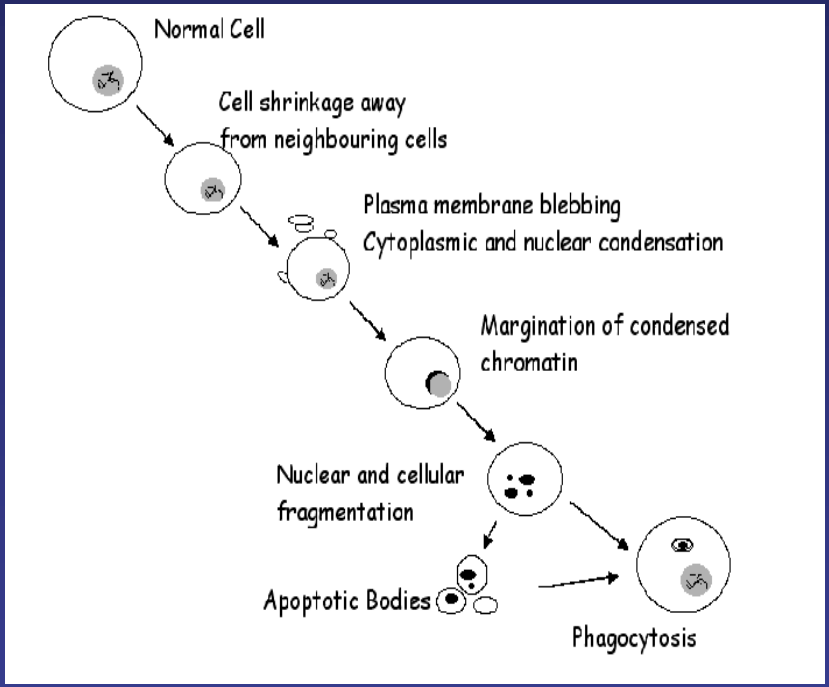
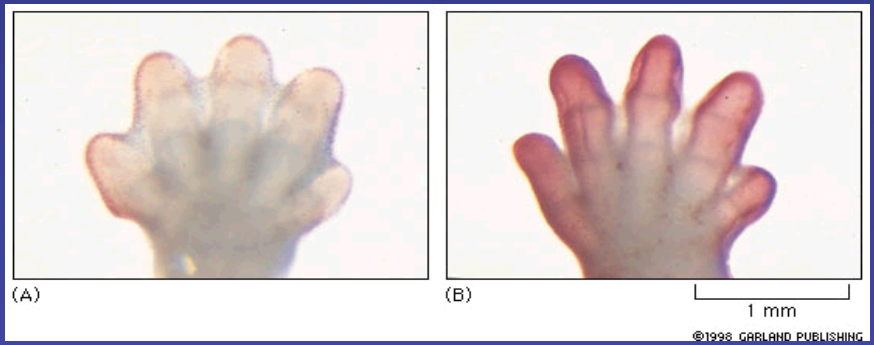
Cellular defenses against cancer: Apoptosis, Senescence and Autophagy

Gerardo Ferbeyre BCM 3512

Programmed cell death



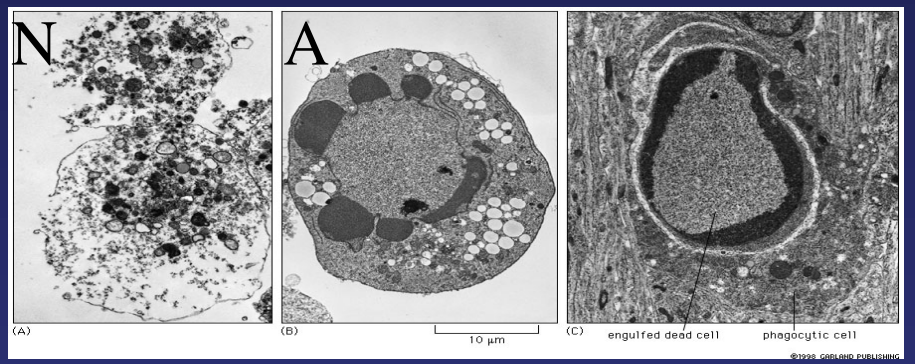


Fingers are formed after programmed cell death of interdigital tissue

Functions of apoptosis

- Control of normal cell number (during development most tissues produce cell sin excess of what is needed, they are eliminated by apoptosis)
- Elimination of damaged cells.
- Programmed in vivo means predictable at specific developmental stages and in specific locations (Lockshin 2016)

Apoptosis vs. necrosis

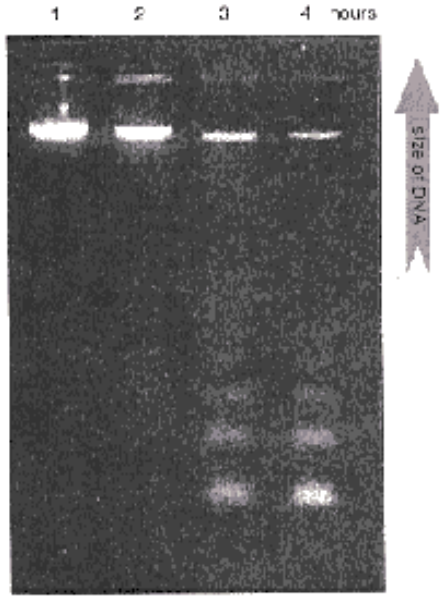


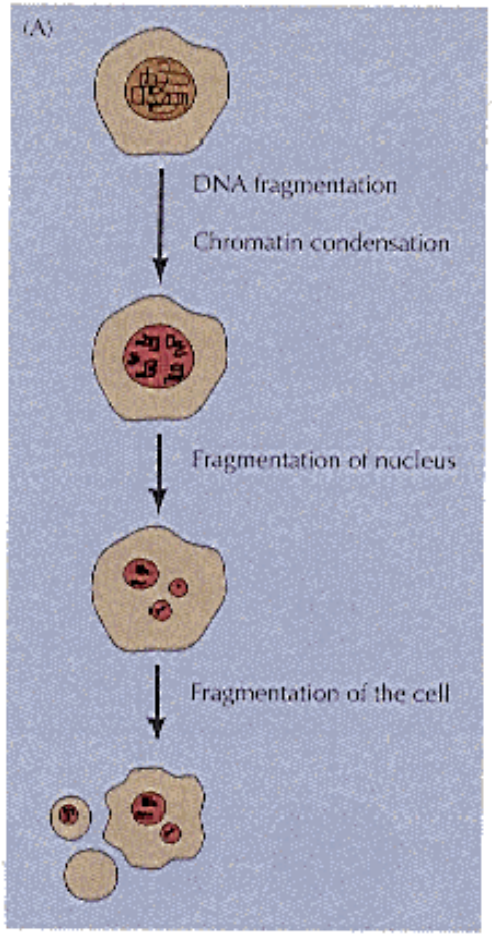
- Programmed
- Regular chromatin condensation
- Intact organelles
- Apoptotic bodies (compact cells)
- Apoptotic cells can be observed besides normal cells
- No leakage and inflammation

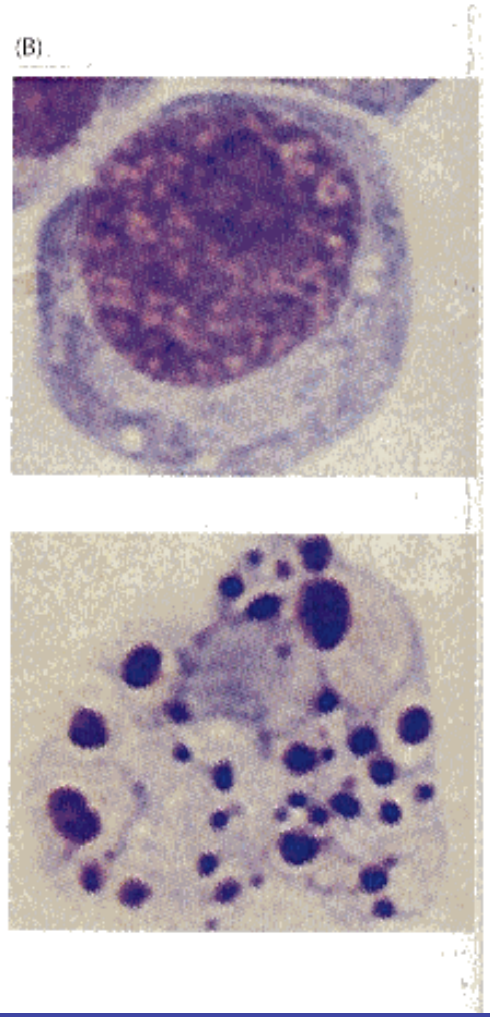
- Accidental
- irregular
- Organelles are destroyed
- Cells are enlarged
- Many cells in proximity are affected
- Leakage of intracellular components that trigger inflammation

Detection of apoptosis

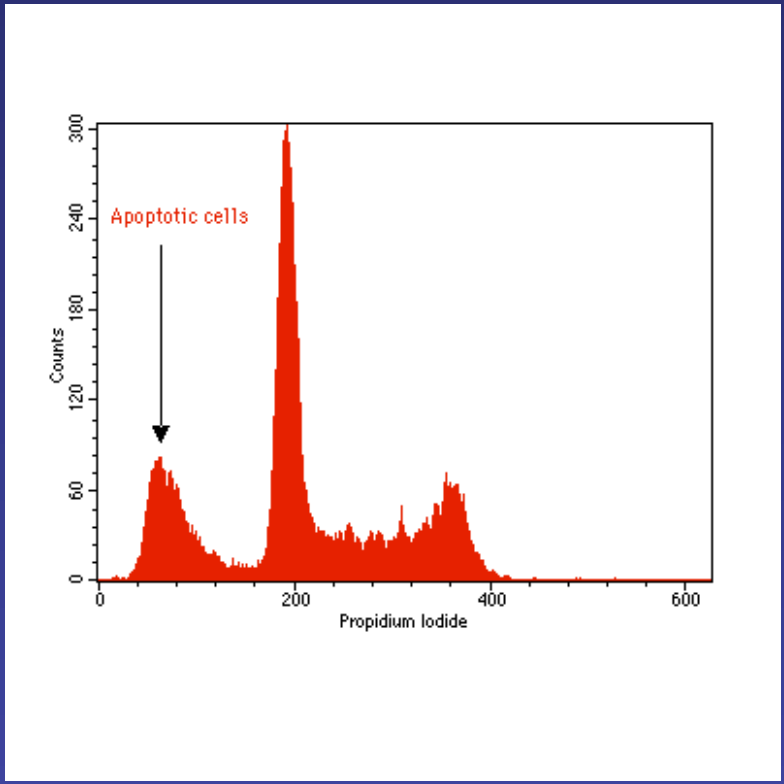
Characteristics:
1. Chromatin condensation
2. DNA fragmentation







Propidium iodide staining



Apoptosis inducers

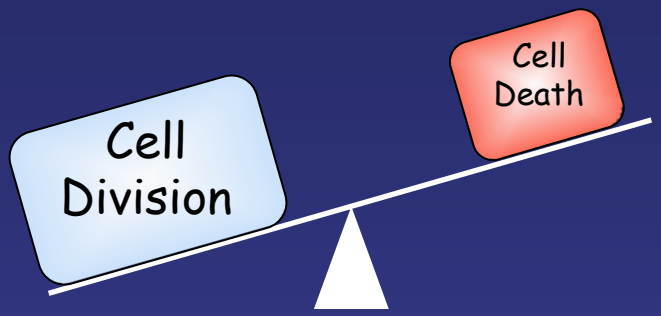
Physiological inducers:

- Cytokines: TNF, TGFß, Fas-ligand
- Neurotransmitters: glutamate, dopamine, NMDA
- Lack of GF stimulation
- Loss of interaction with the ECM (anoikis)
- Hormones: glucocorticoïdes

Stress:

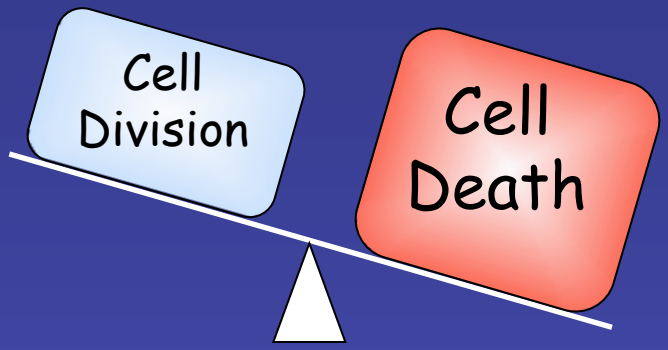
- Oncogenes: myc, E1A
- Heat shock
- Calcium
- Viruses
- Bacteria
- Free radicals
- Drugs (chemotherapy)
- Radiation (DNA damage)

Apoptosis and disease



Low apoptosis

- Viruses
- Cancer
- Autoimmune diseases



High apoptosis

- AIDS
- Neurodegenerative diseases
- Ischemia

Executioners

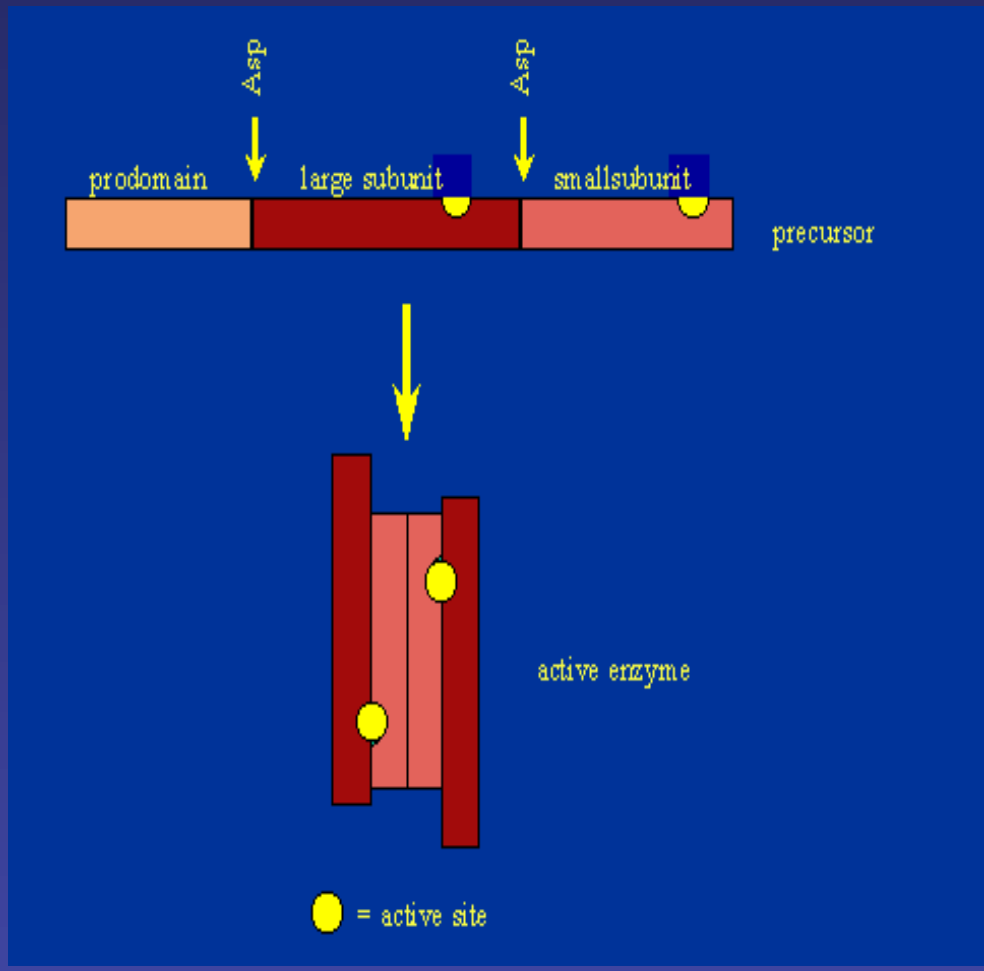
Caspase-dependent

Caspase-independent:

- Cathepsins
- Apoptosis Inducing Factor (AIF)
- EndoG

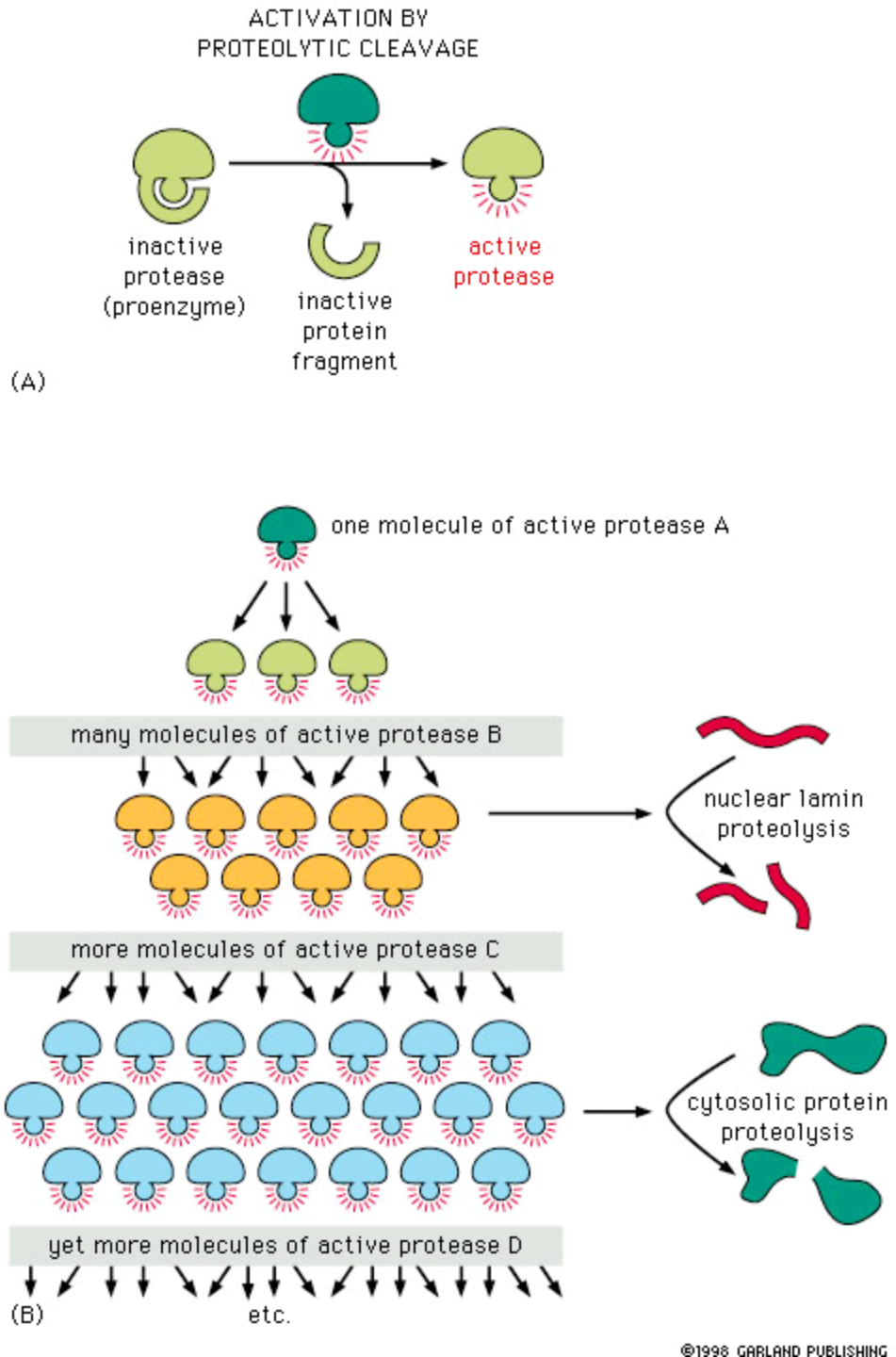
Caspases

- Cysteine proteases cleaving after Asp.
- Synthesized as inactive precursors (zymogen).
- Activated by proteolysis
  - At Asp residues
  - Elimination of N-terminal pro-domain
  - Form tetramers.
- Adaptor proteins concentrate procaspases to start a catalytic cascade reaction.
- 14 caspases: 1, 4, 5, 11 and 12 non apoptotic.
- Regulatory Caspases : Caspases 2, 8, 9 and 10.
- Executioner Caspases : 3, 6, 7



Protease cascade

- Apoptotic stimuli induce activation of one or more of the initiator caspases through specific oligomerization platforms.
- The initiators then trigger a cascade-like proteolytic stimulation of effector caspase zymogens.

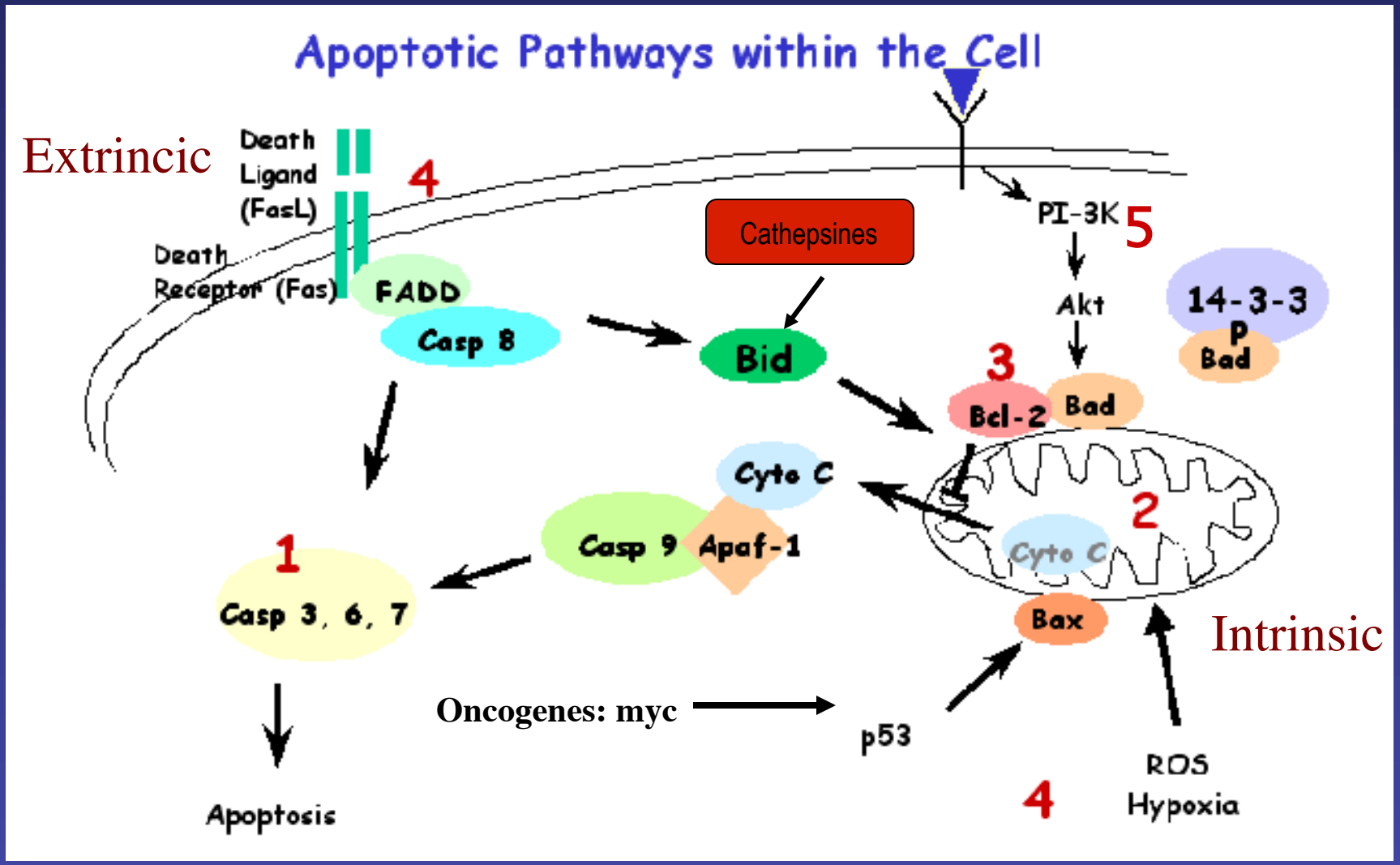


Caspases functions in apoptosis

- Execution phase of the apoptotic death program by cleaving hundreds or even thousands of structurally and functionally critical proteins within the cell
- Inactivation of anti-apoptotic proteins (i.e., ICAD).
- Lamin degradation: lamins provide attachment point for DNA known as lamin attachment domains. Lamin degradation exposes DNA to nucleases.
- Activation of DNAses
- Cytoskeleton degradation (FAK, PAK).

http://bioinf.gen.tcd.ie/casbah/
http://www.scripps.edu/cravatt/protomap/

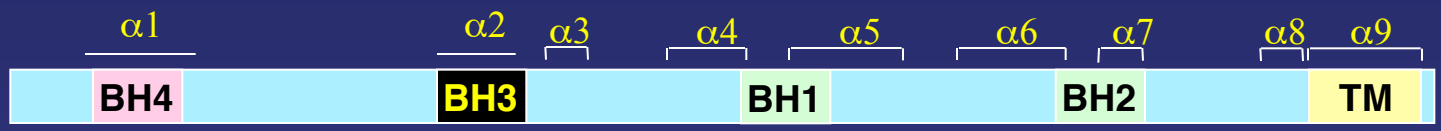
Apoptosis pathways



1) Caspases, 2) Mitochondria, 3) Bcl2 family, 4) Death signals, 5) Survival signals

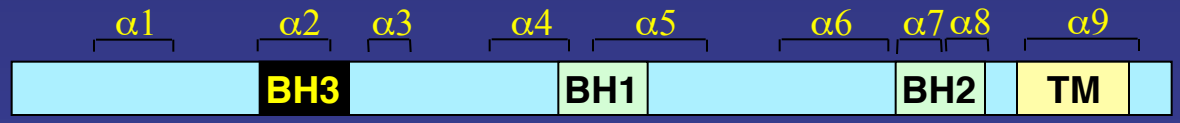
The BCL2 family and the BCL-2 homology domain (BH)

- Anti-apoptotic



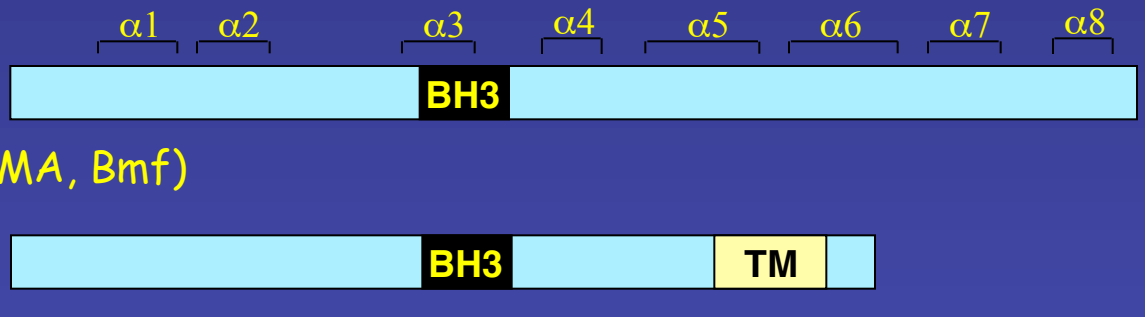
(Bcl-x_L, A1, Boo, Bcl-w, Mcl-1, NR13, Bcl-B)

- Pro-apoptotic



(Bak, Bok, Bcl-X_s, Bcl-rambo)

Multi-BH motif

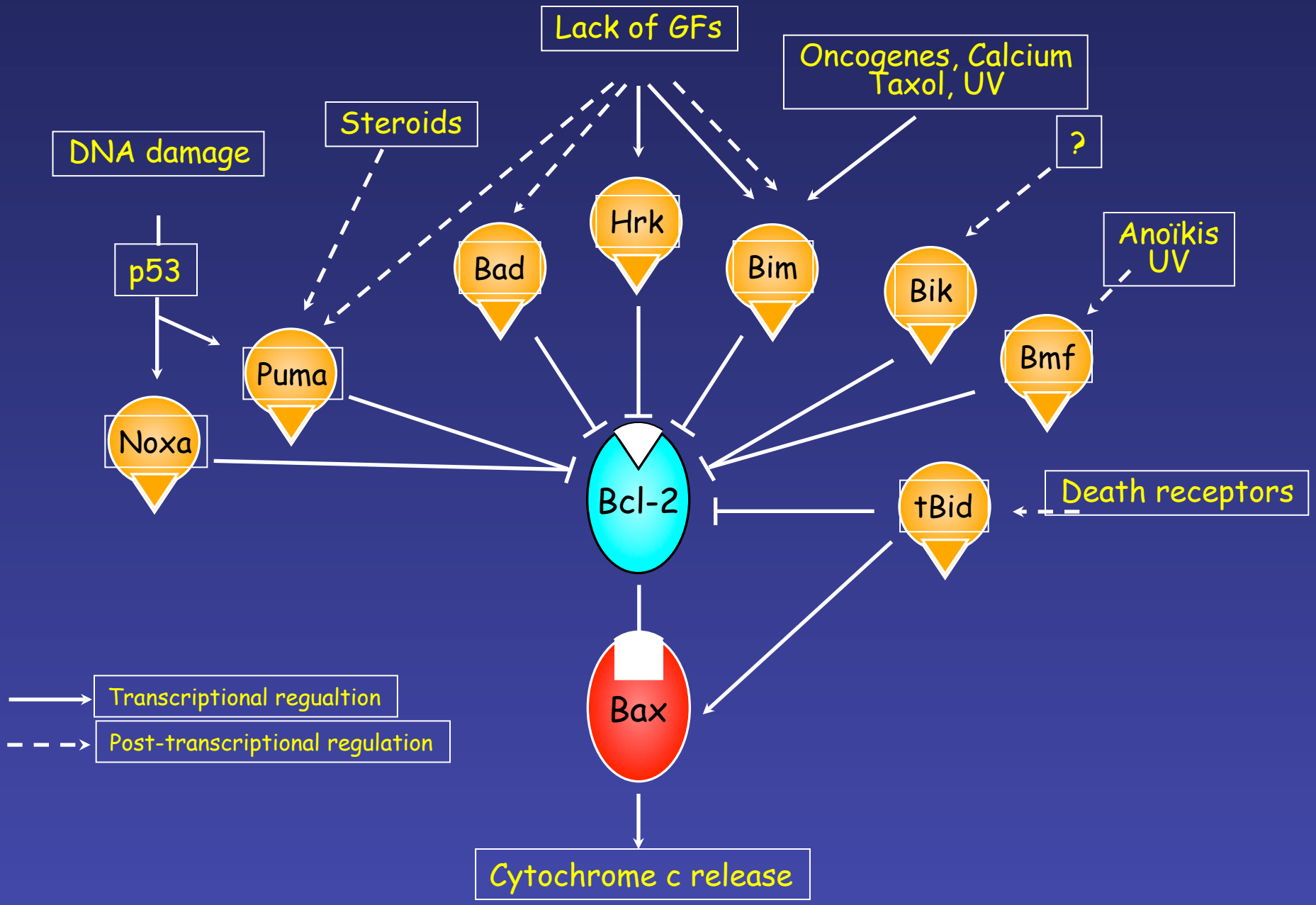


(Bad, Noxa, PUMA, Bmf)

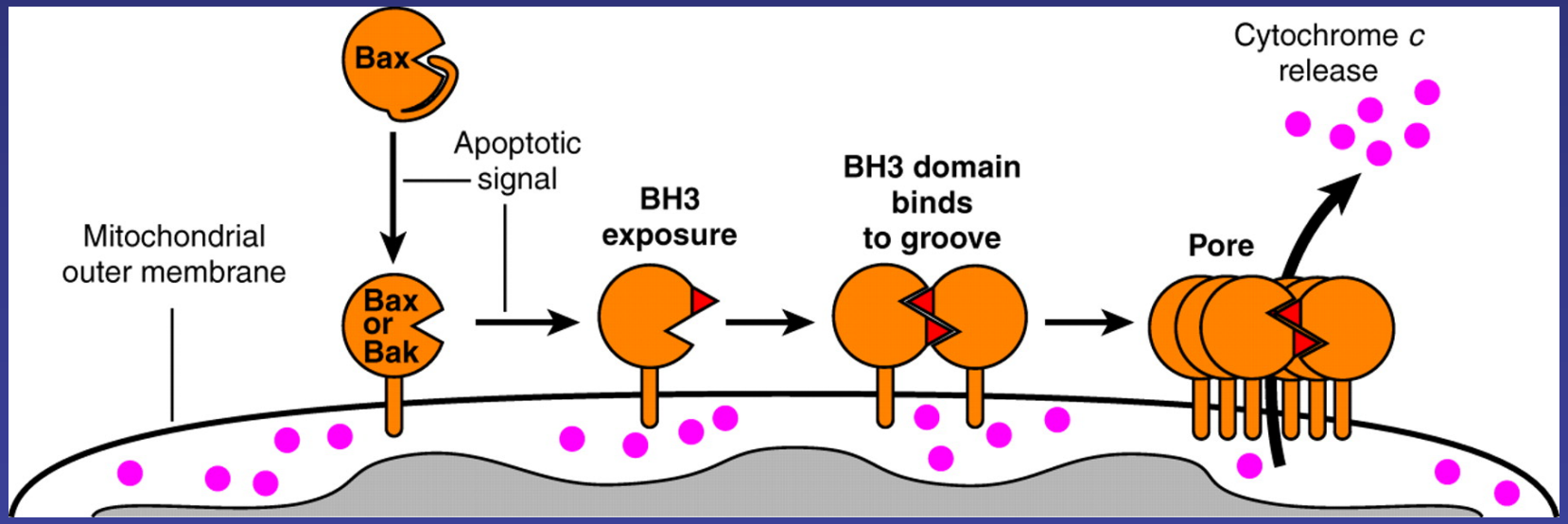
(Bik, Hrk, Blk, NIP3, BNIP3, Bbc3)

BH3-only

BH3-only as sensors of apoptotic stimuli



The BH3:groove model of Bak and Bax conformational change and oligomerisation during apoptosis.



Grant Dewson, and Ruth M. Kluck J Cell Sci 2009;122:2801-2808

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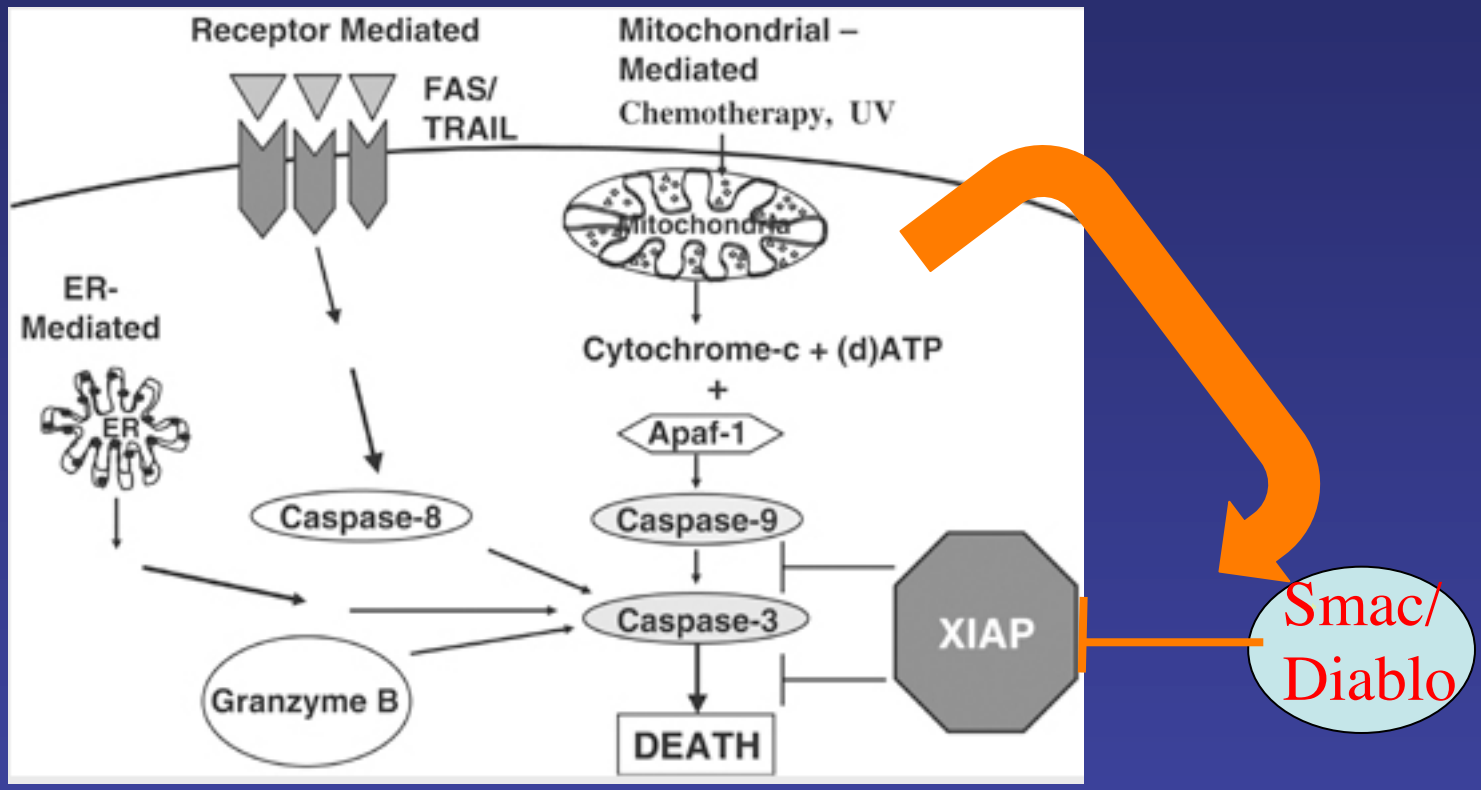
XIAP inhibits apoptosis in response to multiple stimuli.



XIAP inhibits active caspases-3, -7 and -9

Cell Death and Differentiation (2006) **13,** 179–188.

Necroptosis

Programmed necrosis with membrane permeabilization and inflammation

Necrosis-like morphology of the dying cells.

independent of caspase activity

required receptor-interacting protein 1 (RIP1, also called RIPK1)—a serine/threonine kinase previously known to be involved in mediating nuclear factor-κB (NF-κB) activation by TNF receptor 1 (TNFR1)

reactive oxygen species by mitochondria, as well as lysosomal leakage and lipid peroxidation, plays a role in necroptotic cell demise

Regulated necrosis triggered by death receptor ligands TNFα, FasL or Toll receptor ligands (damage associated molecular patterns, i. e. LPS)

Protect cells from intracellular pathogens

RIPK1 and programmed necrosis

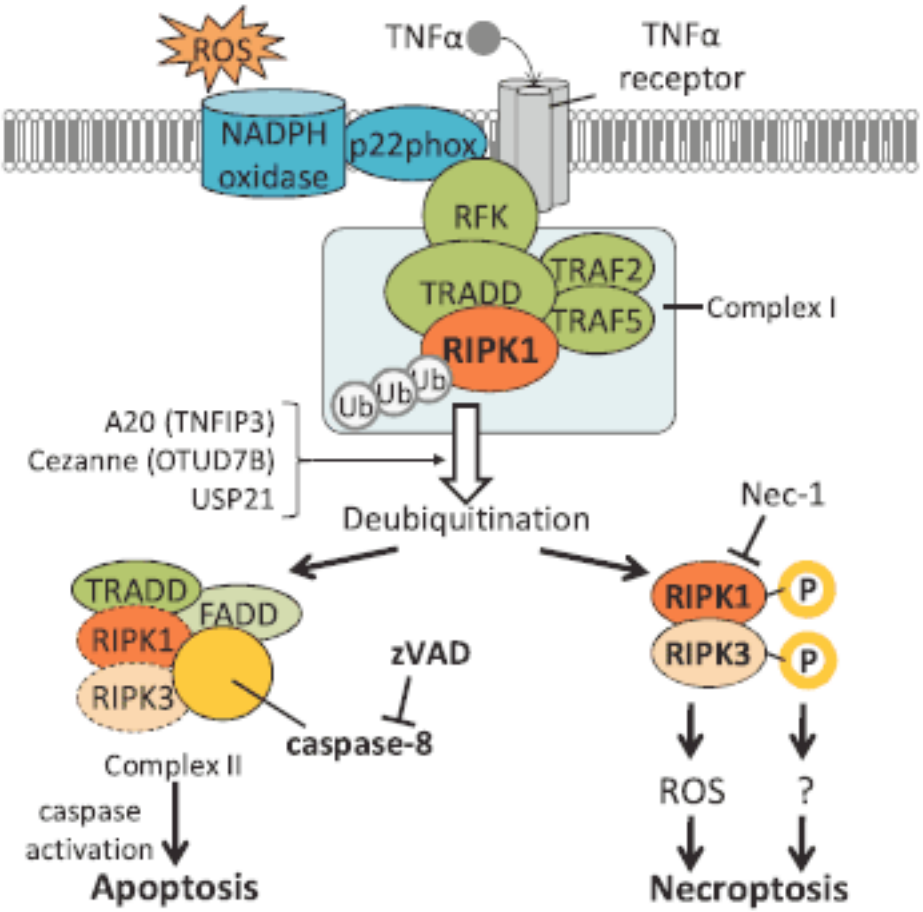


図３　アポトーシスとネクロトーシスの分子機構

Apoptosis quiz

What roles in regulating the intrinsic pathway of apoptosis are played by the Bcl-2 protein family members Bax and Bcl-2?

a) Bax inhibits apoptosis while Bcl-2 stimulates apoptosis.

b) Bax stimulates apoptosis while Bcl-2 inhibits apoptosis.

c) Both Bax and Bcl-2 inhibit apoptosis.

d) Both Bax and Bcl-2 stimulate apoptosis.

Which of the following proteins is a death receptor which triggers the extrinsic pathway of apoptosis?

a) caspase-8

b) FADD

c) Fas

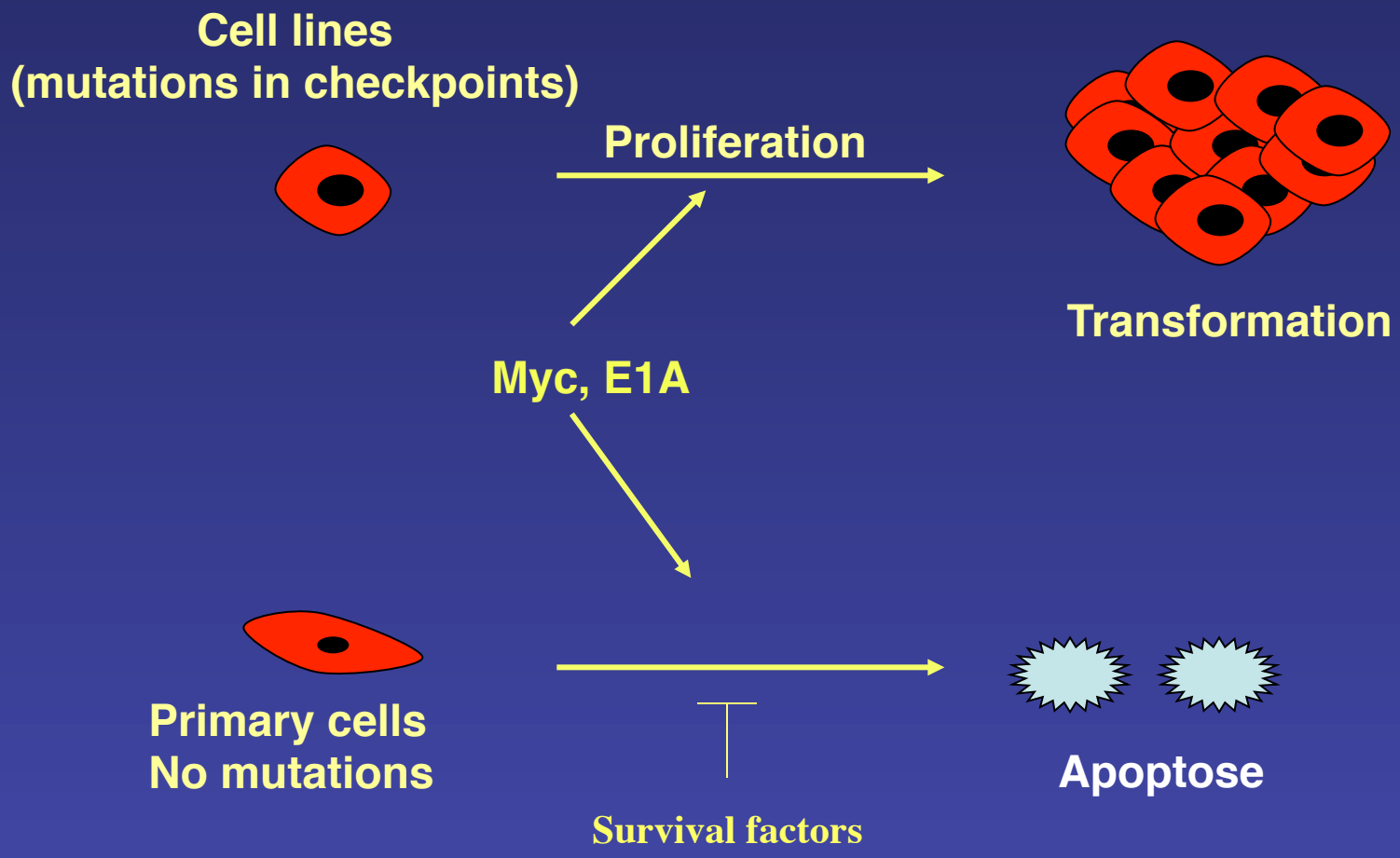
d) Fas ligand

Final exam

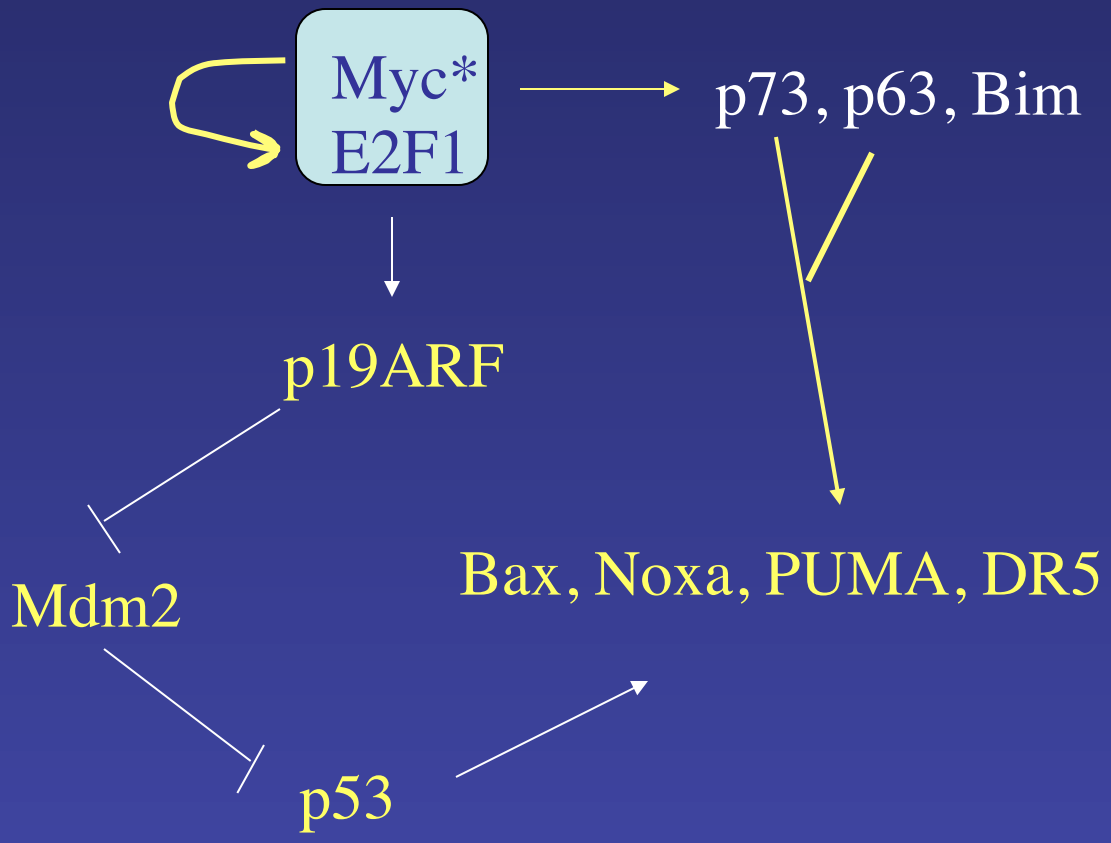
- 1- Propose a project to test a molecular model for the chronic p53 response

- Propose a project to discover the mechanism of action of RIPK1 in necroptosis

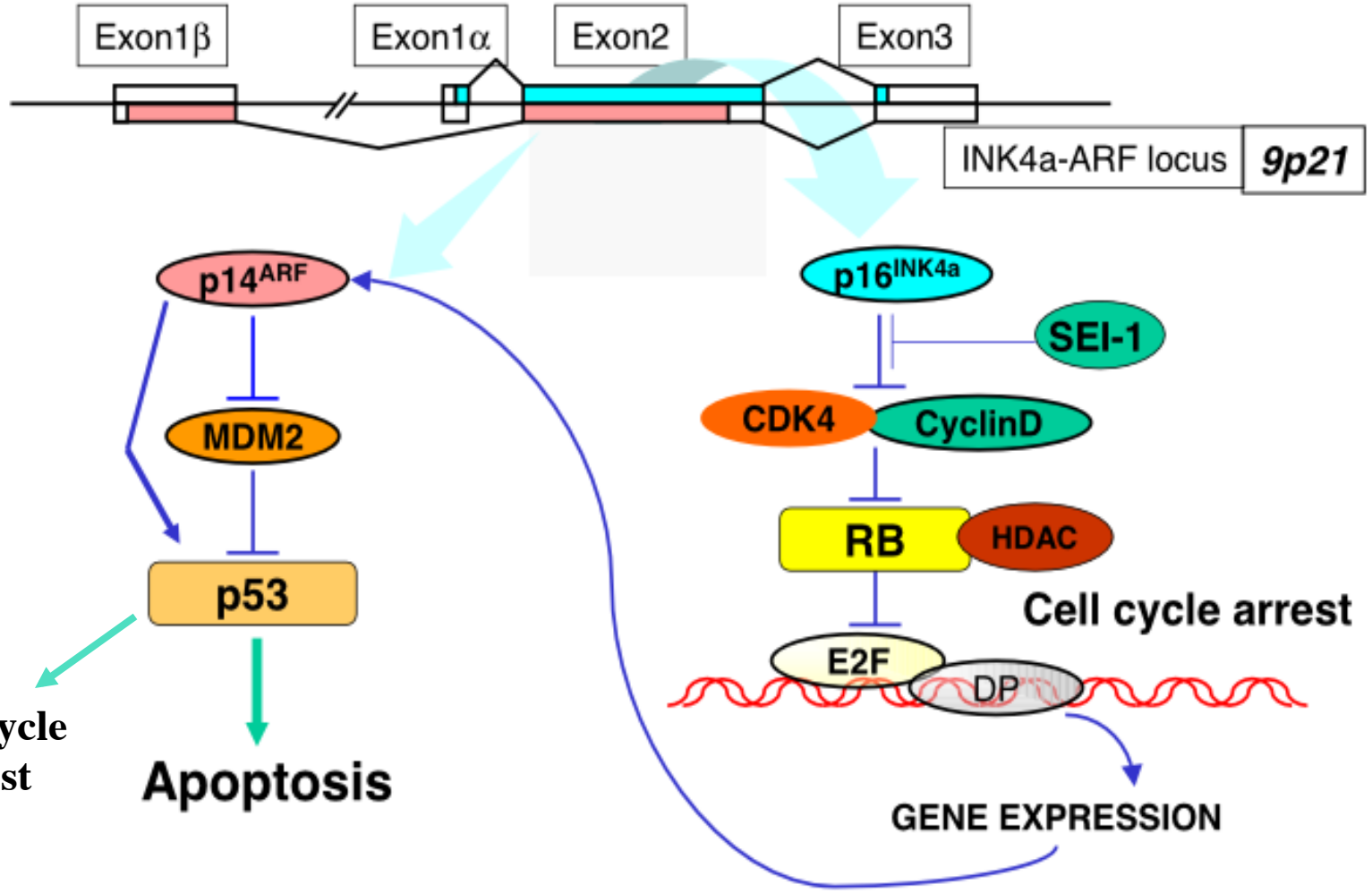
Cancer checkpoints



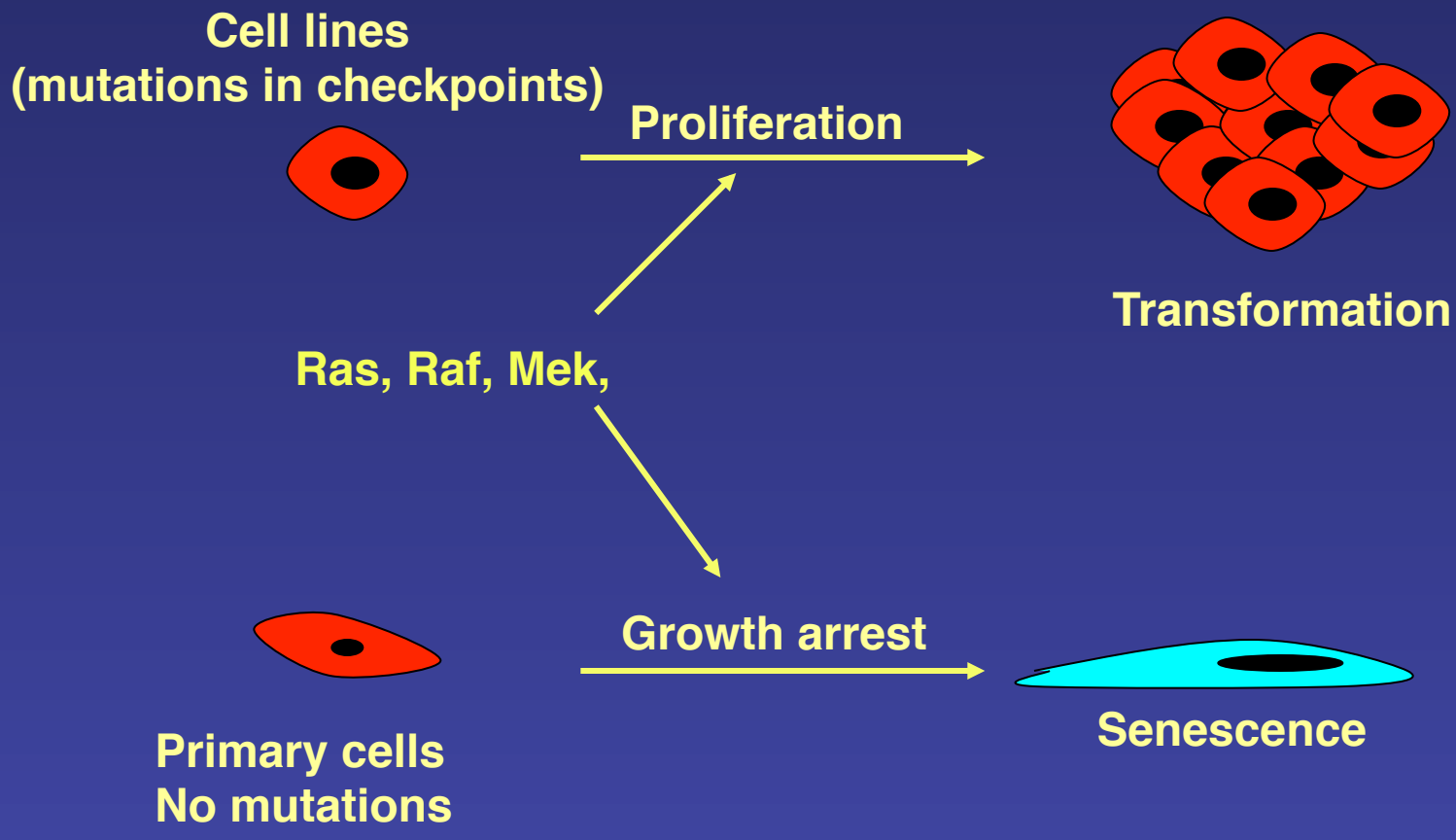
Oncogene induced apoptosis



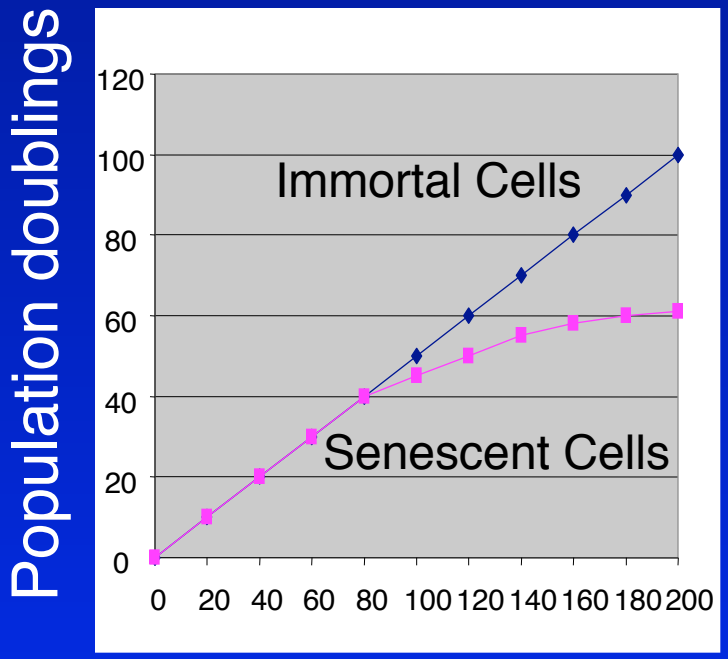
INK4a-ARF → RB / p53 pathway

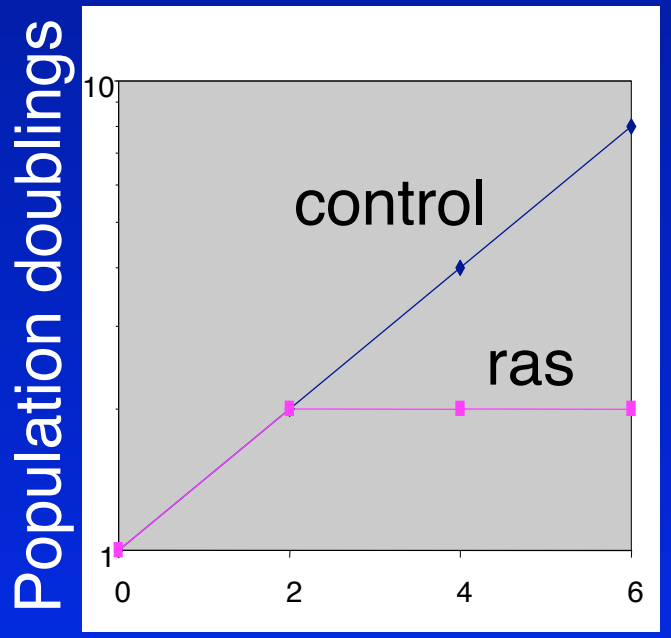


Senescence, another checkpoint against cancer

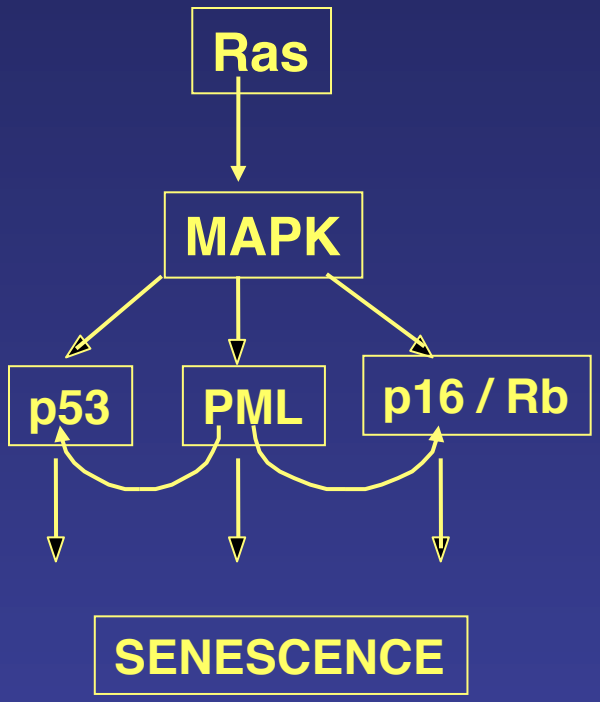


Replicative senescence and oncogene-induced senescence (OIS)

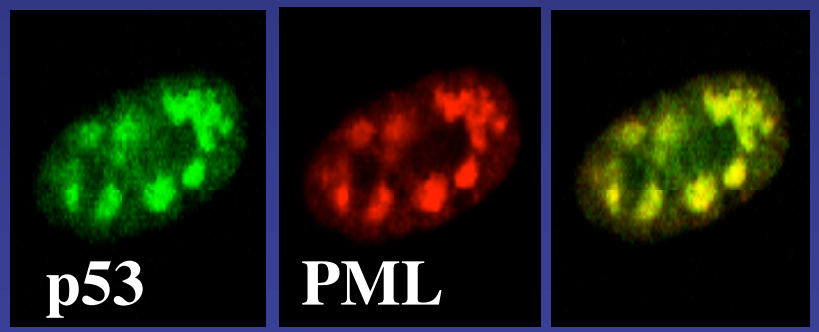




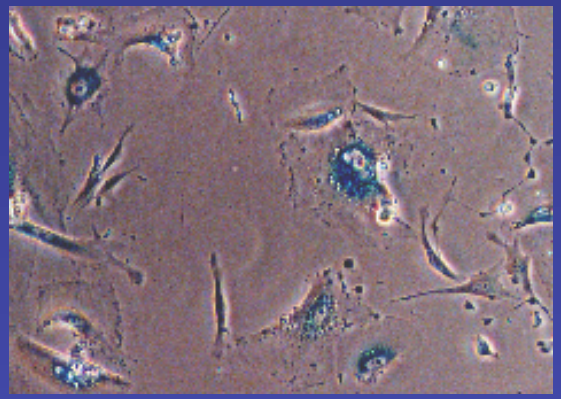
Senescence mechanisms



- Stable arrest of cell proliferation
- SASP= senescence associated inflammatory cytokine
- Tumor suppressors: p53, p16, RB, PML

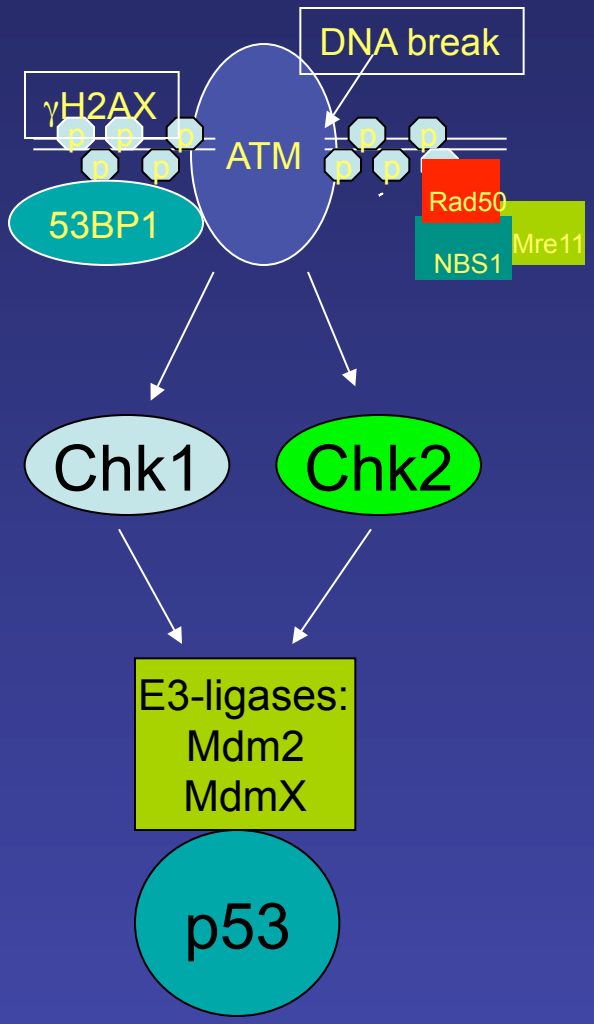


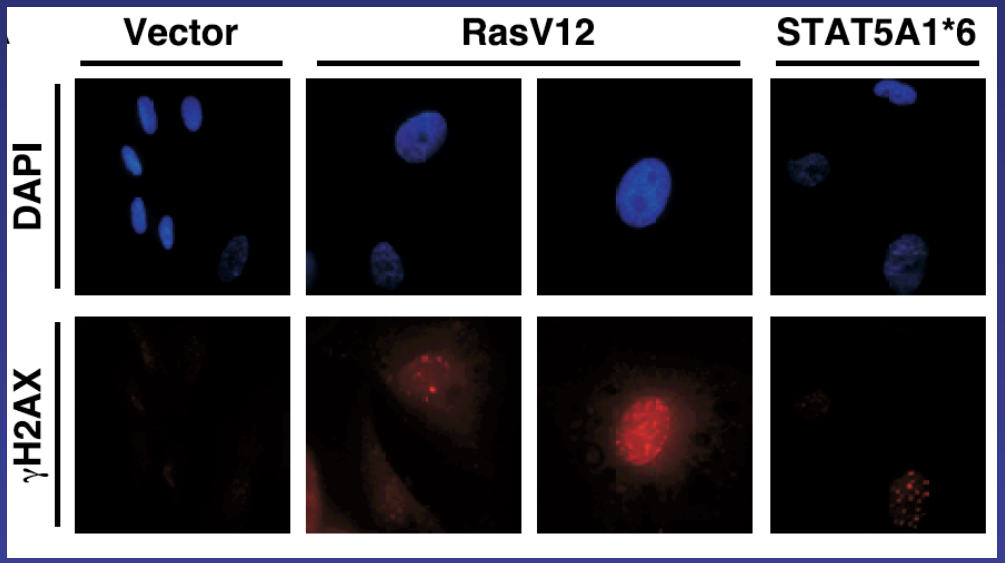
- PML controls both p53 and RB



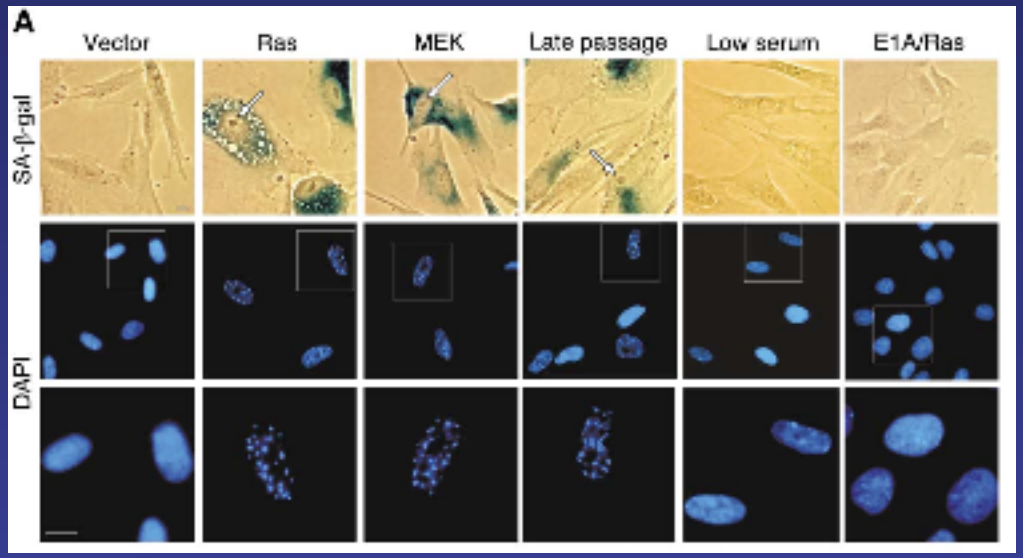
Senescence associated beta galactosidase

Oncogenes induce DNA damage and the DDR

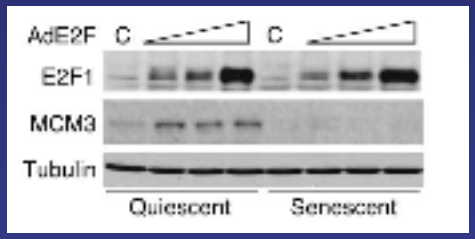




Heterochromatin, Rb and senescence

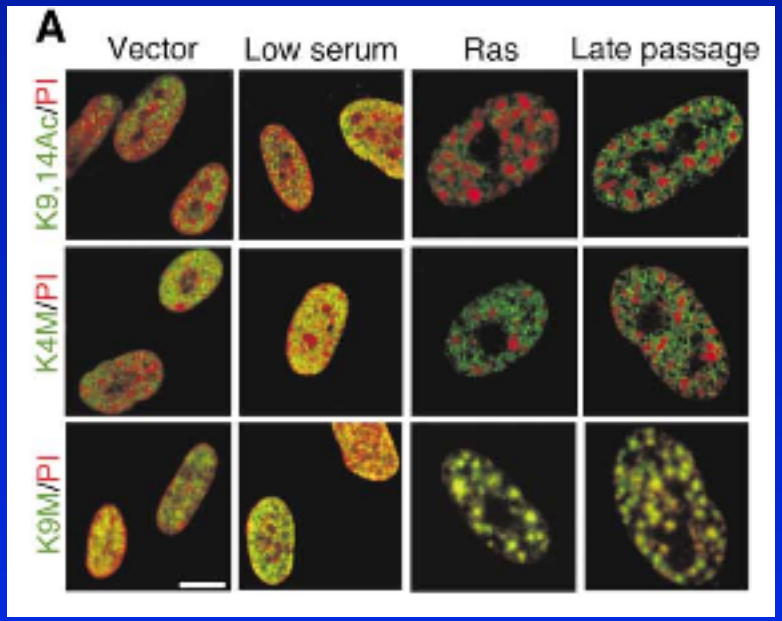


SAHFs

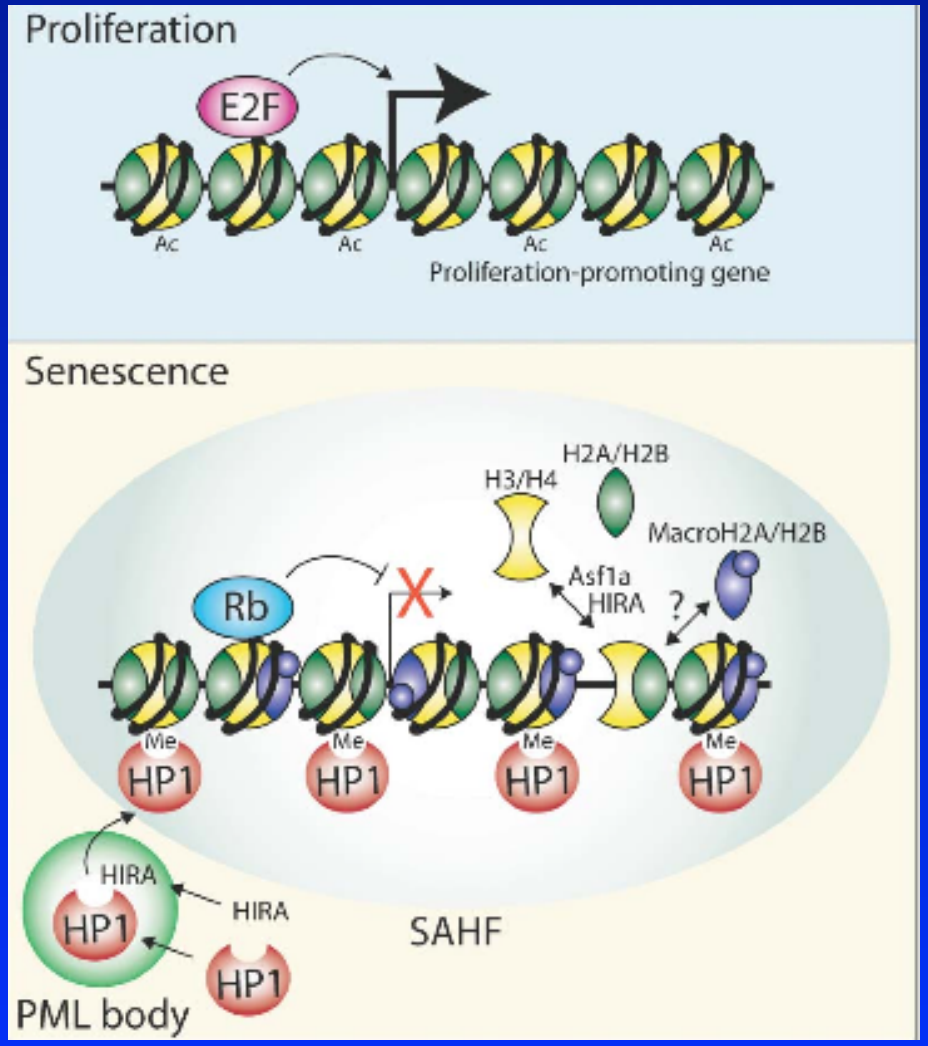


During senescence RB and PML help to catalyze heterochromatin Structures that include E2F target genes. Genes remain locked

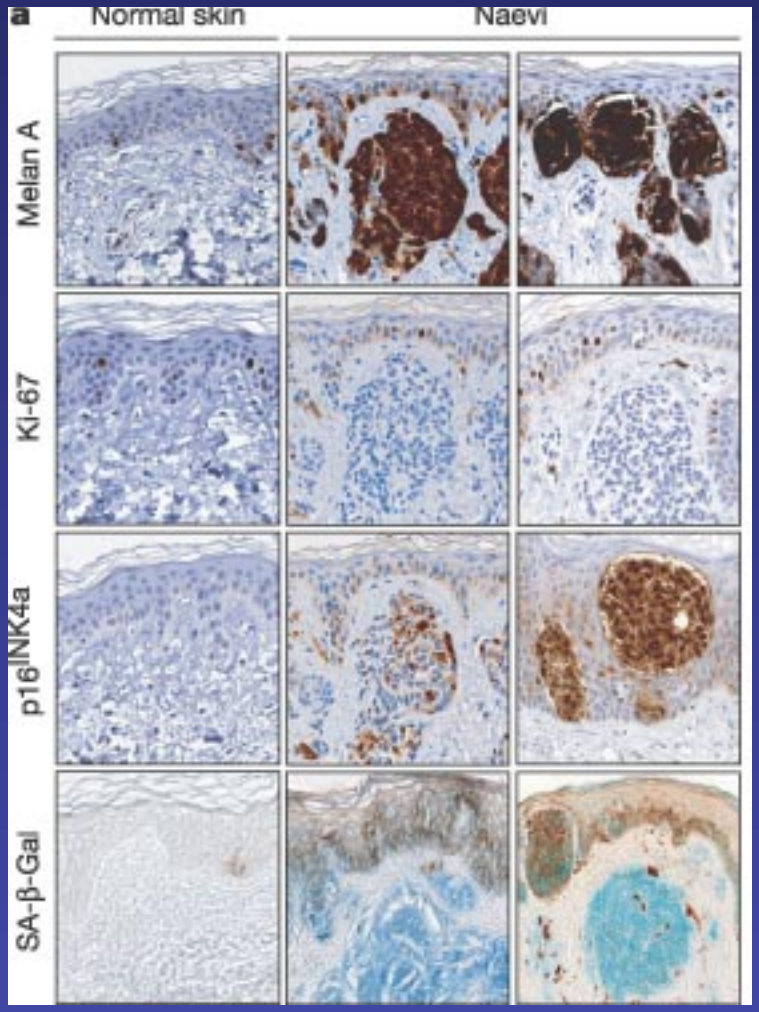
“SAHFs” special kind of heterochromatin



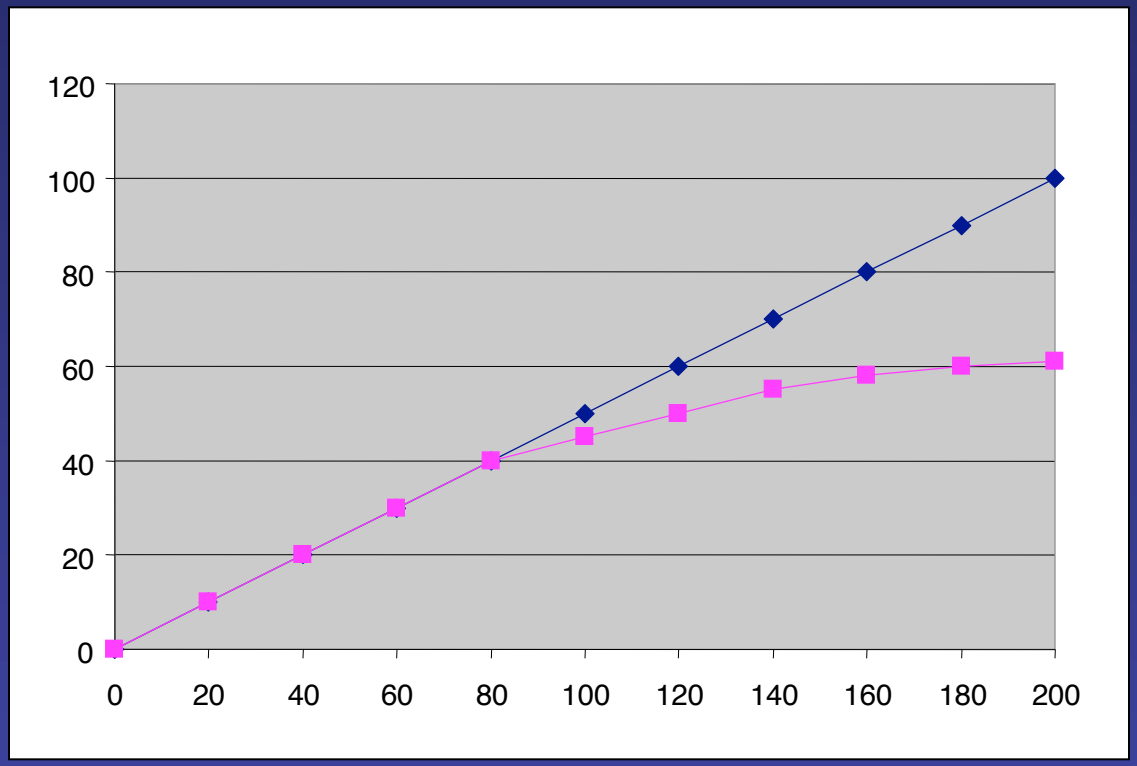
SAHF contain methyl K9H3
HP1 proteins
MacroH2A/H2B



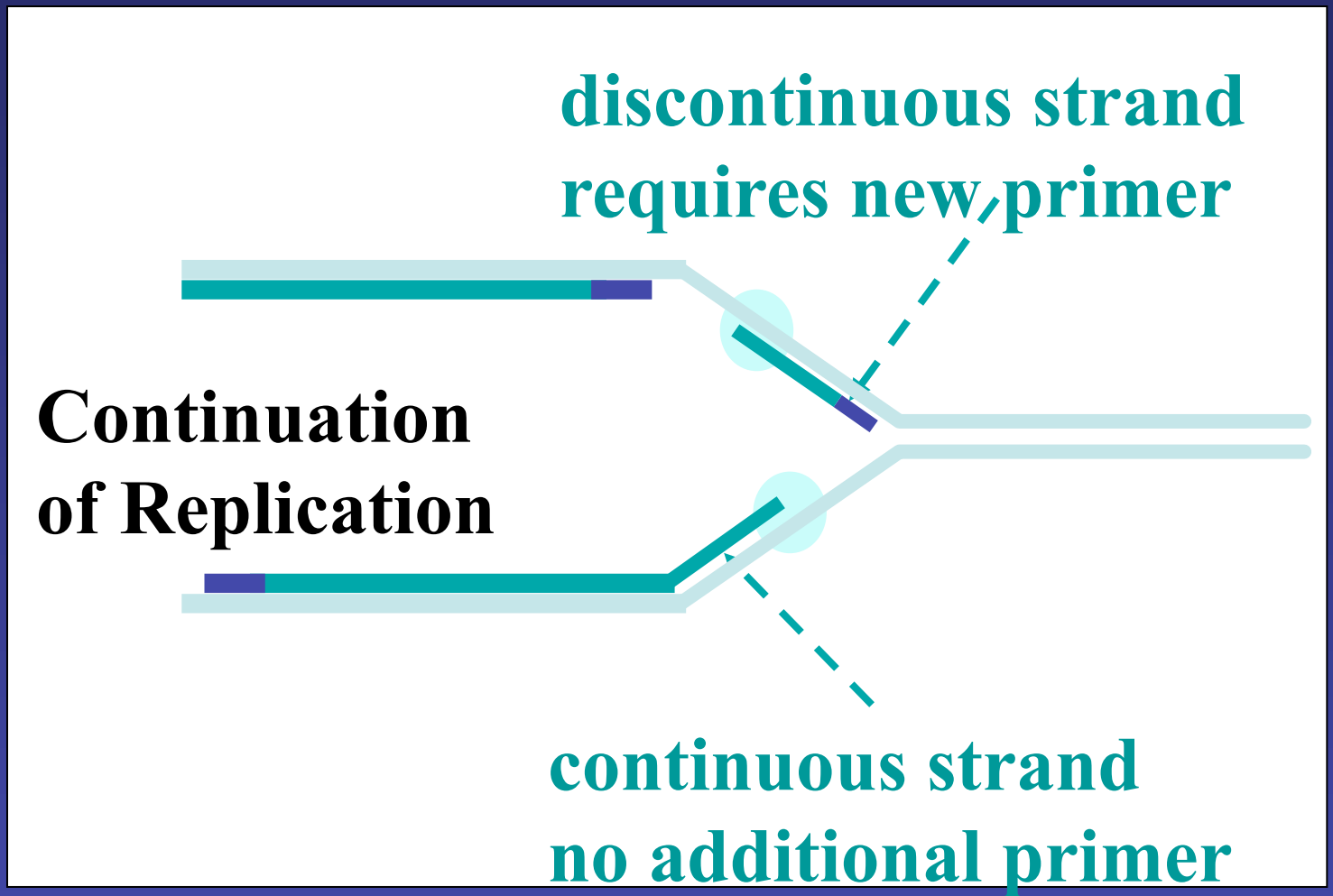
Bening tumors accumulate senescent cells



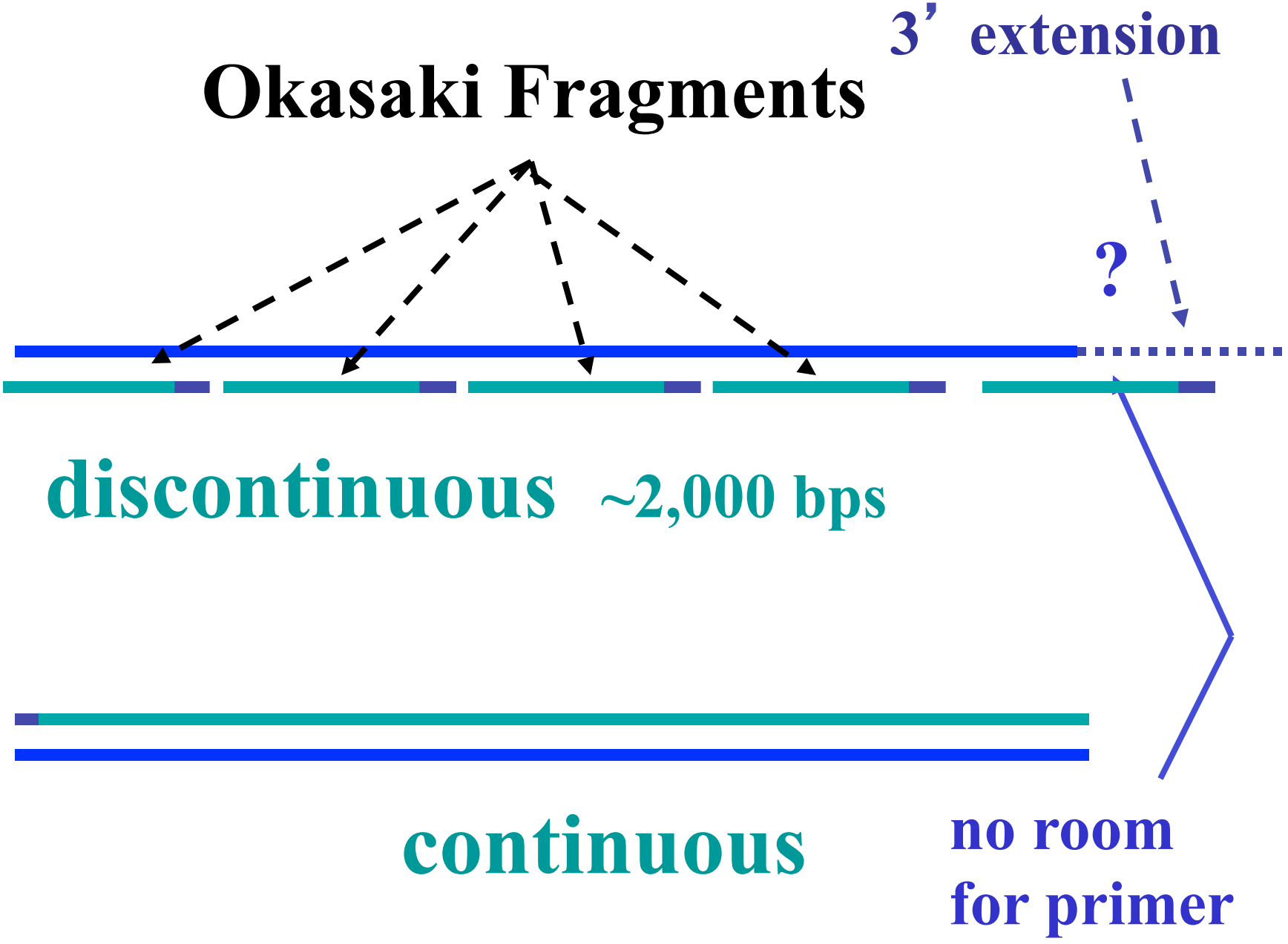
Replicative senescence



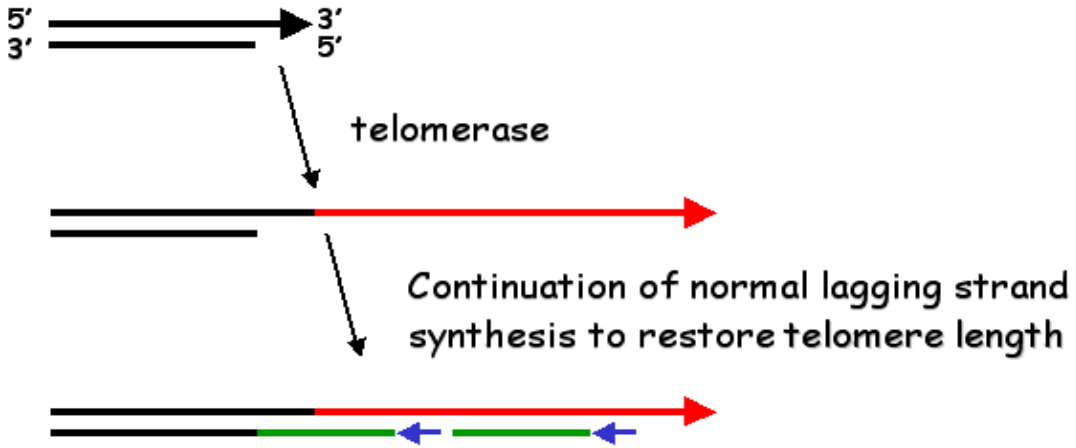
Replication end problem



Okasaki Fragments

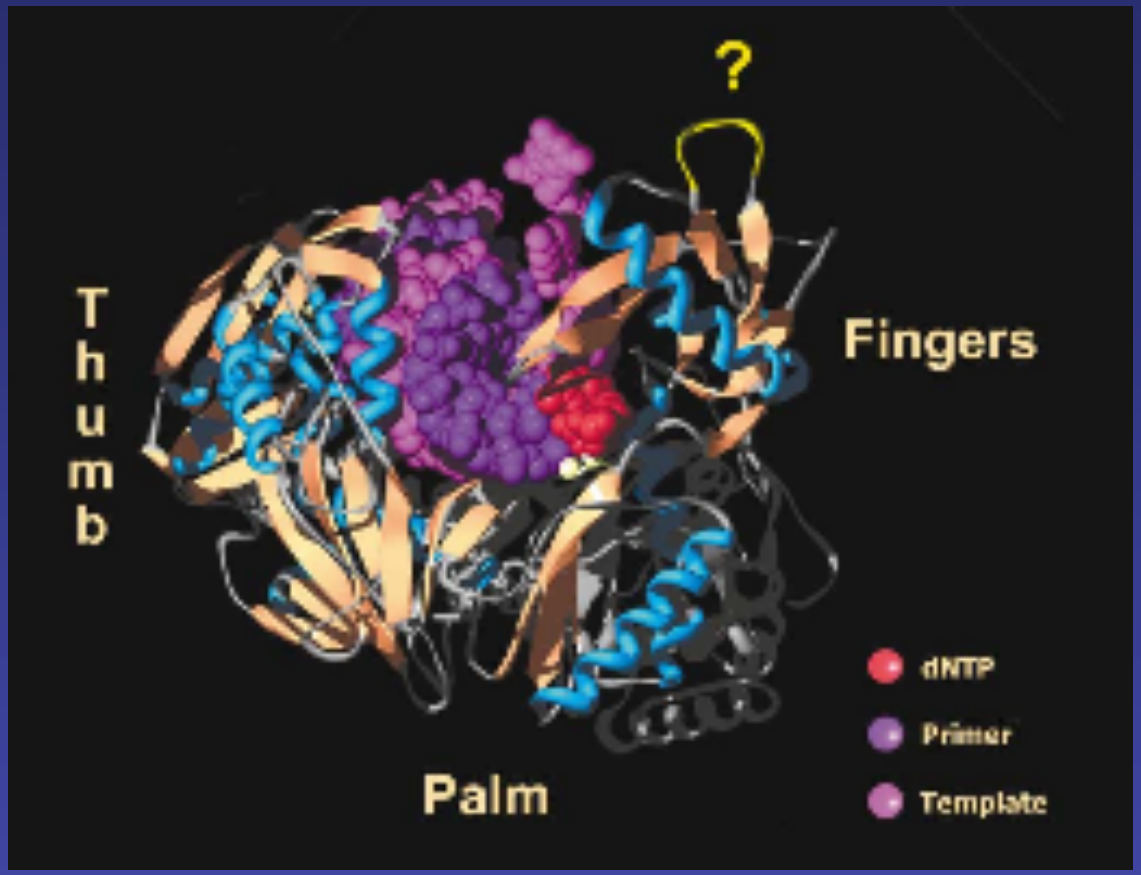


Telomerase



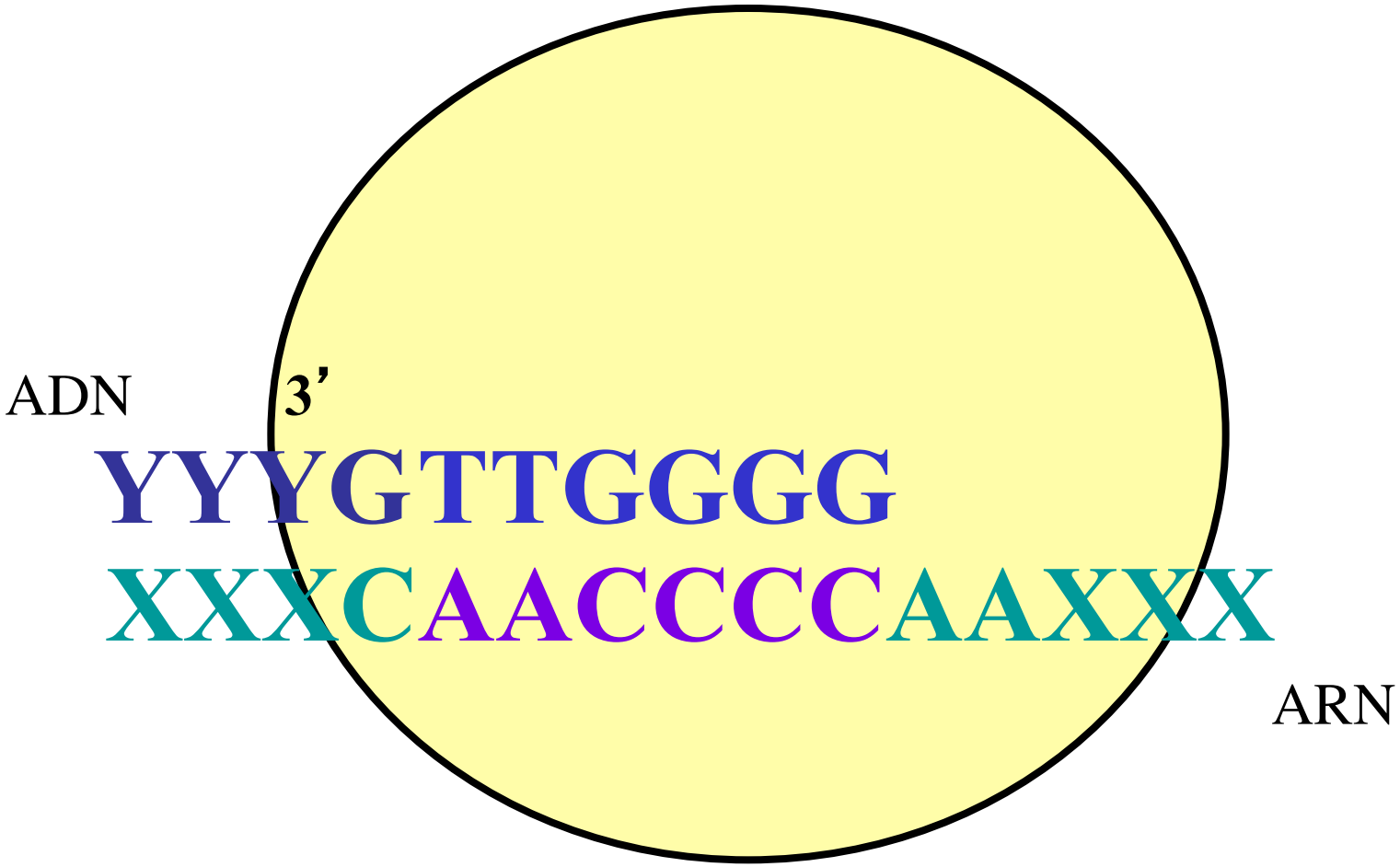
The end-replication problem is never really solved; telomerase overcomes the problem by restoring overall length

Telomerase is a reverse transcriptase

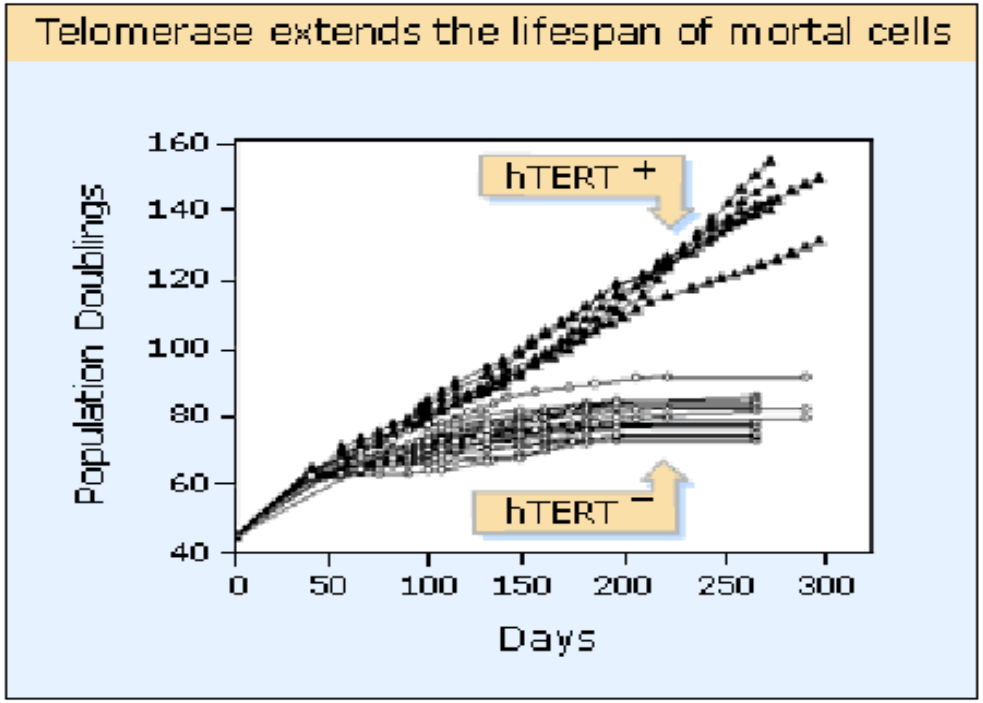


- Ribonucleoprotein
- RNA 159 nts
- CAAUCCCAA
  TTAGGG

Reverse transcription des telomeres par la telomerase

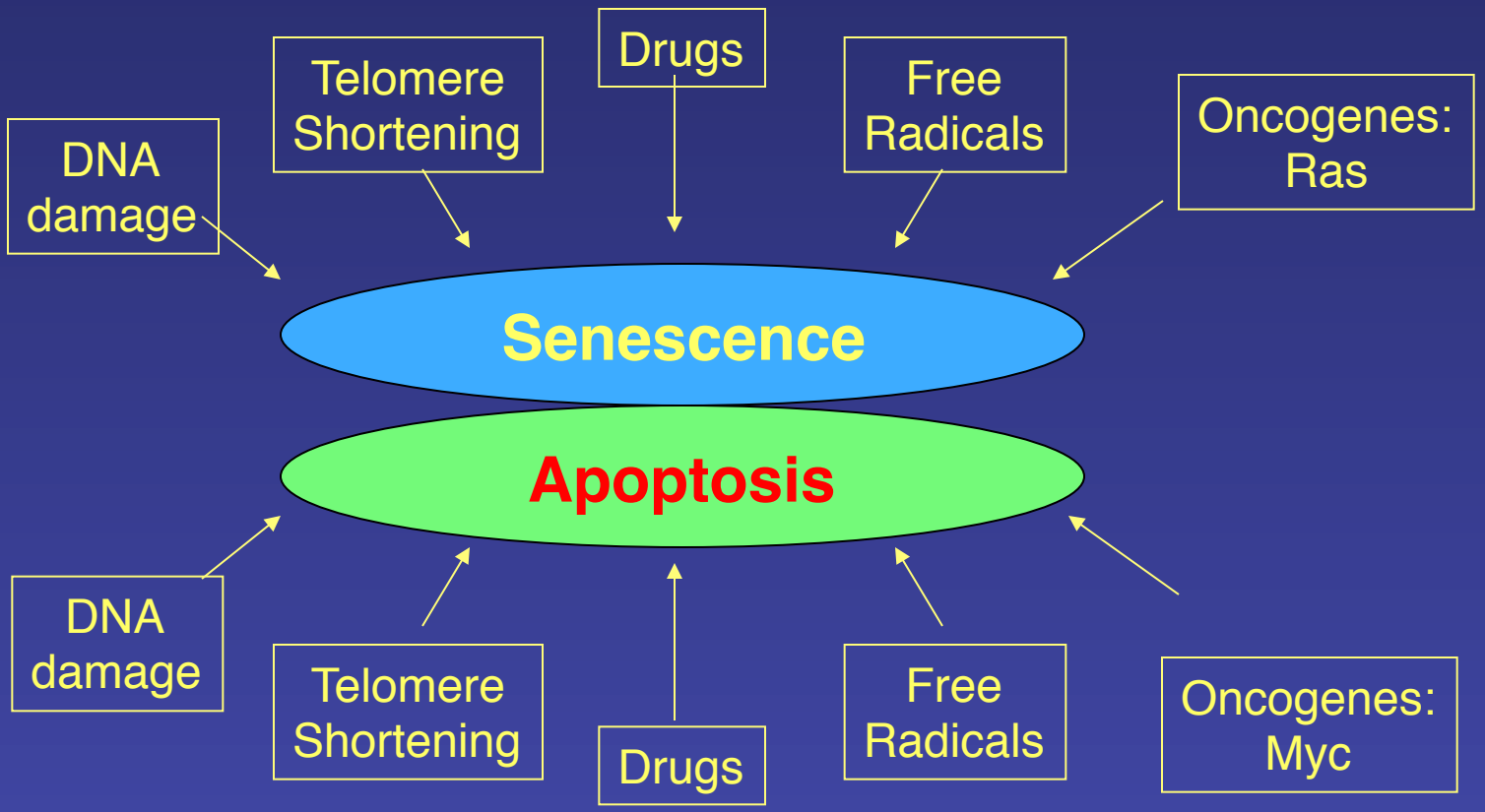


RS is due to telomere shortening

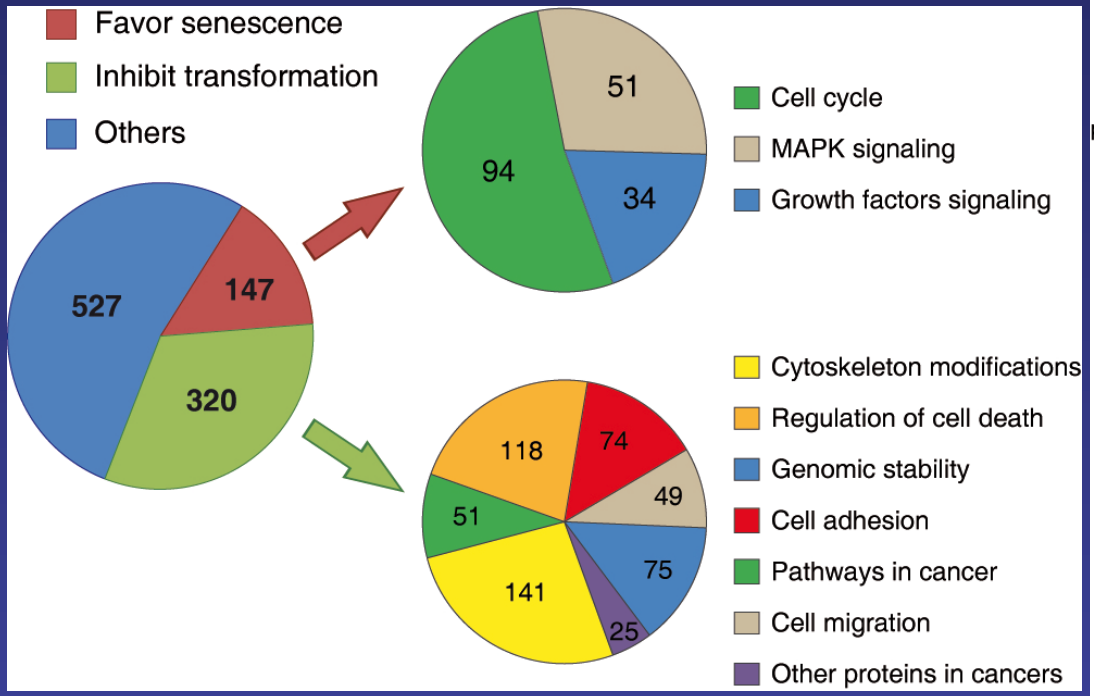


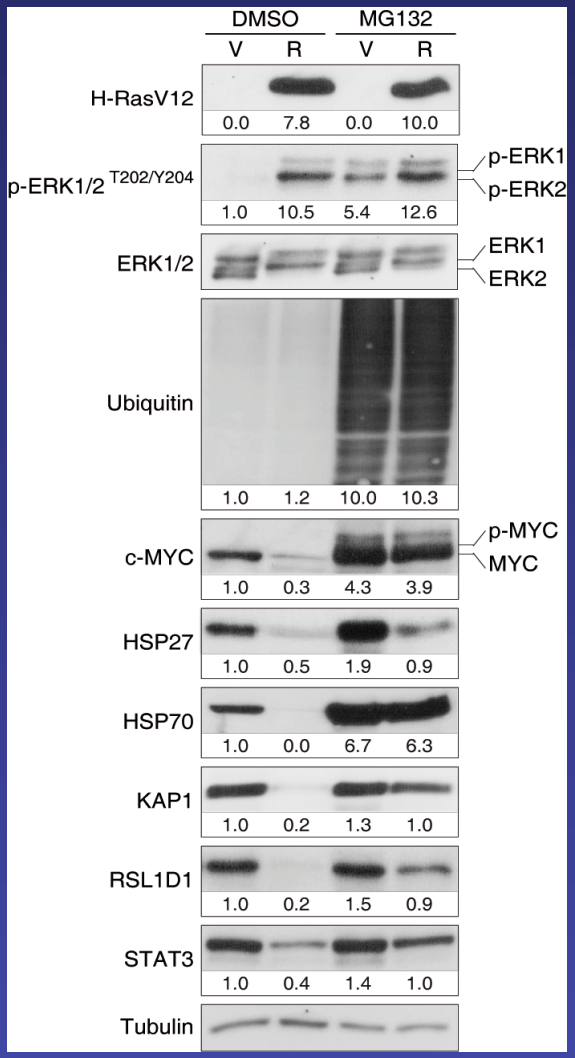
- Most cancers overexpress telomerase
- Human somatic cells repress telomerase expression

Senescence and apoptosis downstream multiple oncogenic stress



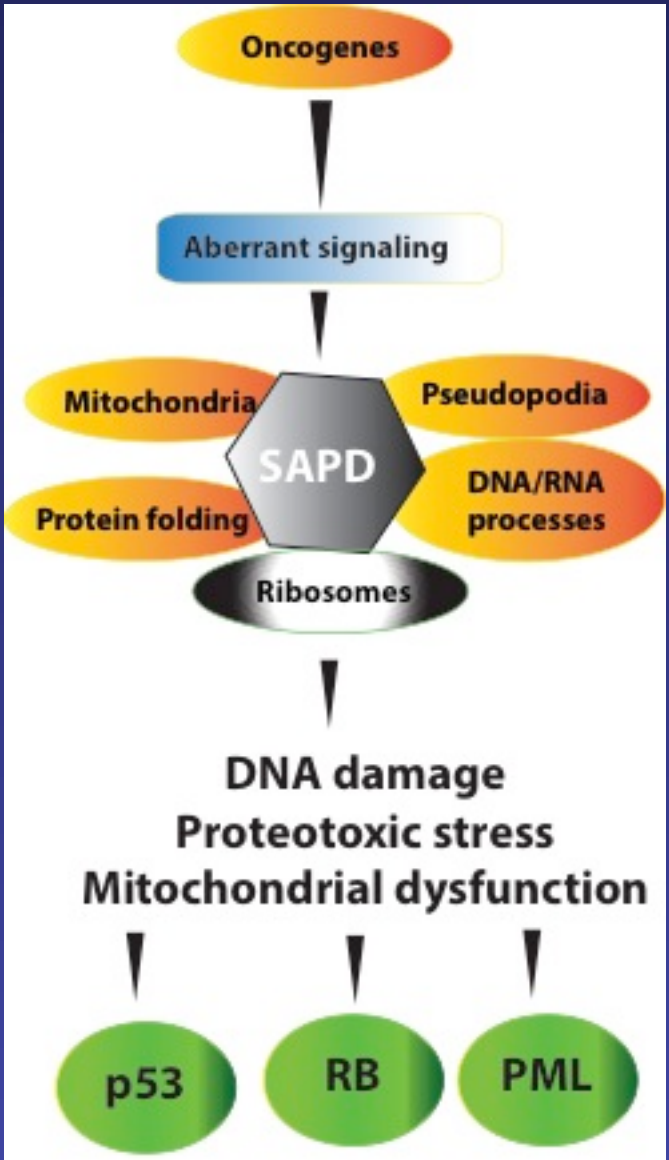
RasV12 promotes selective protein degradation in senescent cells (SAPD)





A: Phosphoproteomic (LC-MS-MS): 2995 peptides for 1018 proteins stabilize by MG132 in cells expressing H-RasV12
B: Validation of protein degradation in H-RasV12-expressing cells

Deschênes-Simard & al. (2013). Genes Dev, 27: 900-915.



SAPD potentially explains all stress responses associated to senescence

Senescence quiz

1- True or false

a. Oncogenes always induce cell proliferation
b. Oncogenes induce DNA damage
c. P53 and RB regulate senescence
d. Senescence cells are death cells
e. Senescent cells are alive and actively secrete inflammatory mediators

2- Which is these statements about telomerase is false

a. Is reverse transcriptase
b. Use an RNA molecule as a template
c. Immortalize cells
d. Poorly expressed in most cancer cells

Autophagy (self eating)

Parts of the cytoplasm and intracellular organelles are sequestered in double-membraned autophagic vacuoles (autophagosomes) and are finally delivered to lysosomes for degradation.

Autophagy is also a process by which cells adapt their metabolism to starvation, generating metabolic substrates that meet the metabolic needs of cells

Dual nature:

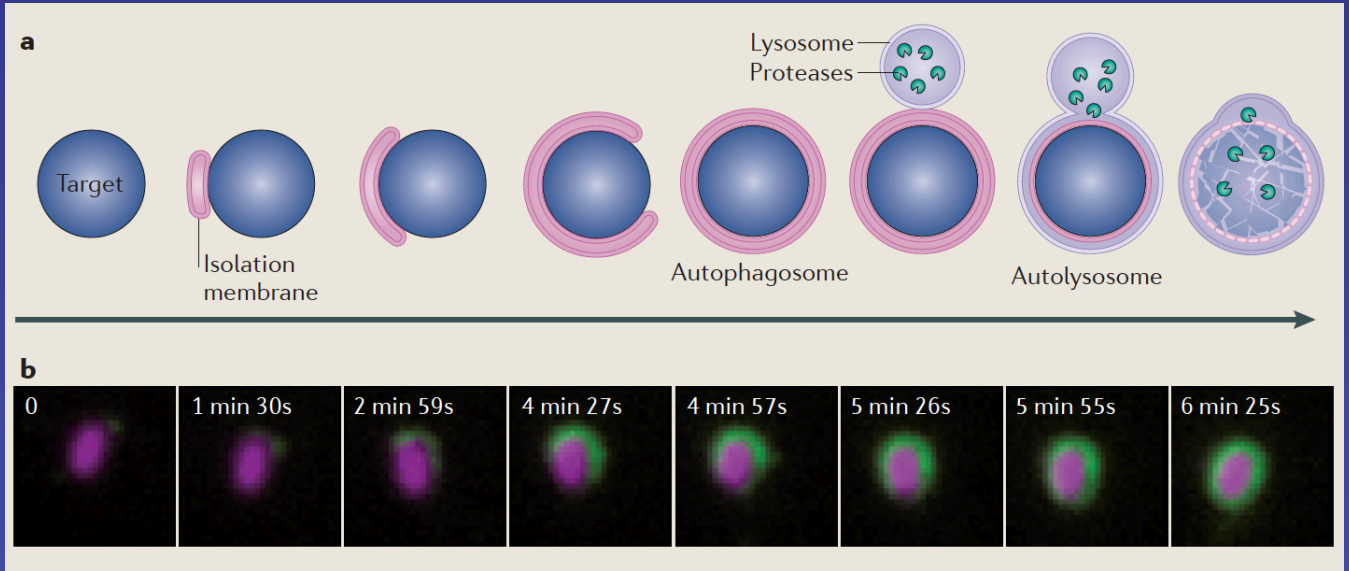
- constitutes a stress adaptation that avoids cell death
- constitutes an alternative pathway to cellular demise that is called autophagic cell death (or type II cell death)

Autophagosome formation

- Initiation: transmission of the signal to the membrane source at which the nucleation of the isolation membrane occurs, which results in the recruitment of key initiating complexes.

- Nucleation leads to the formation of the isolation membrane from the membrane source and the recruitment of ATG proteins.

- Expansion of the isolation membrane occurs until the autophagosome fully forms and closes.



ATG

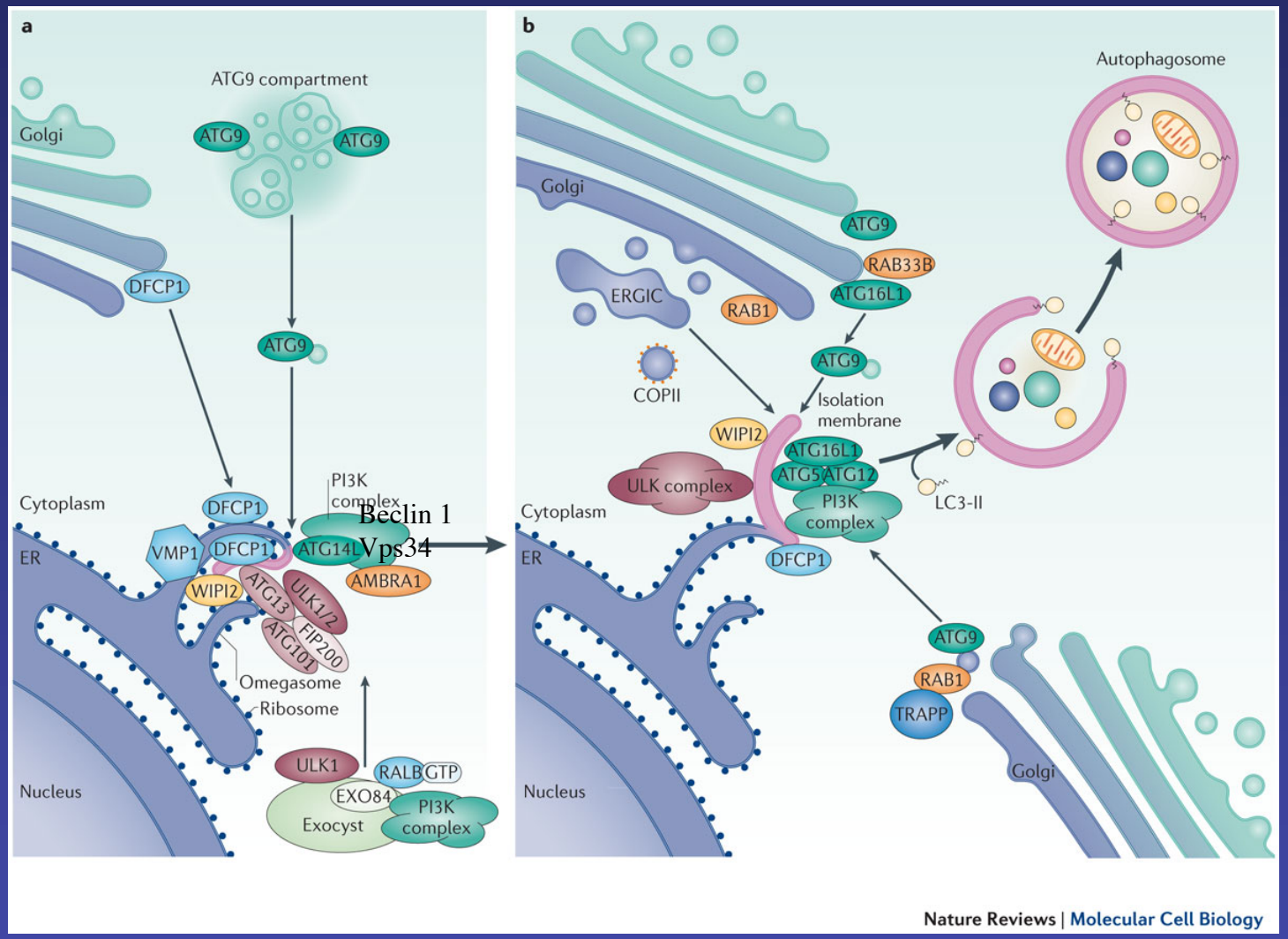
Initiation

Expansion

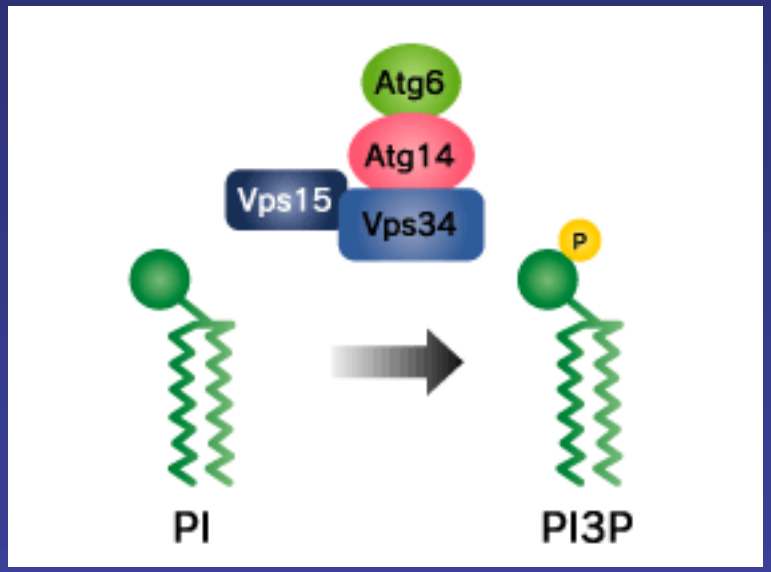
Protein	Position in autophagic pathway	Alternative name* and function
ULK1 and ULK2	ULK complex	Atg1 orthologues; Ser/Thr kinases that mediate mTOR signalling and ATG9 cycling[123]
ATG13	ULK complex	ULK1 and ULK2 substrate that also modulates the activity of the ULK complex[26]
FIP200	ULK complex	Atg17 orthologue; ULK1 and ULK2 substrate that also modulates the activity of the ULK complex[124]
ATG101	ULK complex	Interacts with ULK1 and ATG13 (REFS 125,126)
Beclin 1	PI3K complex	Atg6 orthologue; part of the PI3K complex and also has a role in autophagy during initiation, formation and maturation[20]
VPS34	PI3K complex	Catalytically active subunit of the PI3K complex[127]
p150	PI3K complex	Vps15 orthologue; recruits the PI3K complex to membranes[127]
ATG14L	PI3K complex	Atg14 orthologue; directs the PI3K complex to the omegasome; also known as Barkor[50,128,129]
WIPI1 and WIPI2	PtdIns(3)P-binding protein	Atg18 orthologues; bind to PtdIns(3)P on the autophagosome[35]
ATG3	LC3–phosphatidylethanolamine conjugation	Similar to the E2 ubiquitin conjugating enzyme; conjugates LC3 to phosphatidylethanolamine [130]
ATG4	LC3–phosphatidylethanolamine conjugation	Cys protease that cleaves carboxy-terminal Gly residues from LC3 homologues and is also required to recycle LC3 from the autophagosome outer membrane[131]
ATG7	LC3–phosphatidylethanolamine and ATG12 conjugation	Similar to E1 ubiquitin activating enzymes; activates ATG12 and LC3 homologues[132,133]
LC3-A, LC3-B, LC3-C, GATE16, GABARAPL1, GABARAPL2 and GABARAPL3	LC3–phosphatidylethanolamine conjugation	Atg8 homologues; ubiquitin-like proteins that recruit cargo to autophagosomes and may aid in membrane fusion[131,134]
ATG5	ATG5–ATG12 conjugation	Conjugated to ATG12 (REF. 132)
ATG10	ATG5–ATG12 conjugation	Similar to E2 ubiquitin conjugating enzyme; links ATG12 to an internal Lys residue in ATG5 (REF. 132)
ATG12	ATG5–ATG12 conjugation	Ubiquitin-like protein conjugated to ATG5 that functions in the activation of ATG3 (REFS 132,151)
ATG16L1	ATG5–ATG12 complex	Binds to the ATG5–ATG12 conjugate and directs LC3 conjugation at the isolation membrane[135]
ATG9A and ATG9B	Integral membrane proteins	Atg9 orthologues; required for autophagosome formation[91]
ATG2A and ATG2B	Localize to omegasome	Atg2 orthologues; required for closure of isolation membranes to form autophagosomes[36]

*Alternative yeast name is given. ATG, autophagy-related; FIP200, FAK family kinase-interacting protein of 200 kDa; mTOR, mammalian target of rapamycin; PtdIns(3)P, phosphatidylinositol-3-phosphate; ULK, UNC51-like kinase; VPS34, vacuolar protein sorting 34; WIPI, WD-repeat domain phosphoinositide-interacting; GABARAPL, γ-aminobutyric acid receptor-associated protein-like.

The ULK and PI3K complex complex controls the initiation of autophagy at the omegasome



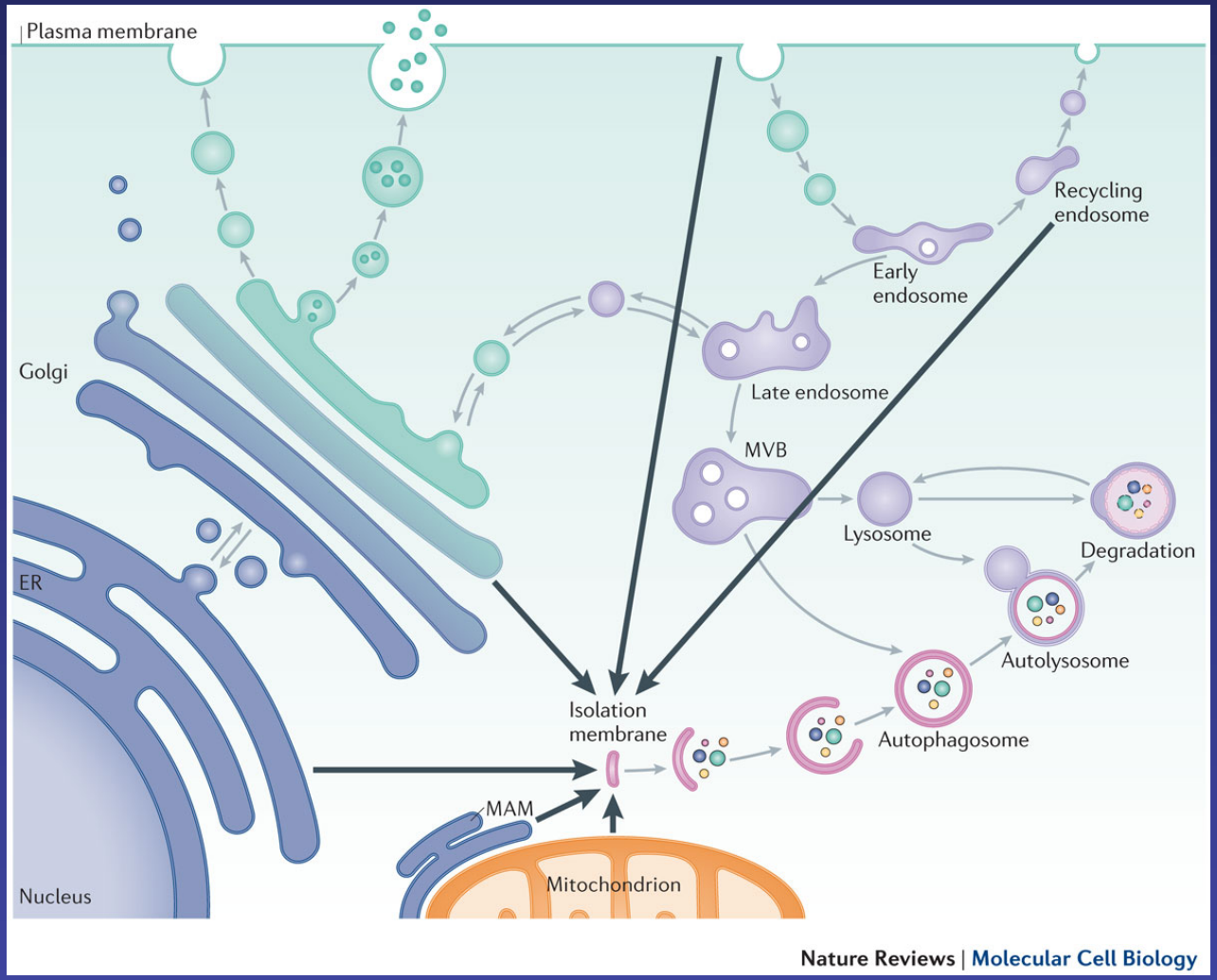
The PI3K complex converts phosphatidylinositol to phosphatidylinositol 3-phosphate



Atg6= beclin1

PIP3 recruits proteins to the isolation membrane
Includes the ATG12–ATG5–ATG16L1 complex and the LC3 complex

Nucleation of the isolation membrane



ATG genes (37). Tsukada, M. & Ohsumi, Y. Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. FEBS Lett. 333, 169–174 (1993).

Initiation

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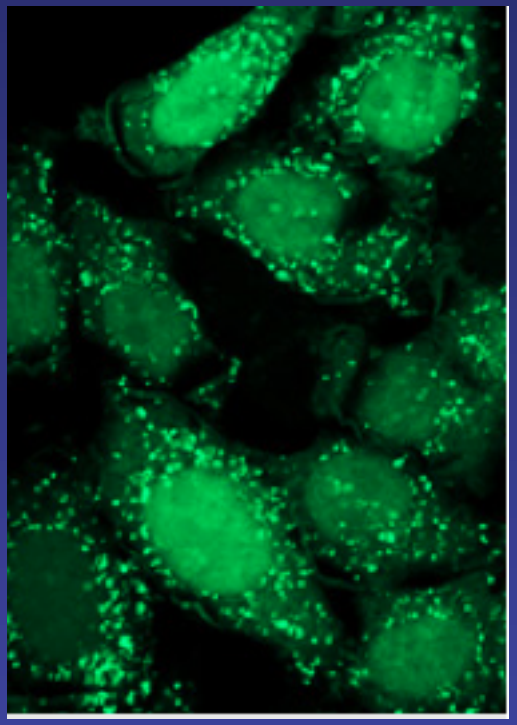
Autophagosome formation

- Initiation: transmission of the signal to the membrane source at which the nucleation of the isolation membrane occurs, which results in the recruitment of key initiating complexes. Requires the ULK complex and the PI3K complex (Vps34, Beclin 1). The ULK complex is controlled by TORC1. The PI3K complex is controlled by hypoxia, the BCL2 family and the ULK complex (Vps34 is a target for the kinase ULK) .

- Nucleation leads to the formation of the isolation membrane from the membrane source and the recruitment of additional ATG proteins.

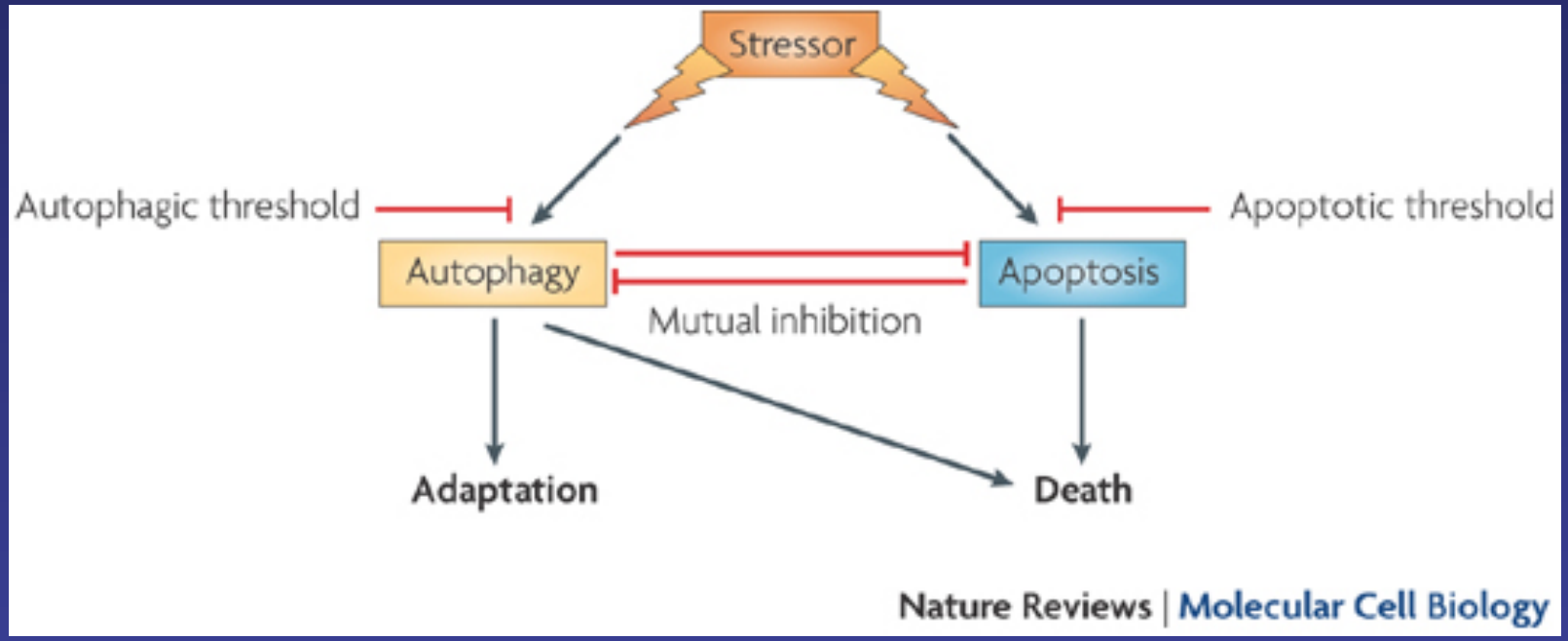
- Expansion of the isolation membrane occurs until the autophagosome fully forms and closes. The ATG12–ATG5–ATG16L1 complex (also called the ATG16L1 complex) is recruited to the membrane, where it functions as an E3-like ligase to mediate the lipidation of LC3

EGFP-LC3: autophagy marker



Sarkar et al. Nature Chemical Biology 3(6):331-338 (2007)

The relationship between apoptosis and autophagy.



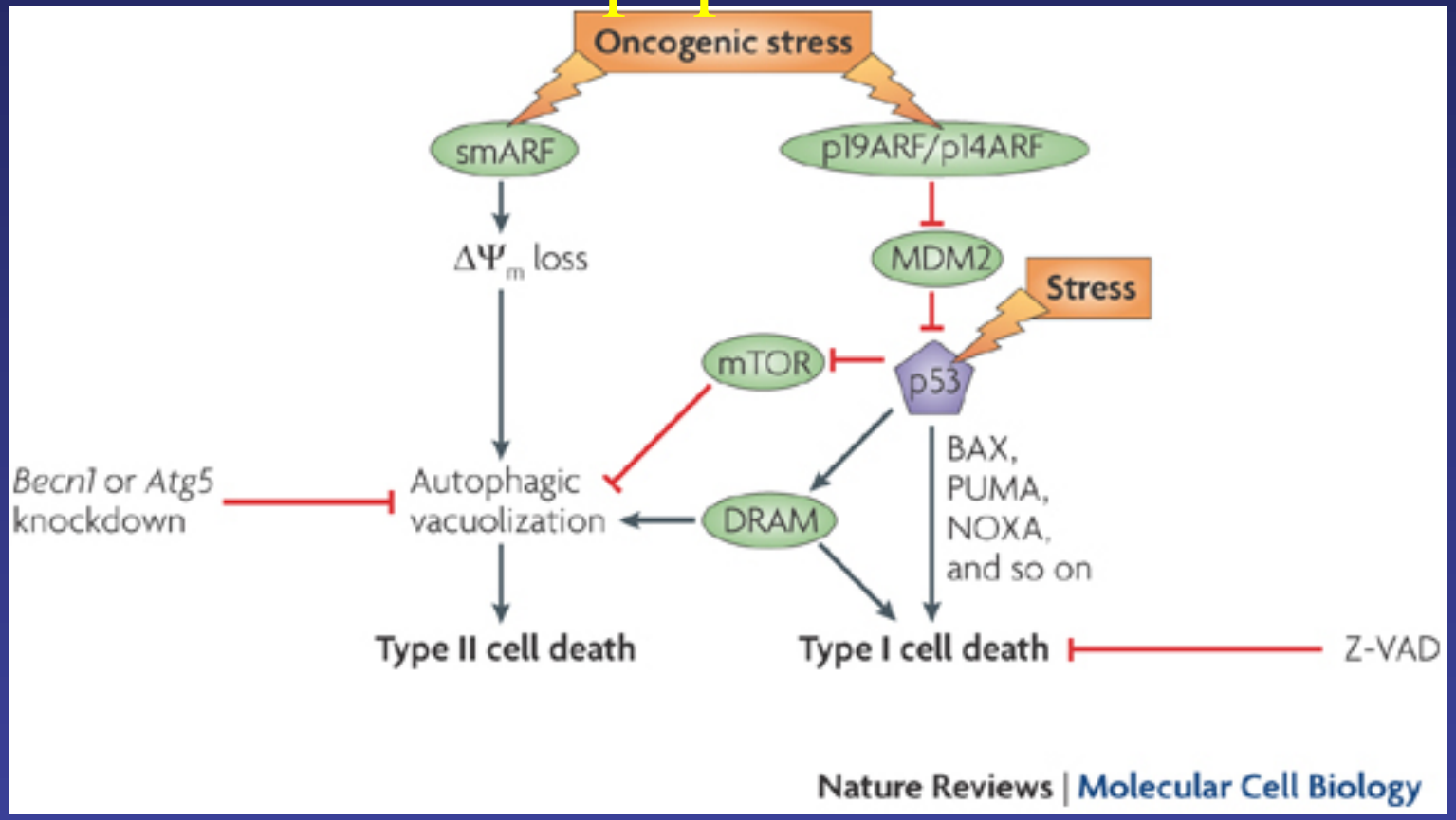
Autophagy-induced cytoprotection is the result of the basic cellular functions of autophagy in eukaryotic cells on the one hand, and the inhibitory effects that autophagy exerts on apoptosis under stress conditions on the other hand.

Nature Reviews Molecular Cell Biology 8, 741-752 (September 2007) |

Autophagy mediated cytoprotection

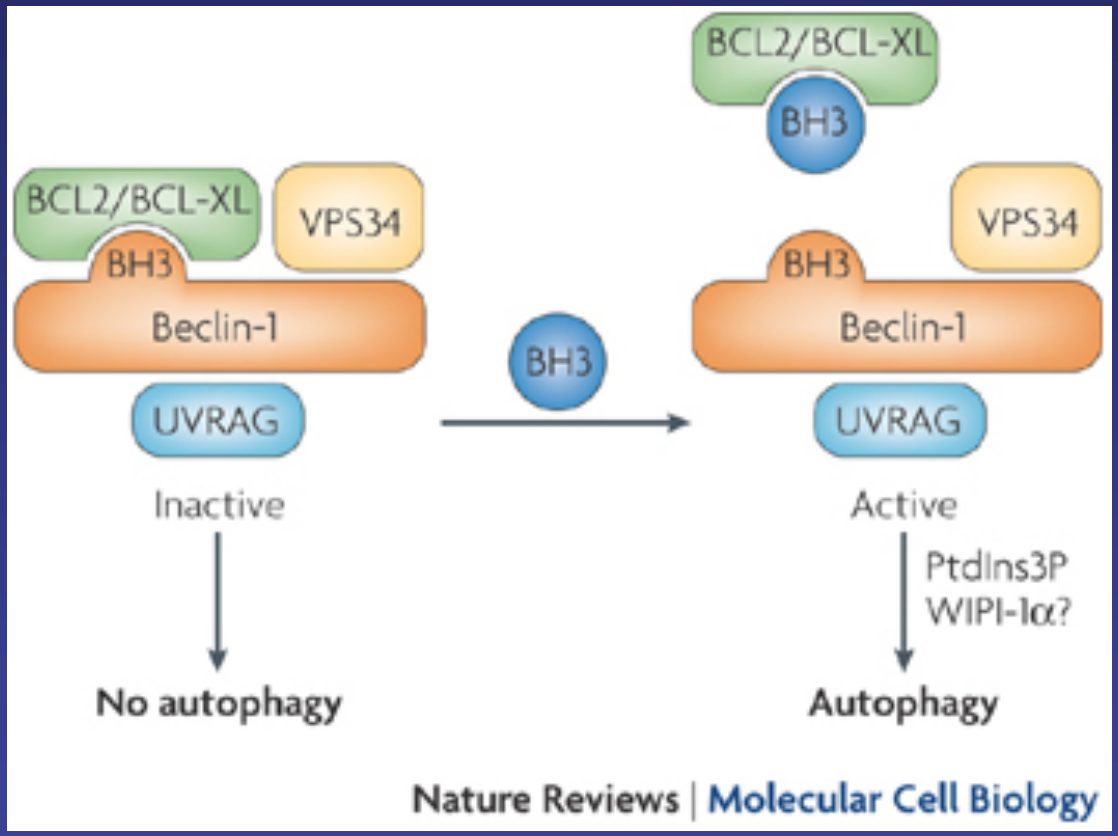
- Autophagy and apoptosis often occur in the same cell, mostly in a sequence in which autophagy precedes apoptosis
- Protein aggregates clearance
- Mitophagy: stimulated by GAPDH

P53 regulates both autophagy and apoptosis



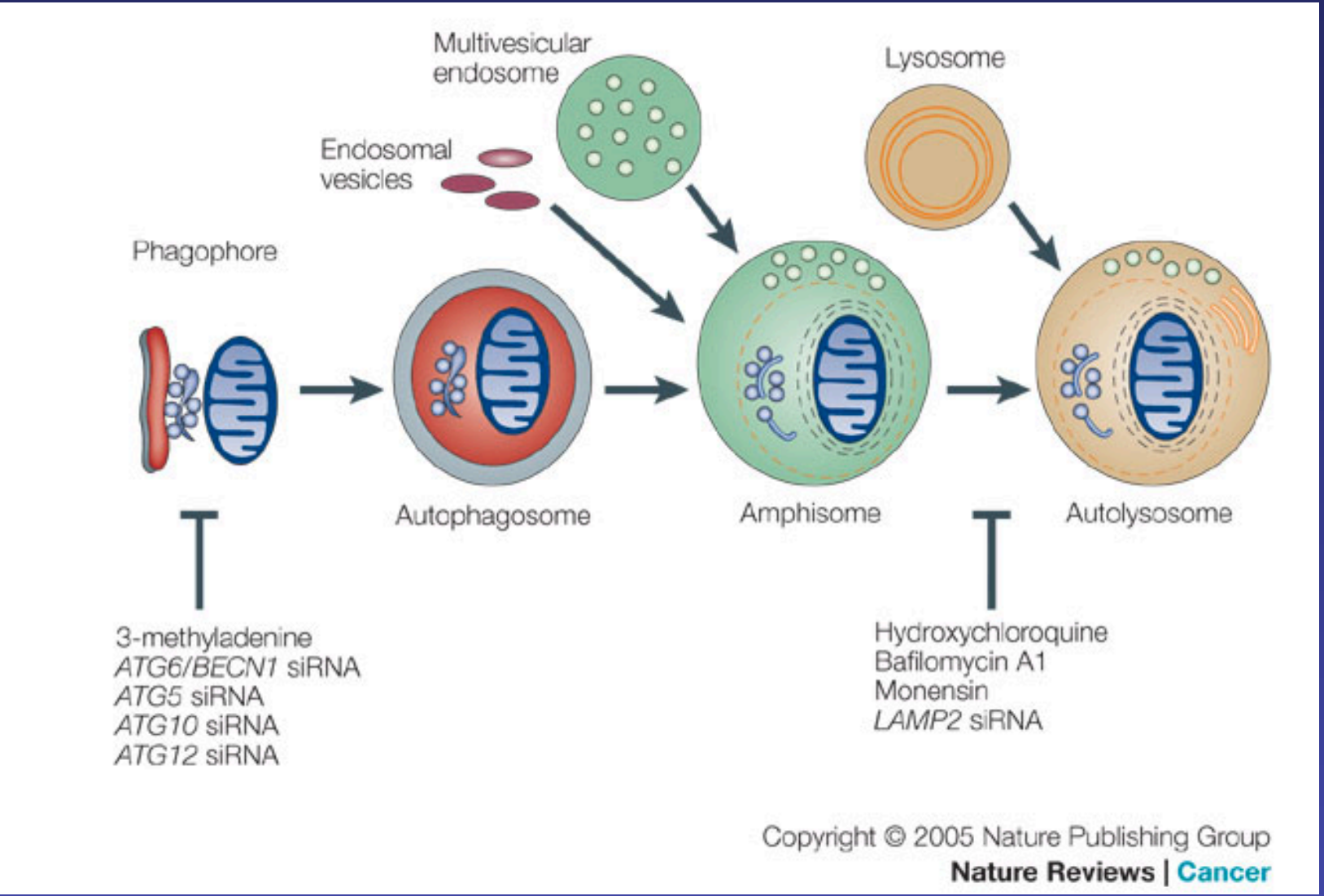
- DRAM (damage-regulated autophagy modulator), a lysosomal protein that can stimulate the accumulation of autophagic vacuoless
- smARF: shorter form of ARF which results from alternative initiation of translation and translocate to mitochondria

Multidomain BH3 proteins inhibit the autophagy BH3 only protein beclin 1



Several BH3 (BCL-2 homology 3)- only proteins have the dual capacity to activate autophagy and apoptosis

Autophagy and its inhibitors



Guido Kroemer & Marja Jäättelä. Nature Reviews Cancer 5, 886-897 (November 2005)

Autophagy quiz

1- True or False

a. Autophagy always leads to cell death
b. The core molecular machinery that forms an autophagosome consists of proteins termed autophagy-related genes (ATGs)
c. High nutrient levels and growth factor stimulation will lead to the activation of mTOR, which in turn inhibits autophagy by phosphorylating and thus inactivating a complex containing the two kinases ULK1 or ULK2
d. cell death that is dependent on successful autophagy has been described following the inhibition of apoptosis, implying a role as a backup once classic cell death has been abrogated

2- Which is these statements about the ULK complex is false

a. ULK1 is a serine threonine kinase
b. ULK activates the PI3K complex
c. ULK is required for autophagosome formation
d. ULK is a lipid kinase

Final exam

- 1- Propose a project to test a molecular model for the chronic p53 response
- Propose a project to discover the mechanism of action of RIPK1 in necroptosis
- Propose a project to investigate how autophagy regulates senescence