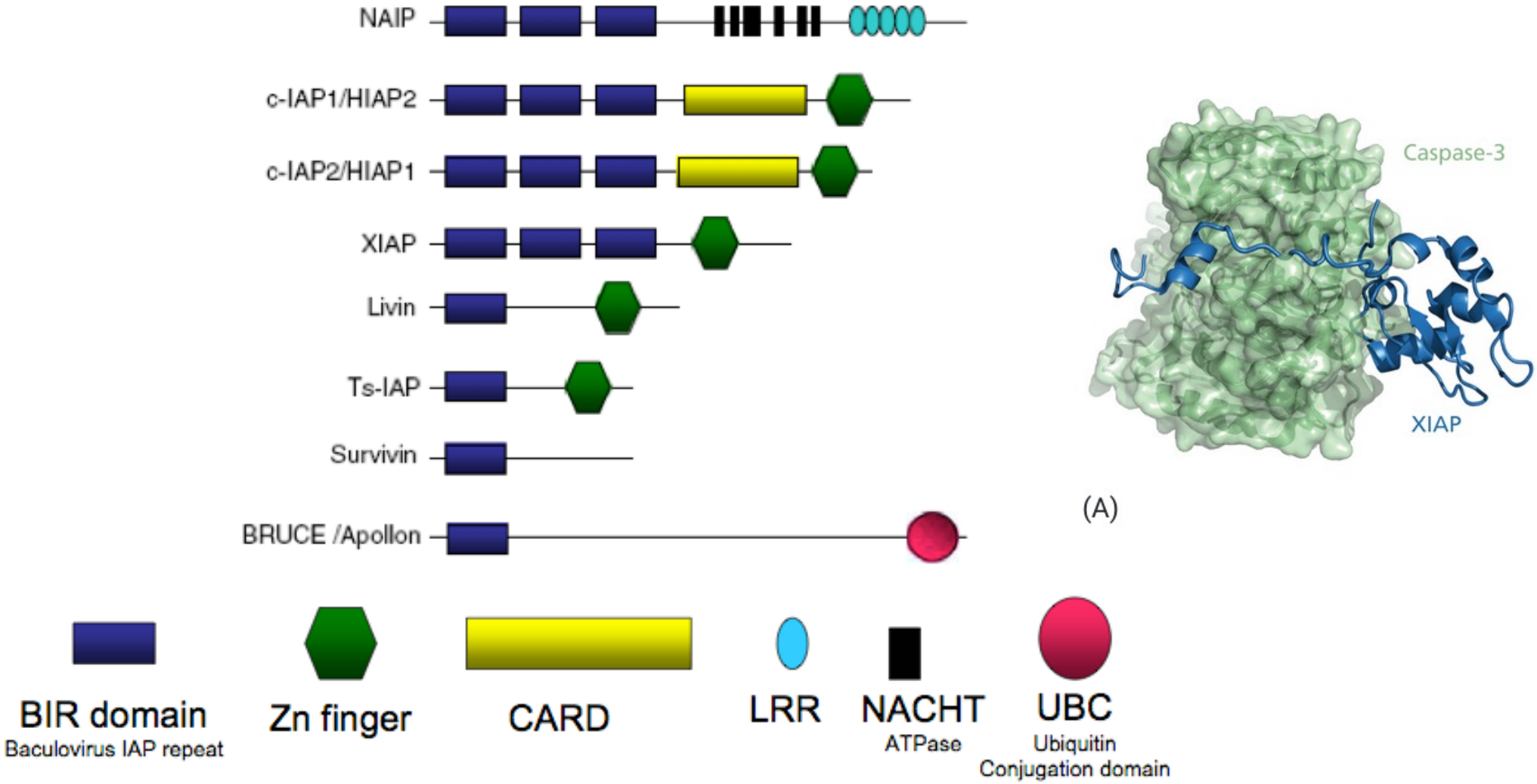


The activities of pro-apoptotic proteins are controlled in multiple ways

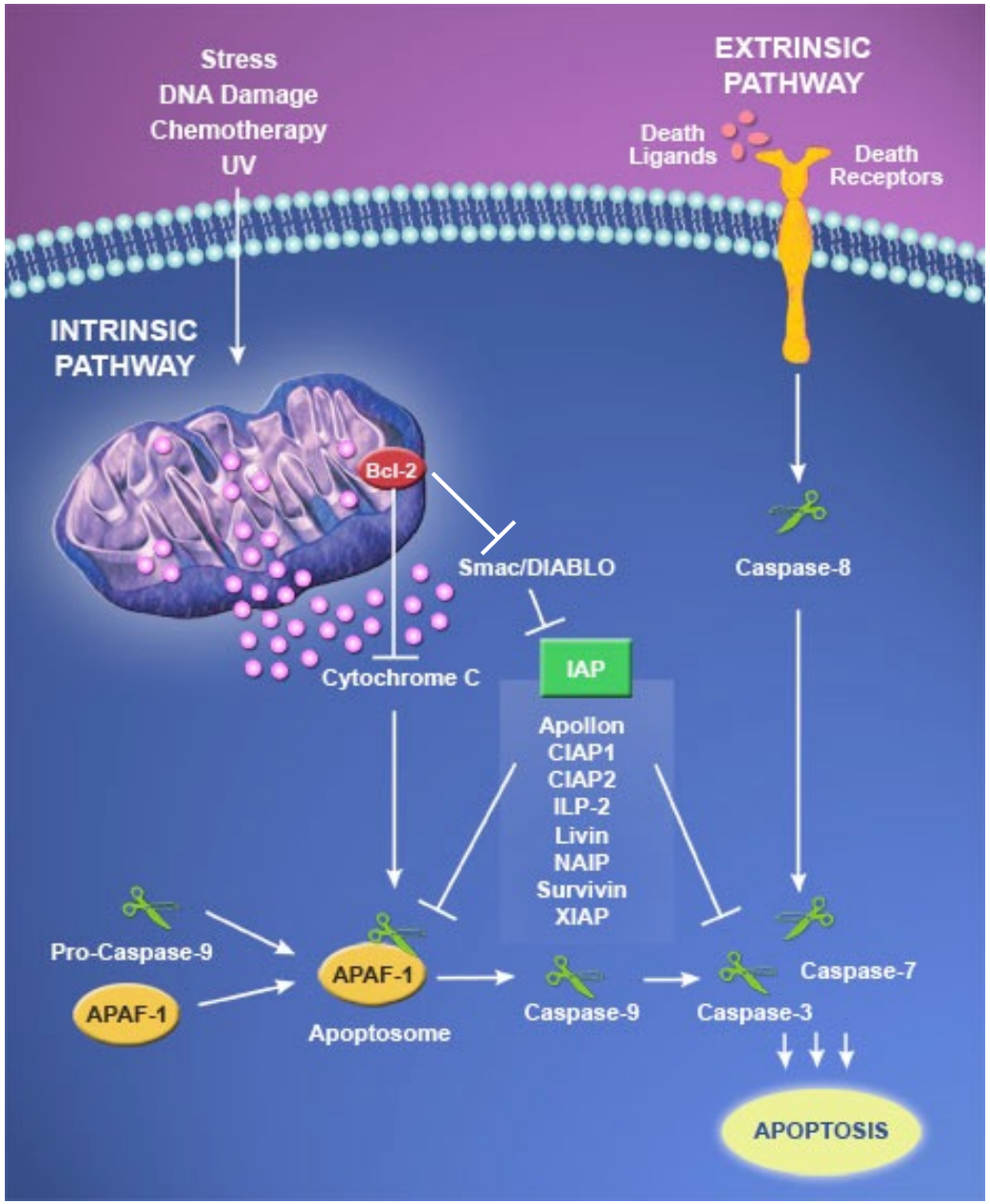


IAP proteins bind to and inhibit caspases via their BIR domain

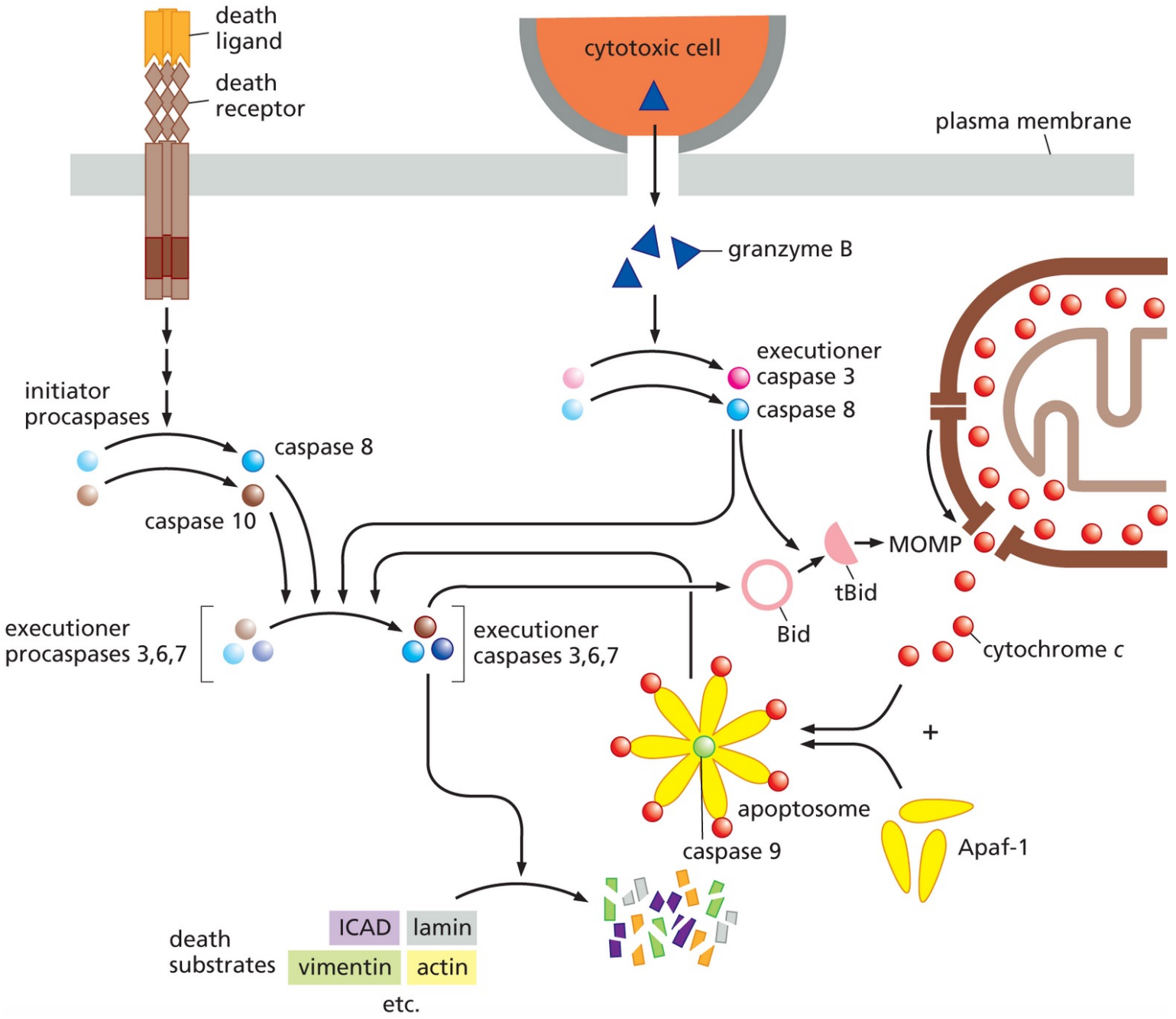
Members of IAP family in humans



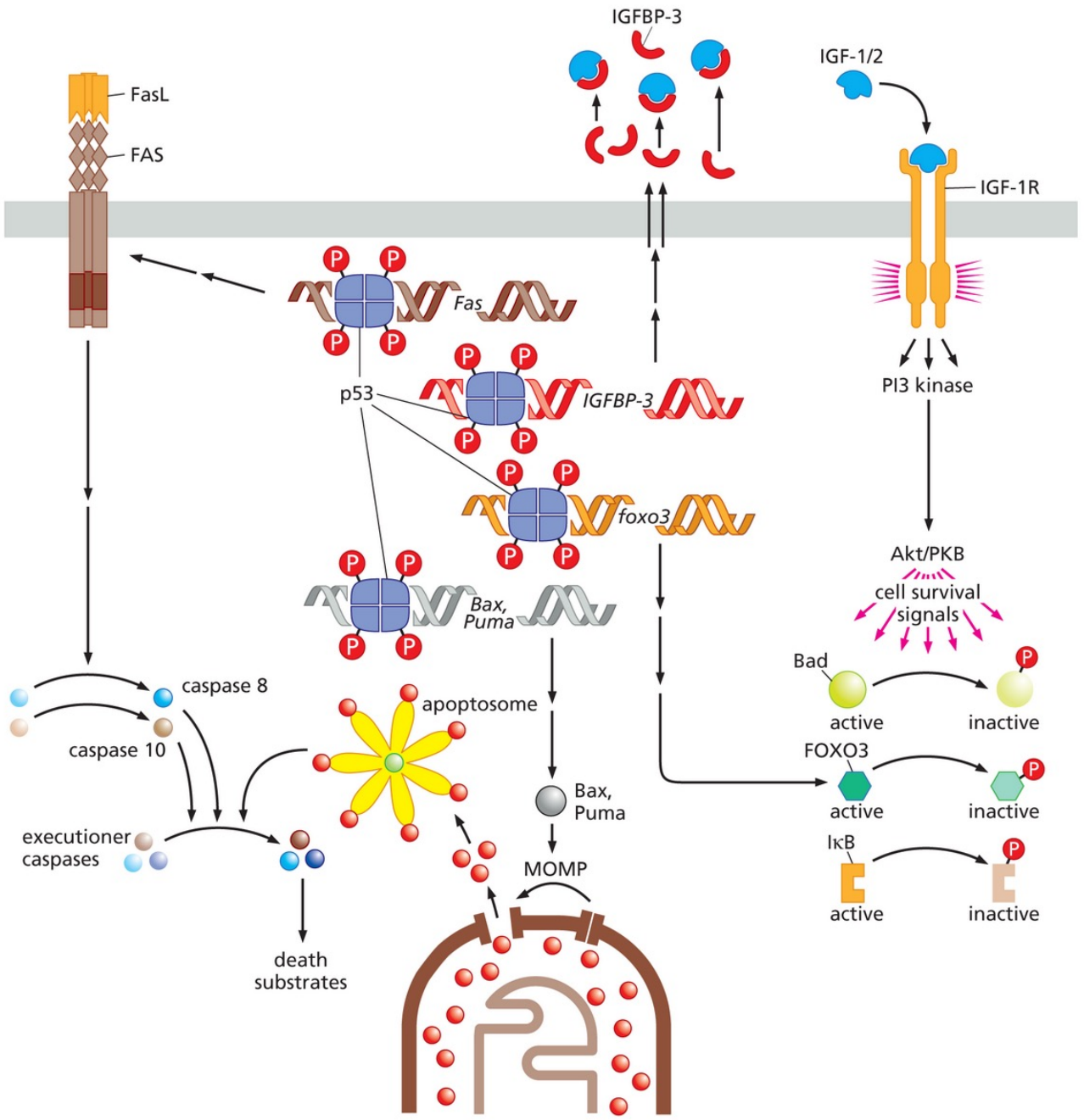
Smac/Diablo is a mitochondrial pro-apoptotic protein that is released during apoptosis and inihibits IAP proteins



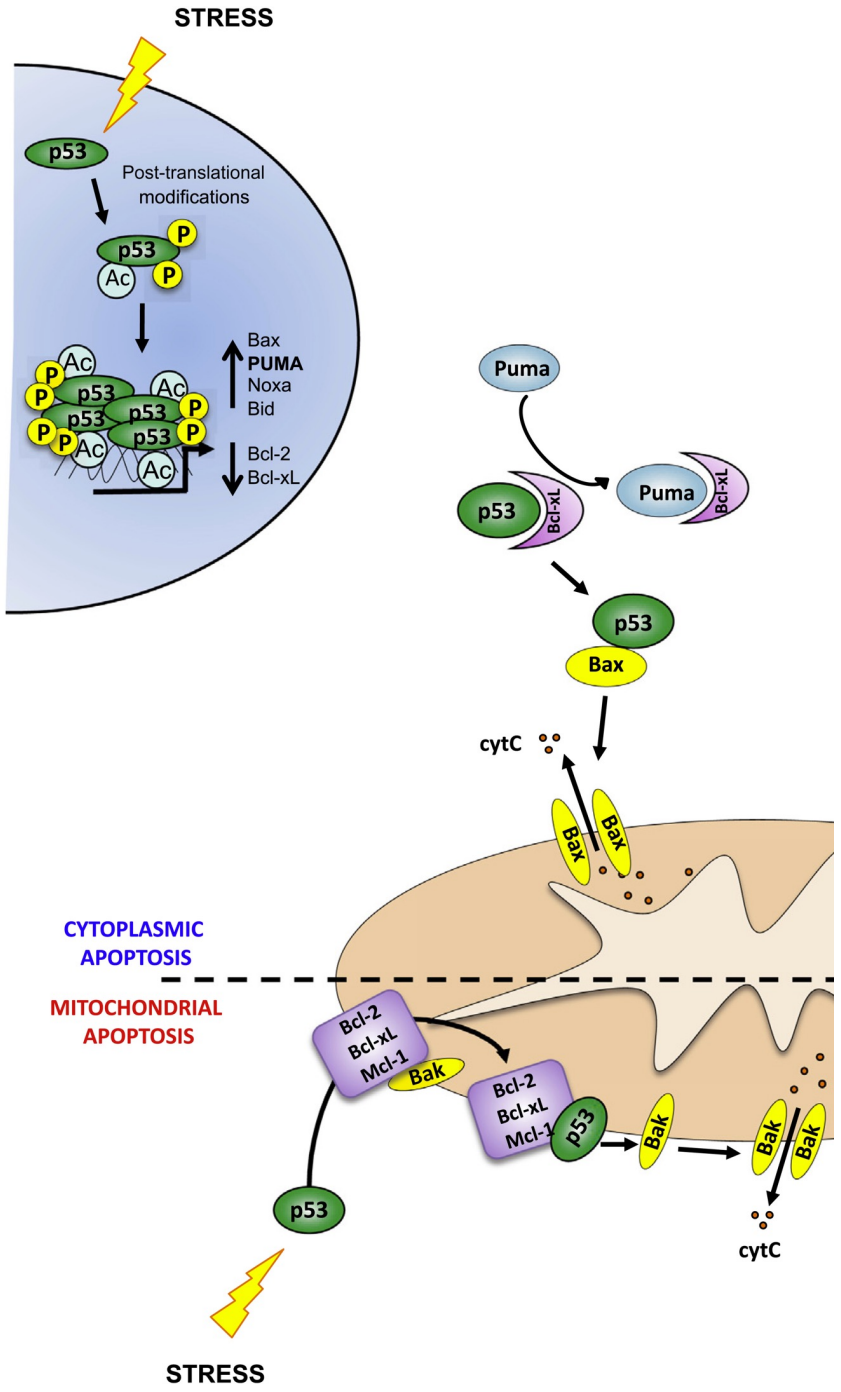
Convergence of intrinsic and extrinsic apoptotic pathways



Activation of apoptosis by p53

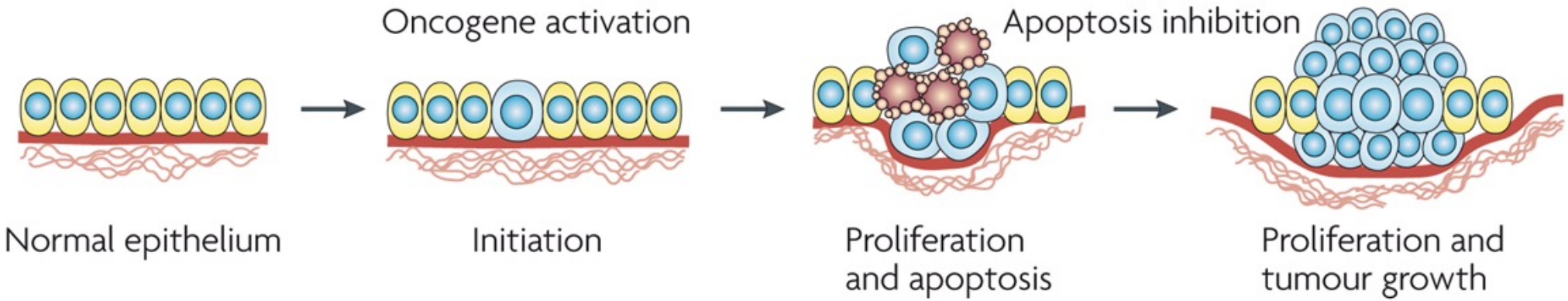


Regulation of the mitochondrial pathway by p53



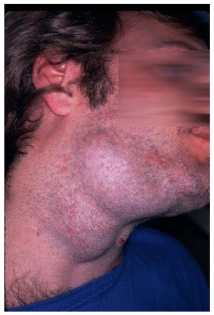
Apoptosis & Cancer

Apoptosis as a barrier against tumor development



Apoptosis as a barrier against tumor development : First example

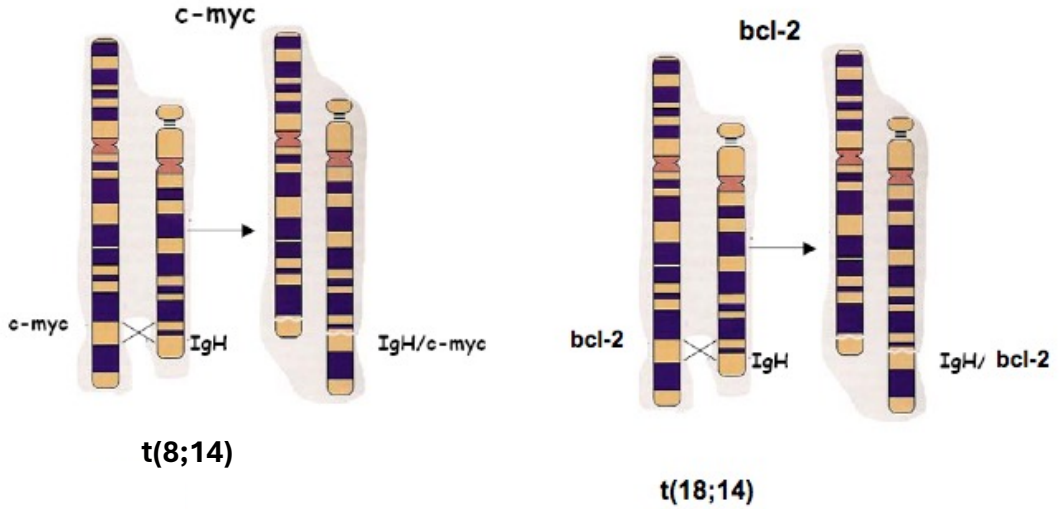
Follicular lymphoma



-Deregulated growth of mature B lymphocytes

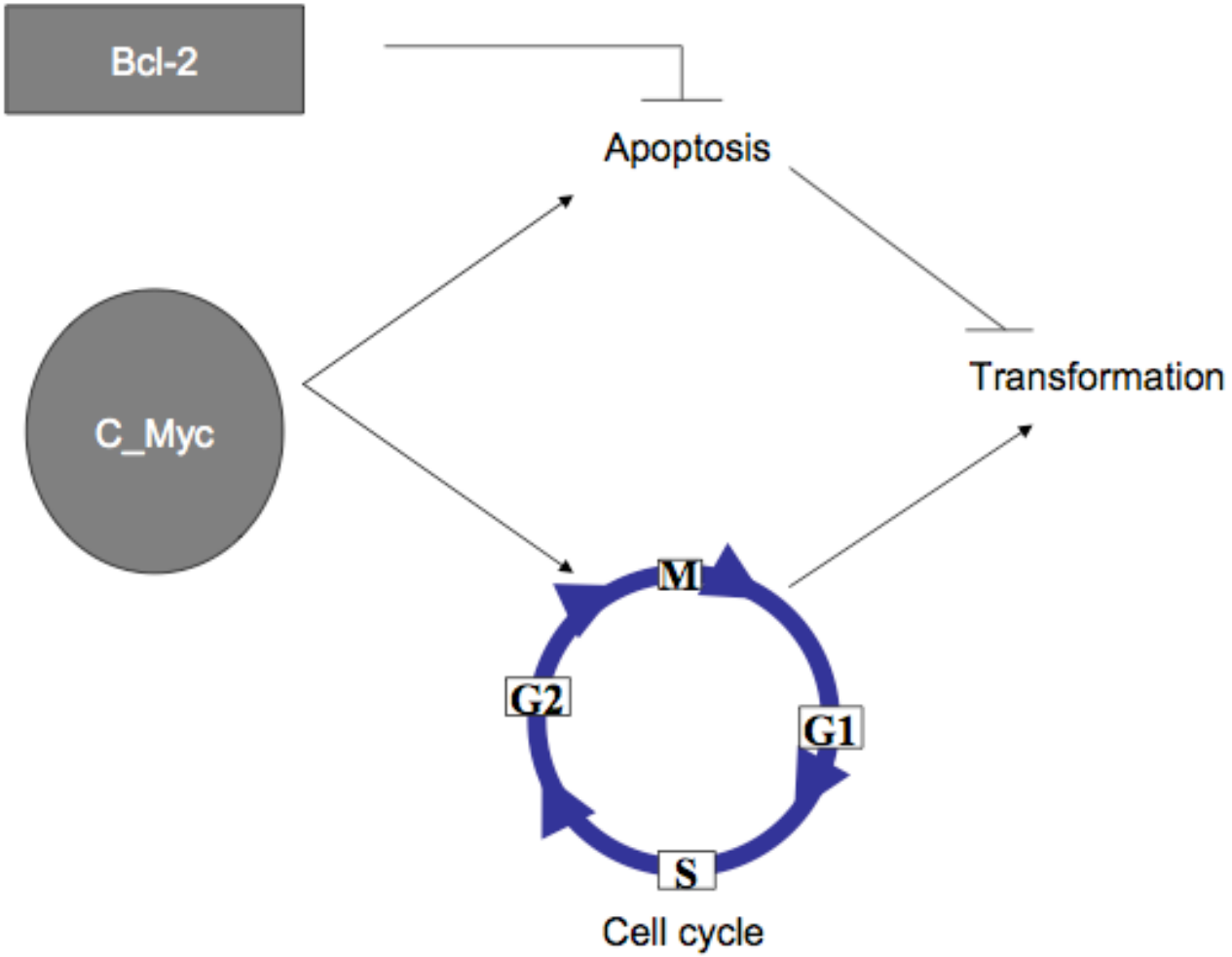
➢Lymphatic node hyperplasia

- Associated chromosome translocations :
t(8;14); t(14;18); t(12;15)



- Lymphomas with both t(8;14) and t(18;14) translocations are more aggressive.

Collaboration between Bcl-2 and c-Myc in cell transformation (Evan, 1992)



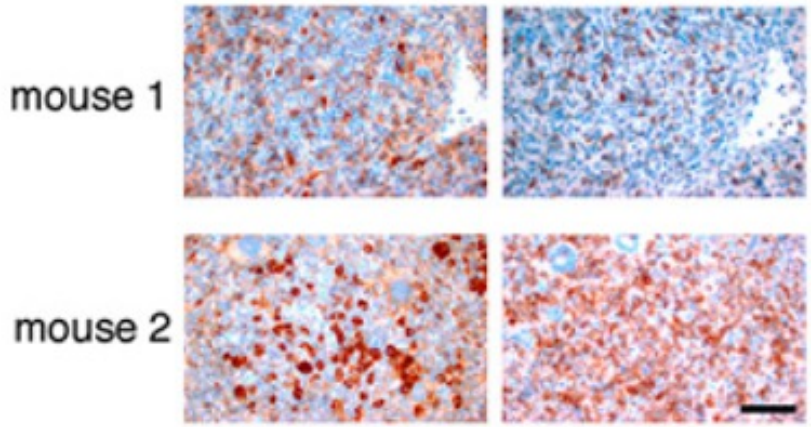
Apoptosis as a barrier against tumor development : Second example

Deletion of the mouse *sept4* gene which encodes the IAP antagonist ARTS leads to spontaneous hematopoietic malignancies.

A

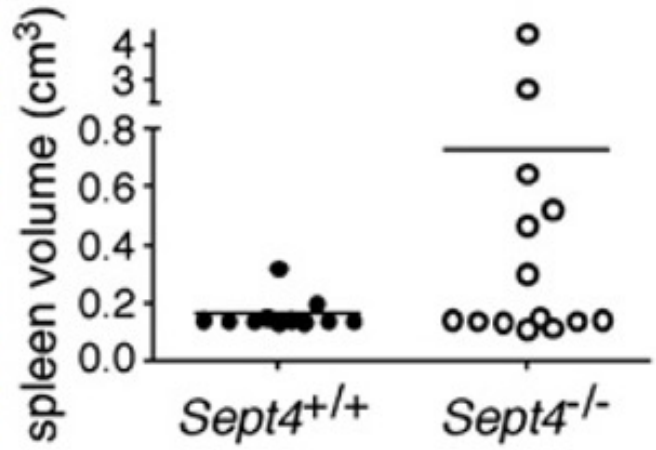
Genotype (n)	No significant findings (%)	Lymphoid pathology		Others (%)
		Mild/mod (%)	Severe	
Sept4$^{+/+}$ (10)	30	70	0	0
Sept4$^{+/-}$ (12)	16.6	66.7	8.4	8.3
Sept4$^{-/-}$ (13)	15.3	46.2	38.5	0

B



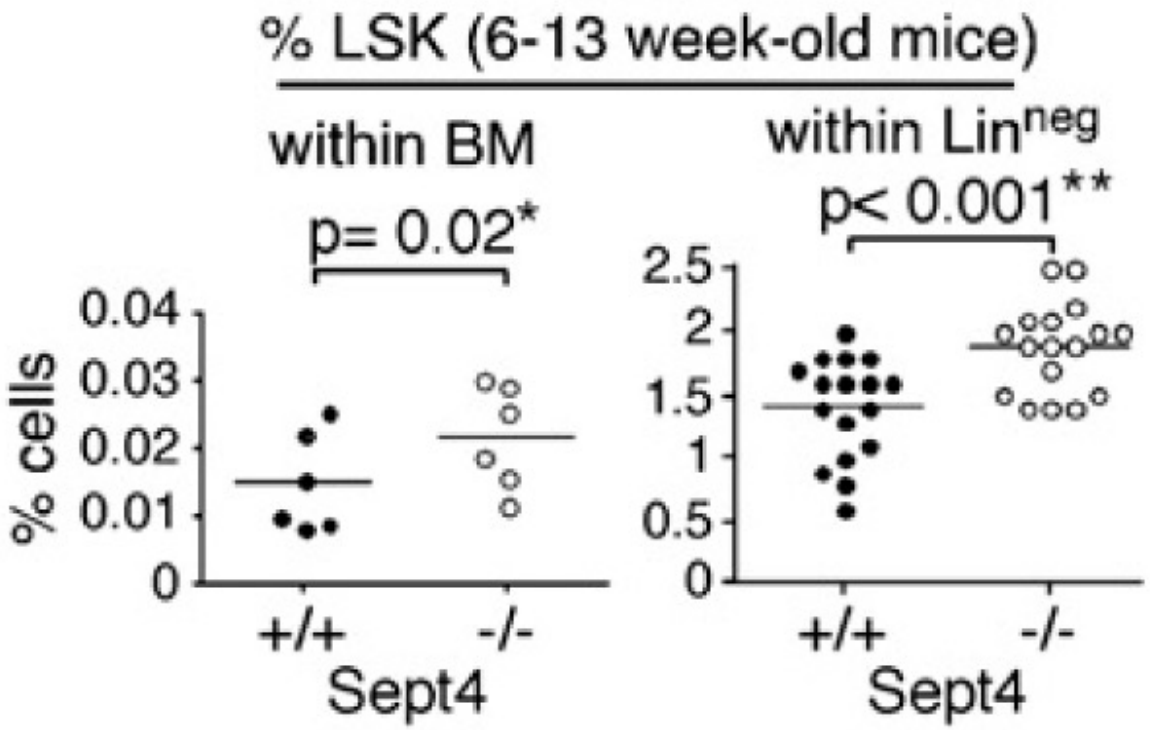
C

D



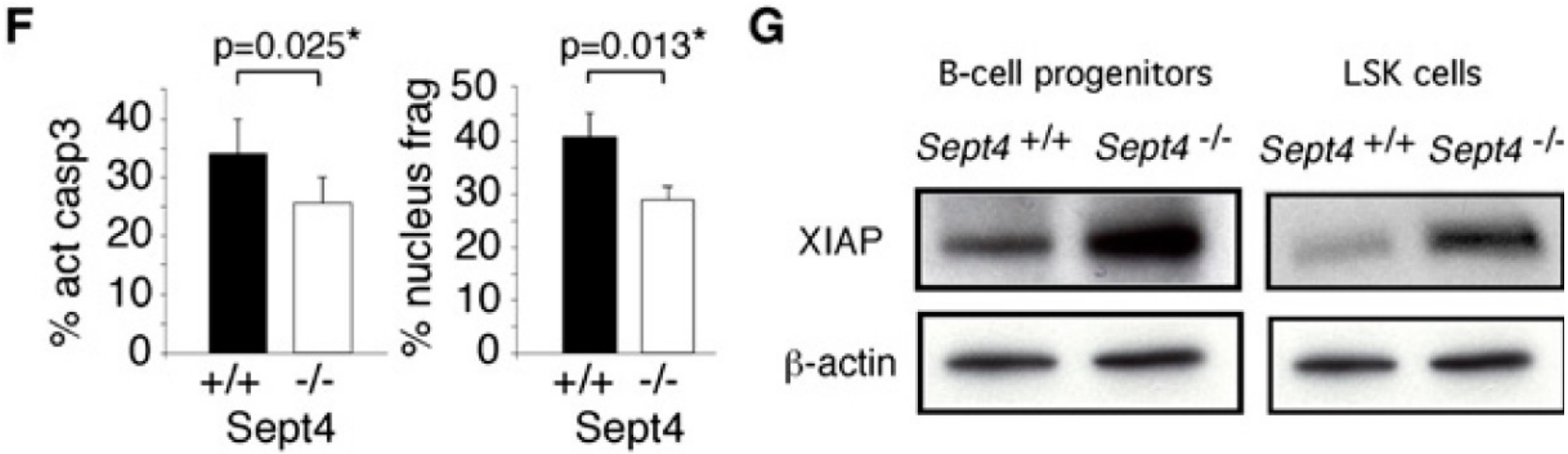
Garcia-Fernandez et al, Genes & Dev, 2010

Sept4-null mice have increased numbers of hematopoietic stem and progenitor cells.



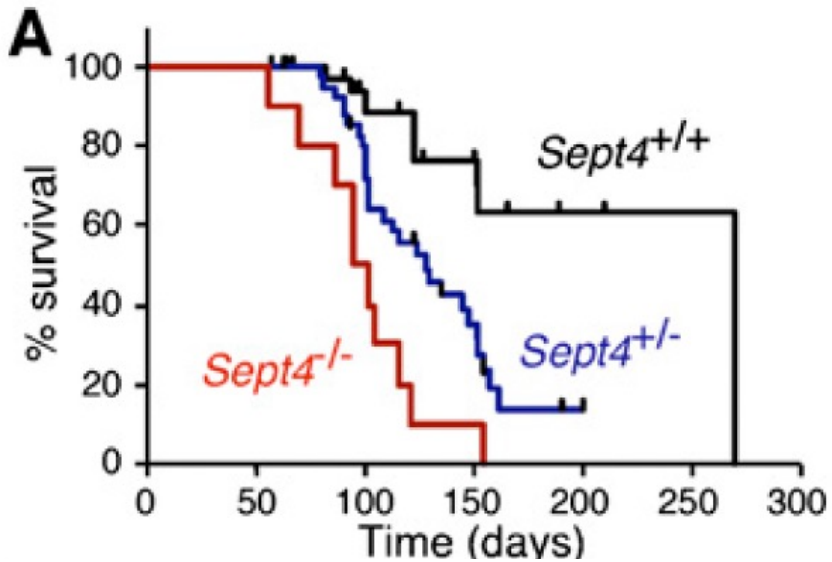
Garcia-Fernandez et al, Genes & Dev, 2010

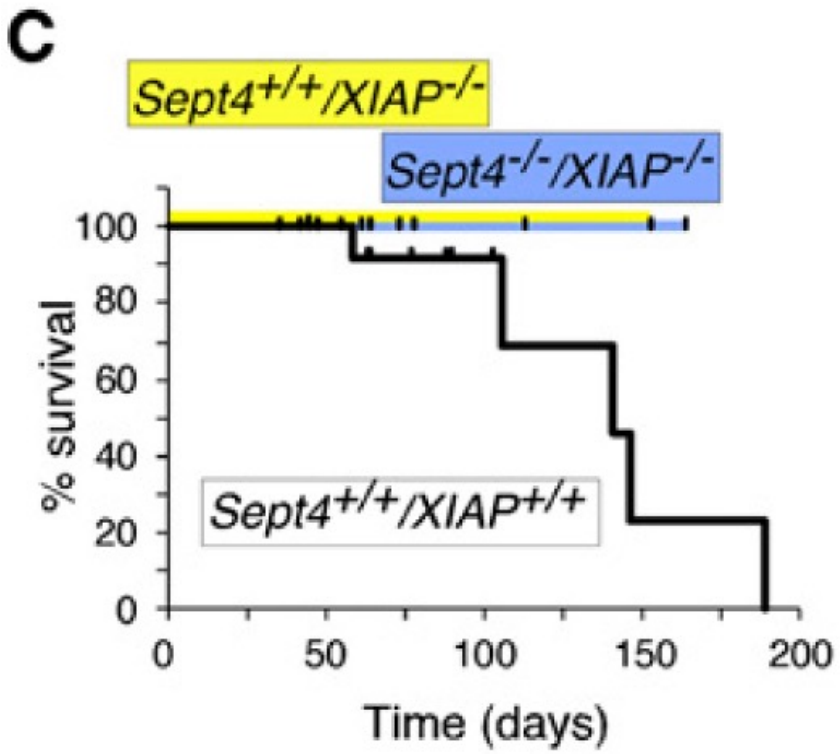
Sept4-null progenitors exhibit elevated XIAP protein and increased resistance to cell death.



Garcia-Fernandez et al, Genes & Dev, 2010

Sept4-null mice exhibit accelerated tumor development in an Eμ-Myc background.

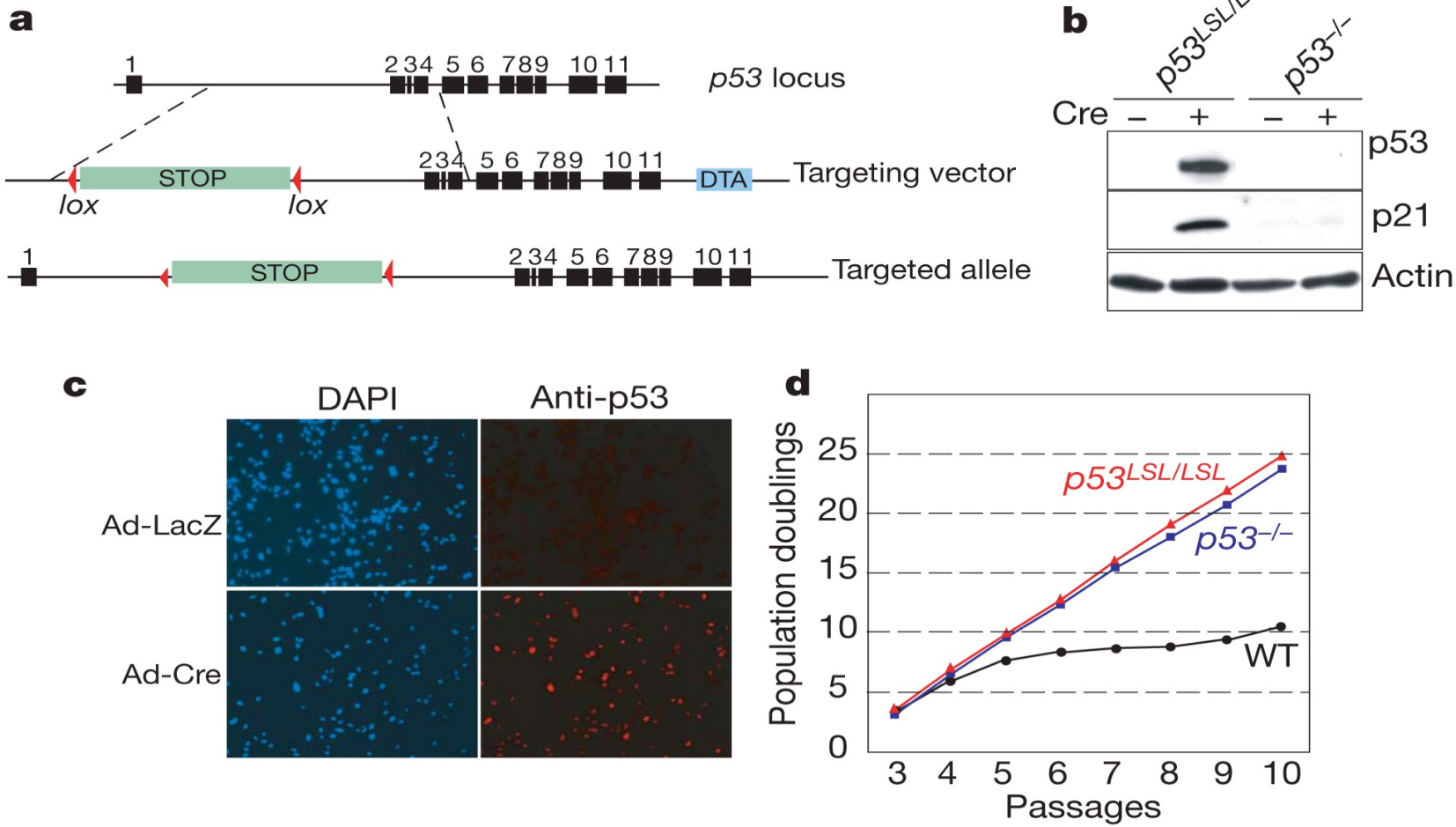




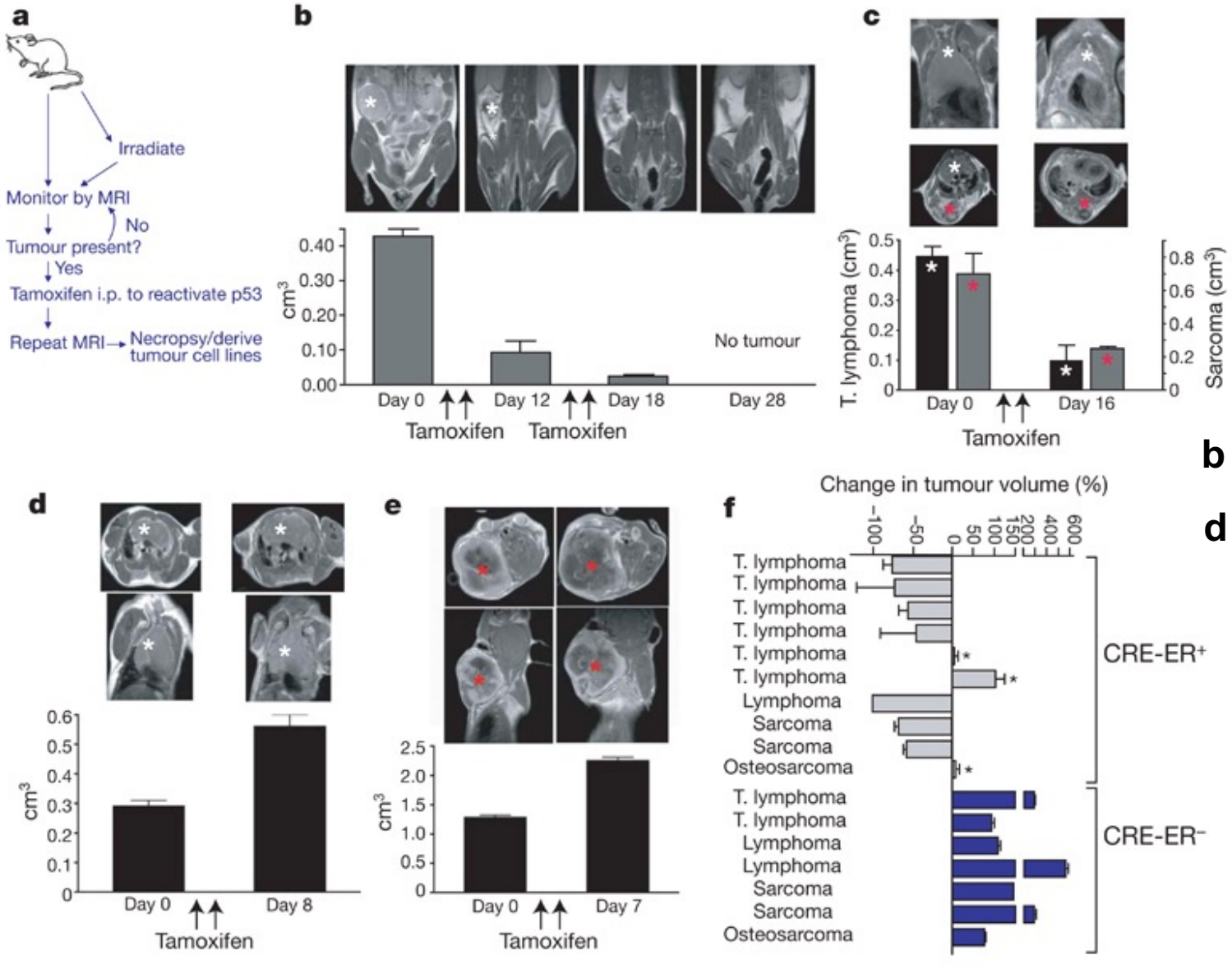
Garcia-Fernandez et al, Genes & Dev, 2010

Apoptosis as a barrier against tumor development : Third example

A reactivable allele of p53 in mice



In vivo tumor regression after p53 reactivation



b & c: p53-LSL; Cre-ER^{T2}

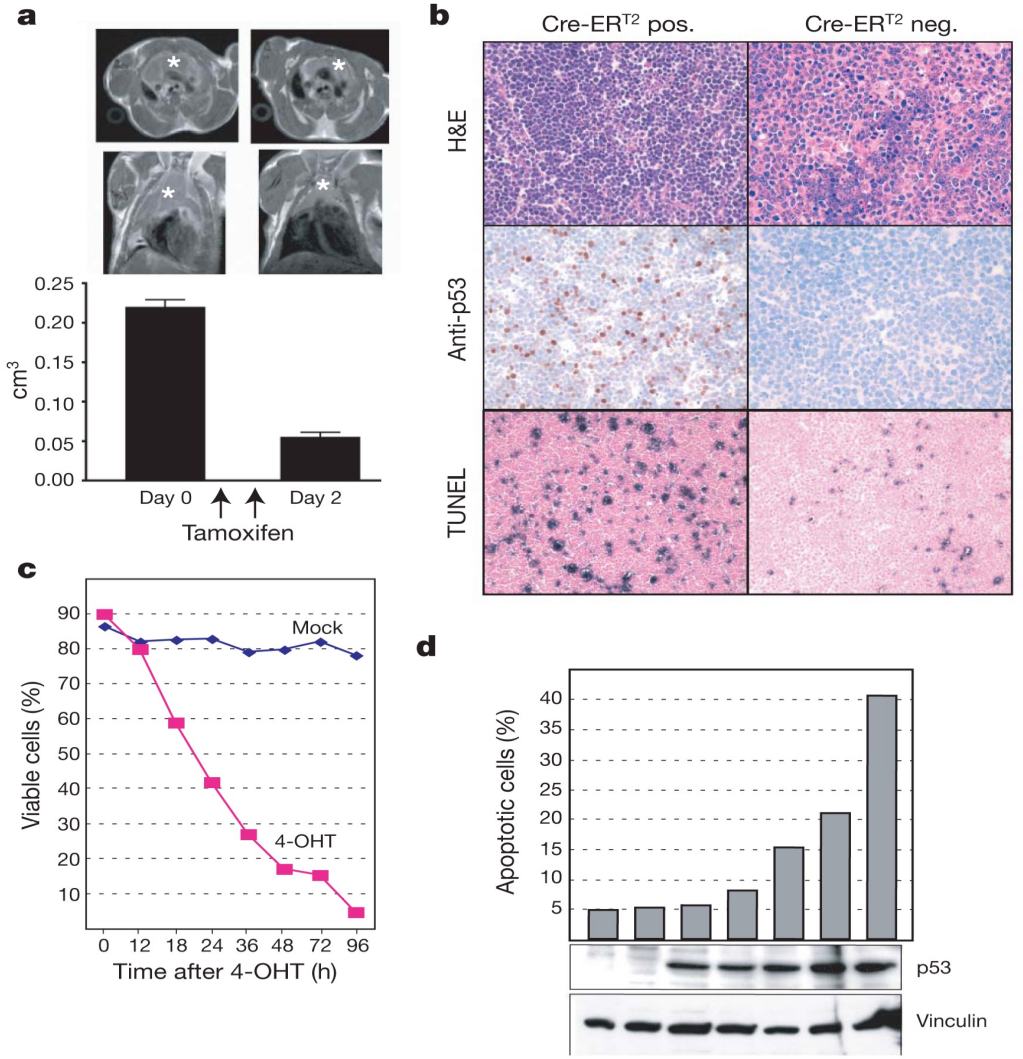
d & e: p53-LSL

*** lymphome**

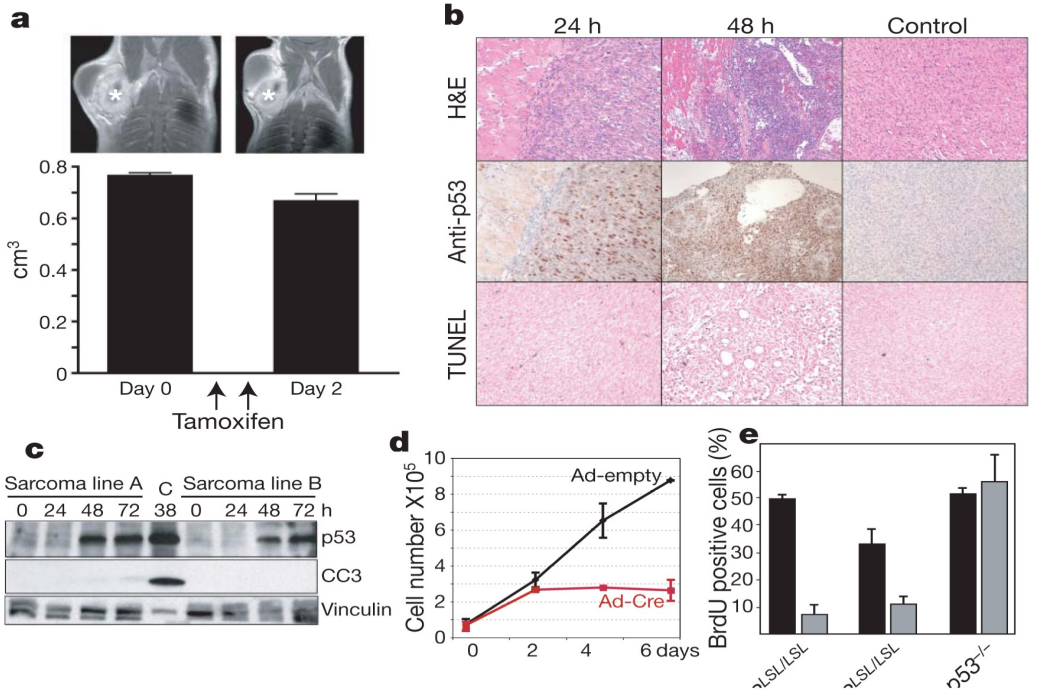
*** sarcome**

Tiré de Ventura et al, Nature, 2007.

Regression mechanism depends on the tumor type.

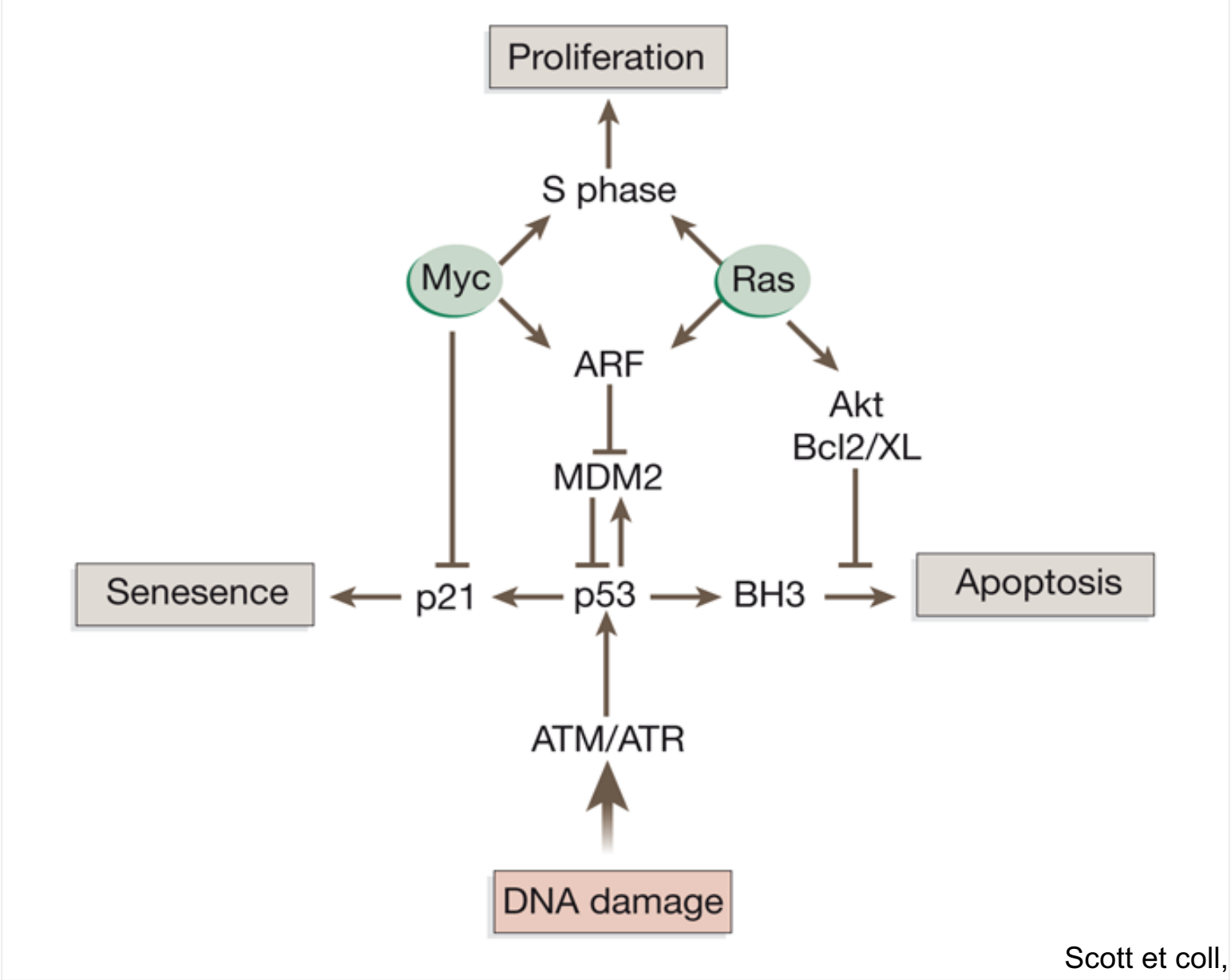


p53 restoration in lymphomas leads to apoptosis.



p53 restoration in sarcomas leads to growth suppression

Cooperation between Myc and Ras



Scott et coll, *Nature* 2004.

Anti-apoptotic strategies used by cancer cells

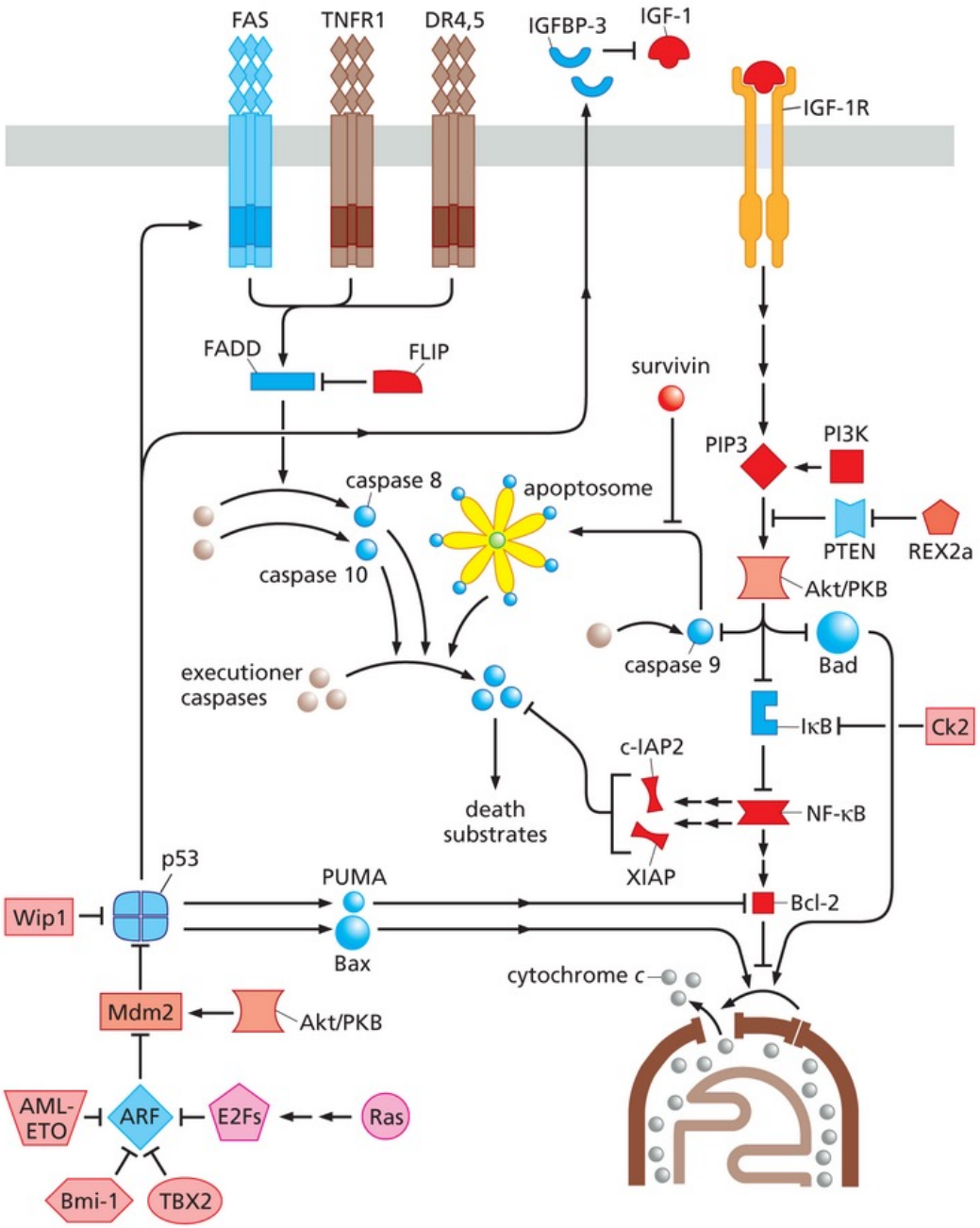
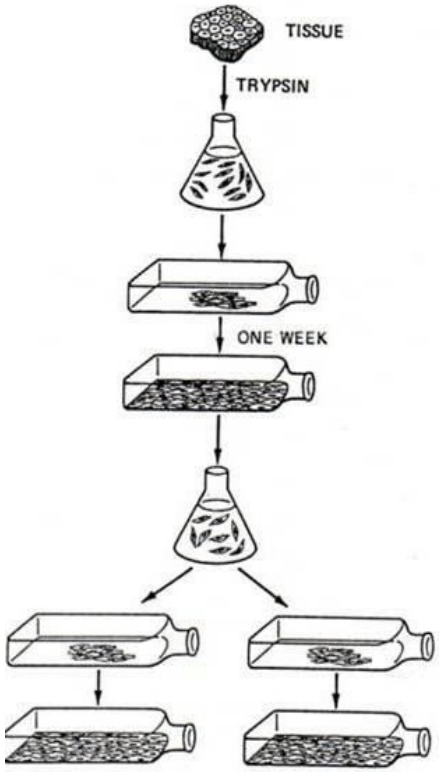


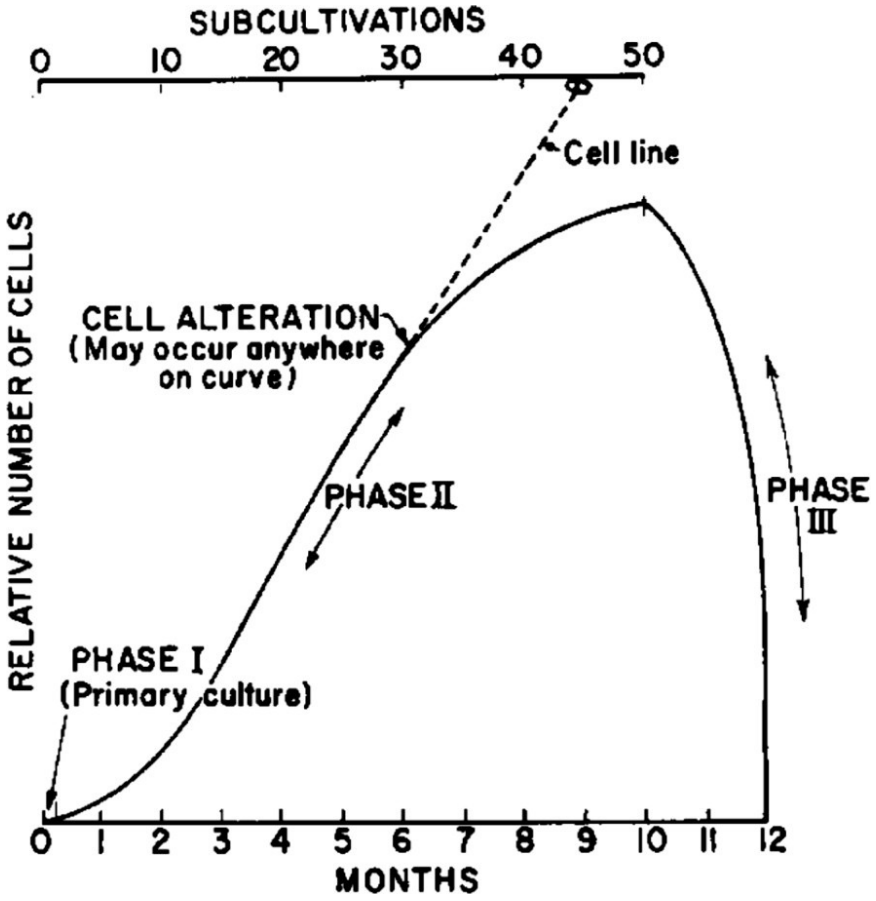
Table 9.4 Examples of anti-apoptotic alterations found in human tumor cells

Alteration	Mechanism of anti-apoptotic action	Types of tumors
CASP8 promoter methylation	inactivation of extrinsic cascade	SCLC, pediatric tumors
CASP3 repression	inactivation of executioner caspase	breast carcinomas
Survivin overexpression[a]	caspase inhibitor	mesotheliomas, many carcinomas
ERK activation	repression of caspase 8 expression	many types
ERK activation	protection of Bcl-2 from degradation	many types
Raf activation	sequestration of Bad by 14-3-3 proteins	many types
PI3K mutation/activation	activation of Akt/PKB	gastrointestinal
NF-κB constitutive activation[b]	induction of anti-apoptotic genes	many types
p53 mutation	loss of ability to induce pro-apoptotic genes	many types
$p14^{ARF}$ gene inactivation	suppression of p53 levels	many types
Mdm2 overexpression	suppression of p53 levels	sarcomas
IAP-1 gene amplification	antagonist of caspases 3 and 7	esophageal, cervical
APAF1 methylation	loss of caspase 9 activation by cytochrome *c*	melanomas
BAX mutation	loss of pro-apoptotic protein	colon carcinomas
Bcl-2 overexpression	closes mitochondrial channel	~½ of human tumors
PTEN inactivation	hyperactivity of Akt/PKB kinase	glioblastoma, prostate carcinoma, endometrial carcinoma
IGF-1/2 overexpression	activates PI3K	many types
IGFBP repression	loss of anti-apoptotic IGF-1/2 antagonist	many types
Casein kinase II overexpression	activation of NF-κB	many types
TNFR1 methylation	repressed expression of death receptor	Wilms tumor
FLIP overexpression	inhibition of caspase 8 activation by death receptors	melanomas, many others
Akt/PKB activation	phosphorylation and inactivation of pro-apoptotic Bcl-2-like proteins	many types
USP9X overexpression	deubiquitylates Mcl-1	lymphomas
STAT3 activation	induces expression of $Bcl\text{-}X_L$	several types
TRAIL-R1 repression	loss of responsiveness to death ligand	small-cell lung carcinoma
FAP-1 overexpression	inhibition of FAS receptor signaling	pancreatic carcinoma
XAF1 methylation[c]	loss of inhibition of anti-apoptotic XIAP	gastric carcinoma
Wip1 overexpression[d]	suppression of p53 activation	breast and ovarian carcinomas, neuroblastoma

II/ Echapper à la sénescence réplicative

SÉNESCENCE RÉPLICATIVE : LA LIMITE DE HAYFLICI



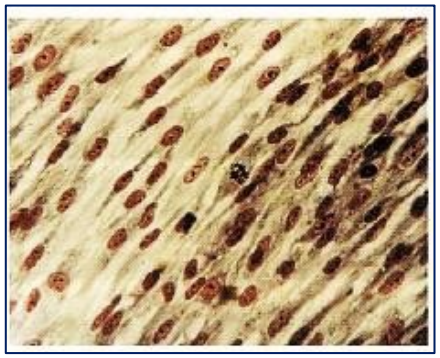


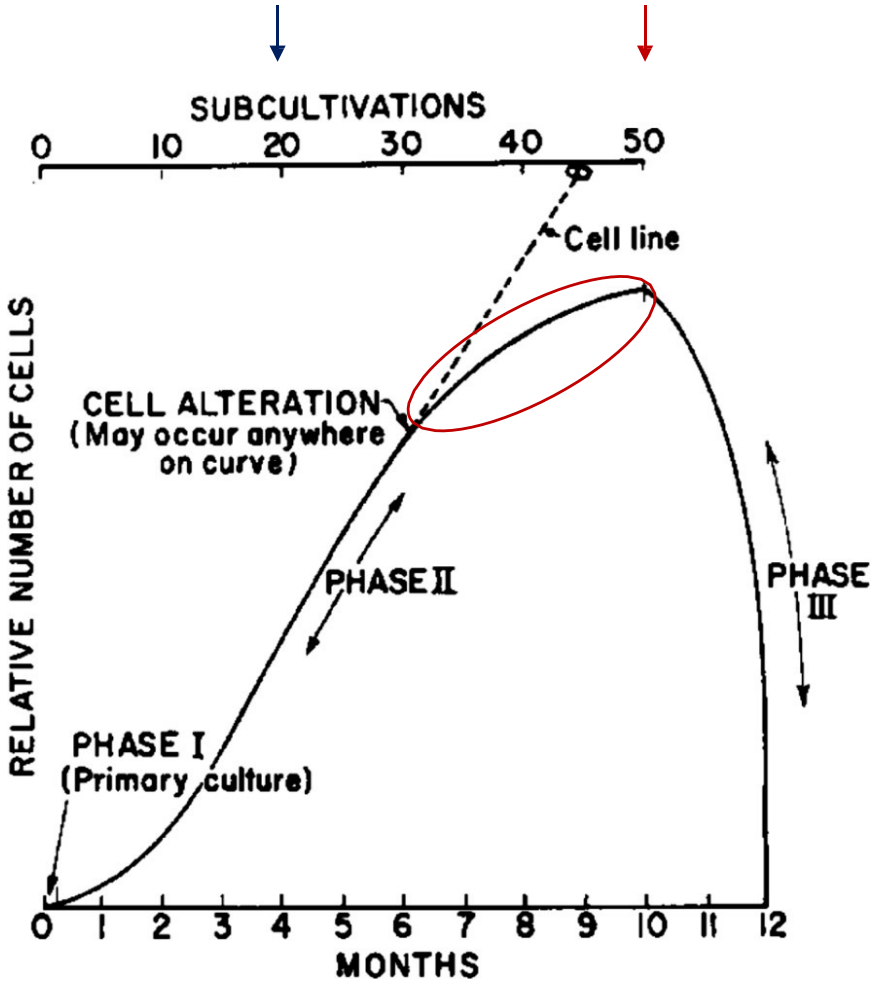
Hayflick & Moorhead, 1961

LES CELLULES PRIMAIRES ONT UN POTENTIEL DE DIVISION LIMITÉ (HORLOGE MOLÉCULAIRE ?

SÉNESCENCE RÉPLICATIVE : CARACTÉRISTIQUES DES CELLULES SÉNESCENTES

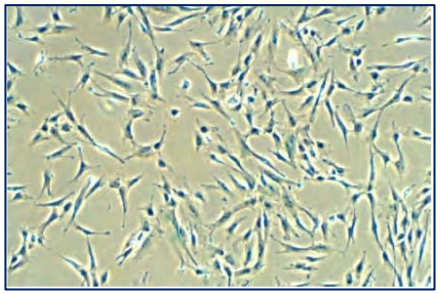
Sudan Black B

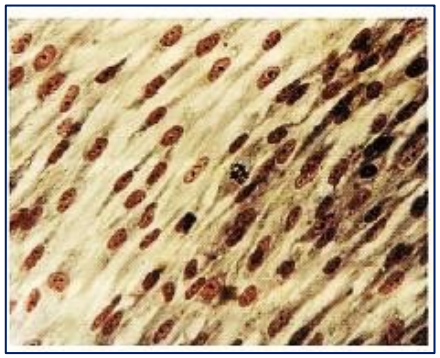


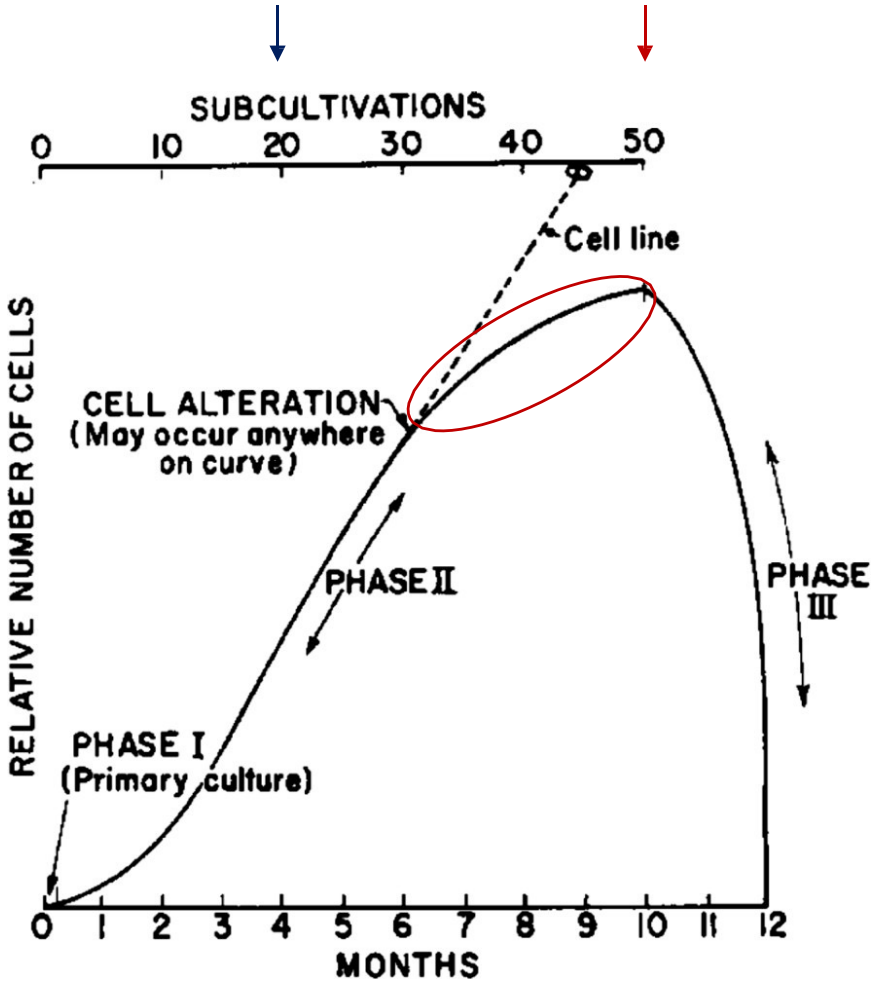


- Non prolifération
- Changements morphologiques

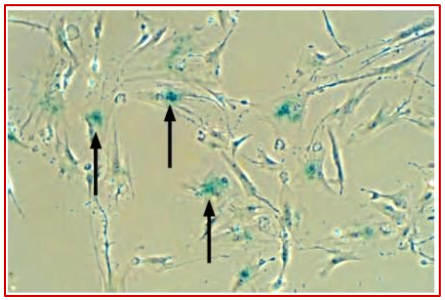
SÉNESCENCE RÉPLICATIVE : CARACTÉRISTIQUES DES CELLULES SÉNESCENTES







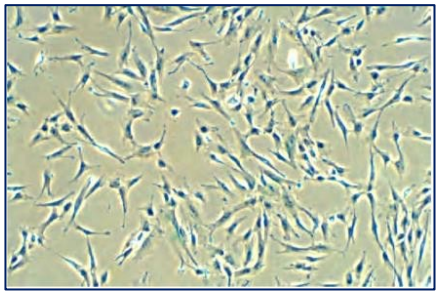
Activité
β-Galactosidase
(pH 6,0)

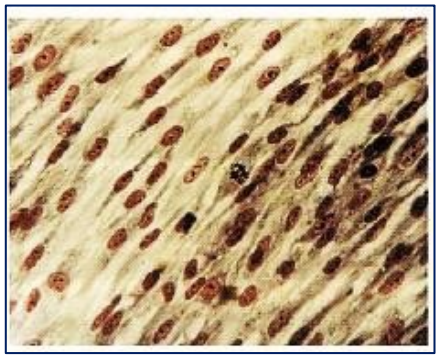


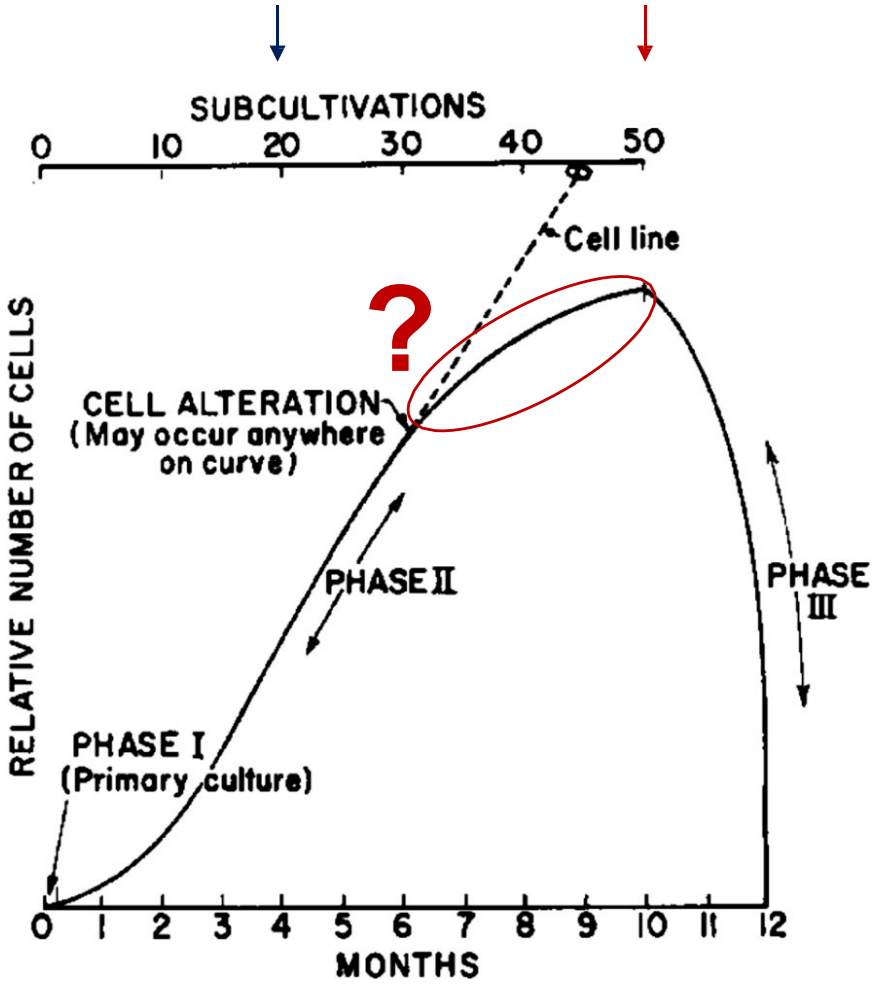
- **Non prolifération**
- **Changements morphologiques**
- **Changements lysosomiques**

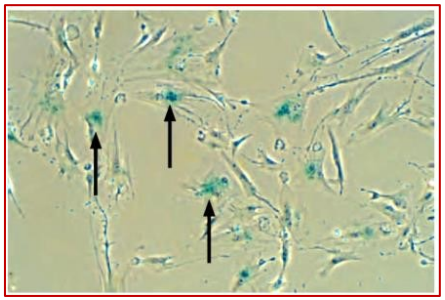
Fig.10.3 *The Biology of Cancer, 2014*

SÉNESCENCE RÉPLICATIVE : CARACTÉRISTIQUES DES CELLULES SÉNESCENTES



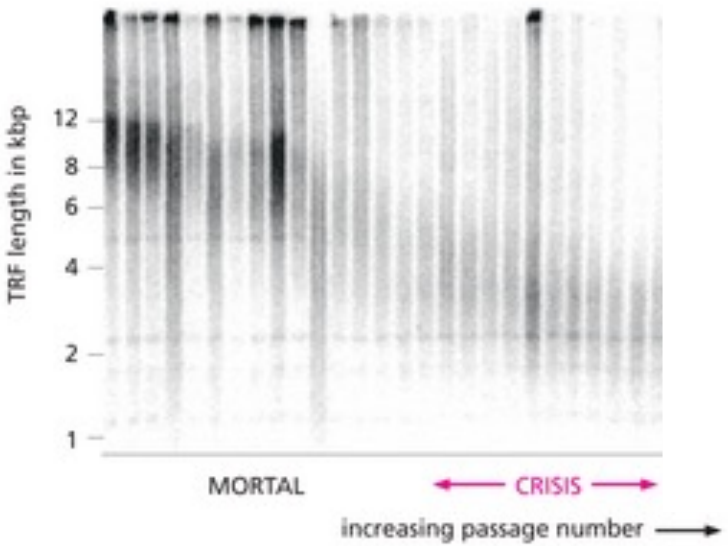




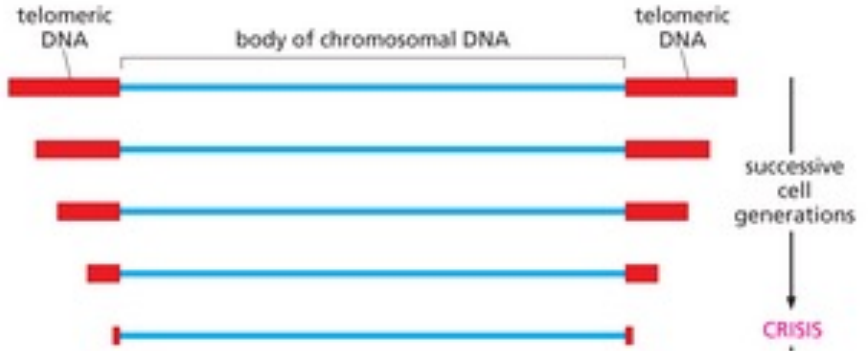


- Non prolifération
- Changements morphologiques
- Changements lysosomiques

Les télomères raccourcissent au fur et à mesure des cycles de divisions cellulaires



(A)



(B)

Les télomères protègent les extrémités des chromosomes

Les télomères sont composés de séquences d'ADN répétées associées avec des Protéines spécifiques des télomères (Shelterin complex), des histones et des protéines régulatrices qui protègent les extrémités de la voie de réponse aux dommages de l'ADN (DDR)

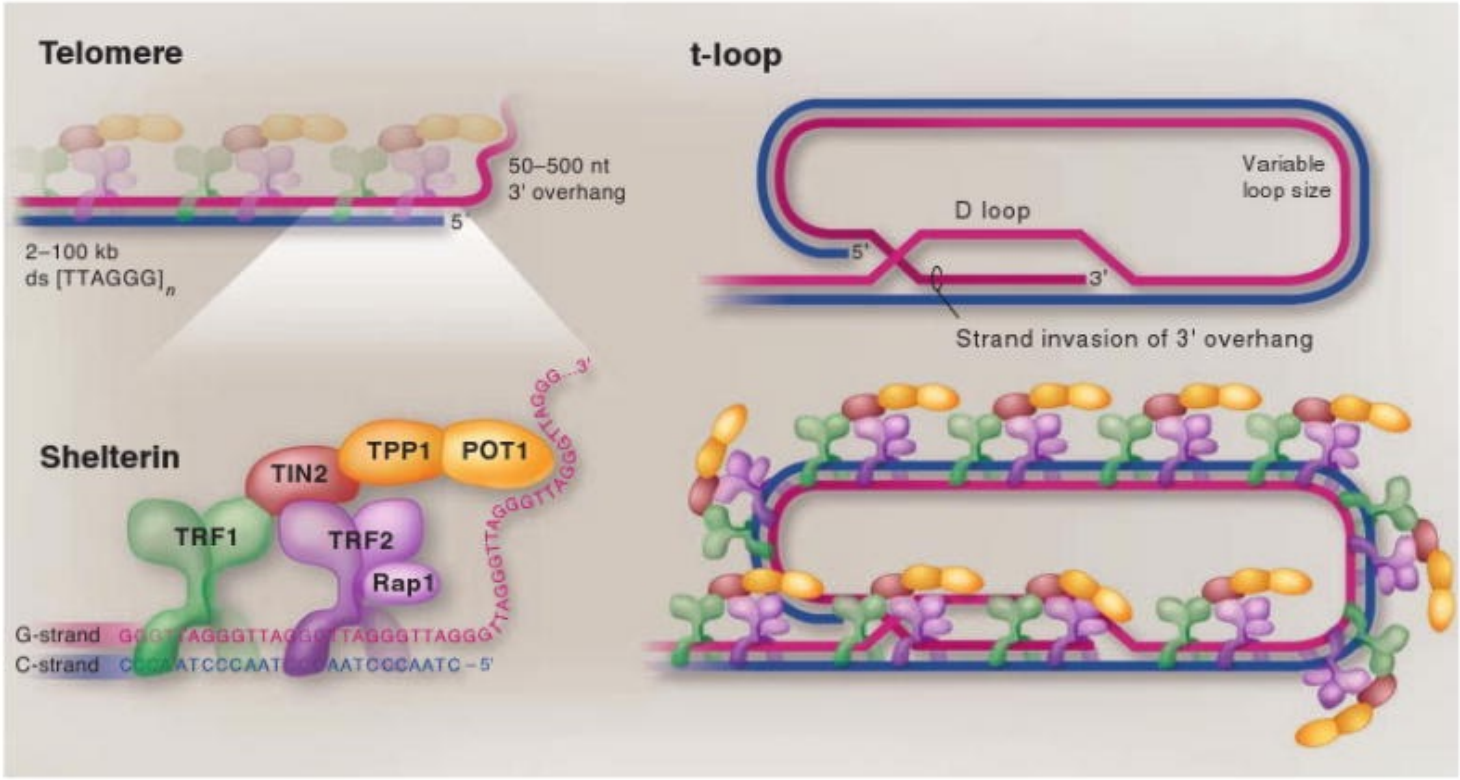
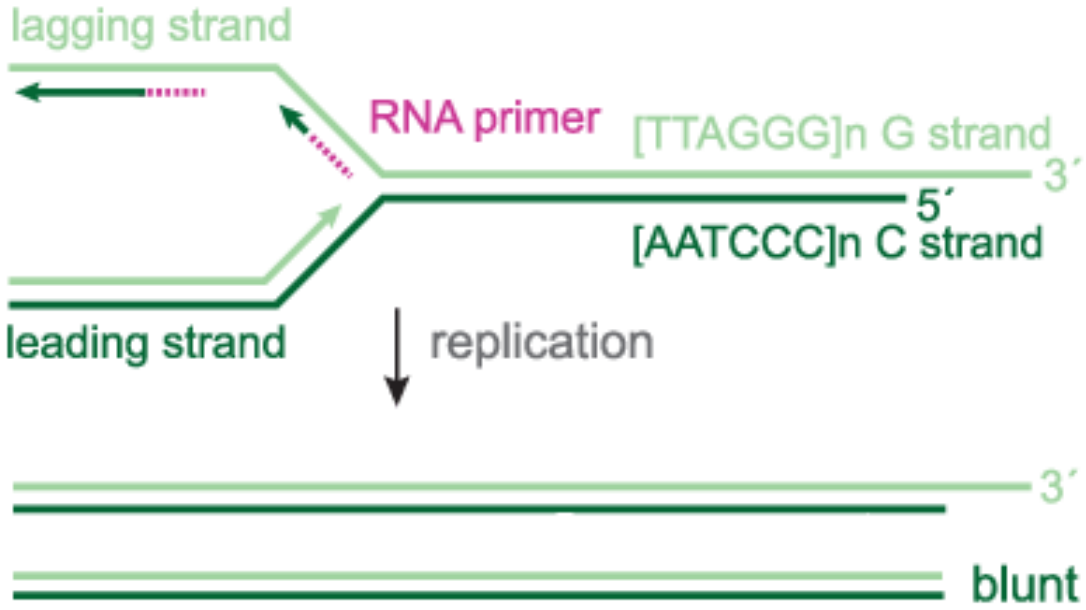


Figure: Mammalian telomeres are protected by the shelterin complex composed of 6 proteins: TRF1, TRF2, RAP1, TIN2, TPP1 and POT1.
The T-loop structure present at some telomeres in dividing cells also contributes to the capping function since it hides the 3' extremity in the heteroduplex.
Not shown is the telomeric RNA (TERRA) which can associate with telomeres and may provide a structural/protective role.

de Lange, Science 2009

Mécanismes d’érosion des télomères liés à la réplication

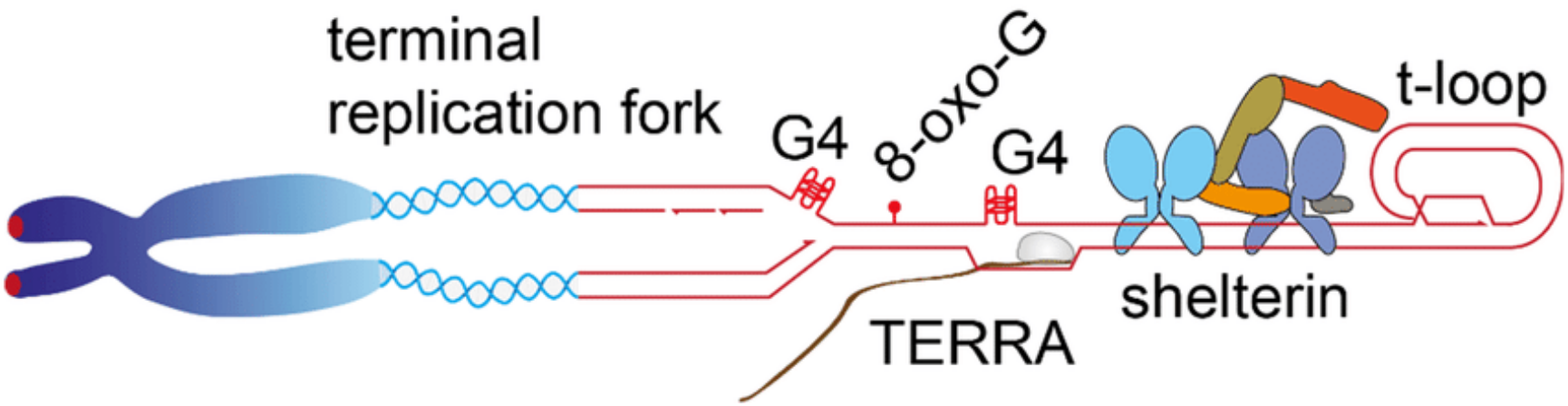
Telomere end-replication problem



La synthèse du brin discontinu est incomplète.

Télomères raccourcissent d'environ 25-50 pb par cycle cellulaire.

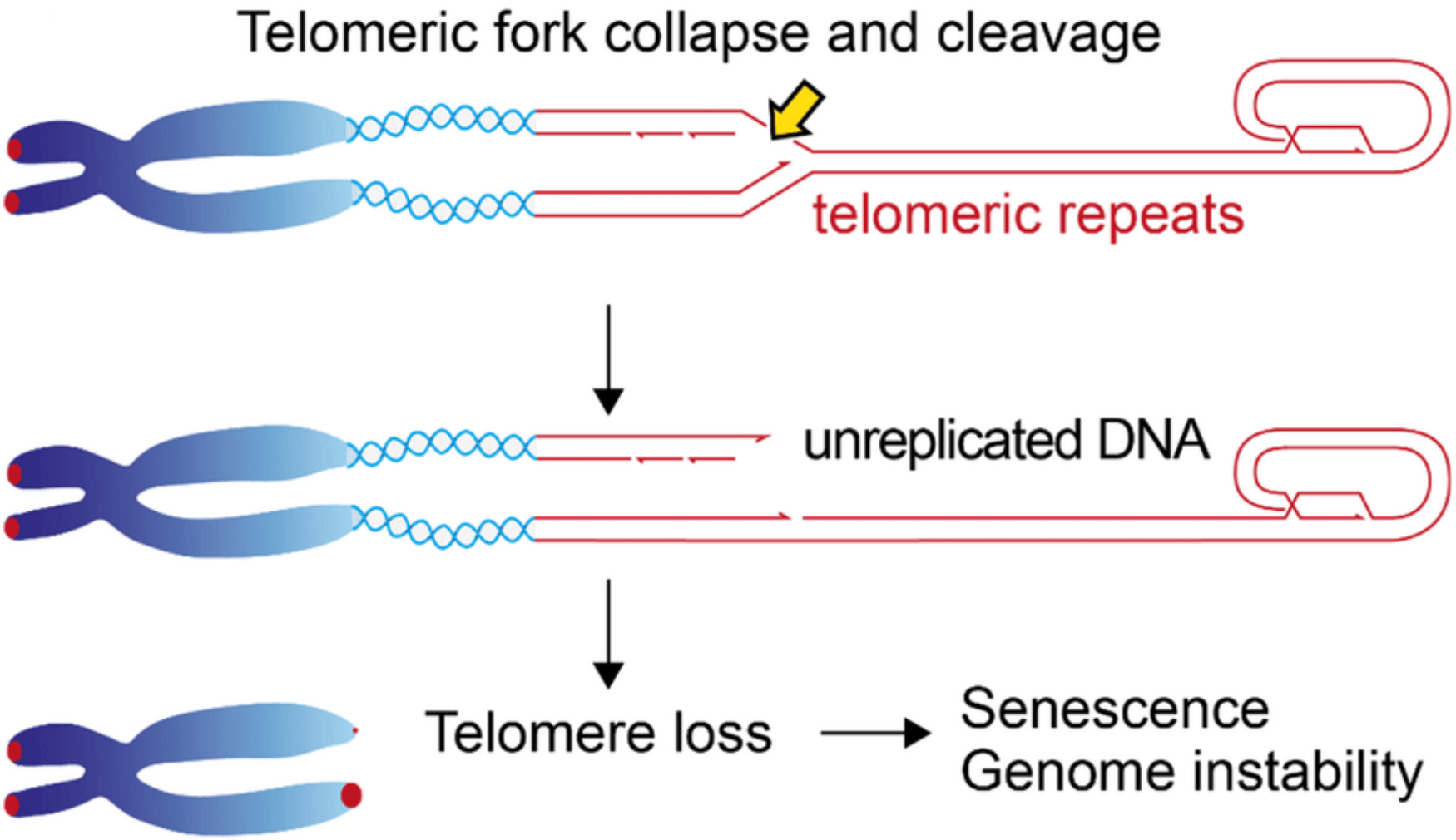
La réplication des télomères est difficile



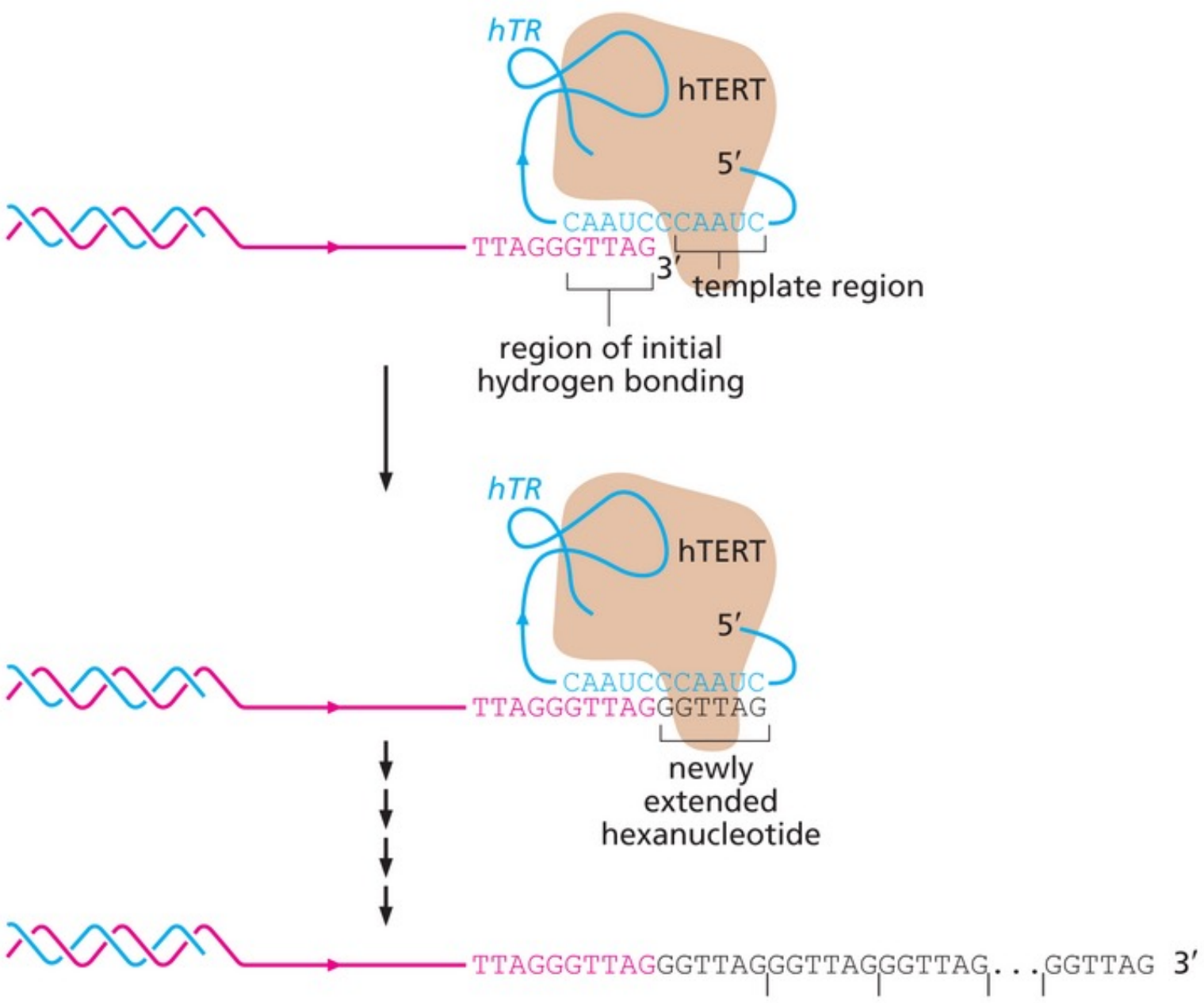
Telomères sont des séquences répétitives et G-riches
Présence des structures secondaires: G-quadruplex, t-loop
Présence du shélterin
Présence de TERRA

→ Collapse de la fourche

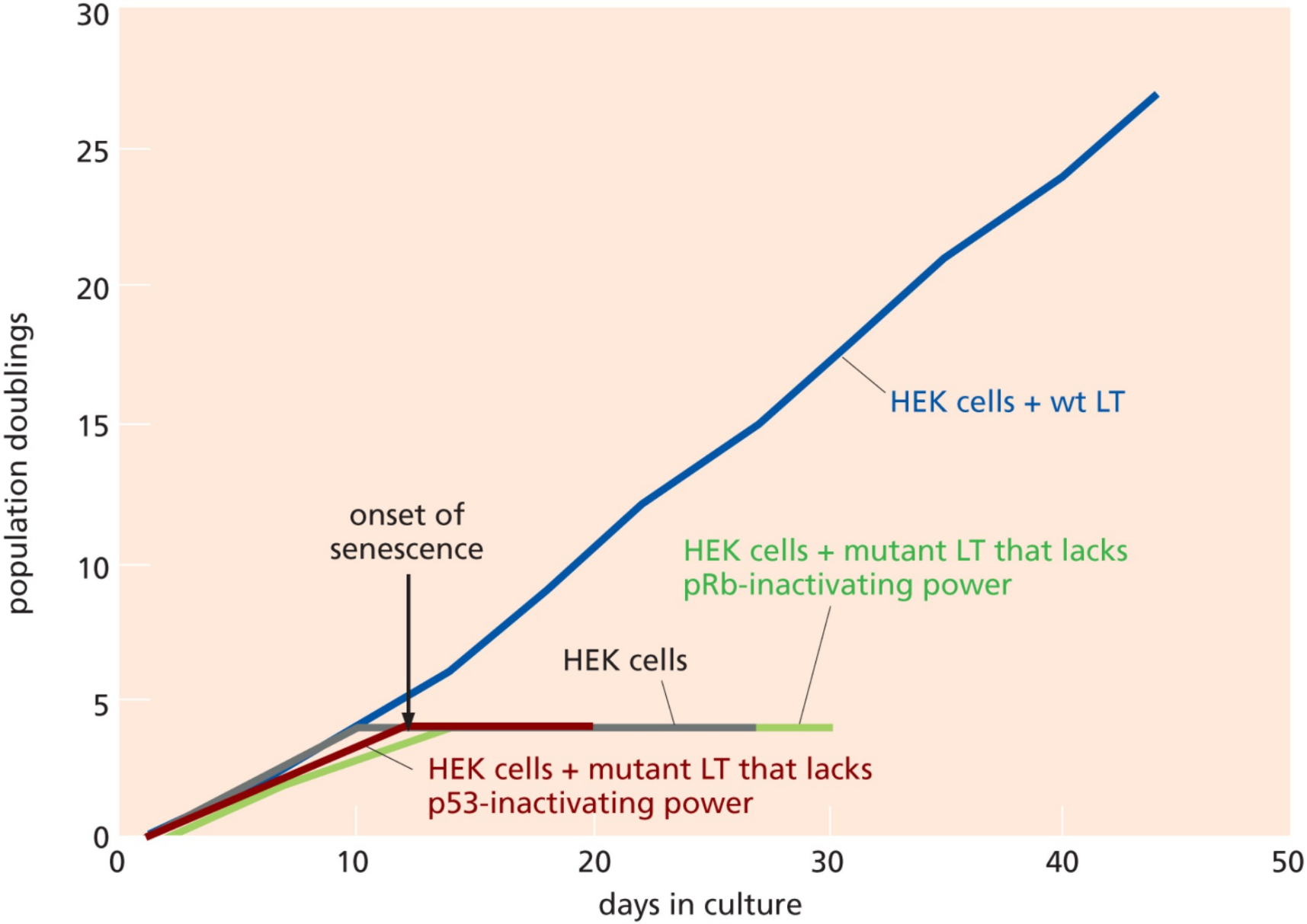
Collapse de la fourche et le raccourcissement télomérique brusque



L’érosion des télomères peut être contrebalancée par l’activité de la télomérase

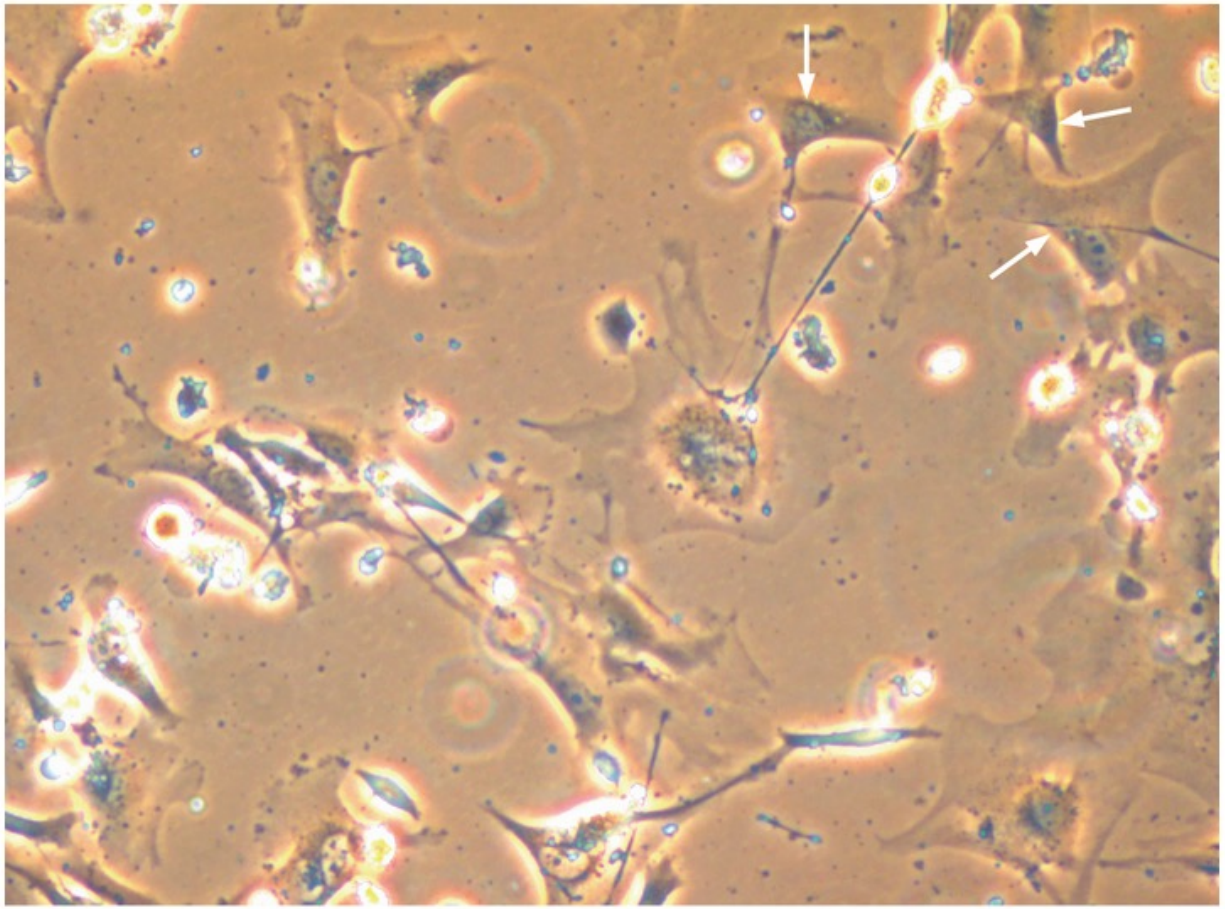


Rôles de l’antigène T de SV40 dans l’échappement à la sénescence réplicative

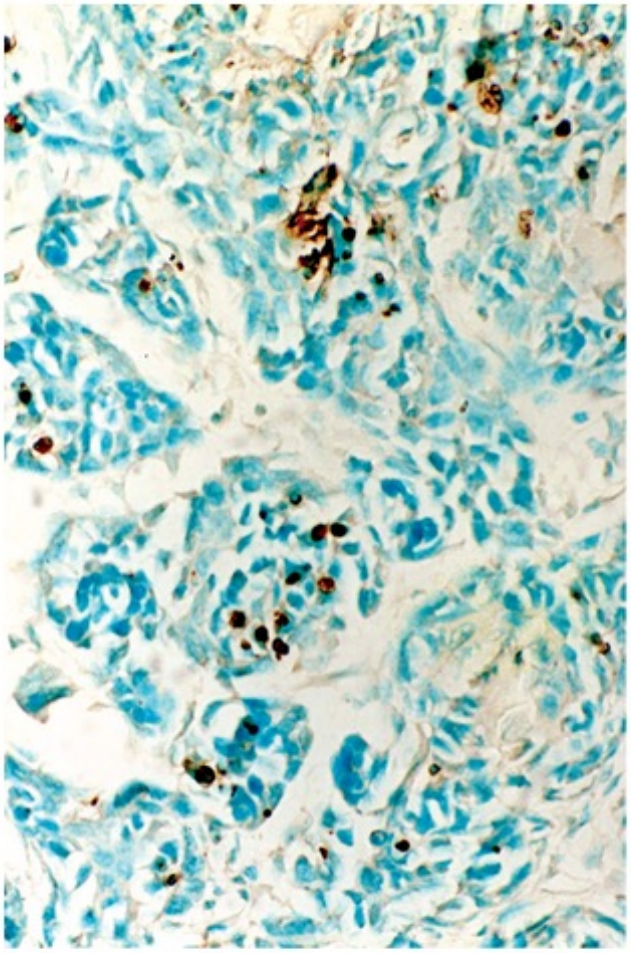


Crise & immortalisation

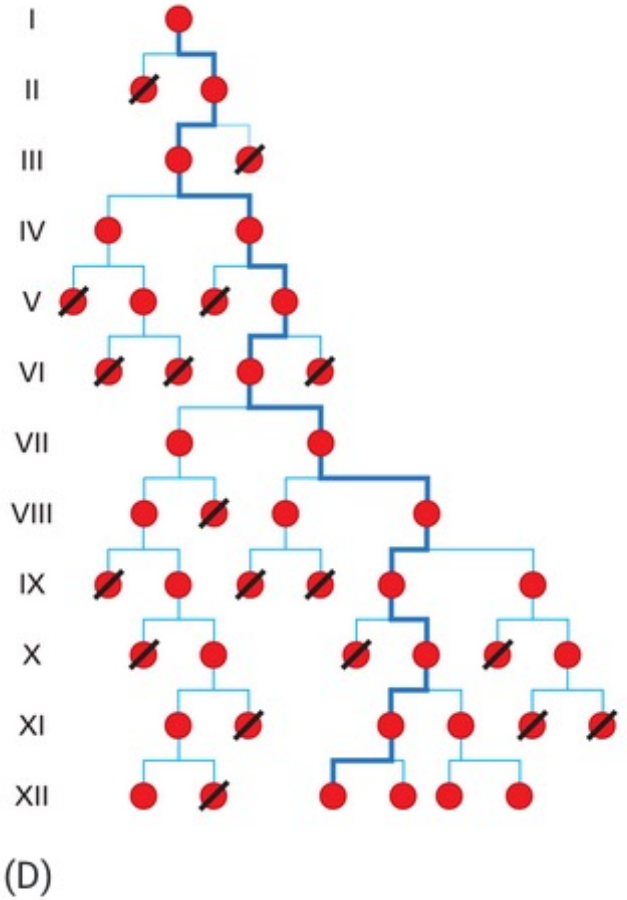
La crise est associée avec une forte induction de l’apoptose in vitro



Les cellules cancéreuses ont besoin de devenir immortelles pour former une tumeur!

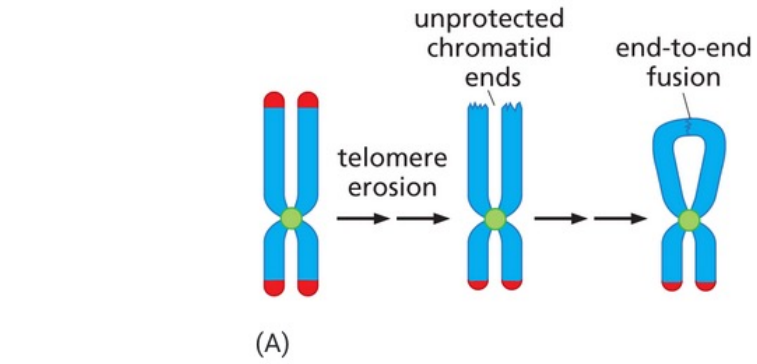


(C)

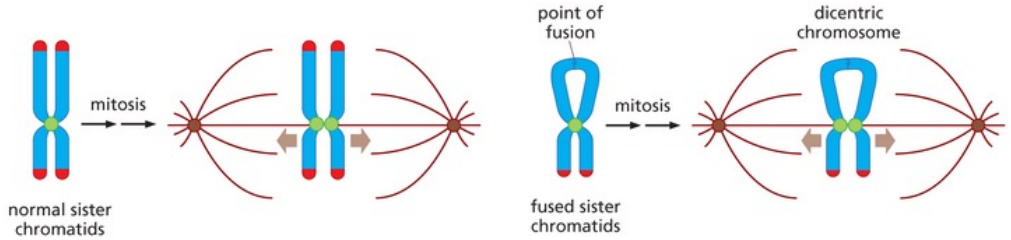


(D)

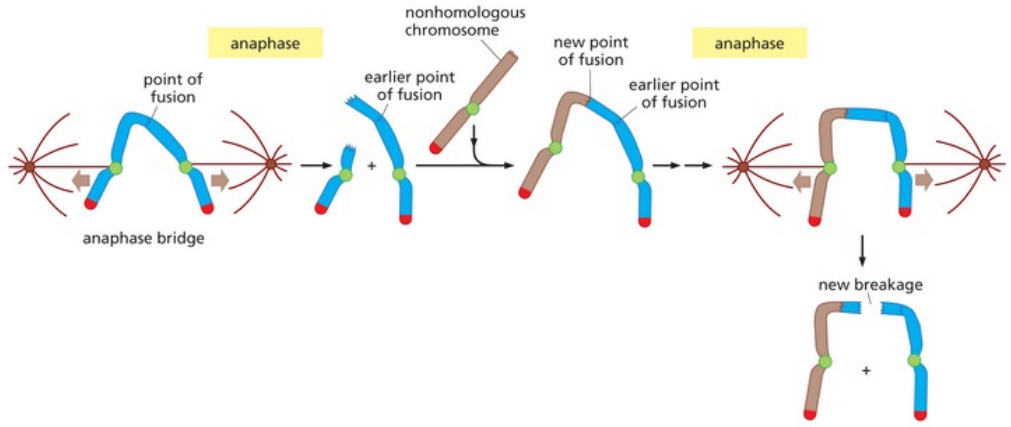
L’instabilité télomérique conduit aux rearrangements chromosomiques



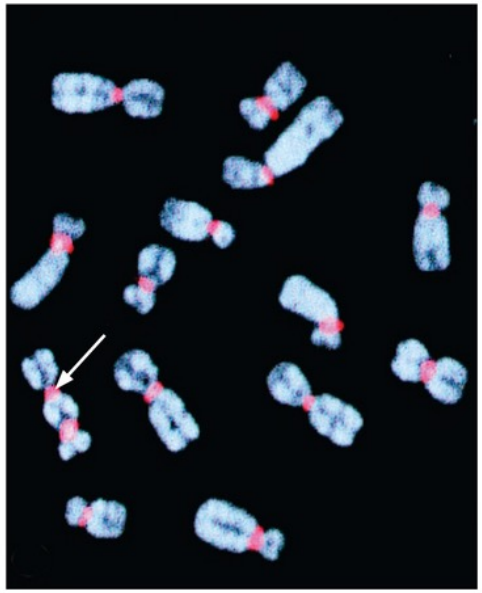
(A)



(B)

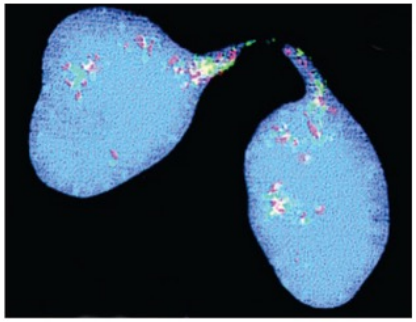


(C)



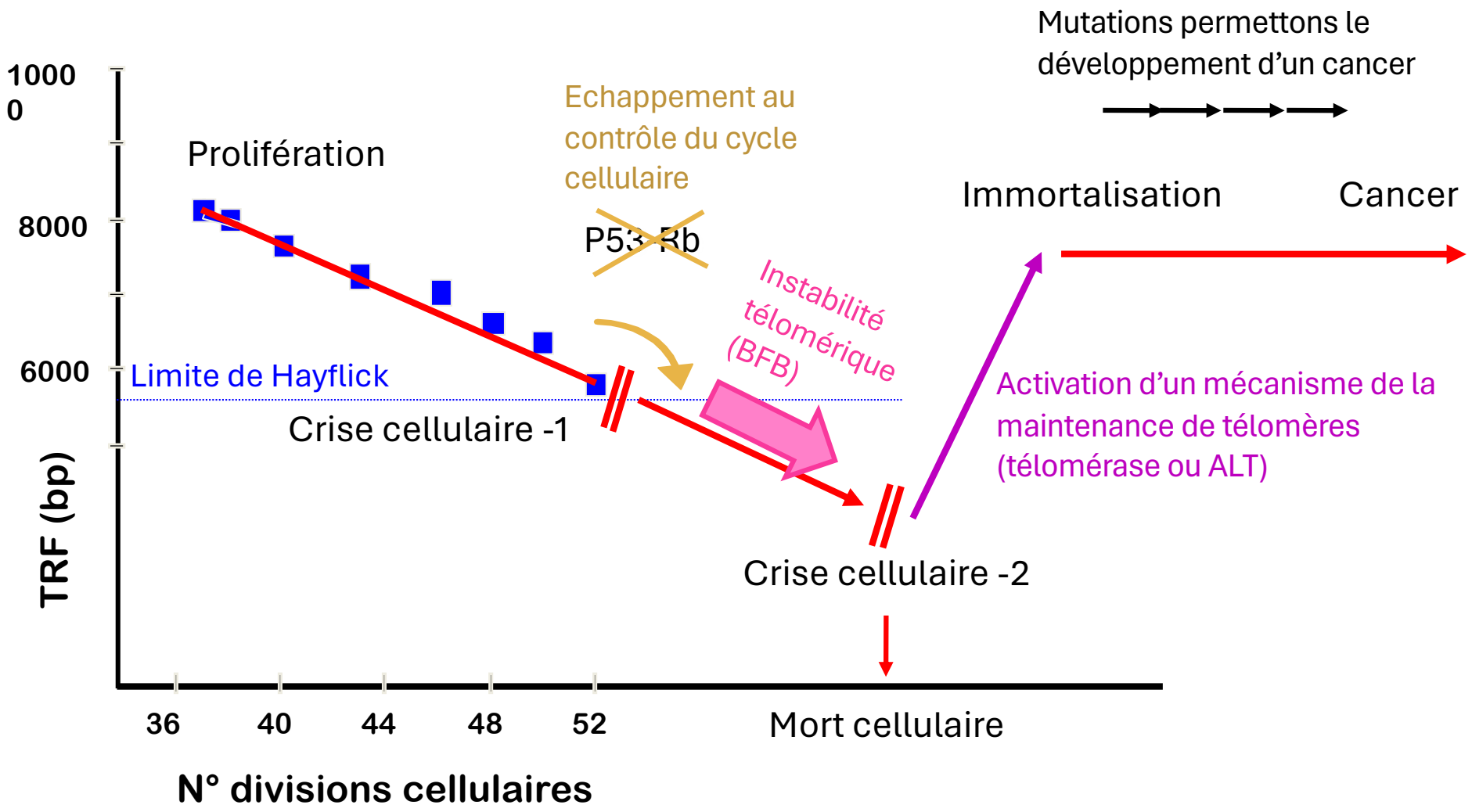
(A)

(B)

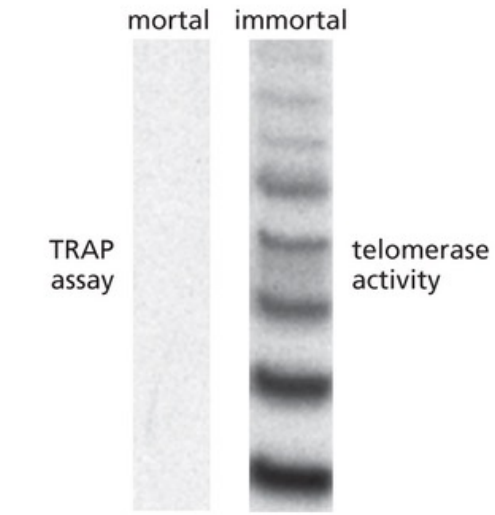


(C)

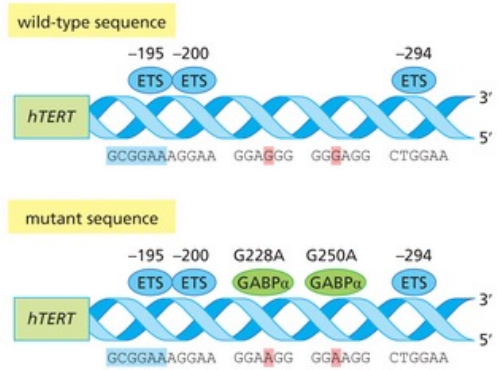
Instabilité télomérique conduit aux rearrangements chromosomiques



Réactivation « spontanée » de la télomérase dans des Lymphocytes T

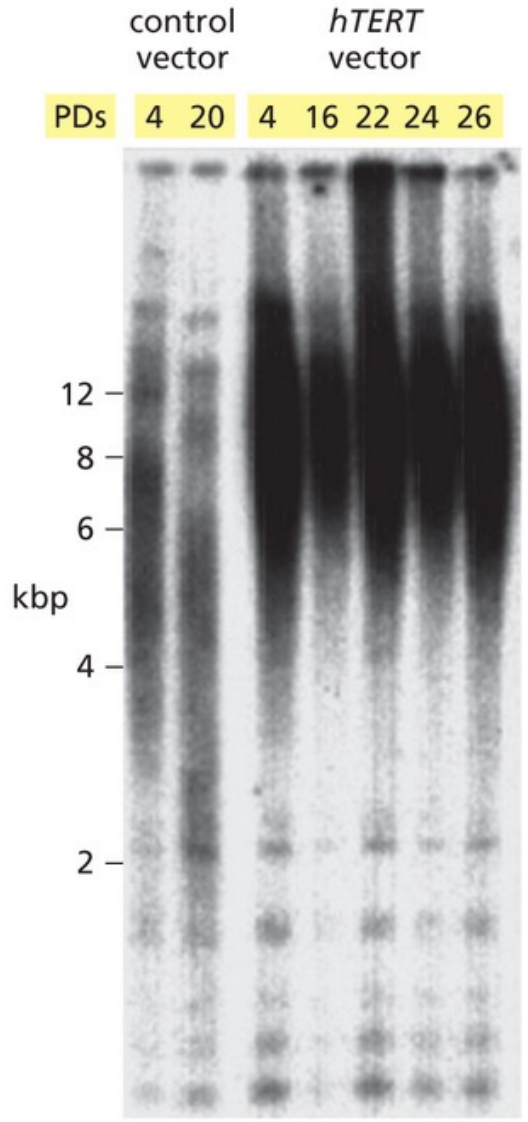


(A)

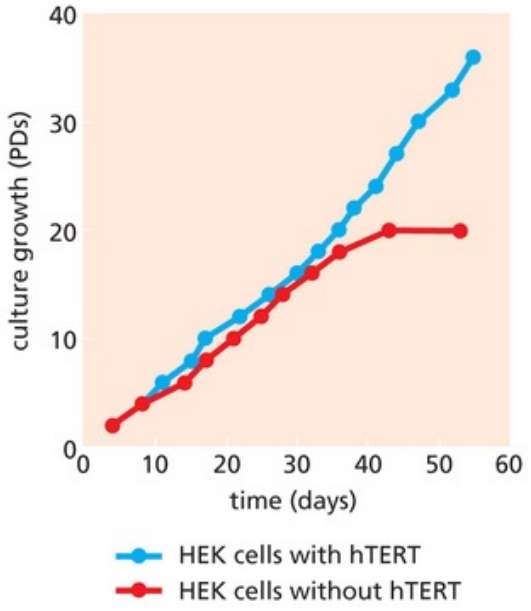


(B)

L’expression de la télomérase peut empêcher la crise et conduire à l’immortalisation des cellules

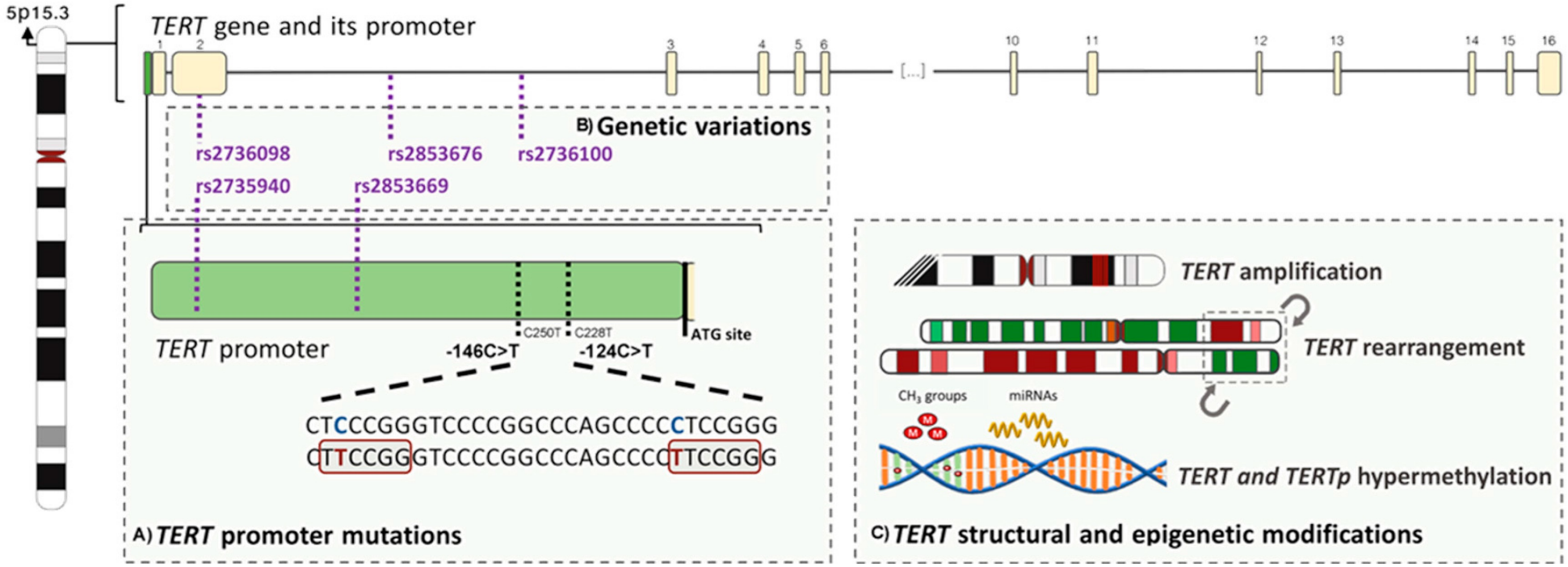


(A)

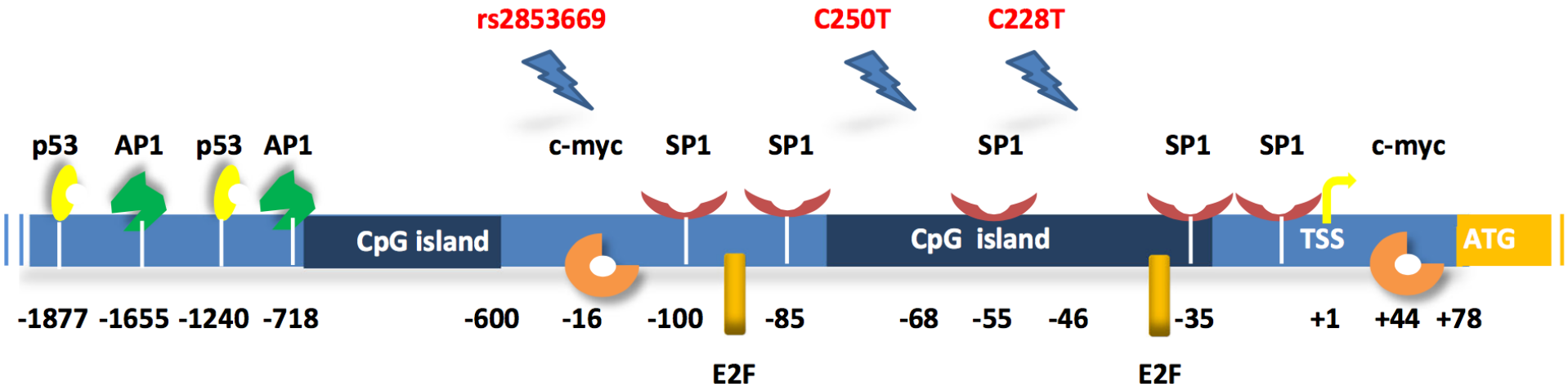


(B)

Re-activation de la télomérase dans les tumeurs



Mutations ponctuelles dans le promoteur du hTERT



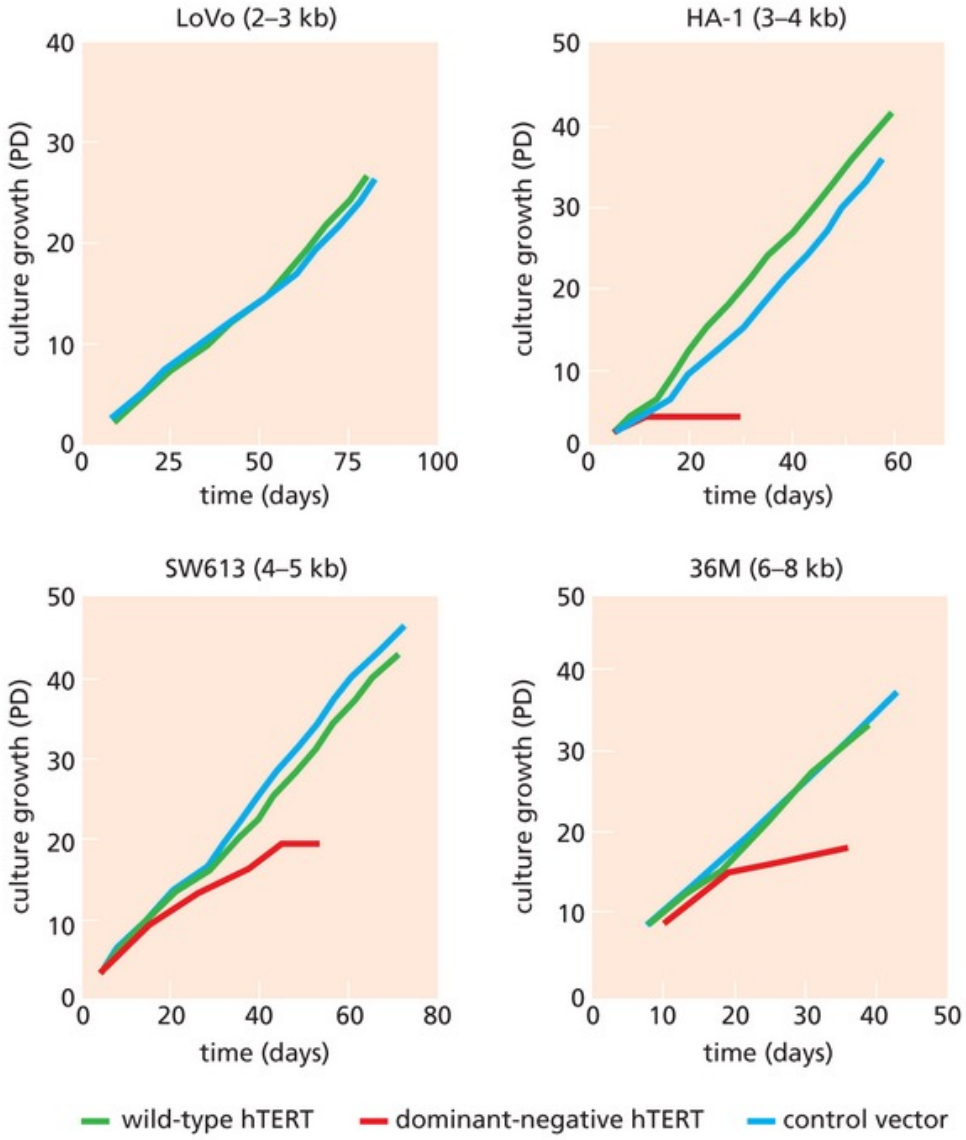
C228T (-124 pb avant l'ATG)
C250T (-146 pb avant l'ATG)
Création de la séquence CCCGGAAGGG (11 pb)
→ site de la liaison du facteur de la transcription EST
Mutations normalement hétérozygotiques et mutuellement exclusives.

Mutations ponctuelles dans le promoteur du hTERT

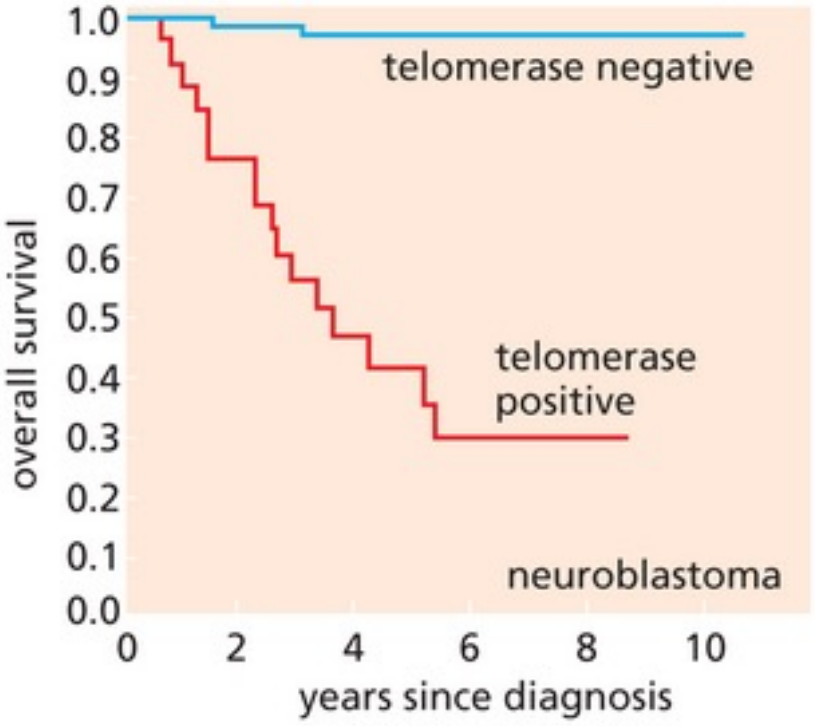
Les plus fréquentes dans les tumeurs suivantes:
83% glioblastomes
71% mélanomes
66% tumeurs de la vessie
47% hepatocarcinomes

Identifiées dans 50 cancers différents

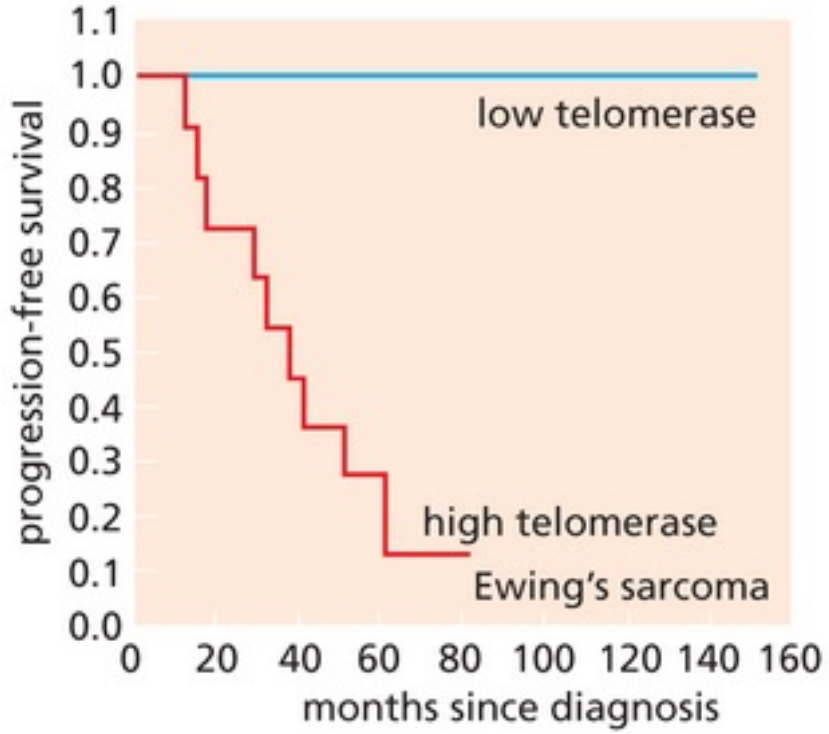
La suppression de l'activité télomérase dans les cellules tumorales peut conduire à l'arrêt de la prolifération



L’activité télomérase est un facteur de mauvais pronostic dans les tumeurs pédiatriques

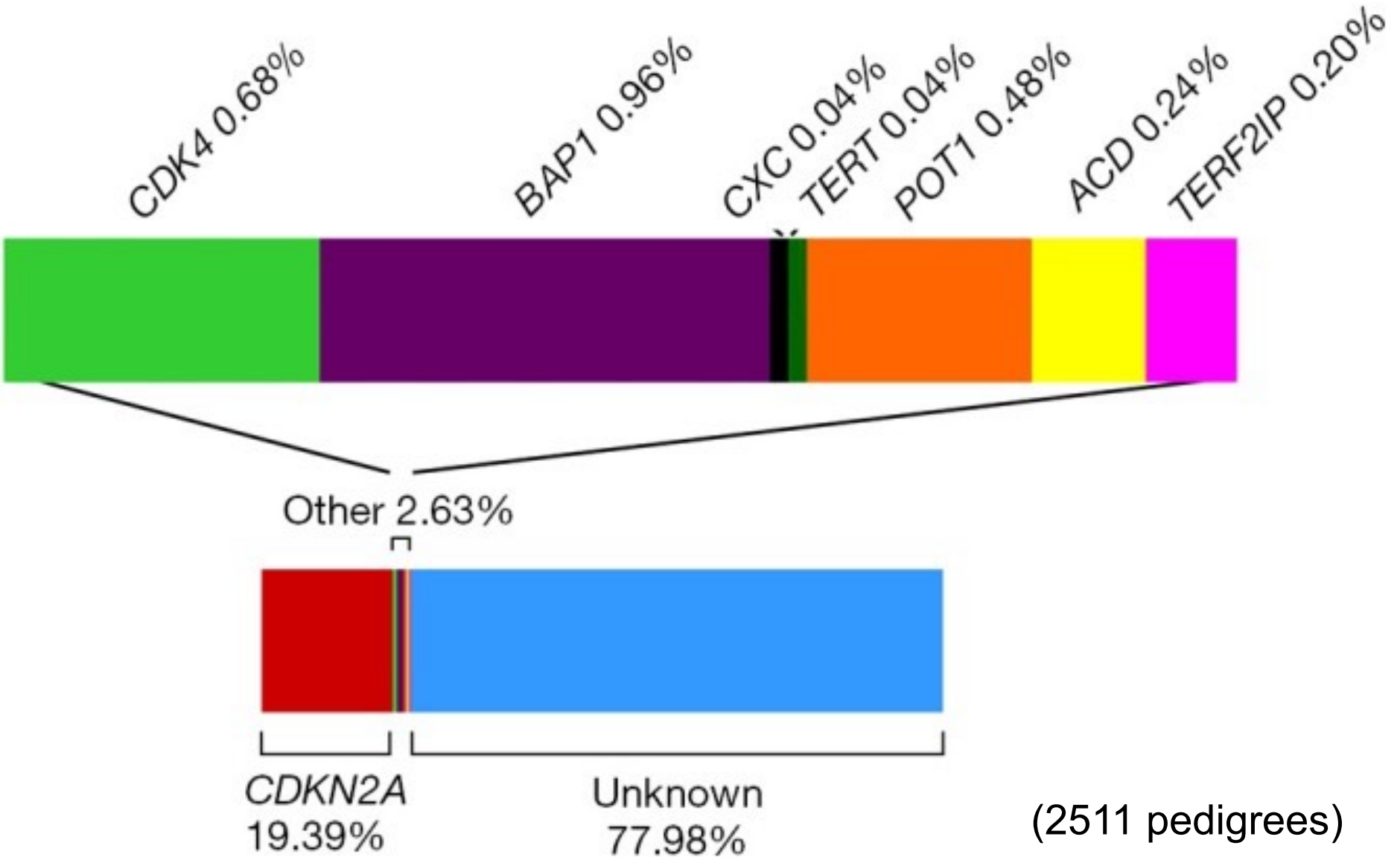


(A)



(B)

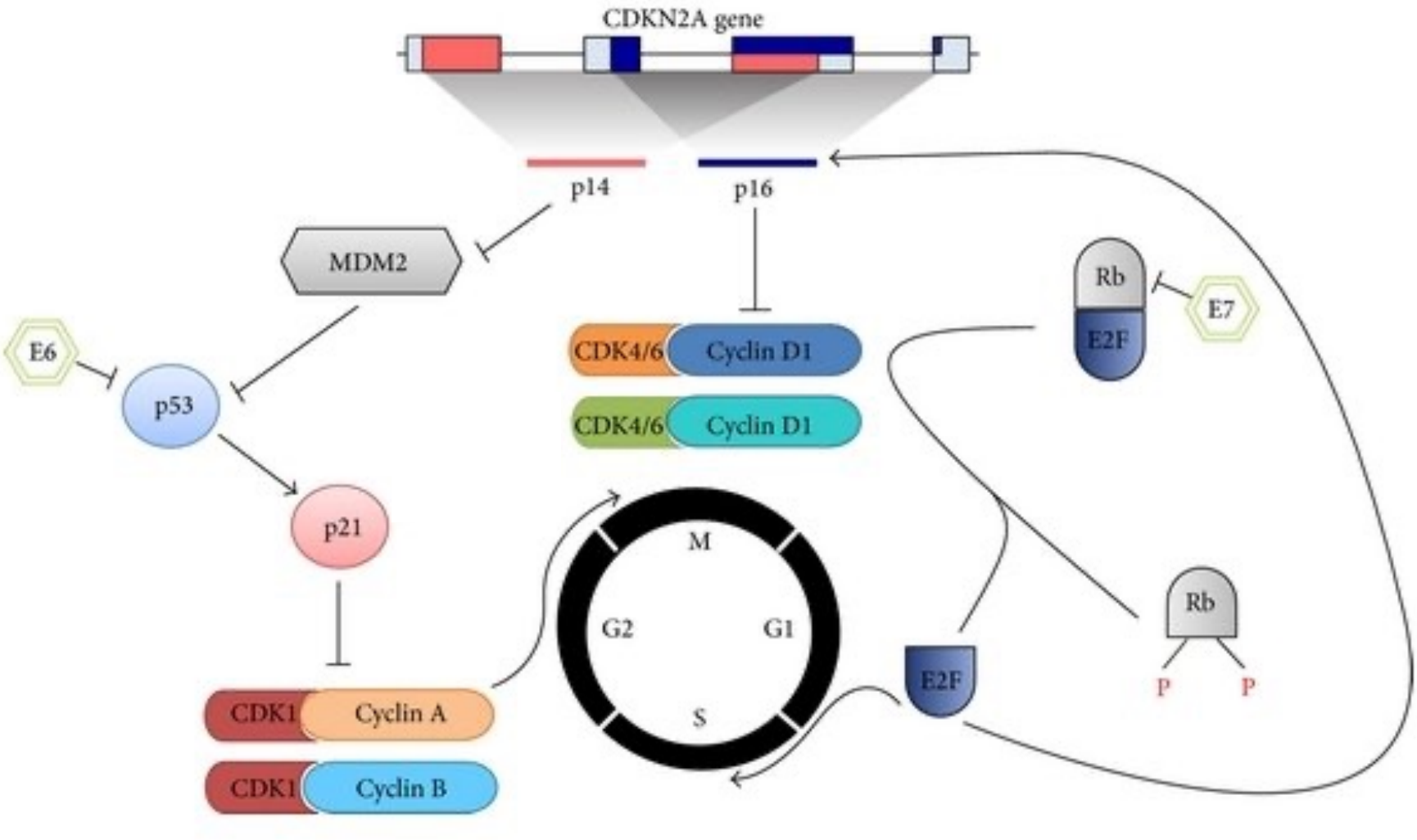
Mélanome Familial: Gènes associés



(2511 pedigrees)

Potrony et al. 2016.

RÔLE CLÉ DU GÈNE CDKN2A



Al-Kaabi et al, Disease Markers, 2014

L'activité CDKN2A est requise pour induire la sénescence dans un contexte de stress oncogenique!