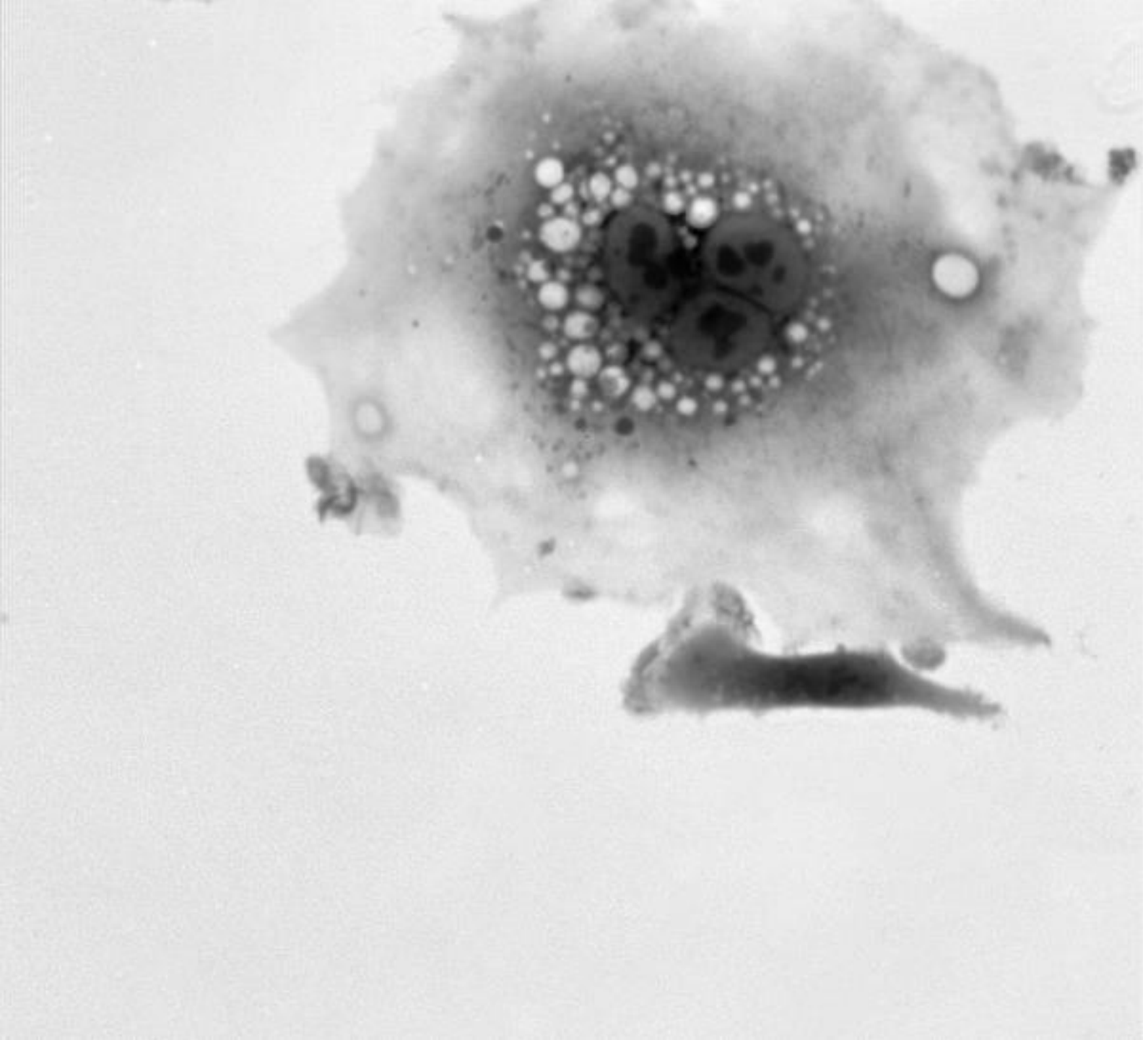


Cellular senescence, cell death

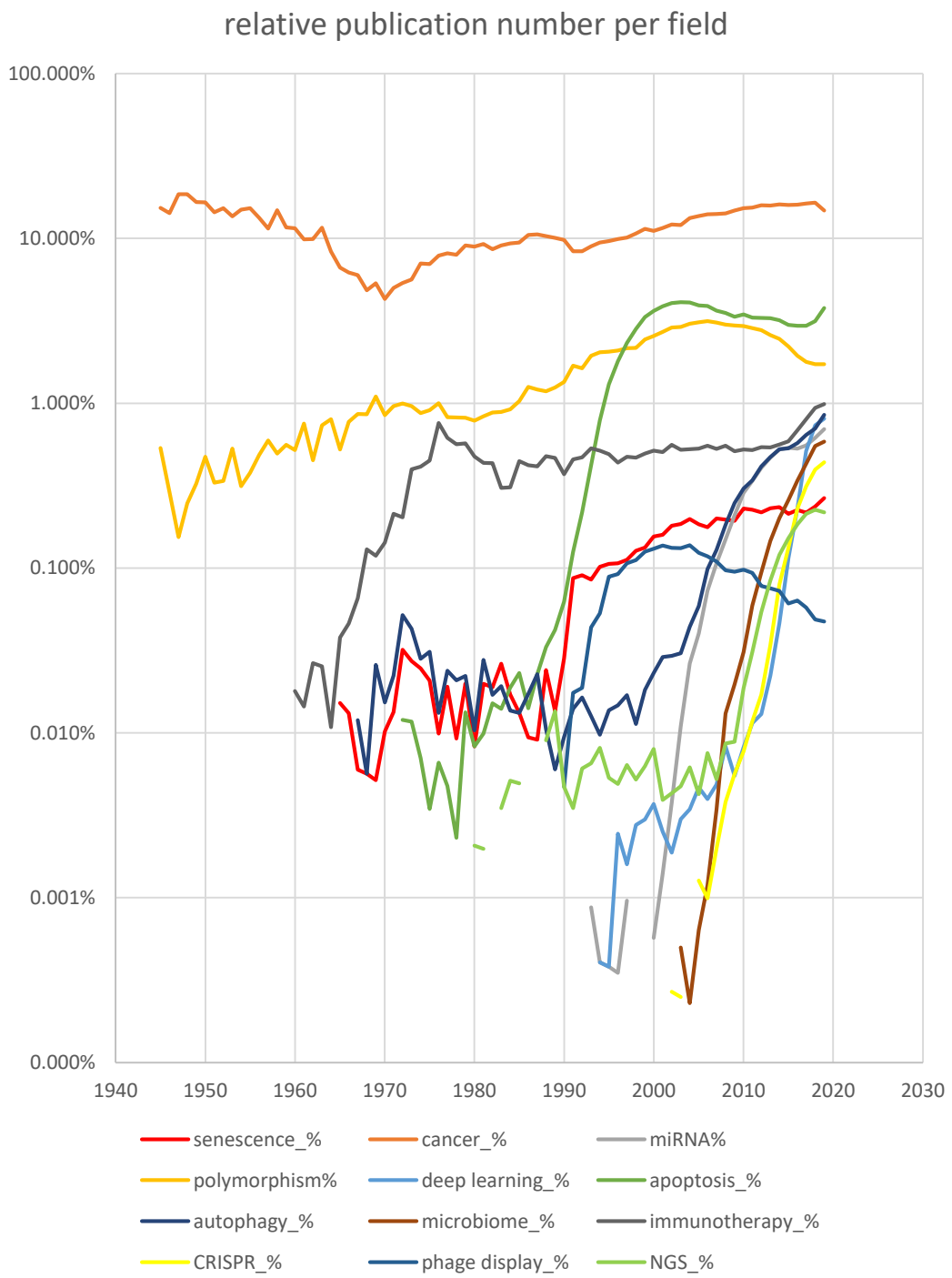
Jaromir Gumulec,

Dept. of Physiology,

Masaryk University

Cellular senescence

- What is it?
- What causes it?
- Why is it important (cancer and aging)?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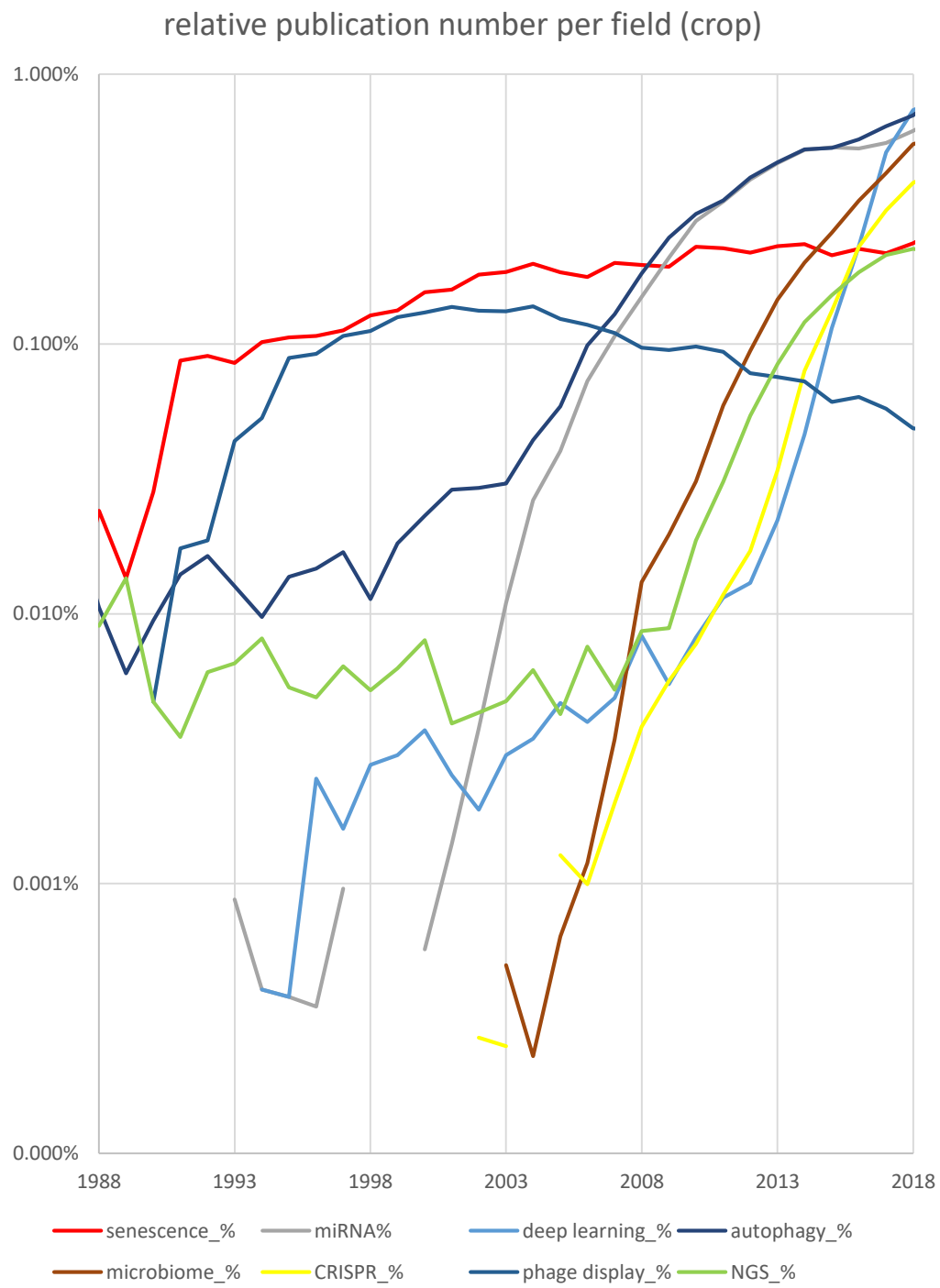
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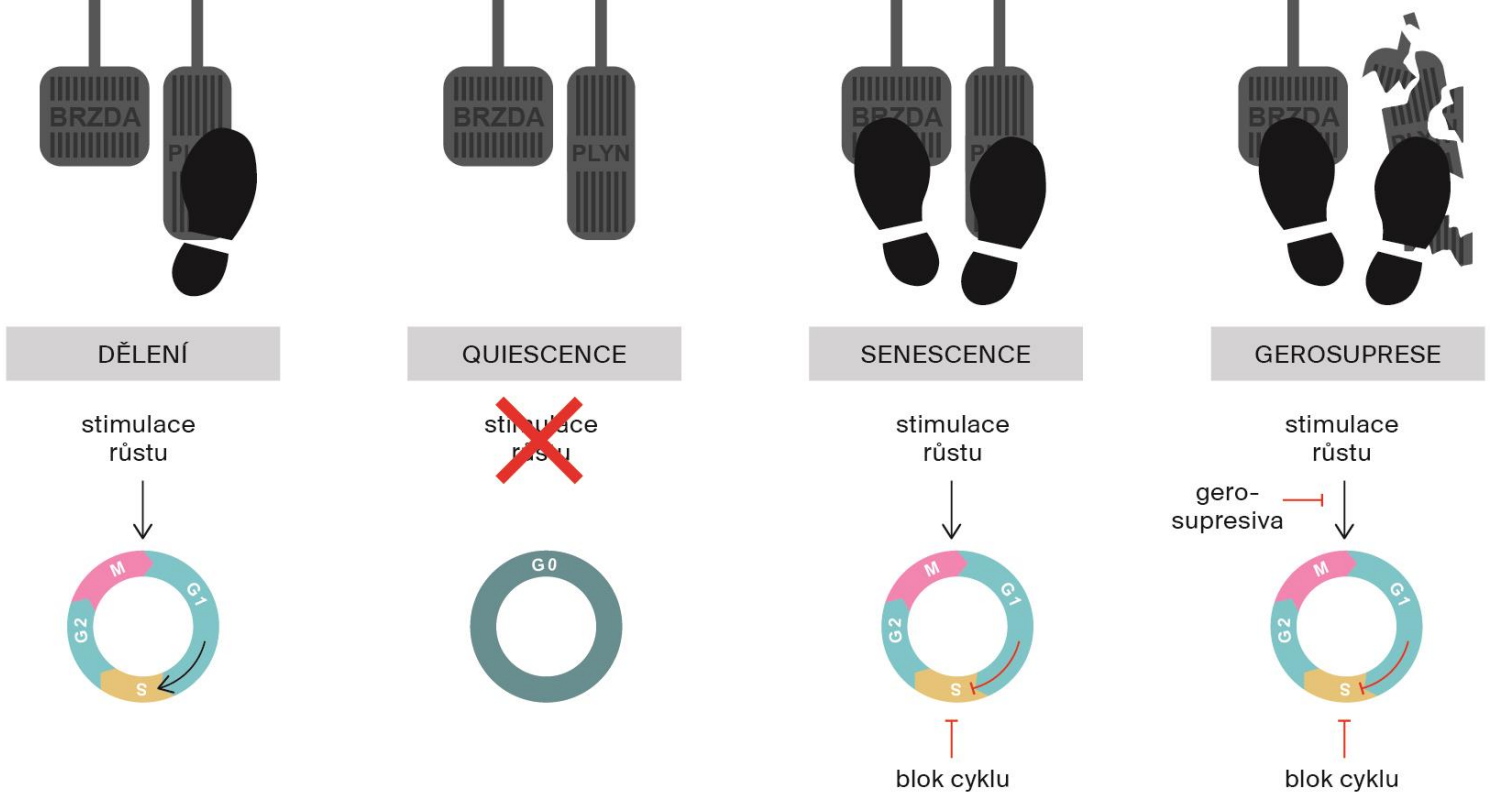


Cellular senescence
What it is?

- proces causing arrest of proliferation of cells in response to stressors
- Important proces for physiology and pathology
- Contributor to aging and age-related deseases
- attractive therapeutic target

G0 arrest ≠ senescence

Senescence vs. quiescence vs. terminal differentiation

reversible G0 arrest = (quiescent)

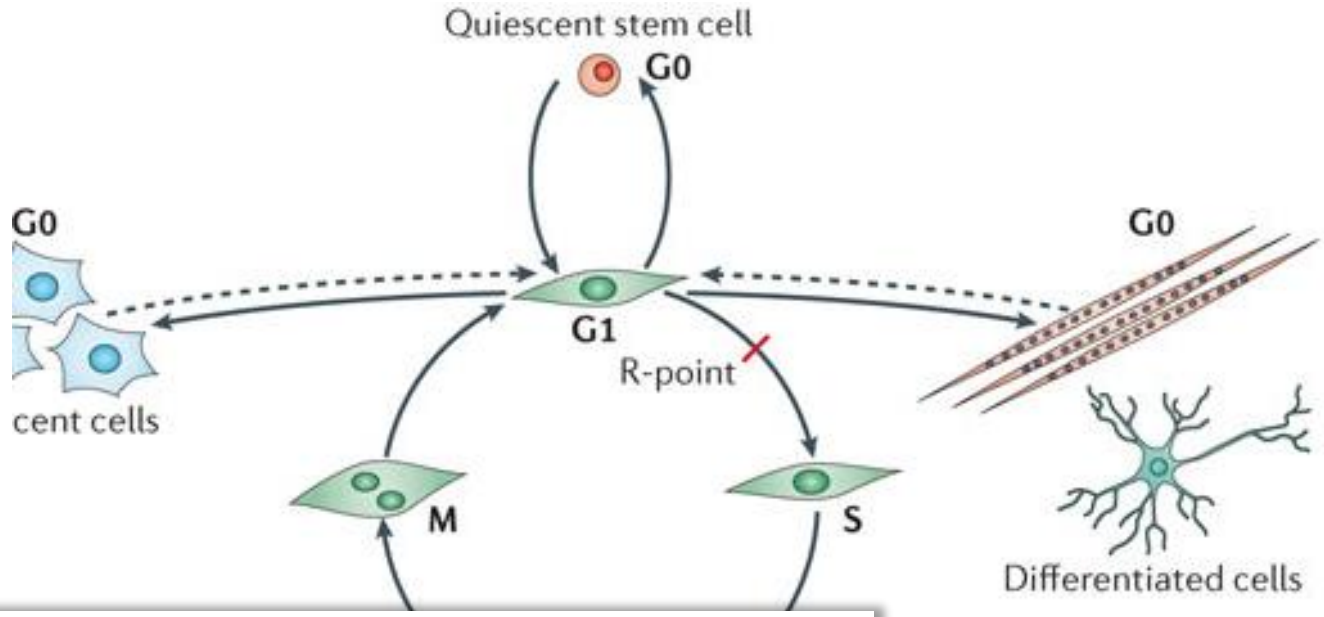
stem cells reside in the *quiescent* state and enter the cell cycle upon activation

irreversible „G0 arrest“ =

terminally differentiated cells

G1, S, G2 irreversible arrest = (senescent)

Senescent cells are dysfunctional cells that have ceased proliferation and are permanently withdrawn from the cell cycle



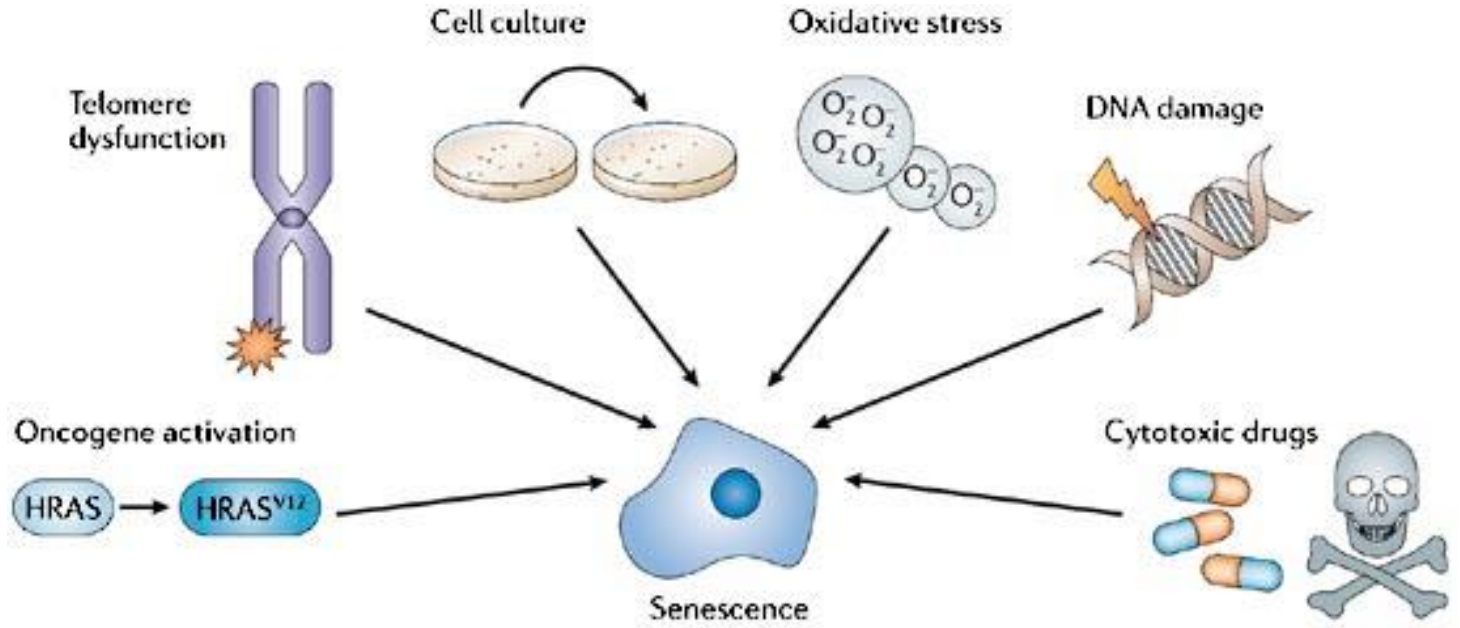
SENESCENCE

mechanism of regulation of damaged cells
mechanism in physiology (embryogenesis)

ws | Molecular Cell Biology

Causes of cellular senescence

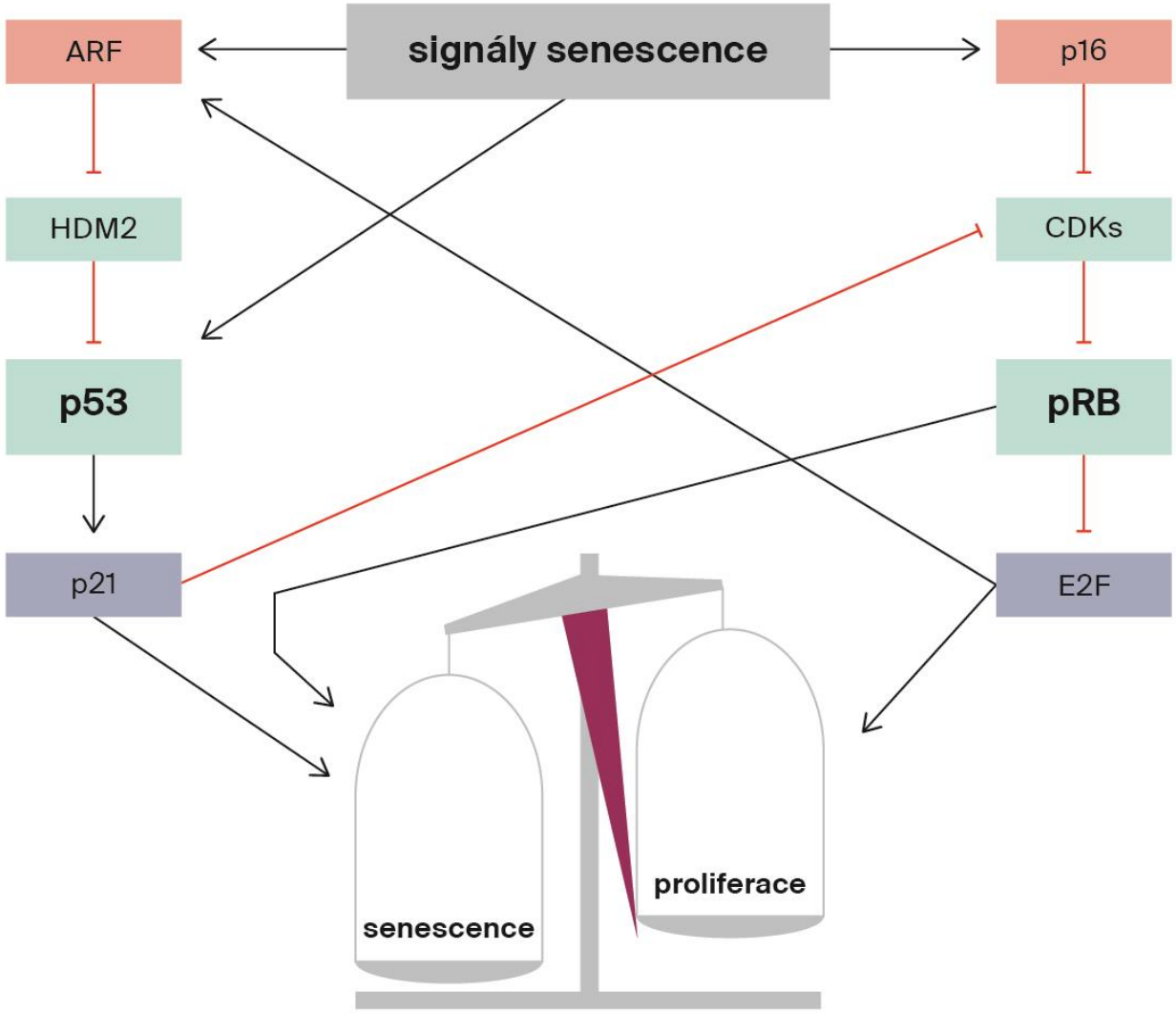
- Caused by multiple factors

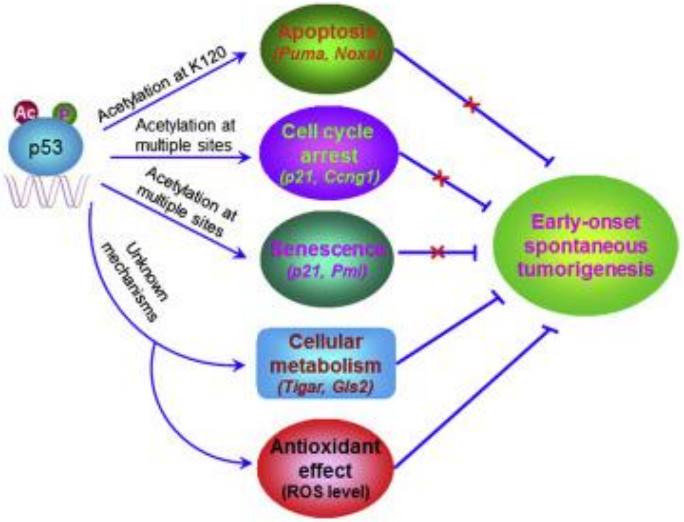


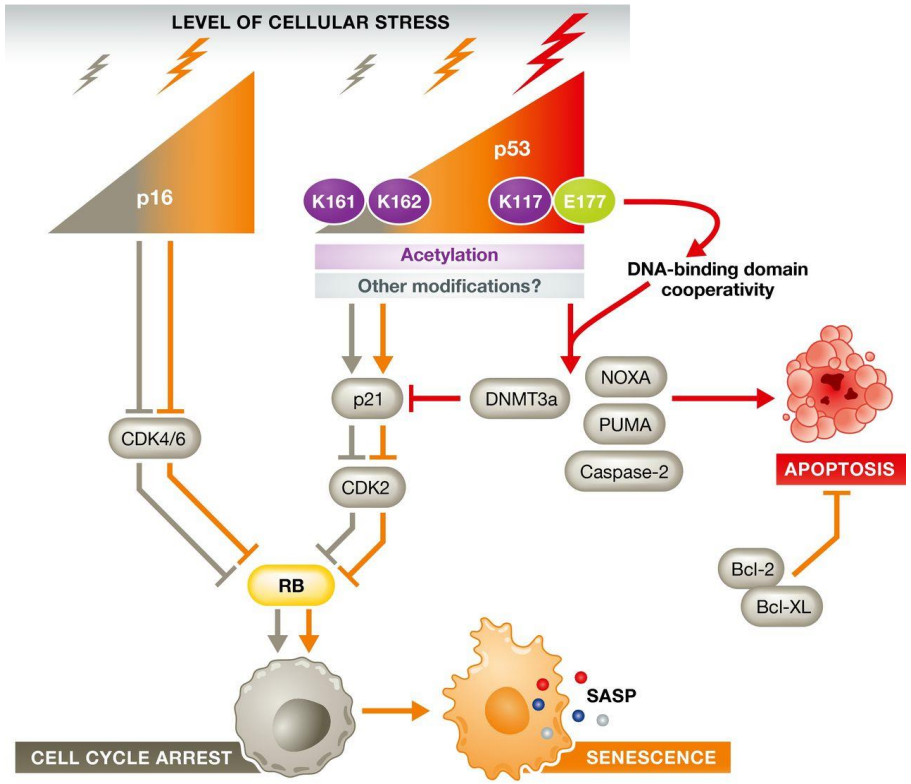
Collado *et al. Nature Reviews Cancer* **6**, 472–476 (June 2006) | doi:10.1038/nrc1884

Mechanisms of senescence: What causes it?

- Telomere shorteing (replicative senescence)
- CDKN2A locus derepression
- Stress-induced senescence
- Oncogene-induced senescence
- Senescence-associated secretory phenotype





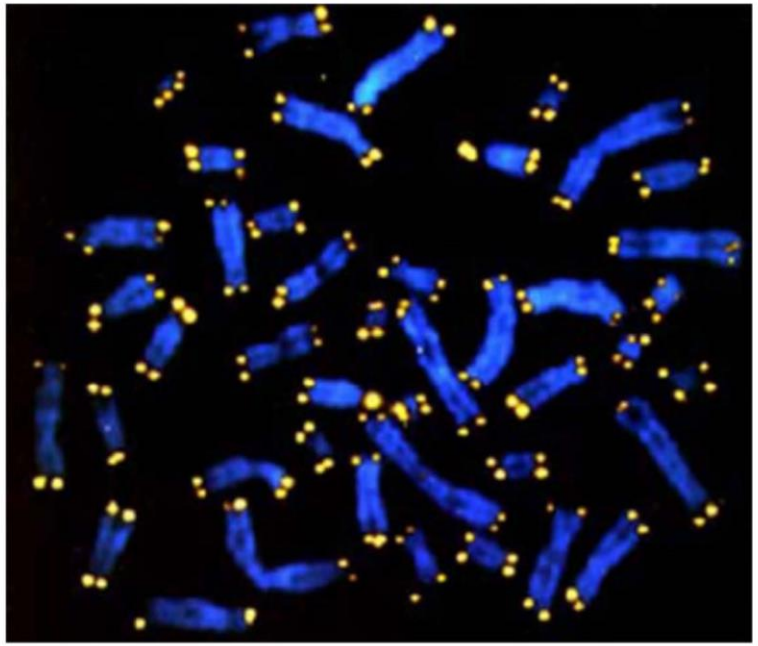


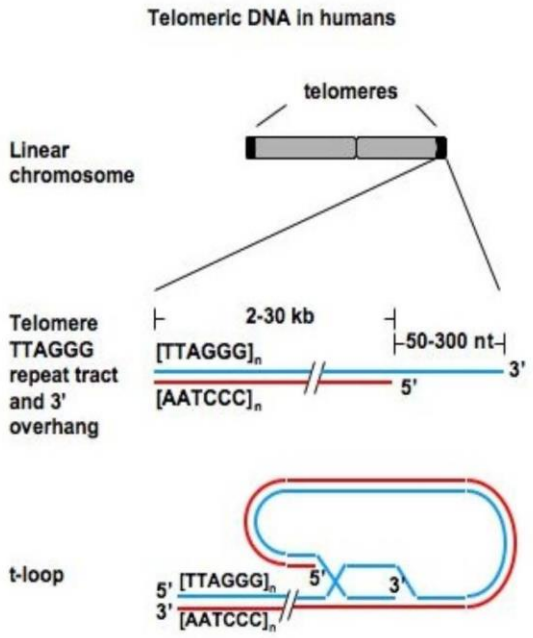
p53 „decision making“

- supressor regulating DNA damage protection and uncontrolled growth, and others
- DNA damage triggers p53 stabilization and activation
- not completely clear what direct to apoptosis vs senescence
  - Fibroblasts and epithelial cells > senescence
  - lymphocytes > apoptosis
  - p53 acetylation-related mechanisms

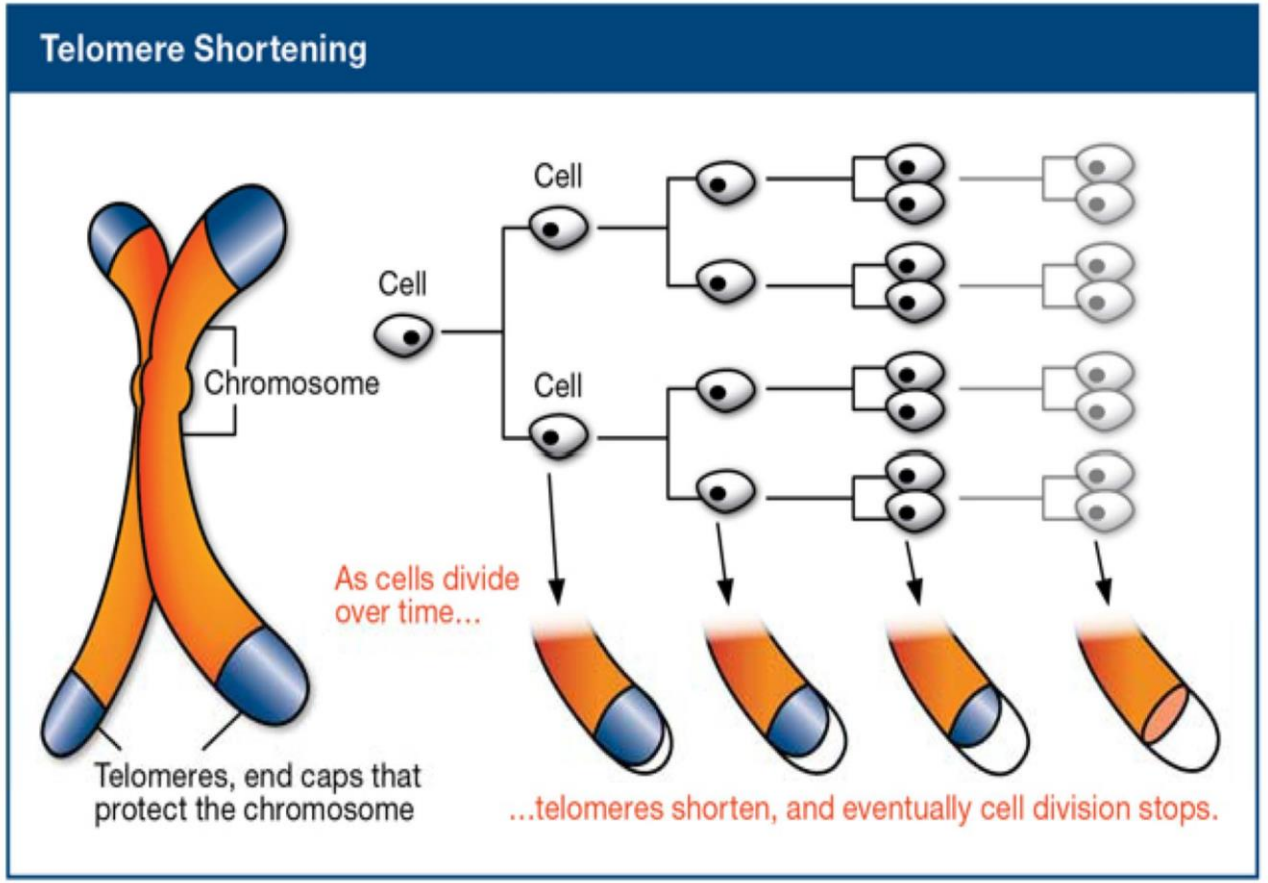
Telomere

- A telomere is a region of repetitive nucleotide sequences at each end of a chromosome.
- It protects the endof chromosome from deterioration of fusion with neighbouring chromosomes





Telomere shortens as cells divide

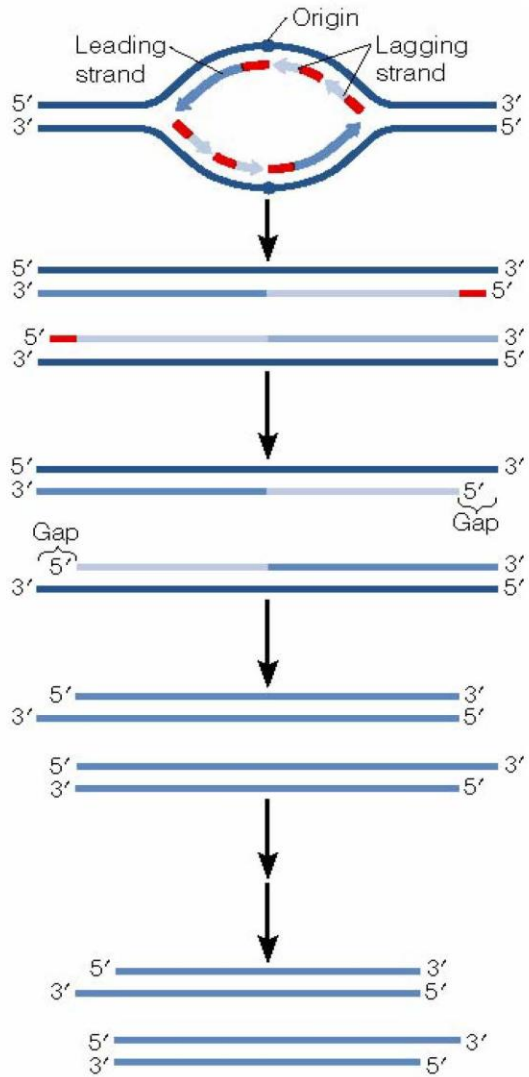


1. DNA replication is initiated at the origin; the replication bubble grows as the two replication forks move in opposite directions.

2. Finally only one primer (red) remains on each daughter DNA molecule.

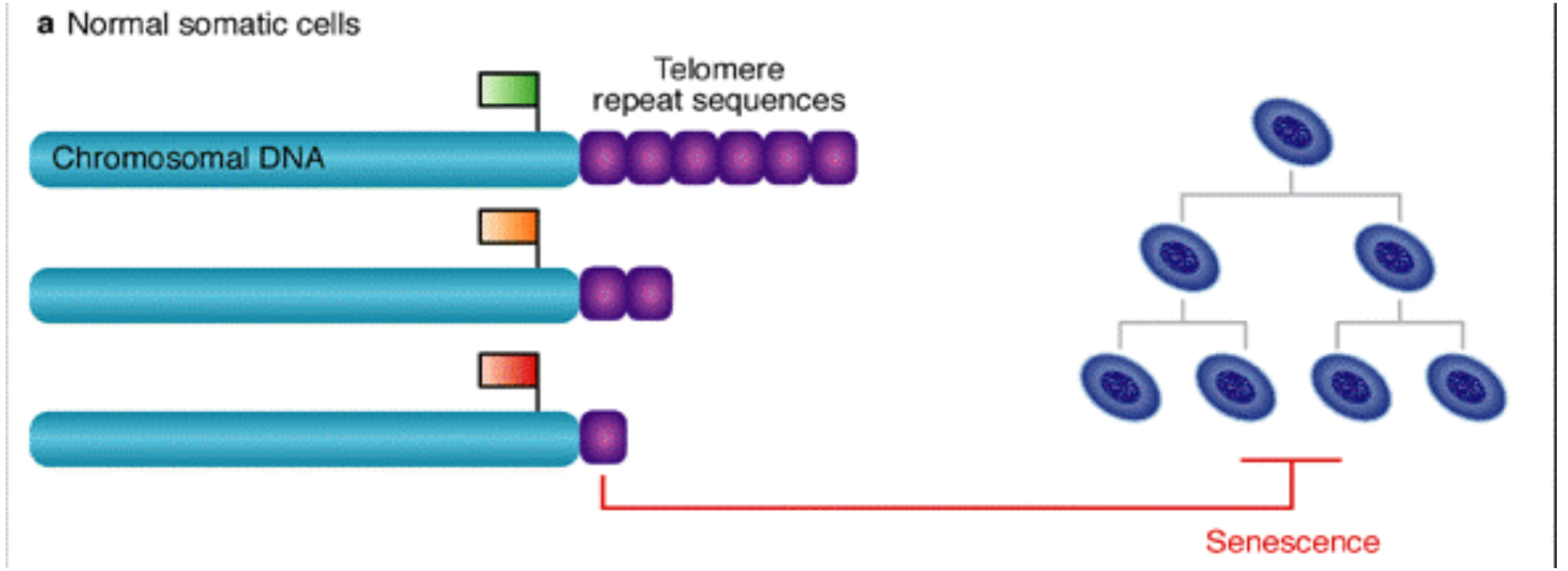
3. The last primers are removed by a 5′→3′ exonuclease, but no DNA polymerase can fill the resulting gaps because there is no 3′ OH available to which a nucleotide can be added.

4. Each round of replication generates shorter and shorter DNA molecules.



Telomerase and Senescence

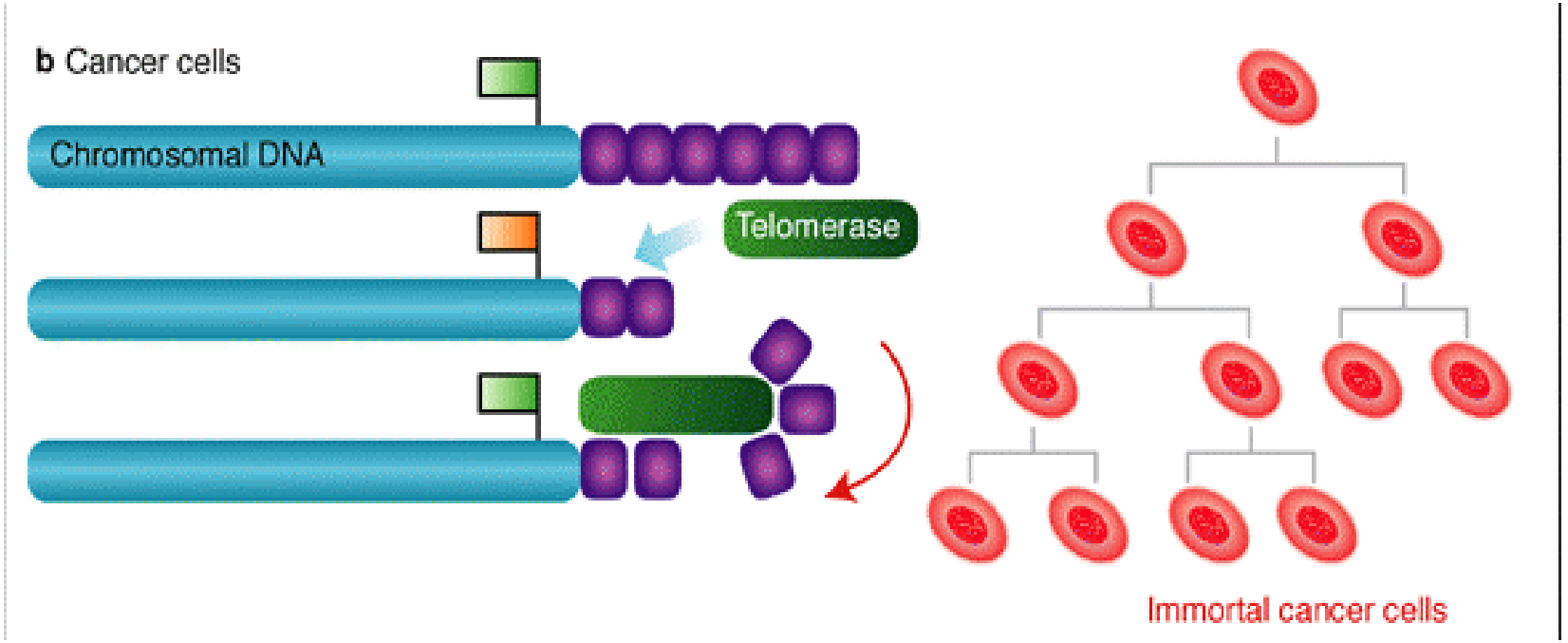
In most somatic tissues, telomerase is expressed at very low levels or not at all -- as cells divide, telomeres shorten



Short telomeres signal cells to senesce (stop dividing)

Telomerase and Cancer

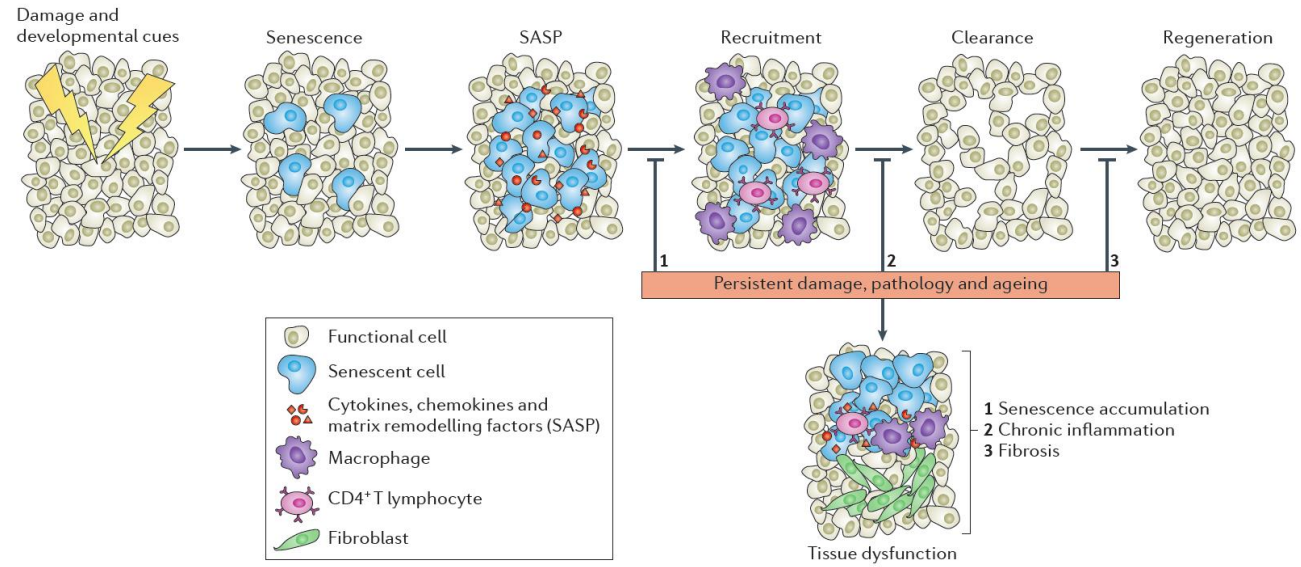
The presence of telomerase in cancer cells allows them to maintain telomere length while they proliferate



When telomeres reach a critical minimal length, their protective structure is disrupted. This triggers a DNA damage response (DDR)

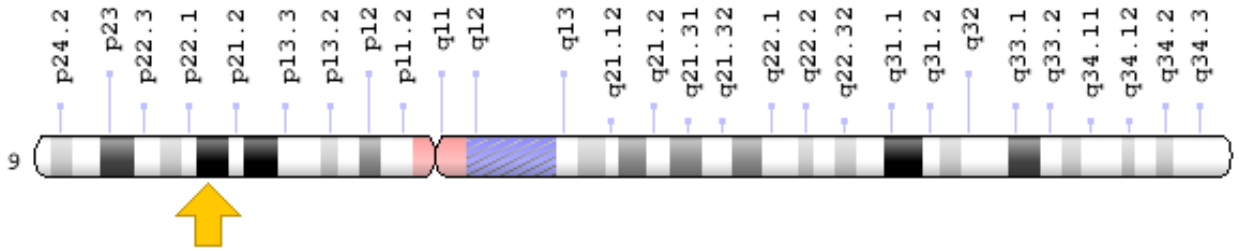
Senescence-associated secretory phenotype

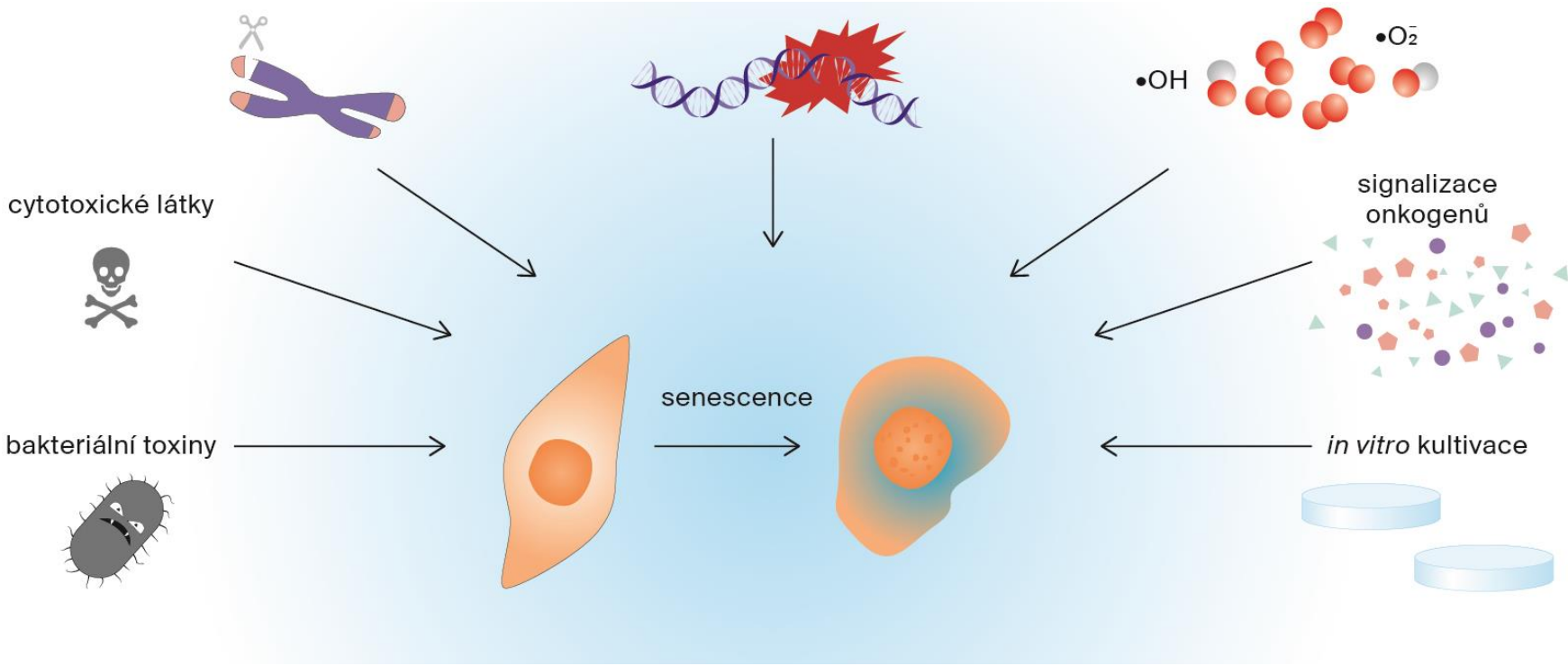
- Complex pro-inflammatory response
- Senescent cells produce various factors, including IL-6, NF-kB, TGF-B, GM-CSF, etc.)
- This causes inflammation with subsequent phagocytosis of senescent cells



CDKN2A locus derepression

- = tumor supressor
- Normally expressed at very low levels
- Derepressed with ageing (indicator of biological age)

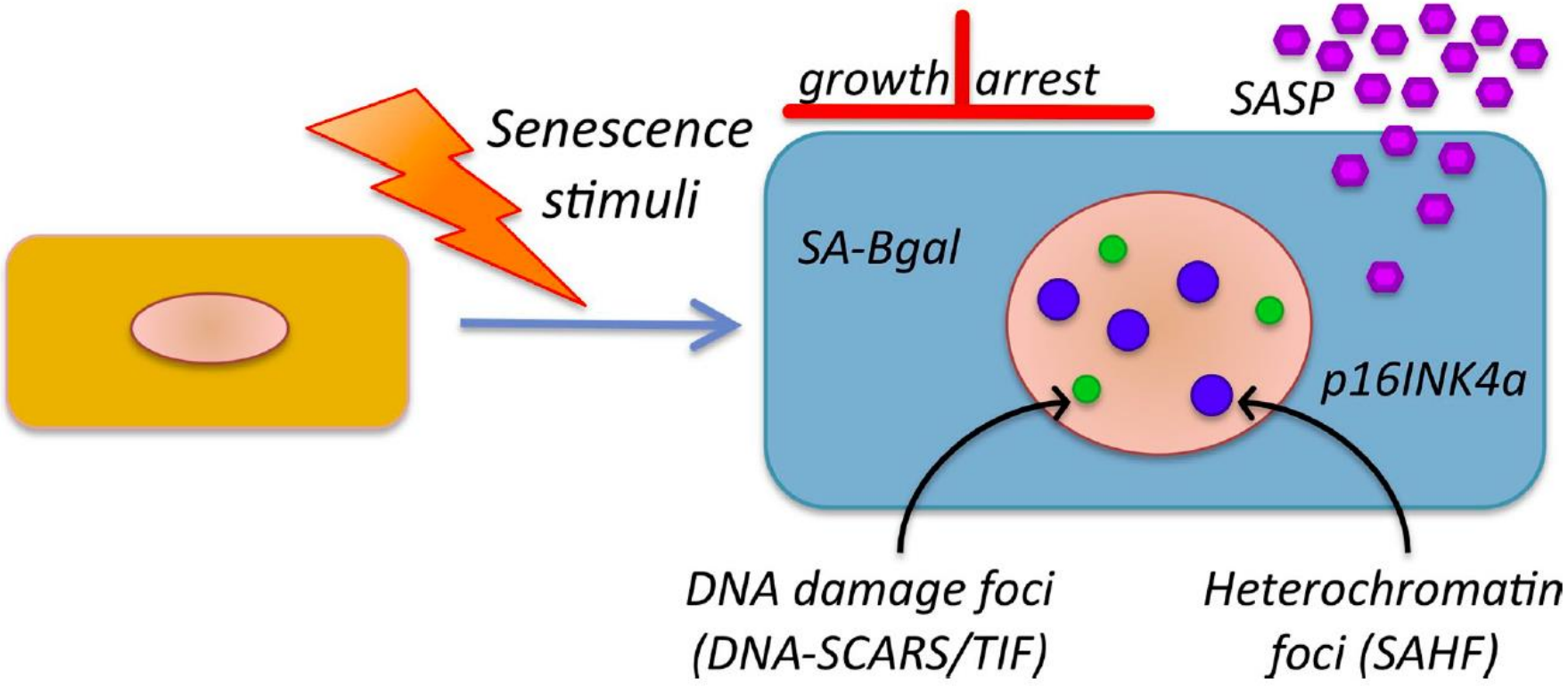


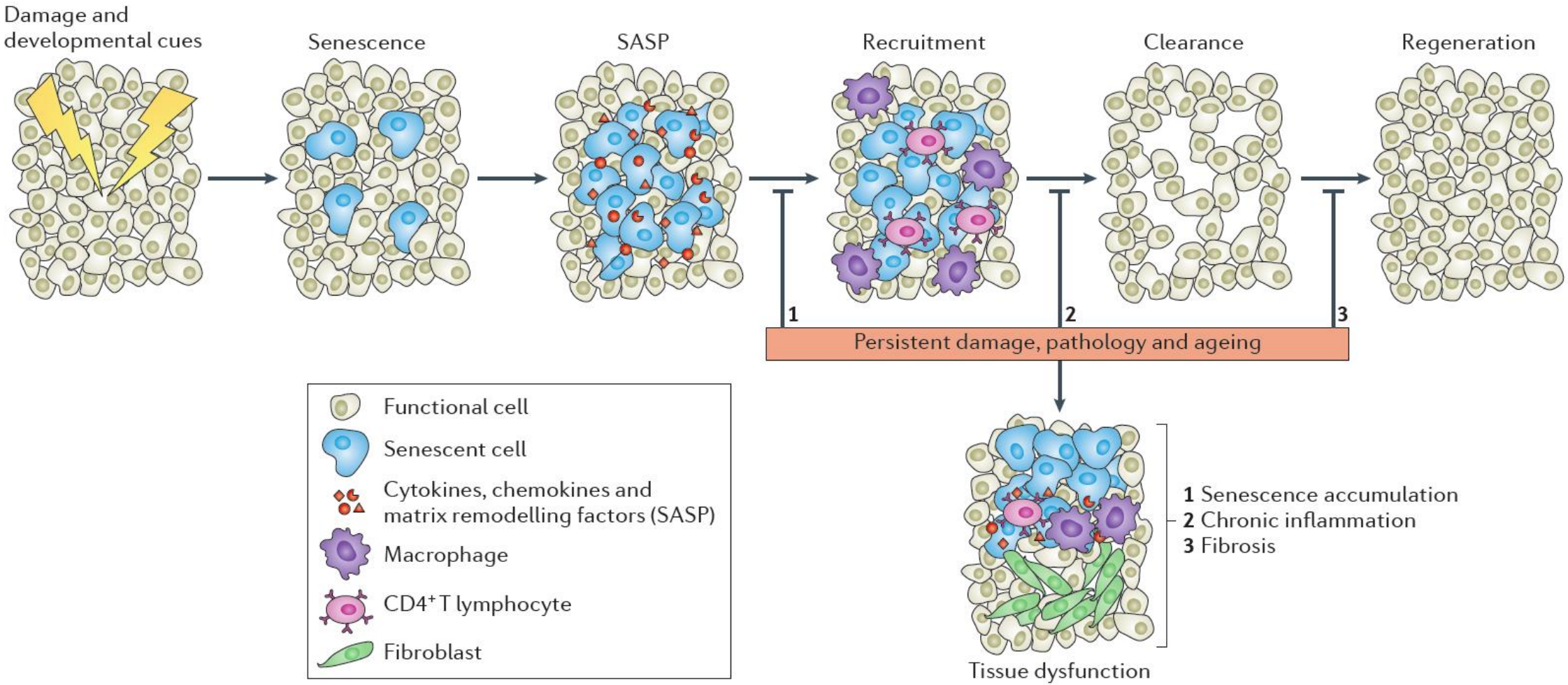


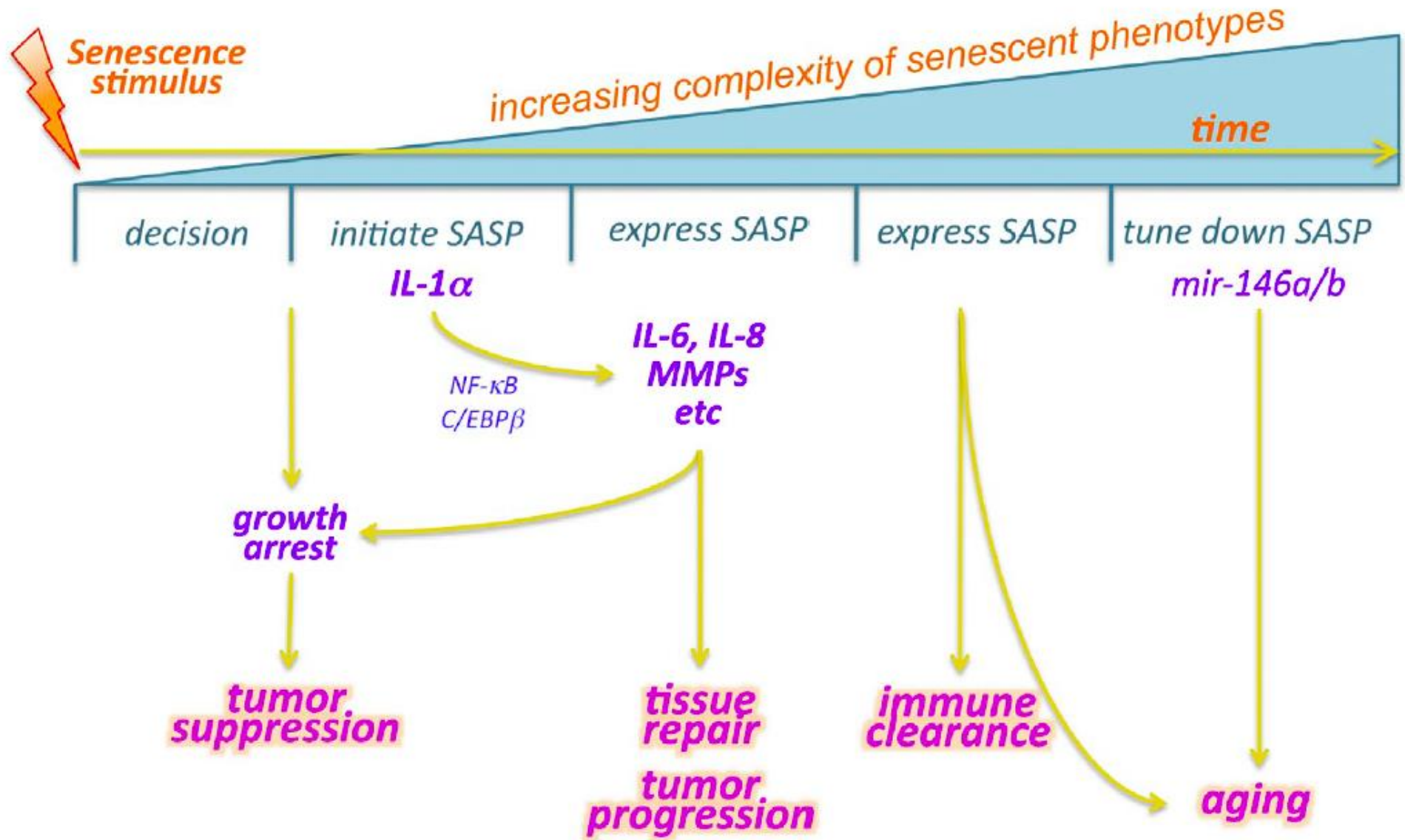
Stress-induced sensescence

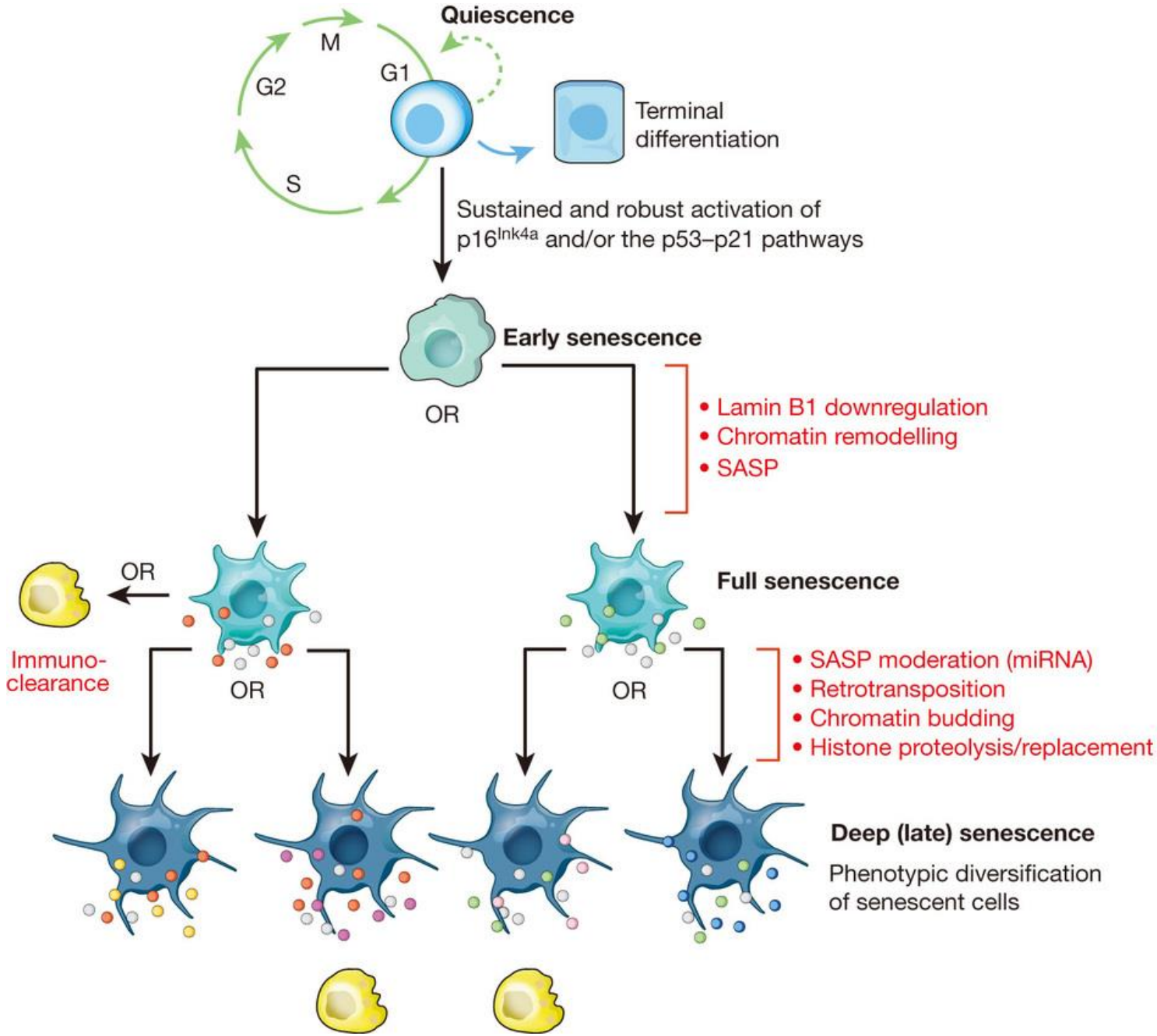
- Reactive oxygen species increase after various stresses, incl. Chemotherapy
- Antioxidant treatment prevents senescence

DNA-damage response (DDR): cell fate following senescence triggering



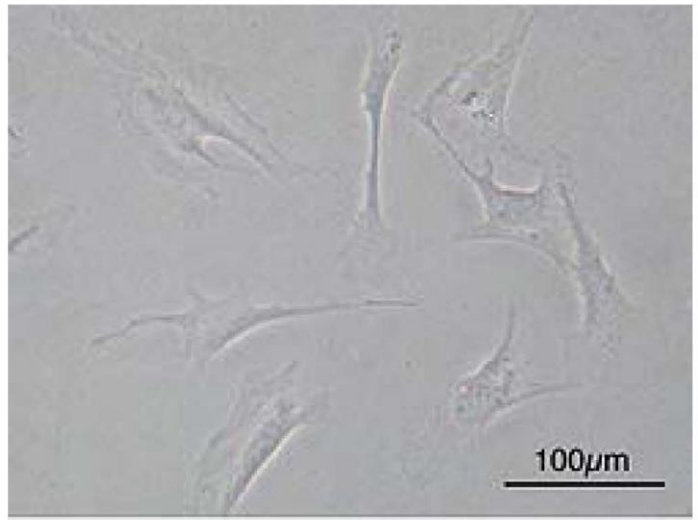

Damage and developmental cues
Senescence
SASP
Recruitment
Clearance
Regeneration
1
2
3
Persistent damage, pathology and ageing
Functional cell
Senescent cell
Cytokines, chemokines and matrix remodelling factors (SASP)
Macrophage
CD4+ T lymphocyte
Fibroblast
1 Senescence accumulation
2 Chronic inflammation
3 Fibrosis
Tissue dysfunction


Senescence stimulus
increasing complexity of senescent phenotypes
time
decision
initiate SASP
express SASP
express SASP
tune down SASP
IL-1α
mir-146a/b
IL-6, IL-8
MMPs
etc
NF-κB
C/EBPβ
growth arrest
tumor suppression
tissue repair
tumor progression
immune clearance
aging

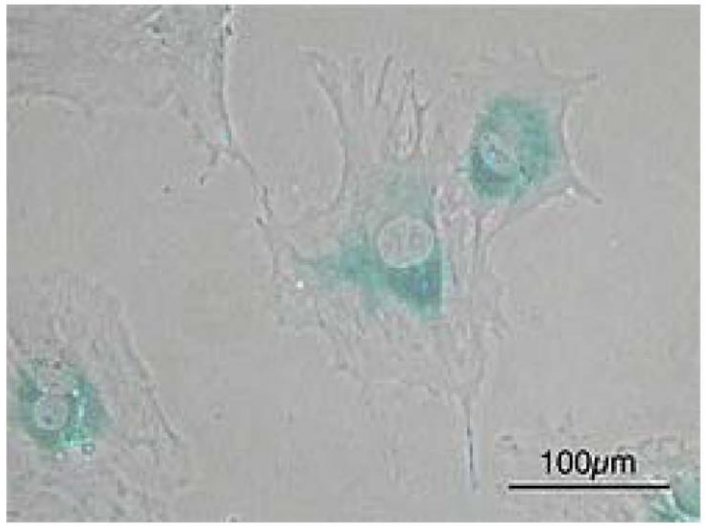

Quiescence
M
G1
G2
S
Terminal differentiation
Sustained and robust activation of p16Ink4a and/or the p53–p21 pathways
Early senescence
OR
• Lamin B1 downregulation
• Chromatin remodelling
• SASP
OR
Immuno-clearance
OR
Full senescence
OR
• SASP moderation (miRNA)
• Retrotransposition
• Chromatin budding
• Histone proteolysis/replacement
Deep (late) senescence
Phenotypic diversification of senescent cells

What does a senescent cell look like?

Primary mouse embryonic fibroblasts (MEFs)



Before senescence
Spindle-shaped



Senescent after passages
Larger, flatten shape and express senescence-associated β-galactosidase
a maker of cellular senescence

Modified from Wikipedia

How to identify senescence

- **Cell cycle arrest**

  Long-term exit from the cell cycle is the central and only indispensable marker for the identification of all types of cellular senescence both in vitro and in vivo. However, cell cycle arrest is not unique to senescence.

  Cellular senescence is largely irreversible. However, there are multiple ways to reverse the arrest, allowing cells to re-enter the cell cycle.
  For example, inactivation of the p53 pathway permits senescence reversal.

- **Morphological transformation**

  Cell senescence is generally accompanied by morphological changes and senescent cells can become large, flat,and multinucleated, or rather refractile depending on the trigger.

◆ Activation of tumor suppressor networks

The p53 and p16INK4A–RB signal transduction cascades commonly mediate the activation of the senescence program .

Other proteins in the p16INK4A–RB and p53 pathways, notably p21CIP1 and p15INK4B, also often accumulate in senescent cells, and have been used as markers reflecting the activation of these pathways in senescence

◆ Induction of SA-b-GAL activity

SA-b-GAL is a commonly used senescence biomarker.

Its increased activity in senescent cells derives from lysosomal b-D-galactosidase, which is encoded by the GLB1gene.

◆ Senescence-associated heterochromatic foci (SAHF)

Cellular senescence can be associated with an altered chromatin structure, at least in vitro. While DNA dyes display overall homogenous staining patterns in cycling or quiescent human cells, senescent cells often show strikingly different punctate staining patterns.

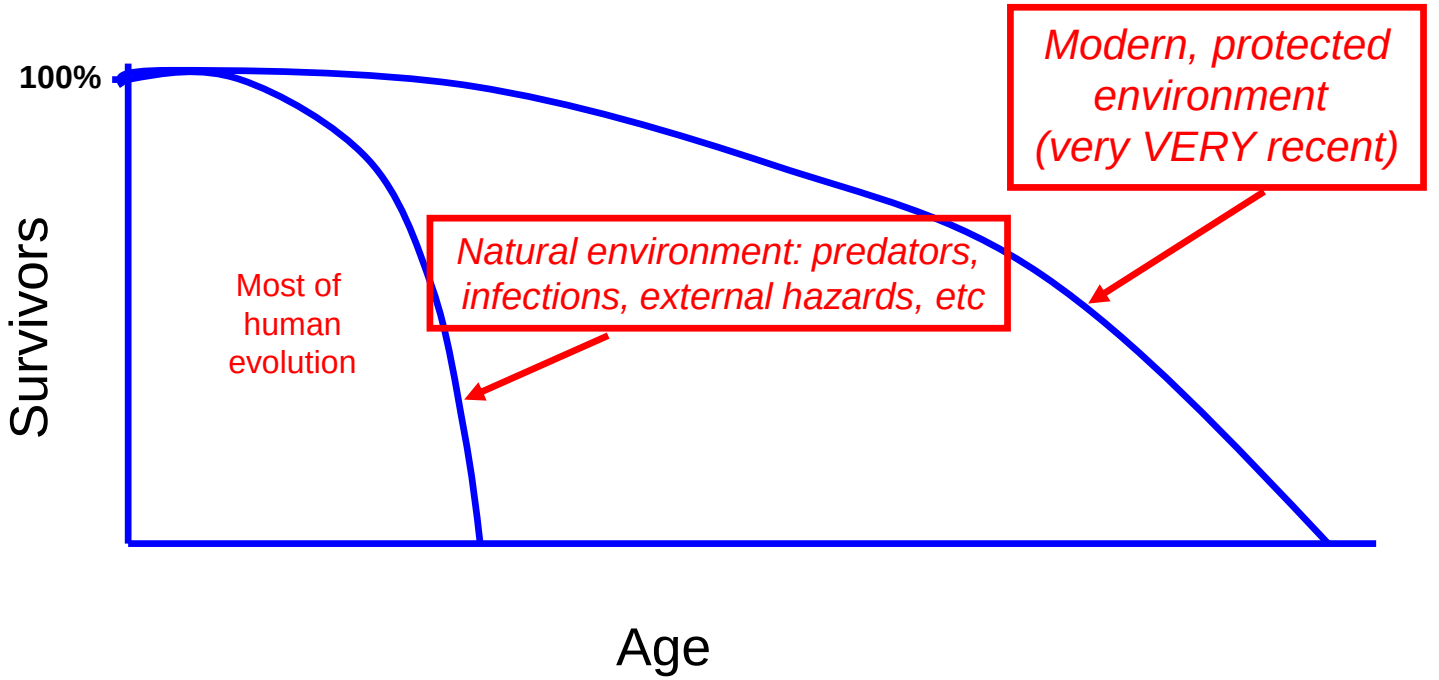
These DNA SAHF (Narita et al. 2003) are specifically enriched in methylated Lys 9 of histone H3 (a modification catalyzedby the histone methyltransferase Suv39h1)

Senescent cells display increased binding of heterochromatin-associated proteins in the promoters of several E2F target genes.
SAHF formation is circumvented by interference with p16INK4A–RB pathway signaling, correlating with bypass of senescence.

- **Senescence-associated secretory phenotype (SASP)**
  Cells undergoing senescence—whether in response to telomere malfunction, DNA damage, or oncogenic alterations—exhibit profound changes in their transcriptomes. A major consequence of this is the secretion of many dozens of factors, including cytokines and chemokines (Campisi 2005).

Cellular senence vs. aging

- **<u>Aging</u>** = progressive loss of organ function over time
- organismal fitness declines
- **Antagonistic pleiotropy**: Some traits selected to optimize fitness in young organisms can have unselected deleterious effects in old organisms
  (what's good for you when you're young may be bad for you when you're old)
  - *genes related to aging were not evolutionary selected*
- **„wear and tear“ theory:** mainatenance of body integrity is extremely dificult to perfom, can only be performed at the expense of growth and reproduction. Species with a high risk of pedators rather vote reproduction strategy



REGISTRATION FORM

Name: The Wanderer

♂ ♀

Strength	4
Perception	6
Endurance	3
Charisma	5
Intelligence	5
Agility	4
Luck	1

0

POINTS AVAILABLE

Next Reset

Perk Chart

Tell us about yourself! Vault-Tec needs to know what kind of citizen you are to ensure your future happiness.

Antagonistic pleiotropy

Cellular senescence

Selected for tumor suppression (growth arrest)

Functional changes unselected, deleterious

FUNCTIONAL CHANGES ASSOCIATED WITH CELLULAR SENESCENCE:

Secretion of molecules that can be detrimental to tissues if not controlled

e.g., senescent fibroblasts secrete proteases, growth factors, inflammatory cytokines

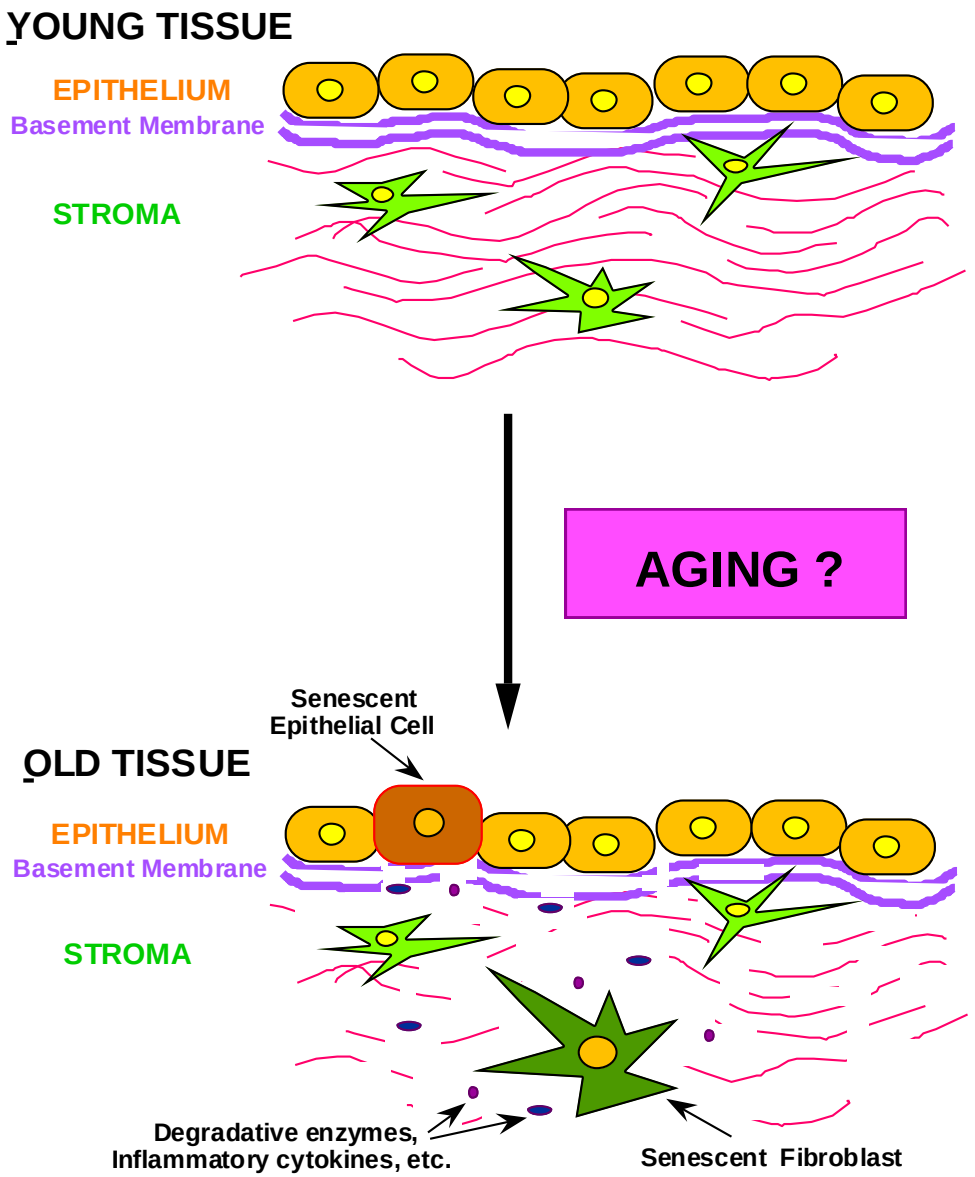
Cellular senescence and aging

•Cells from old donors divide less often than cells from young donors

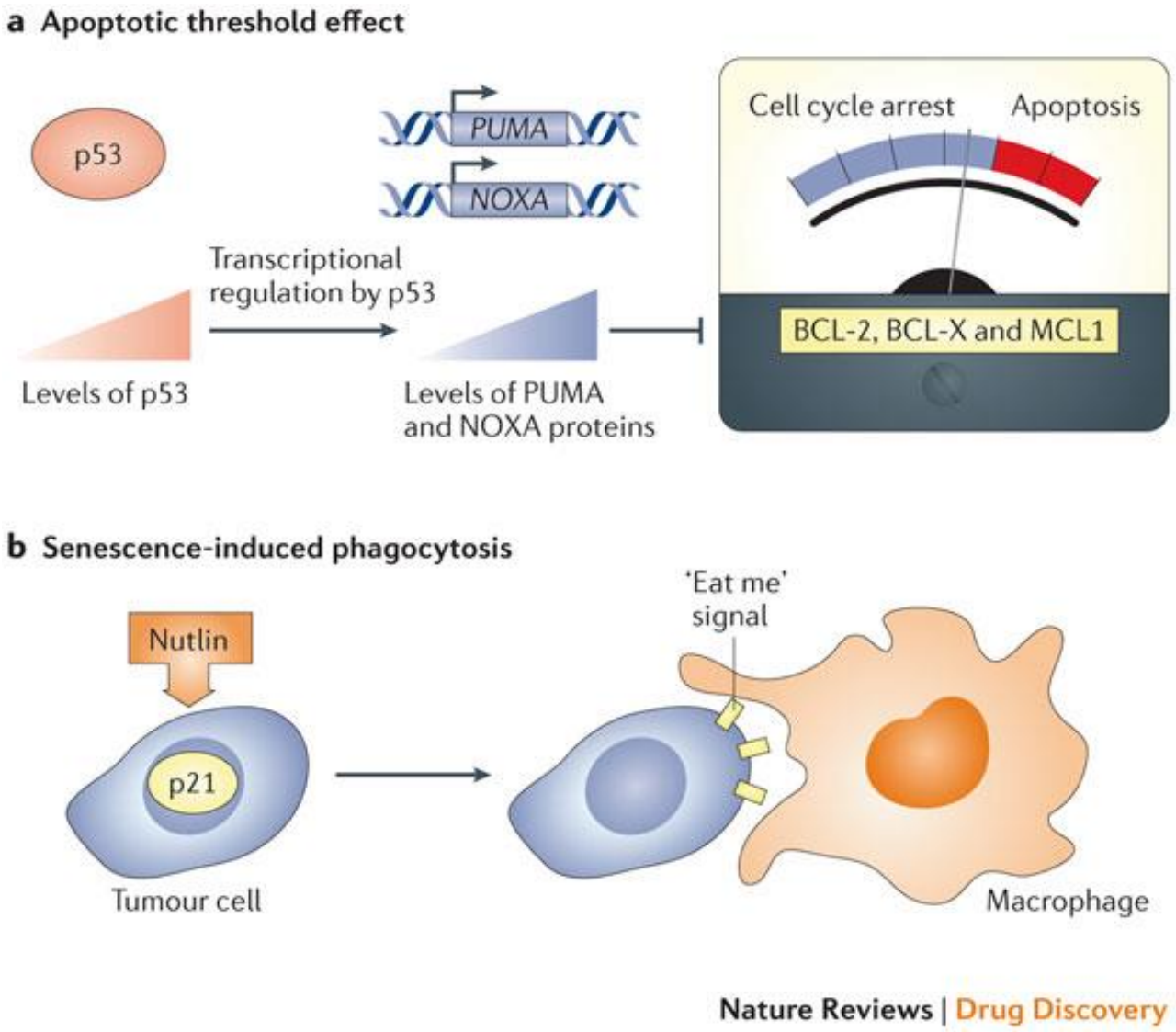
•Cells from short-lived species are more sensitive to senescence-inducers, particularly oxidative stress, than cells from long-lived species

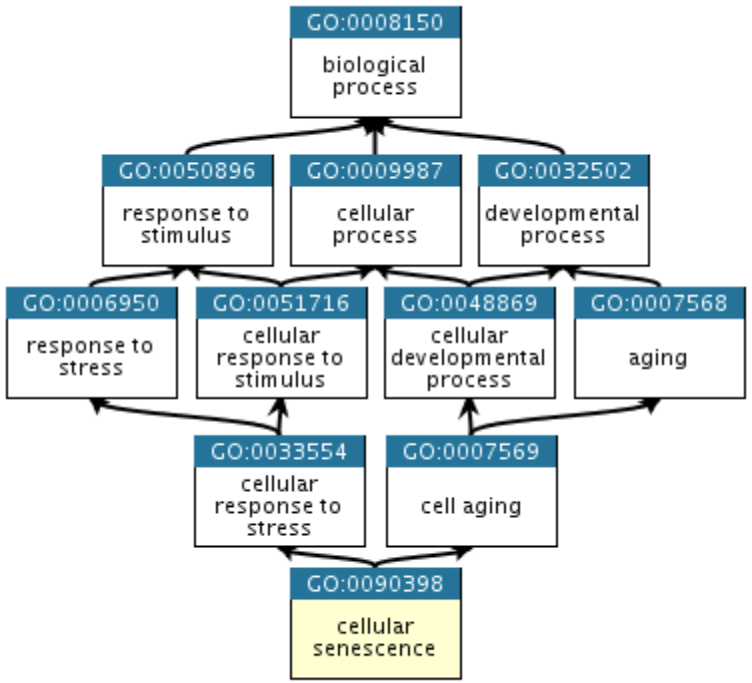
•Cells from donors with premature aging syndromes senesce more readily than cells from normal donors

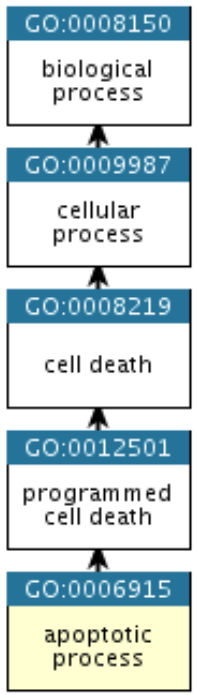
•Senescent cells (expressing a senescence marker) accumulate with age and at sites of age-related pathology


YOUNG TISSUE
EPITHELIUM
Basement Membrane
STROMA
AGING ?
Senescent
Epithelial Cell
OLD TISSUE
EPITHELIUM
Basement Membrane
STROMA
Degradative enzymes,
Inflammatory cytokines, etc.
Senescent Fibroblast

Senescence vs. apoptosis





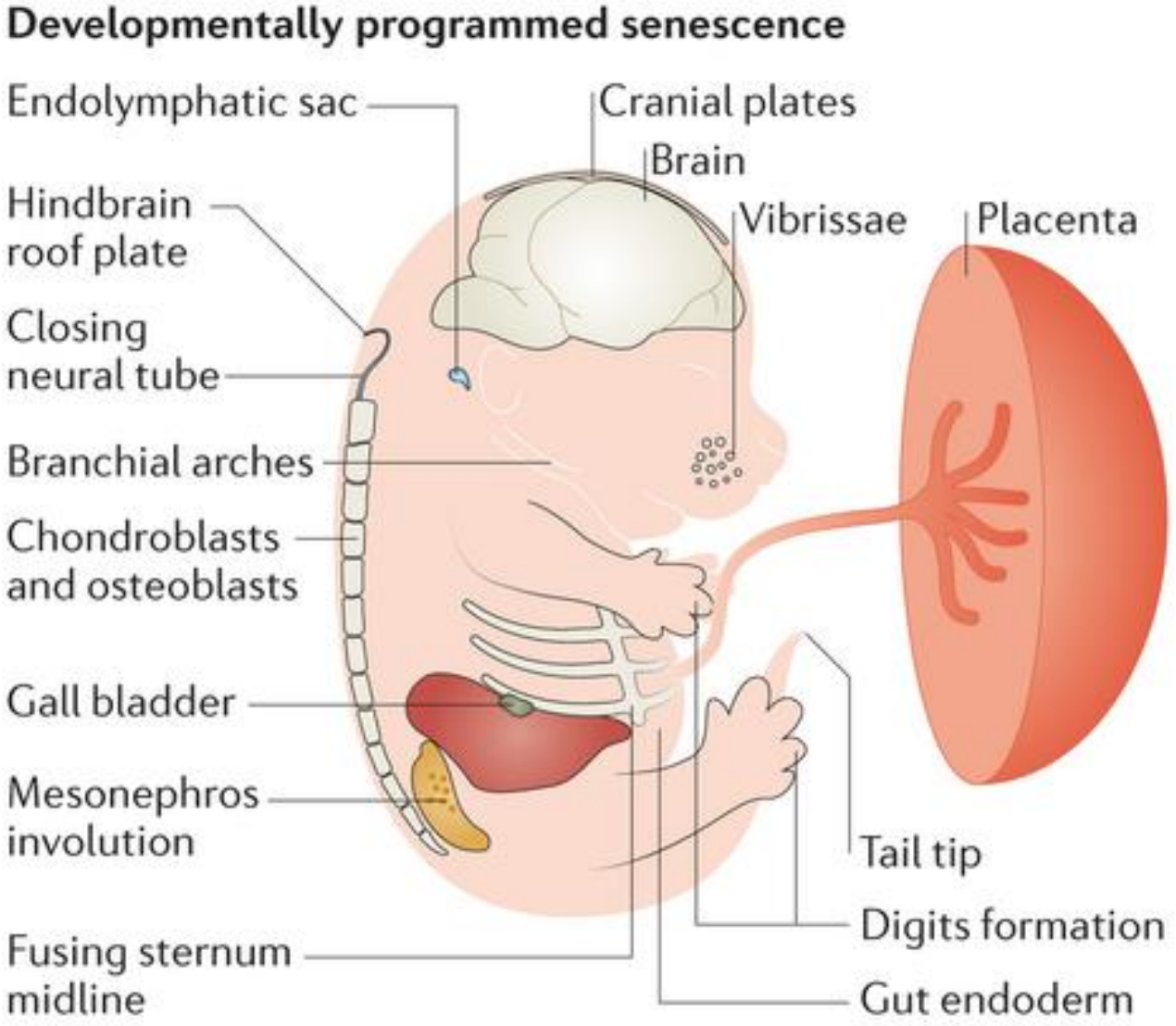


QuickGO - https://www.ebi.ac.uk/QuickGO

Abl1
Abl1
Akt3
Arg2
Arntl
Arntl
Bcl2l12
Bcl6
Bmpr1a
Calr
Calr
Cav1
Cdk6
Cdkn1a
Cdkn2a
Cdkn2a
Cdkn2a
Cdkn2a
Cdkn2b
Cgas
Eef1e1
Fbxo5
H2-M3
Hmga2
Hras
Icmt
Id2
Ing2
Kat6a
Kras
Map2k1
Map3k3
Mapk14
Mapkapk5
Nampt
Nek4
Nsmce2
Nuak1
Opa1
Pawr
Pla2r1
Plk2
Pml
Pnpt1
Prkcd
Prkdc
Prmt6
Rbl1
Rsl1d1
Sirt1
Sirt1
Sirt1
Slc30a10
Smc5
Smc6
Srf
Suv39h1
Tbx2
Tbx3
Terf2
Tert
Trp53
Trp53
Trp63
Twist1
Vash1
Wnt16
Ypel3
Zfp277
Zkscan3
Zmpste24
Zmpste24

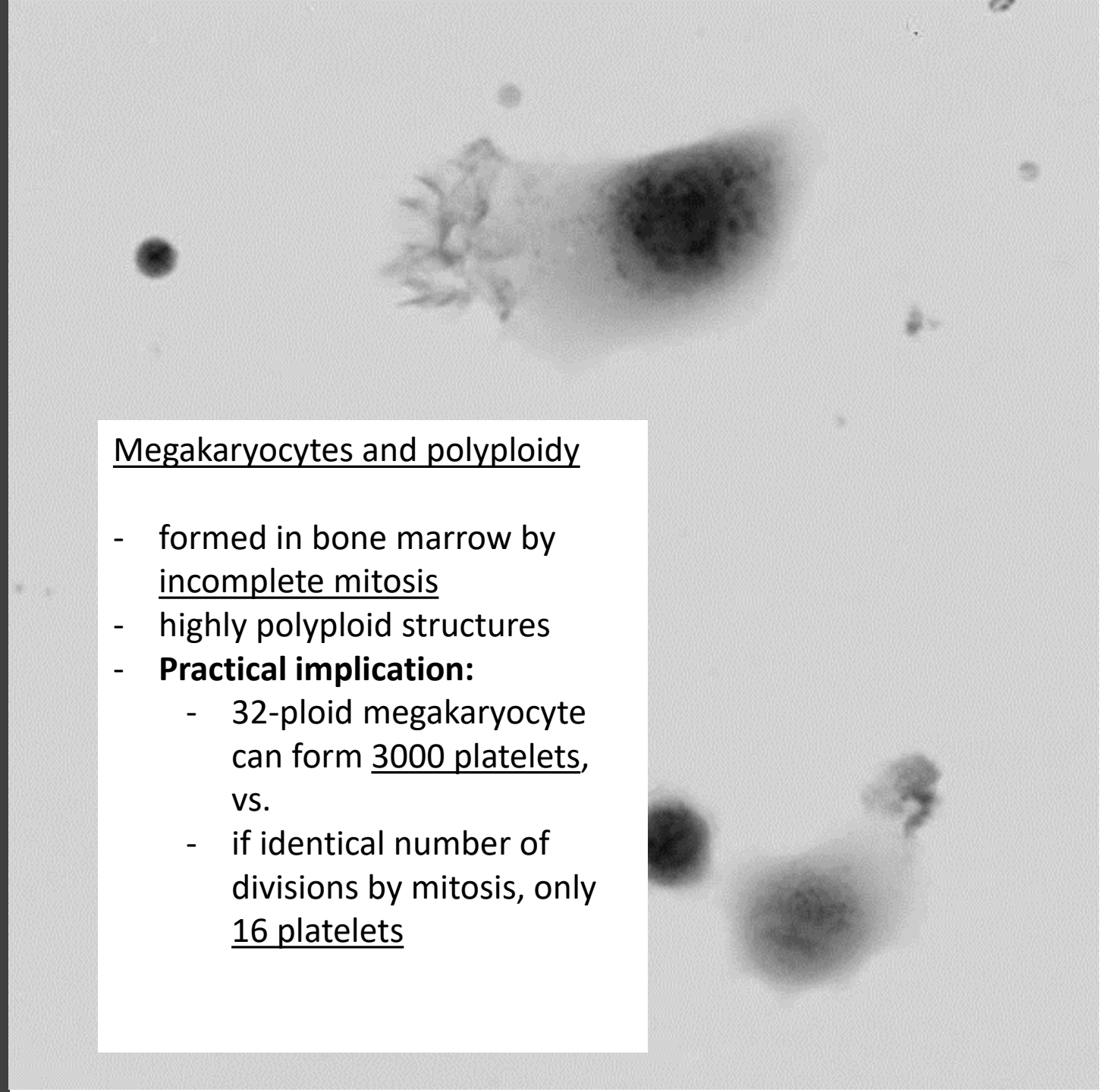
Senescence in physiological processes

- Role in Embryonic development and normal adult cells
- Embryo: mesonephros involution,
- Importance of apoptosis during embyo well defined
- Adult: human placenta = large syncytium - endoreruplicaition



Polyploidy

- during mitosis, cells with diploid chromosome sets are created (46XY)
- polyploidy = number of chromosome sets > 2
  - physiological in plants (higher gene copy numbers)
  - role in pathology in humans (consequence of defects during cell division)
    - organism aging
    - oncogenesis
    - trigering DNA-Damage response pathway
  - physiologically in humans in specialzed cells only
    - *megakaryocytes*
    - *cardiomyocytes* (during hypertrophy)
    - *skeletal muscle cells*
    - during stress response (oxidative stress, toxins exposition)
    - better cell damage- and oxidative stress-resistance
  - in humans physiological and pathological polyploid cells may coexist

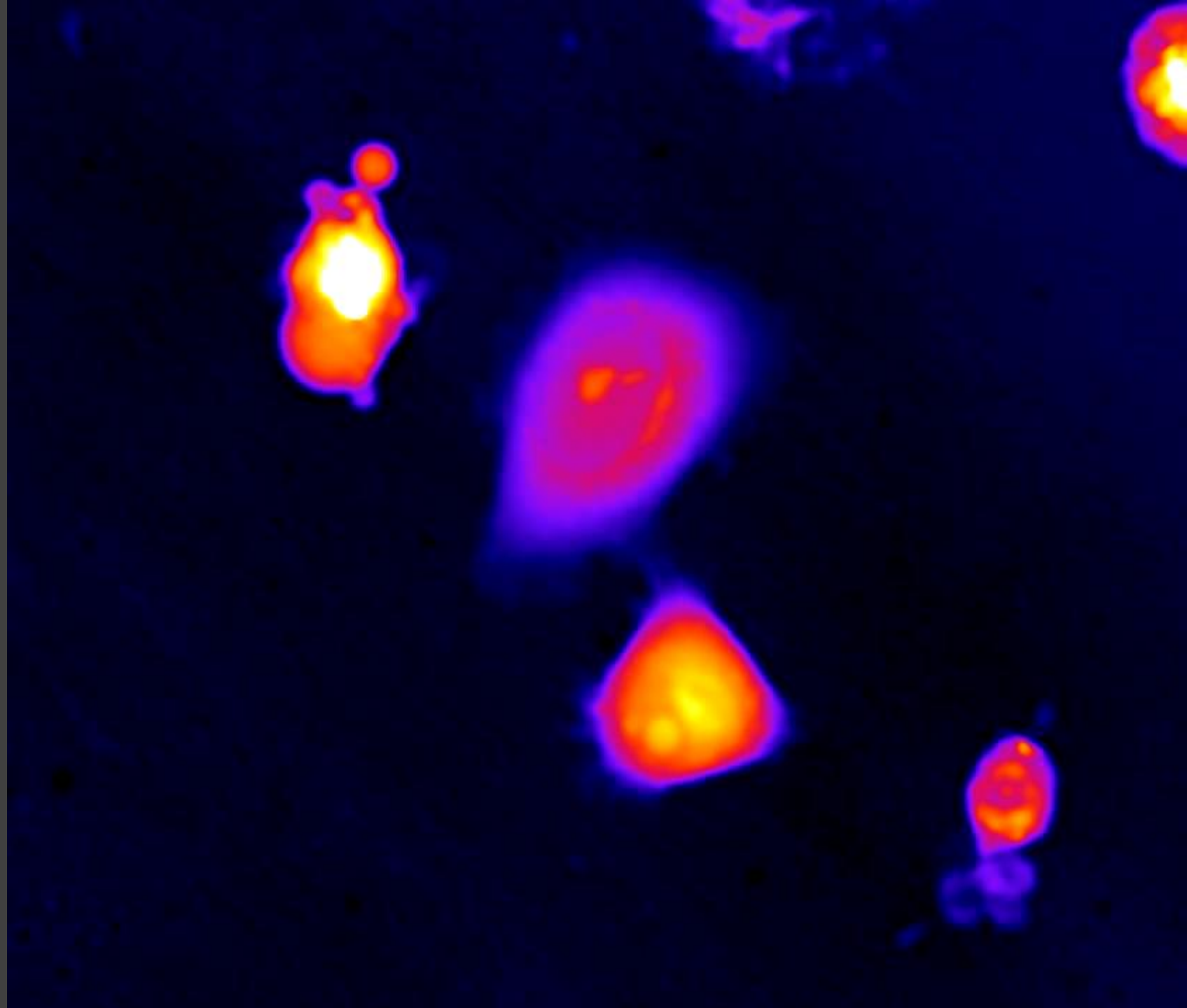


Megakaryocytes and polyploidy

- formed in bone marrow by incomplete mitosis
- highly polyploid structures
- **Practical implication:**
  - 32-ploid megakaryocyte can form 3000 platelets, vs.
  - if identical number of divisions by mitosis, only 16 platelets

Polyploidy formation by cell fusion

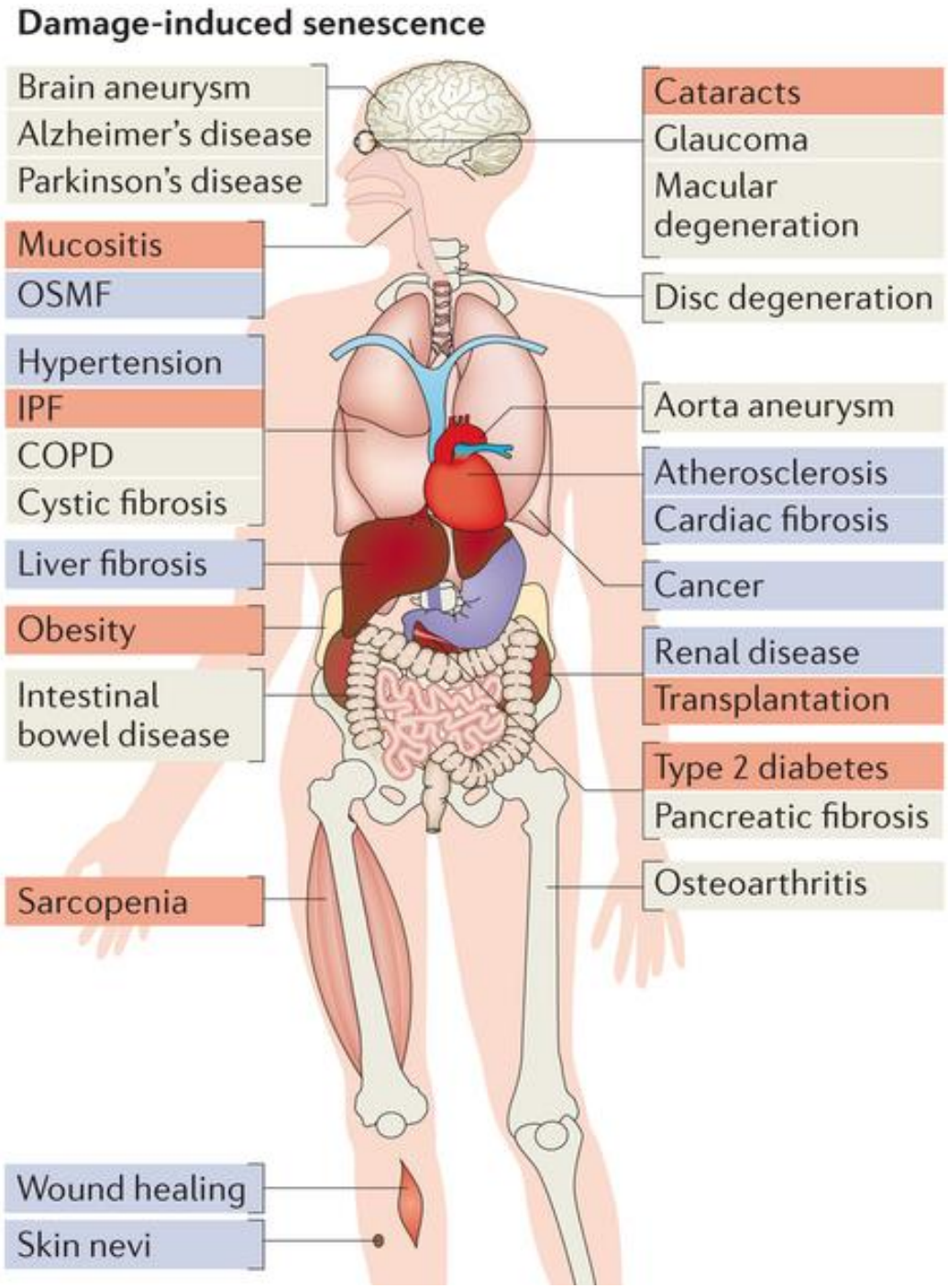
- PC-3 tumor cells under increased stress (chemotherapy treatment)
- evolution-driven pro-survival mechanism



Senescence in pathological processes

- Senescence initiation is beneficial/harmful, based on disease

- Conditions where senescence is beneficial:
  - **Counteracting tumor progression** (when intensity of oncogenic signals reach threshold, p53 pathway activates)
  - **Post-infarction cardiac fibrosis** senescence improves
  - **Liver fibrosis atenuation** senescence restricts fibrosis.
  - **Skin scarring restriction**
- Conditions where senescence is harmful
- Conditions where senescence play a role:



senescence generally beneficial in short-term stress condition.

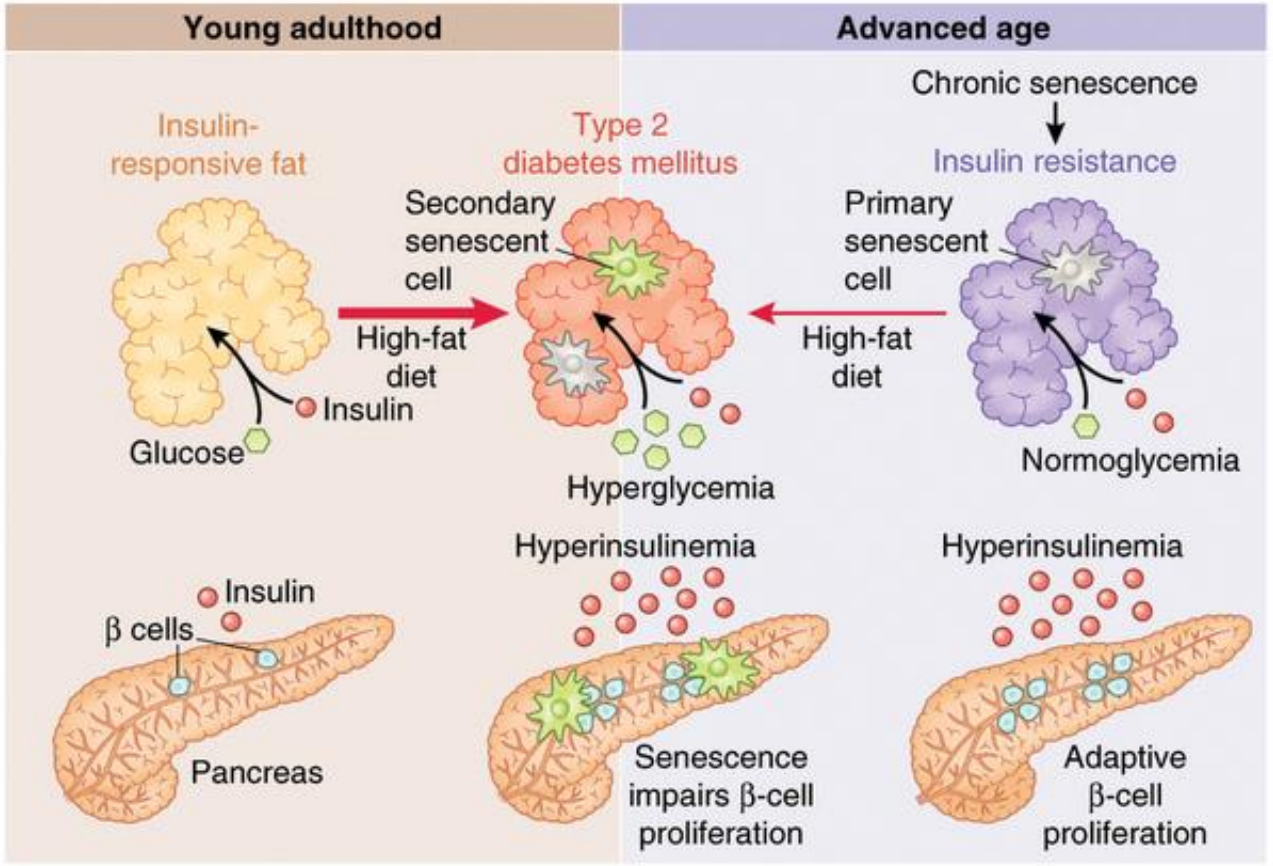
prolonged senescence activation associated with complications

senescence is beneficial

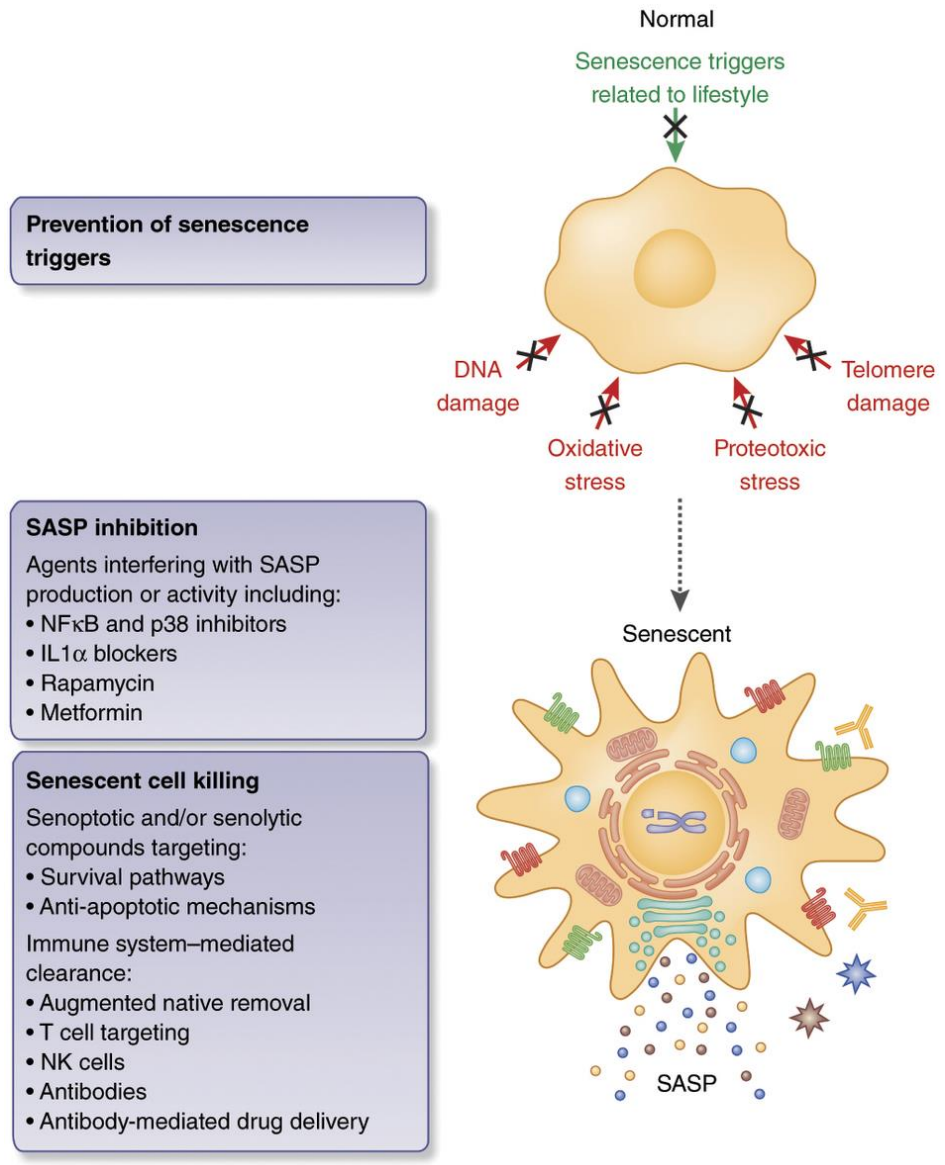
senescence is detrimental

role, but unknown

Diabetes mellitus



Senescence as a therapeutic target



Demonstating cause and effect in biology

- to eliminate gene or proces and determine the phenotype

Knockdown vs Knockout

Gene Knockdown

- Expression level of mRNA was decreased by RNAi (siRNA/shRNA/dsRNA)
- Gene itself are still intact
- SilencingEfficiency is not ~100%
- Sometime residual activities are sufficient for phenotypes
- Relatively easy and fast to perform
- You can do in embryonic lethal gene

Gene Knockout

- Gene locus in chromosome is permanently removed/changed
- Complete loss of genotype
- Difficulty & Time consuming
- Embryonic lethal gene : Conditional Knockout

Dual role of senescence

- „knockout“ strategies does not work to prove senescence:
- organisms which cannot undergo senescence do not live longer,
- rather, they prematurely die of cancer

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