Introduction to Oncology

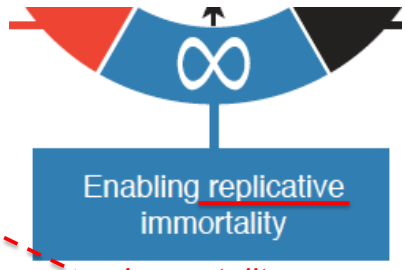
BIO-392

TODAY

Hallmark capability 3: Replicative immortality

The Biology of Cancer, chapter 10

Specific barrier
Hyperproliferation induces replicative stress (« wear and tear »), leading to cellular senescence (≠ cell death), marked by permanent cell cycle arrest, *or* (!) to cell death (≠ senescence)



«Immortality»:
Not simply equivalent to 'cell survival'

Acquired capability:

Evasion of stress signals and avoidance of telomere erosion
through inactivation of checkpoints and upregulation of telomerase activity

Outline

***Part I - Replicative* senescence**

- the chromosome end-replication problem and the "Hayflick limit"
- Telomere uncapping engages DNA damage checkpoint: Role of p53
- Indefinite replication of unstable genomes in cancer cells

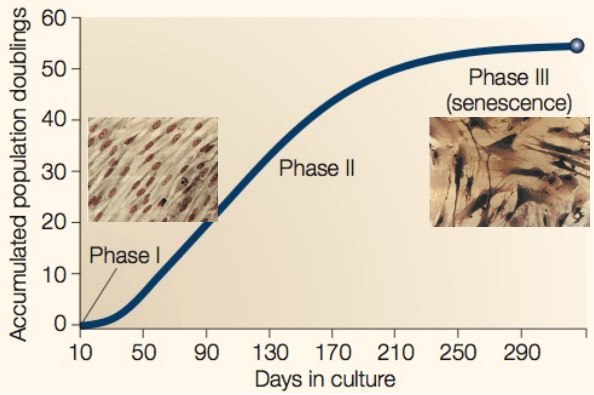
***Part II – Premature* cellular senescence**

- Induced by other DNA stressors, but similar outcome: SASP
  - Induction of premature senescence by oncogenes (Example: H-RasV12)
  - Senescence-associated heterochromatin foci (SAHF): Regulation by RB1
- The INK4a/ARF (CDKN2a) locus encodes two mediators of oncogene-induced premature senescence (OIS)
- Induction of premature senescence by TGFβ/SMAD signaling

Morphological signs of replicative senescence

- August Weismann (1881): *"...death takes place because a worn-out tissue cannot forever renew itself, and because a capacity for increase by means of cell division is not everlasting but finite"*.

- Leonard Hayflick (1961) revived this hypothesis when he observed 3 distinct growth phases in cultures of primary human fibroblasts:



>60-80 population doublings (Phase III):

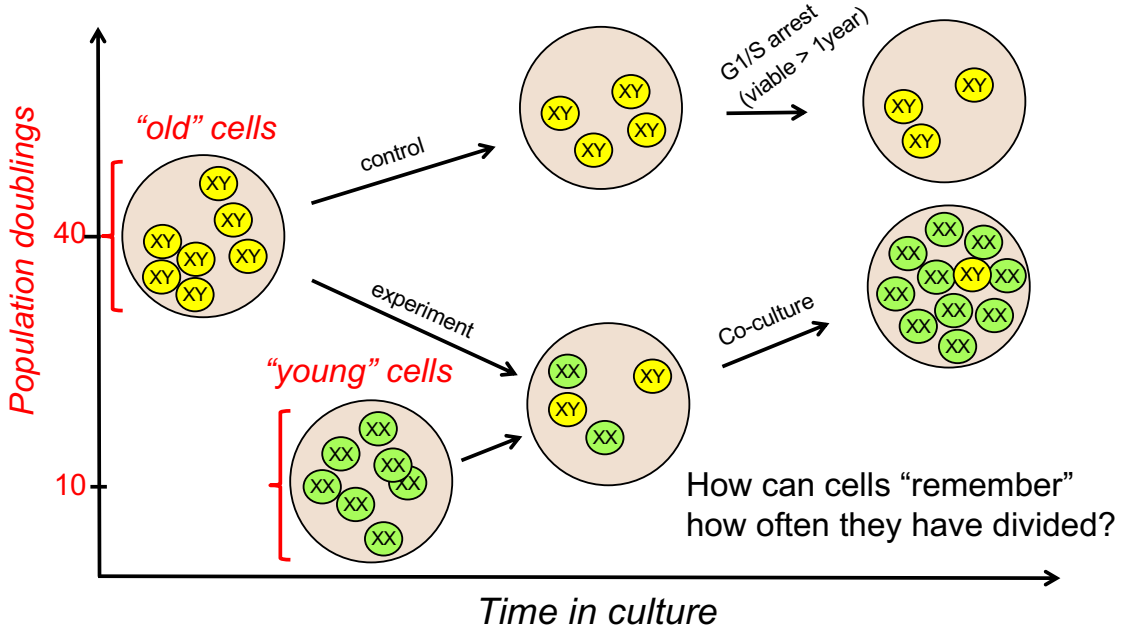
- Cells enlarge and flatten
- Replication ceases even in presence of growth factors
- But metabolism continues

Shay & Wright 2000 Nat. Rev. Mol. Cell Biol.

Demonstration of replicative senescence

Hayflick experiment:

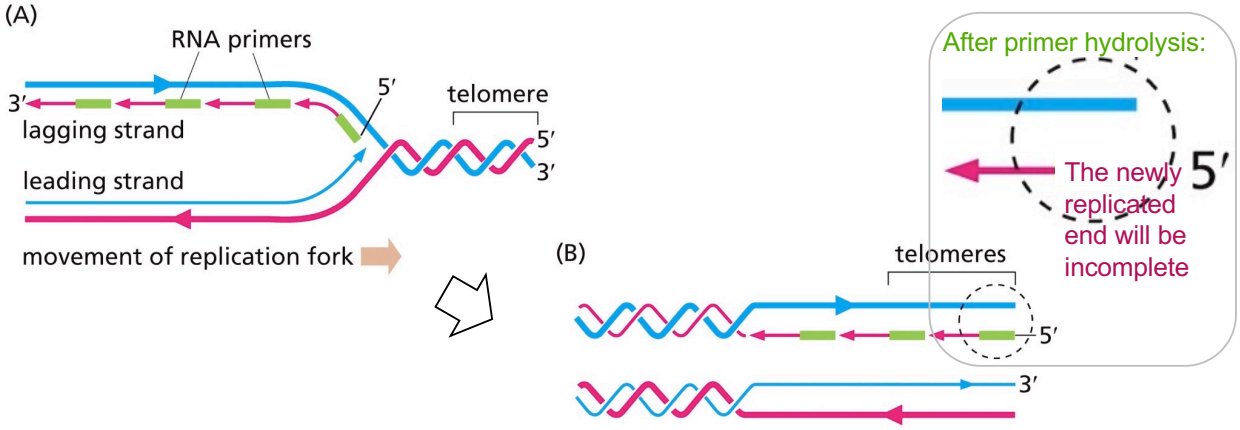
Primary fibroblasts that already doubled *many* times (XY)
are outgrown by a co-cultured population that doubled *few* times (XX)



The problem of replicating chromosome ends

Olovnikov (1971):

- If RNA primers of Okazaki fragments have no template *beyond the 3' end* of the lagging strand, linear chromosomes will only be incompletely replicated at telomeres during each cell division:

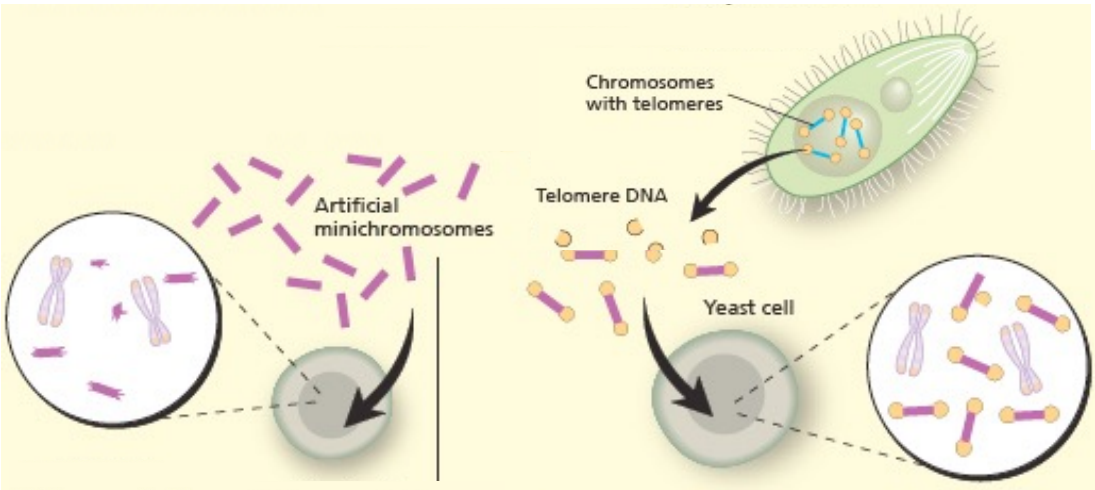


- Progressive erosion of telomeric DNA in each cell division!
- To divide indefinitely, germ cells and cancer cells require a special polymerase that can entirely replicate even telomeres!

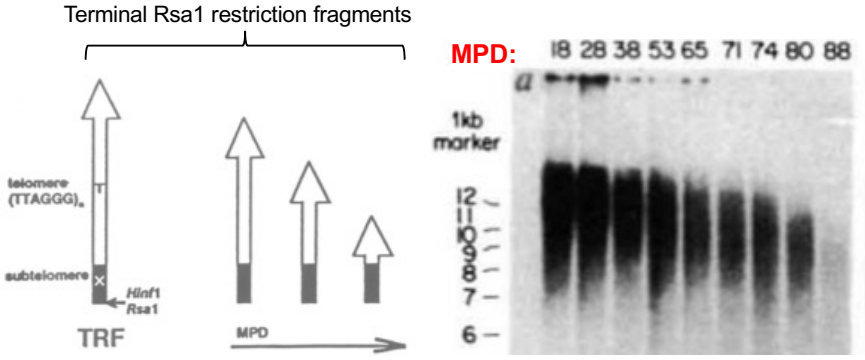
Testing Olovnikov's prediction

1978 Blackburn & Gall, cloned telomeric DNA from *Tetrahymena* (2x10^4 minichromosomes => high density of telomeres)
Discovery of a repetitive telomeric element $(CCCCAA)_{20\text{-}70}$

1982 Szostak & Blackburn: Telomeric repeats allow stable replication of linear DNA (minichromosomes) in yeast



Progressive telomere erosion accompanies cellular ageing

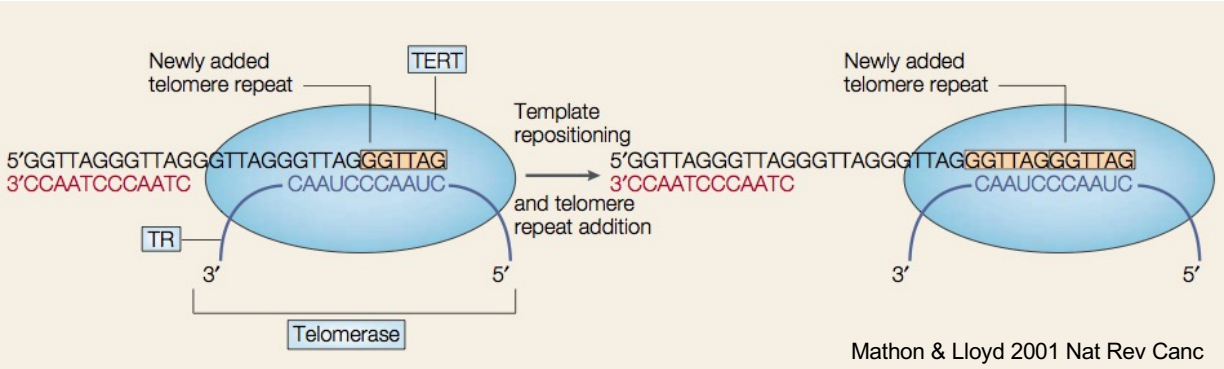


Experiment: Southern blot hybridization of the repetitive telomeric sequence probe $(TTAGGG)_3$ to fetal fibroblast DNA to analyze **Terminal restriction fragment (TRF)** length after the indicated number of mean population doublings (MPD) *ex vivo* in culture:

- No discrete band! => Not all telomeres in a given cell are of the same size
- Revealed *progressive* loss of up to 2 kb telomeric DNA
  => Telomeres shorten 50-100 bp during each round of cell division

Harley, C. B., Futcher, A. B. & Greider, C. W. *Nature* **345**, 458–460 (1990)

Telomere maintenance requires telomerase



Telomerase **holoenzyme** contains:

- Telomerase retrotranscriptase (TERT), catalytic subunit is related to retrotranscriptases
- Telomerase RNA (TR, or TERC for Telomerase RNA component)
- Additional proteins (e.g. for localization in nuclear Cajal bodies)

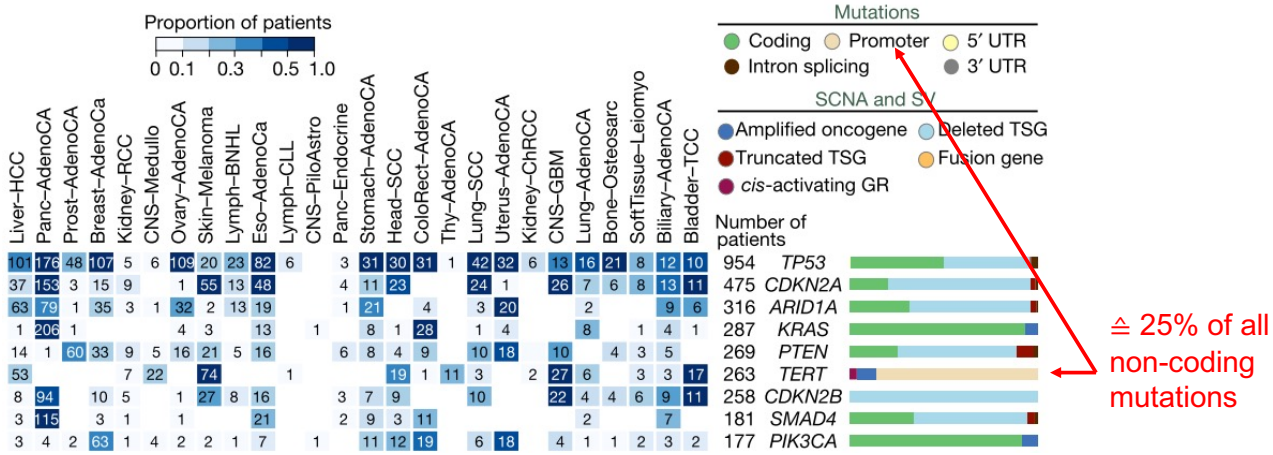
Article

Pan-cancer analysis of whole genomes

https://doi.org/10.1038/s41586-020-1969-6
Received: 29 July 2018
Accepted: 11 December 2019
Published online: 5 February 2020
Open access

The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium

Cancer is driven by genetic change, and the advent of massively parallel sequencing has enabled systematic documentation of this variation at the whole-genome scale[1–3]. Here we report the integrative analysis of 2,658 whole-cancer genomes and their matching normal tissues across 38 tumour types from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium of the International Cancer Genome Consortium (ICGC) and The



Telomerase activity is bad prognosis for some pediatric tumors

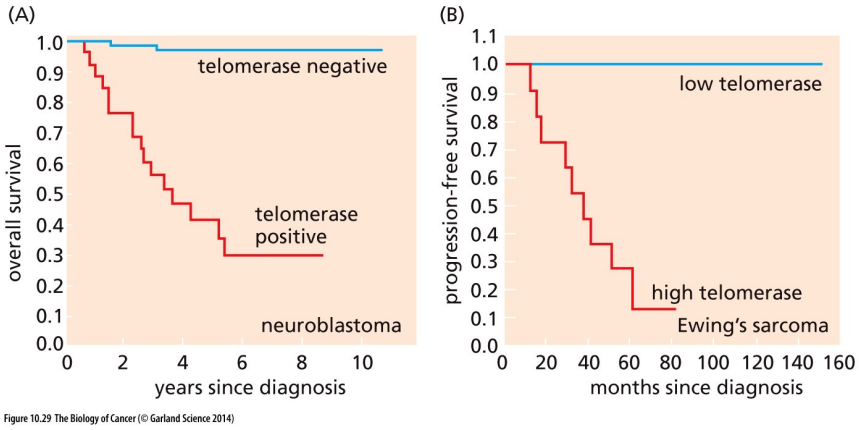
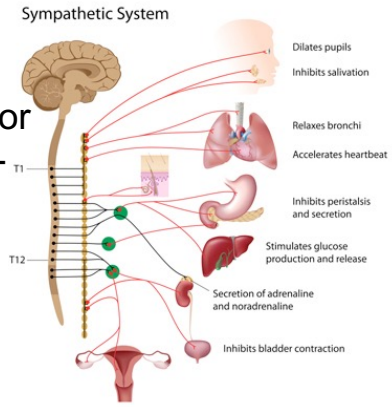
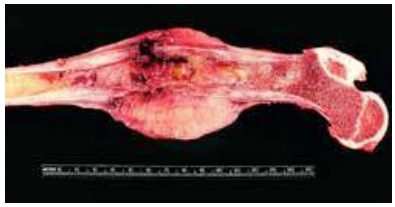


Figure 10.29 The Biology of Cancer (© Garland Science 2014)

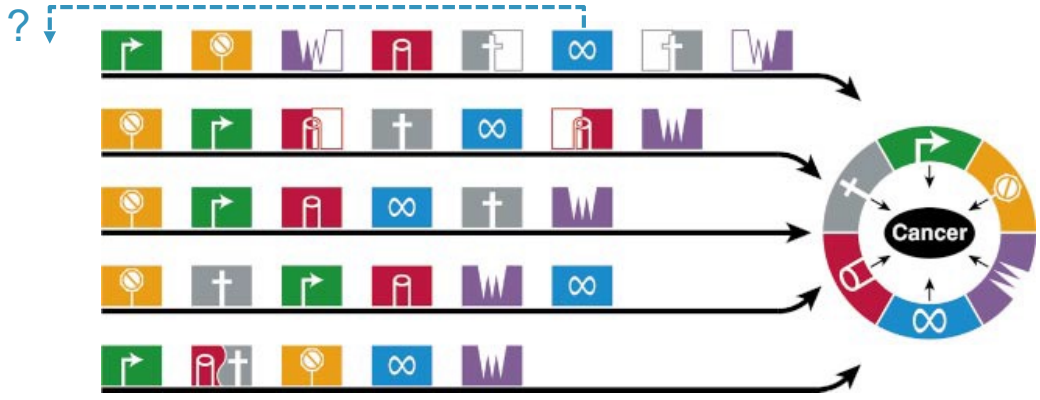
Neuroblastoma from PNS precursor cells (neural crest-derived):



Ewing's sarcoma (from bone marrow-derived MSCs or from neural crest derivatives):



In which order do cancers acquire their hallmark capabilities?



- Alternative paths to cancer
- Telomerase → Replicative Immortality was thought to emerge relatively late. However, hTERT pomoter mutations are found in subventricular zone (glioma ***precursor*** cells)

Telomere replication by RNA-templated DNA synthesis

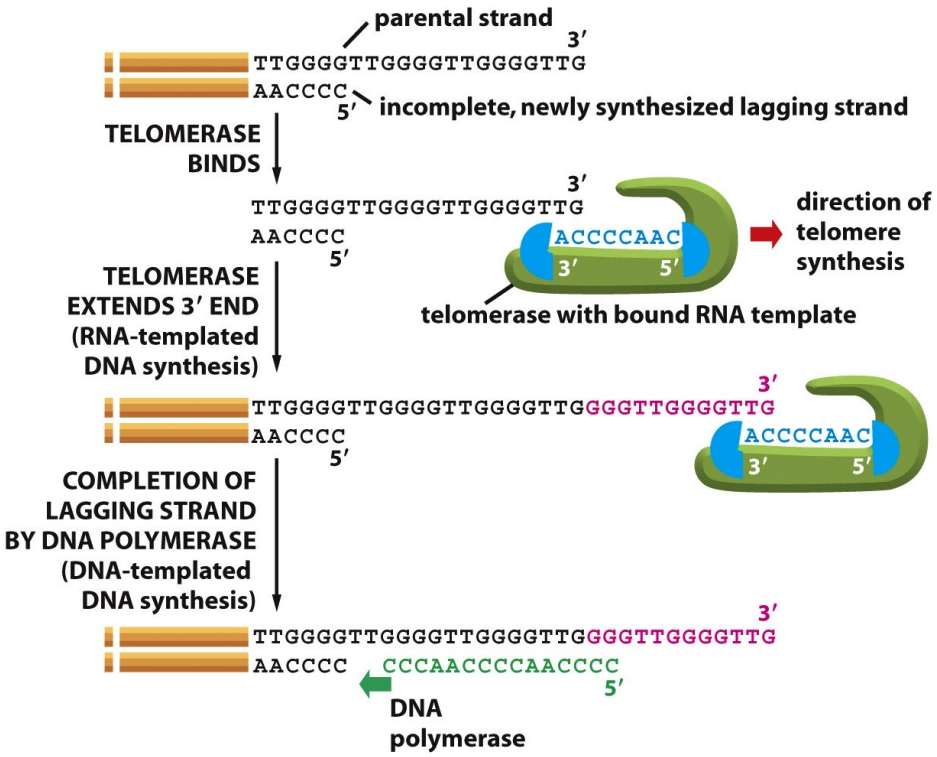
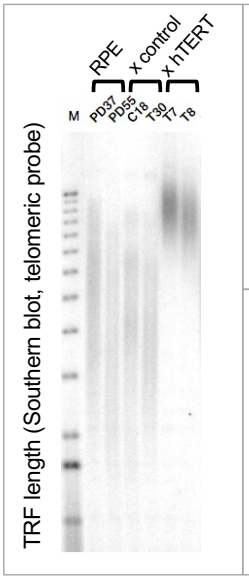
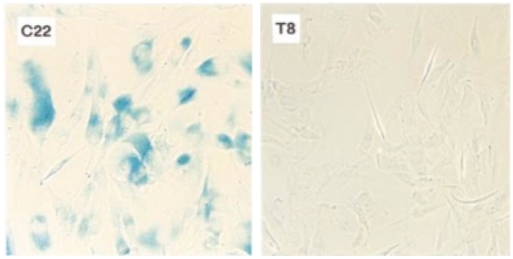


Figure 5-41 *Molecular Biology of the Cell* (© Garland Science 2008)

Is telomere shortening the cause or only a consequence of replicative senescence?

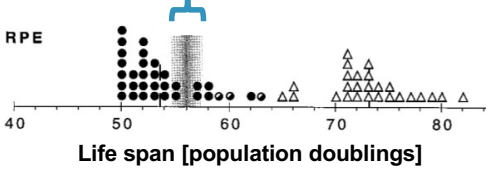
Forced expression of telomerase (hTERT) in retinal pigment epithelial (RPE) cells (similar results were obtained in primary fibroblasts):





Senescence-associated (SA) lysosomal isoform of β-galactosidase, SA-β-Gal (GLB1, a convenient marker)

Typical range of population doublings after which the mass population of untransfected RPE cells senesce:



● RPE cell clones (vector control)

△ stable hTERT – RPE clones

Bodnar et al. 1998 Science 279:349-352

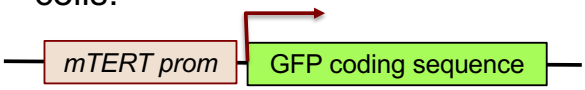
Telomerase retrotranscriptase (TERT) expression levels are low → Difficult to detect !

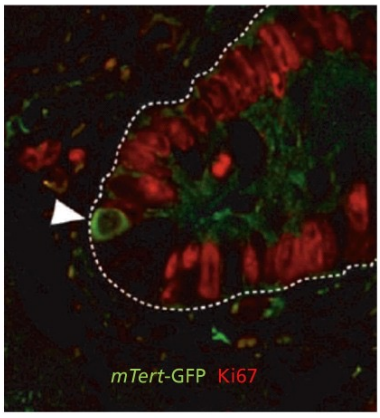
TERT is expressed at extremely **low levels**

- in germ cells, and in stem/progenitor cells of self-renewing tissues
- in "immortalized" cell lines and cancer cells
- but ***absent*** in differentiated and cultured primary cells

Example: Mouse intestinal crypt

- Ki67 immunolabelling, a marker of proliferating cells (max. in S, but also M, G1&G2; absent in G0)
- green: A GFP reporter transgene revealed mouse TERT promoter activity in Ki67-negative crypt cells:





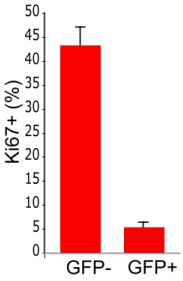
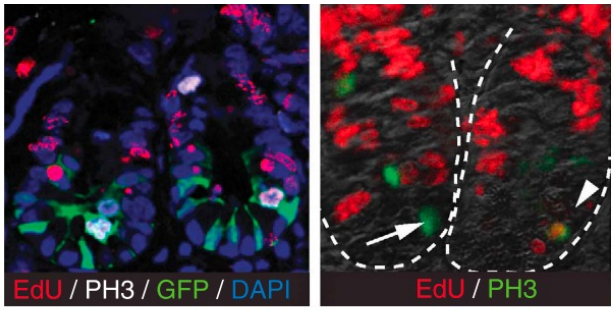


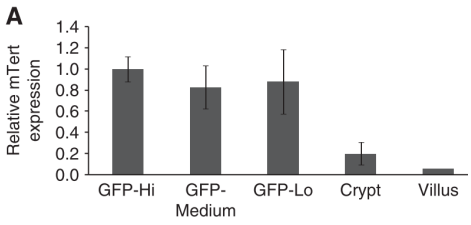
Fig. 10.32 (from Montgomery et al., 2011, Proc Natl Acad Sci USA 108: 179-184)

RT-qPCR analysis of mTert mRNA in Crypt Base Columnar (CBC) stem cells

Proliferation of Lgr5^{GFP+} CBCs:



RT-qPCR on RNA from FACS-isolated Lgr5^{GFP+} CBCs:



Lgr5$^{GFP/+}$: A Crypt Base Columnar stem cell marker

EdU: Similar to BrdU (a thymidine analog to mark DNA synthesis)

PH3: phosphohistone H3, an M-phase protein that marks proliferating cells

DAPI: DNA staining (nuclei)

→ *Endogenous* mTert mRNA *is* detectable also in Lgr5^{GFP+} CBCs

Schepers et al. (2011) Embo J 30: 1104-1109

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Telomeres adopt a lariat structure

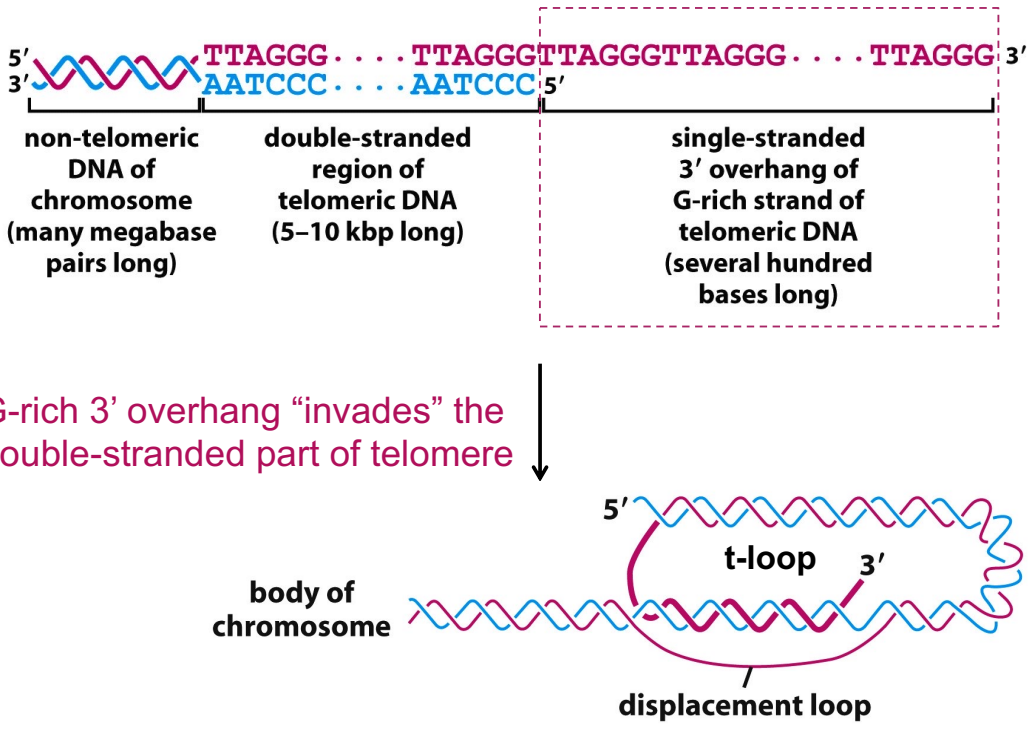
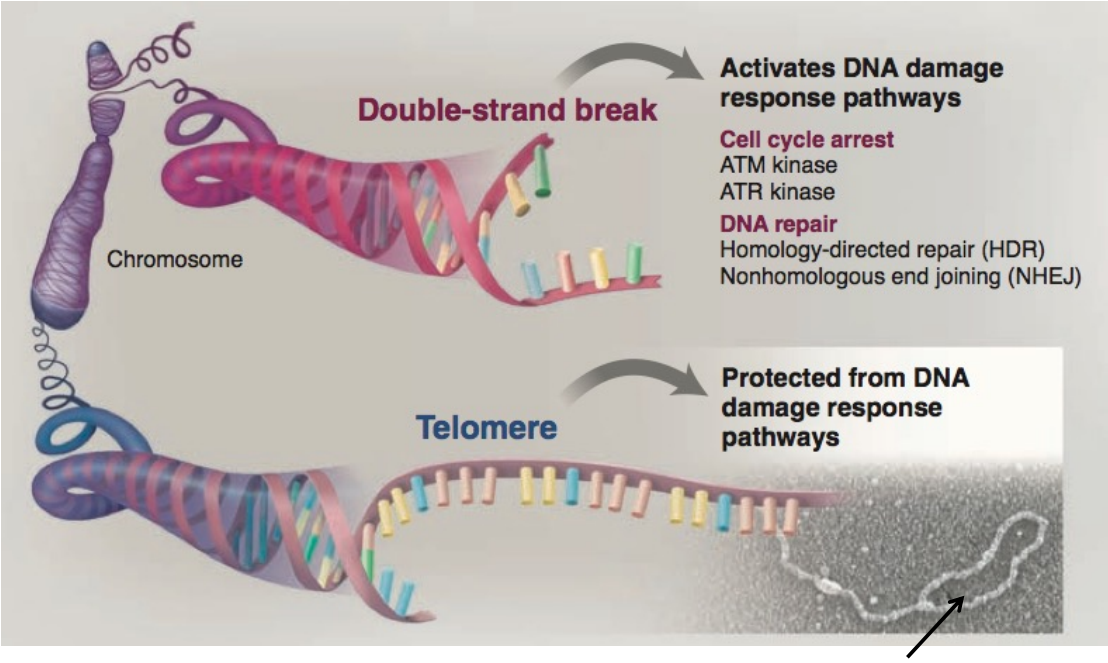


Figure 10.16 *The Biology of Cancer* (© Garland Science 2007)

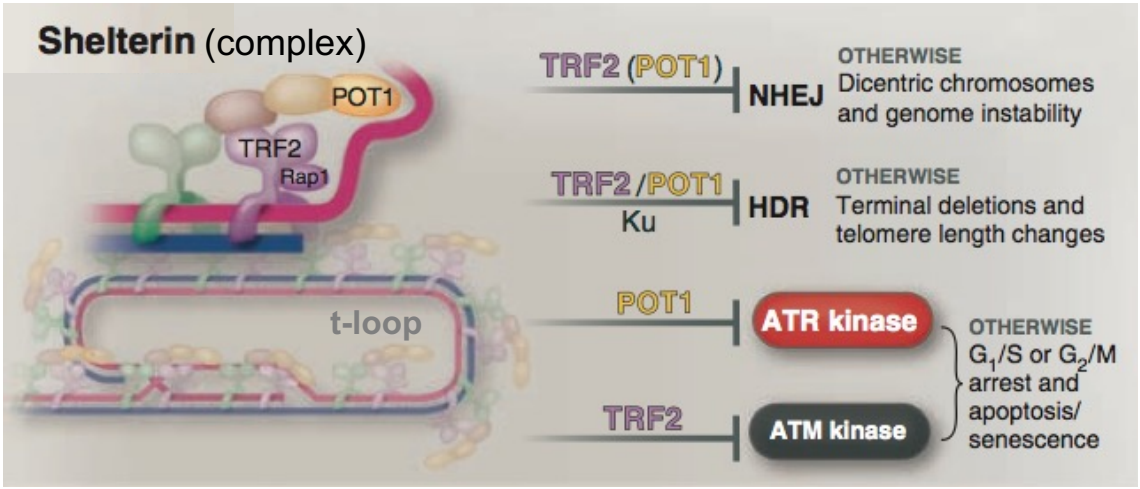
Telomeric t-loops protect chromosomes from end-to-end fusion



t-loops have been imaged by scanning electron microscopy

de Lange, T. (2009). Science 326, 948–952

Shelterin components inhibit DNA damage response pathways

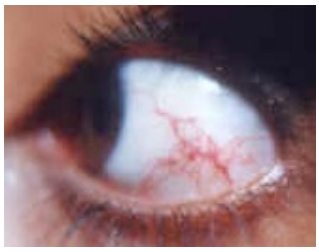


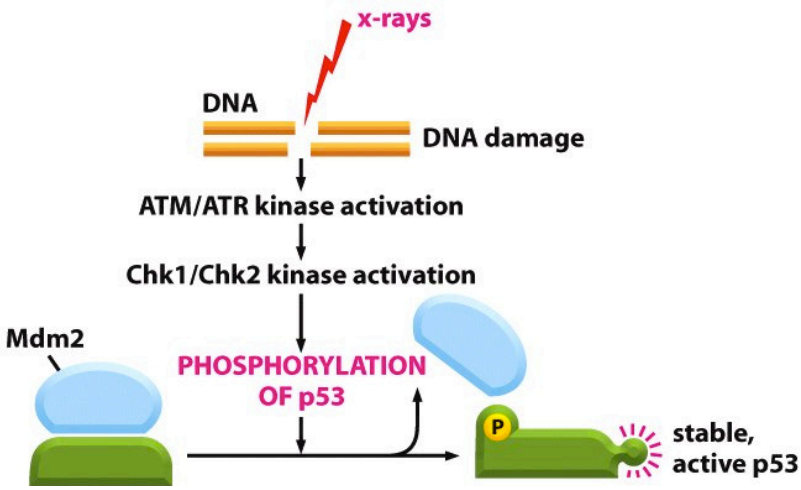
⇒ Functional telomeres concentrate Shelterin factors

⇒ Shelterin complexes inhibit DNA repair enzymes to prevent chromosome end fusions

de Lange, T. (2009). Science 326, 948–952

Loss of ATM function causes **ataxia telangiectasia** syndrome

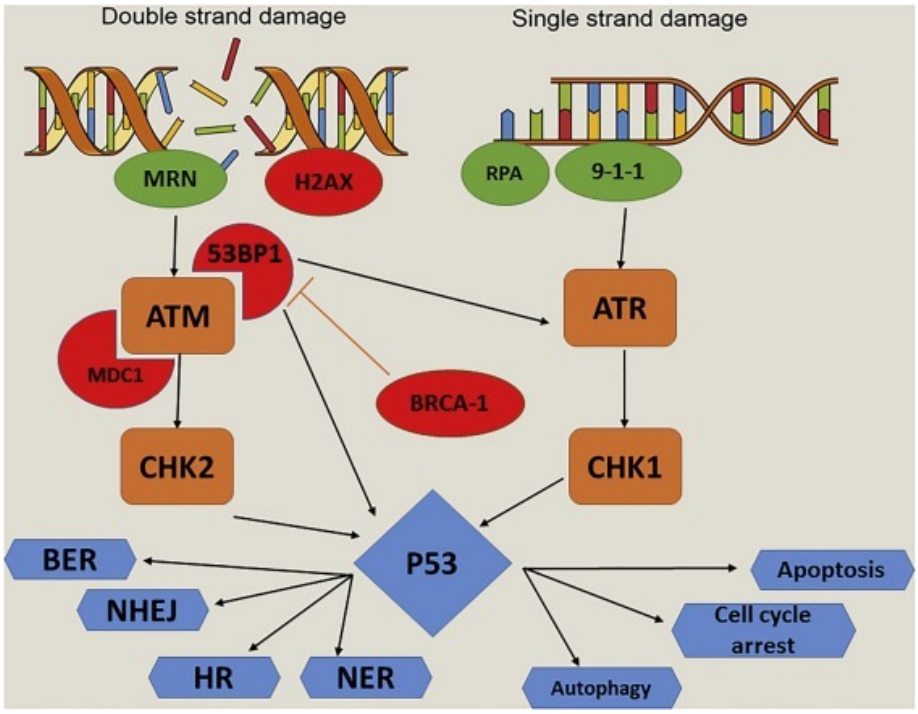




- Dilation of small vessels
- weakened immunity
- motor dysfunction
- 25% life-time risk to develop cancer, especially leukemia and lymphoma

DNA damage response (DDR): Sensors and transducers

e.g. p53 binding protein 1 (53BP1):

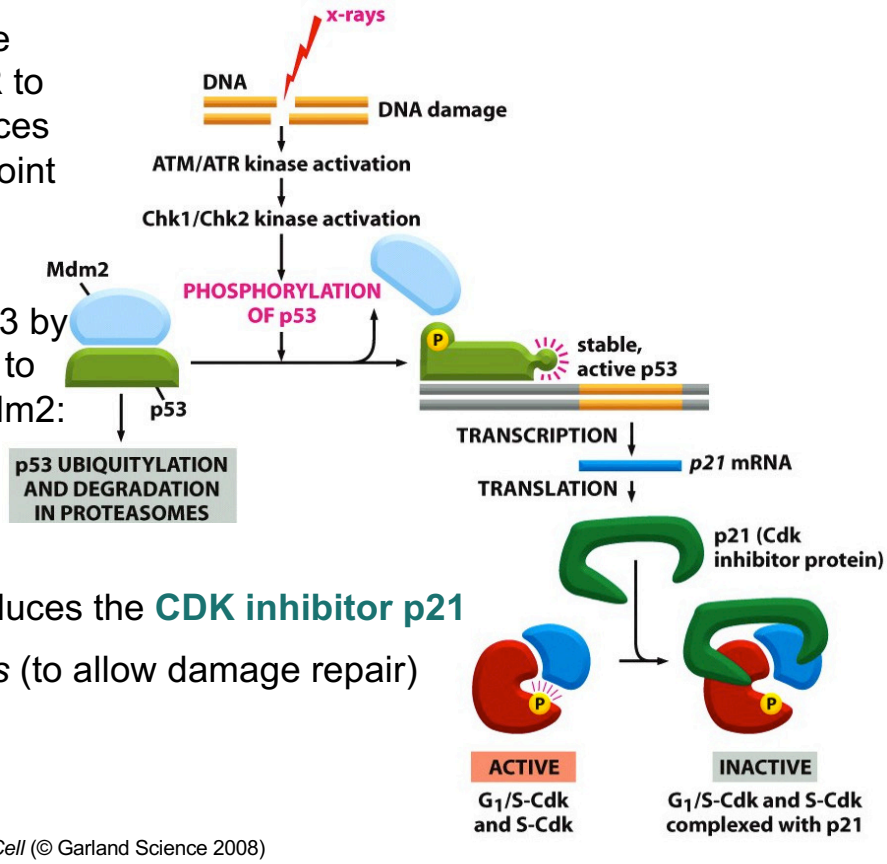


Mirza-Aghazadeh-Attari et al. 2019, DNA Repar 73:110-119

DNA damage leads to activation of p53 and its target p21Cip1

Binding of one of the kinases ATM or ATR to damaged DNA induces activation of checkpoint kinases (Chk1&2)

Chk1&2 stabilize p53 by inhibiting its binding to the E3 Ub ligase Mdm2:

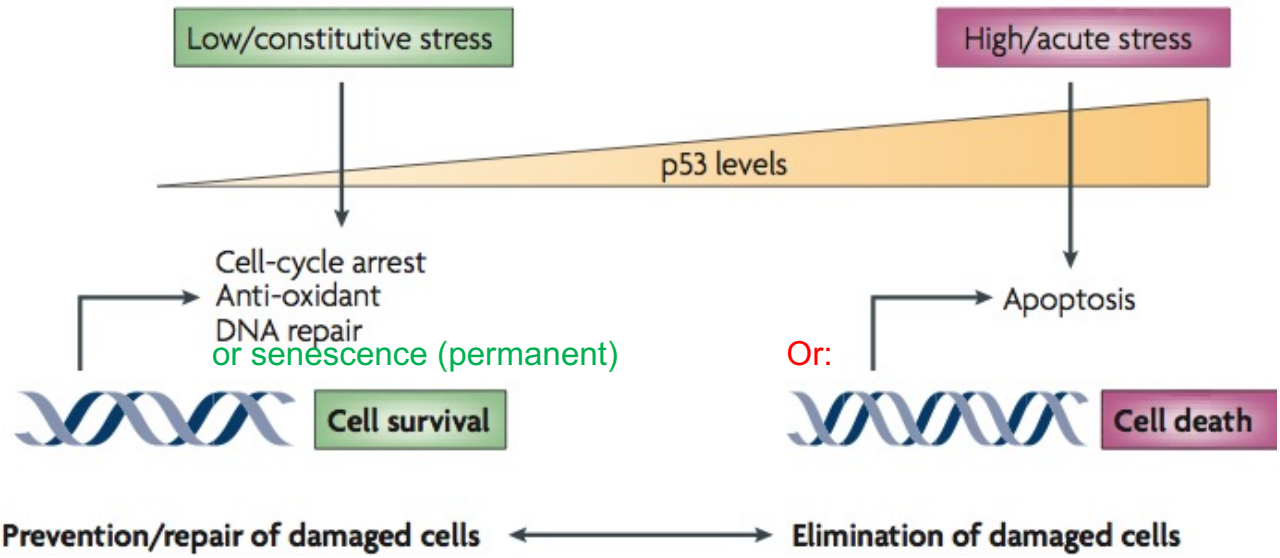


→ Activated p53 induces the **CDK inhibitor p21**

→ Cell cycle *pauses* (to allow damage repair)

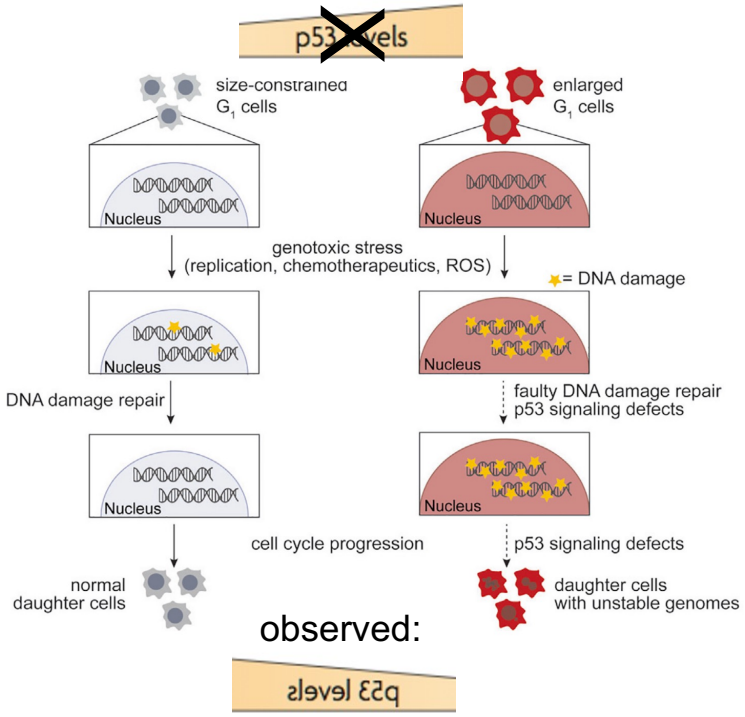
Figure 17-63 *Molecular Biology of the Cell* (© Garland Science 2008)

Context-dependent alternative p53 outputs include transient arrest by p21, *or* senescence, *or* apoptosis



Vousden & Lane 2007 Nat Rev Mol Cell Biol

NEW: Permanent G1 arrest is facilitated by cell size overgrowth, correlating with *weaker* (not stronger) p53 activation



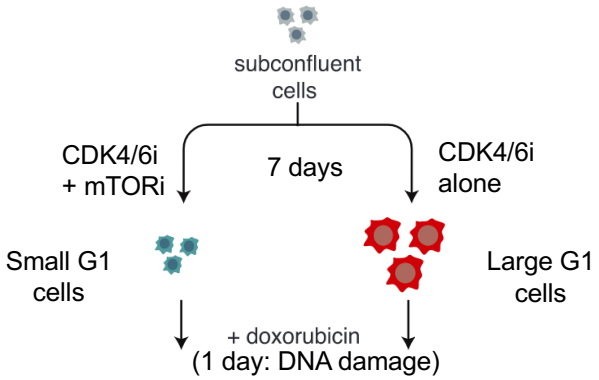
Regulation: of cell size:

- osmosis ?
- mTOR signaling
- Protein homeostasis

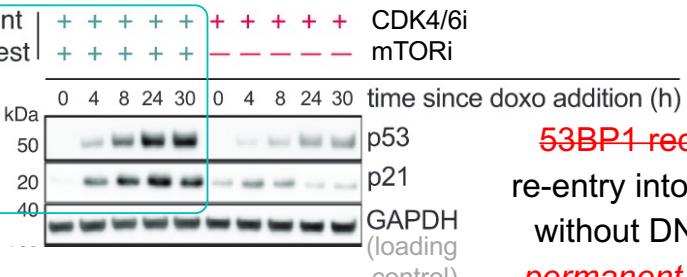
DNA damage still significantly activated p53 also in enlarged cells, but the 53BP1 recruitment to DNA damage sites was impaired

Manohar et al. 2023, Mol. Cell 83:4032-4046.e6

NEW: Permanent G1 arrest is facilitated by cell size overgrowth, correlating with *weaker* (not stronger) p53 activation



53BP1 recruited → DNA repair, re-entry into cell cycle

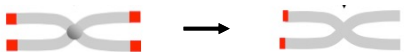


~~53BP1 recruitment~~

re-entry into cell cycle, without DNA repair, *permanent* G1 arrest (p53-dependent)

Manohar et al. 2023, Mol. Cell 83:4032-4046.e6

Telomere shortening activates p53



p53↑

† (apoptosis)

p53↑

If DNA damage checkpoint & p53 are hyperactive!

Premalignant cells:

- G1 arrest (p53, RB1)
- no further telomere erosion

→ **Senescence**
(no need for cell suicide)

How?

CANCER
(*Replicative* 'immortality' = *Indefinite* DNA replication)

Outline

***Part I* - *Replicative* senescence**

✓ the chromosome end-replication problem and the "Hayflick limit"

✓ Telomere uncapping engages DNA damage checkpoint: Role of p53

➢ Indefinite replication of unstable genomes in cancer cells

***Part II – Premature* cellular senescence**

- Induced by other DNA stressors, but similar outcome: SASP
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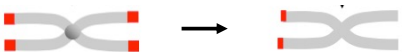
Telomere loss in p53-deficient cells provokes genome instability



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Premalignant cells:

- → G1 arrest (p53, RB1)
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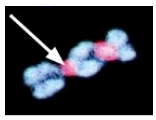
After p53 inactivation:

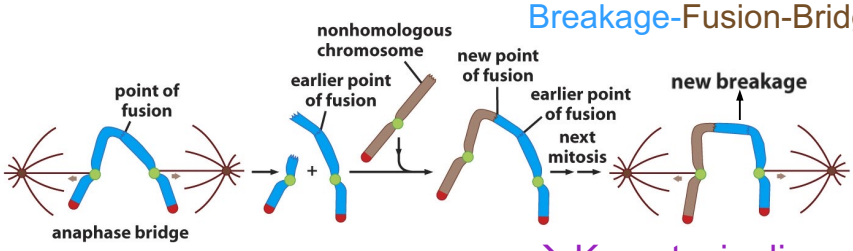
Continued proliferation →
di-centric chromosomes (fusions):

fused sister chromatids & Two non-homologous chromosomes

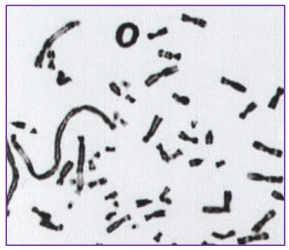


Anaphase bridges:

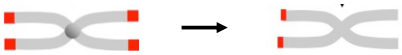
Breakage-Fusion-Bridge (BFB) cycles:



→ Karyotypic disarray:



Karyotypic disarray provokes a crisis



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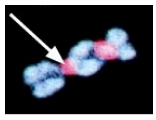
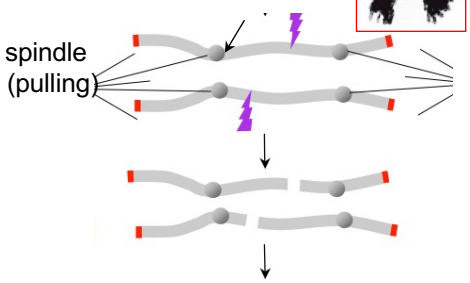
di-centric chromosomes (fusions):

fused sister chromatids & Two non-homologous chromosomes



Anaphase bridges:

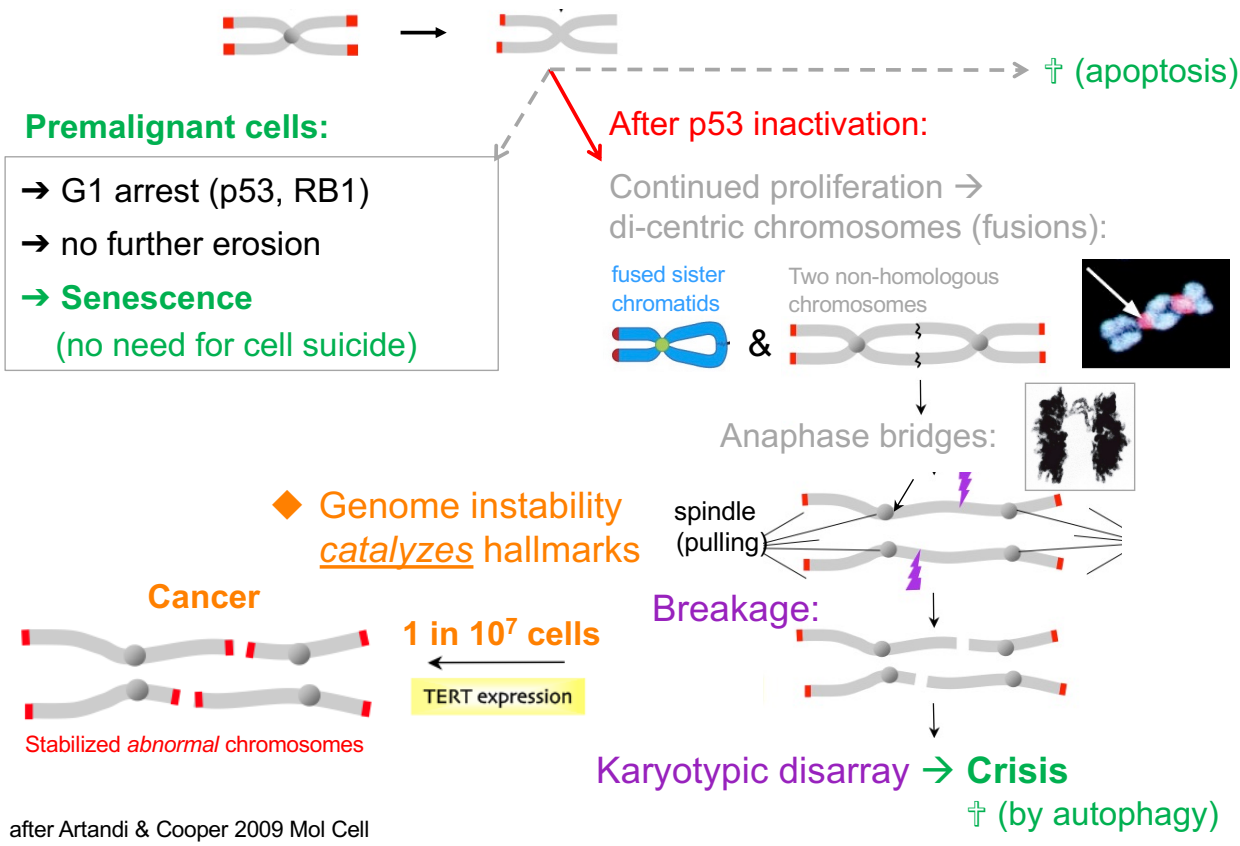
- ◆ Genome instability *catalyzes* hallmarks
- ◆ BFB cycles create karyotypic chaos (McClintock, 1941)



Karyotypic disarray → **Crisis**
† (by autophagy)

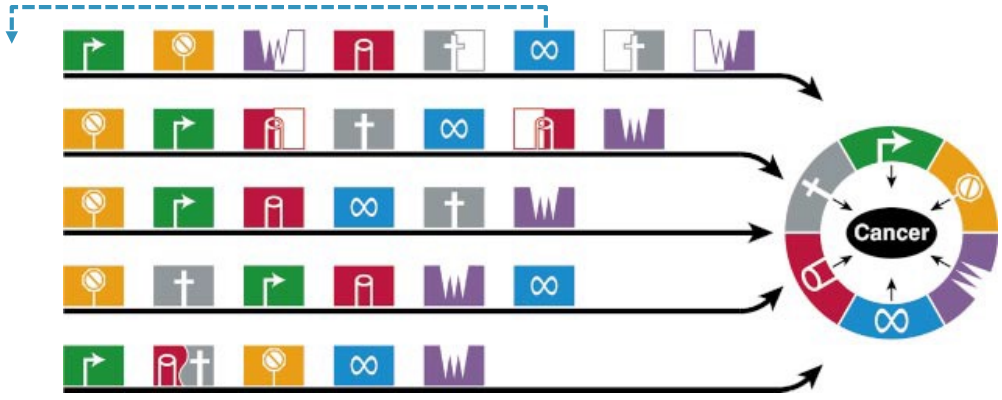
after Artandi & Cooper 2009 Mol Cell

Escape of cancer cells: Role of hTERT



after Artandi & Cooper 2009 Mol Cell

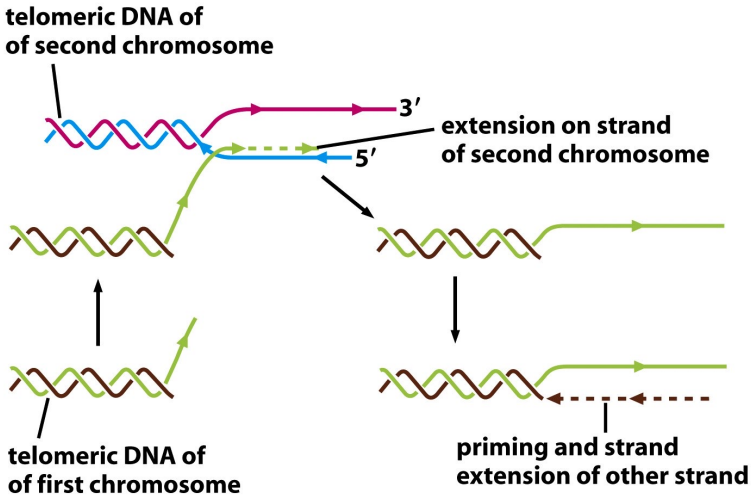
Early hTERT activation: Accelerating tumor progression?



- Alternative paths to cancer
- Telomerase → Replicative Immortality was thought to emerge relatively late. However, hTERT pomoter mutations are found in subventricular zone (glioma ***precursor*** cells)

Some cancers (15%) can maintain telomeres without hTERT

A model of Alternative Lengthening of Telomeres (ALT)
in cancers and ES cells lacking telomerase activity:



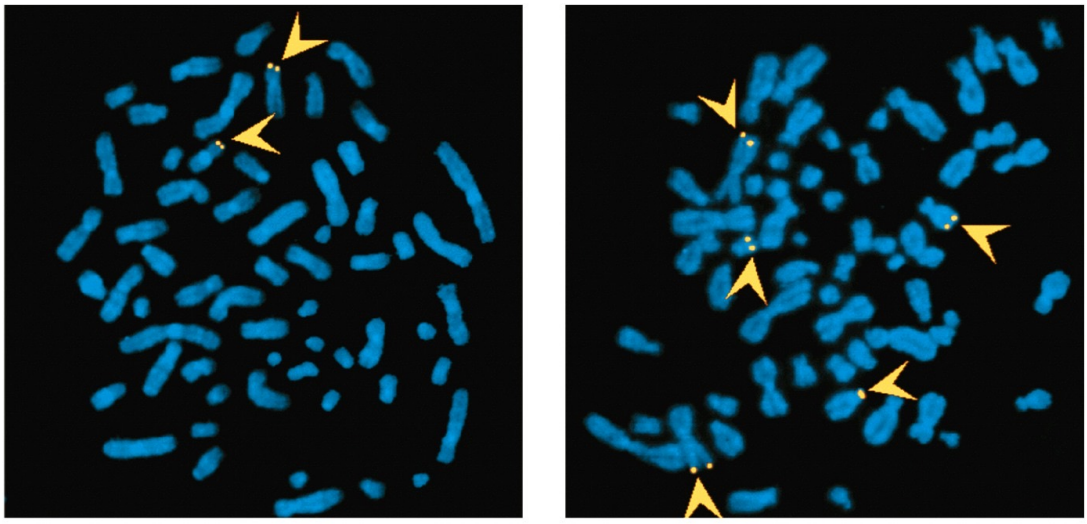
Requires special DNA polymerases, but no telomerase

ALT can be detected by FISH analysis of the resulting sequence exchange between chromosomes

Figure 10.31 *The Biology of Cancer* (© Garland Science 2014)

Detection of Alternative Lengthening of Telomeres

Fluorescent in situ hybridization (FISH) of a genetic marker engineered into telomeres of a human cancer cell line reveals the occurrence of ALT:

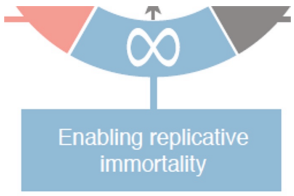


early passage cells (PD23) | 40 population doublings later

- *Number* of stained telomeres per cell increased (from 2 to 10)
- *Recombination* at telomeres can reverse their uncapping

Summary

- Hayflick demonstrated that normal cells do not replicate forever: Telomere research provided an elegant molecular explanation for this phenomenon
- Senescence requires specific *upstream regulators* (DNA damage sensors (ATM, ATR, Chk1&2)
- Evasion of this barrier leads to massive karyotype rearrangements that trigger a crisis



- Rare surviving tumor cells that manage to patch up their telomeres by reactivating hTERT (>85%), or by ALT (<15%), become malignant
- Once karyotypic abnormalities are fixed in the genome, they accelerate tumor evolution

Outline

***Part I* - *Replicative* senescence**

✓ the chromosome end-replication problem and the "Hayflick limit"

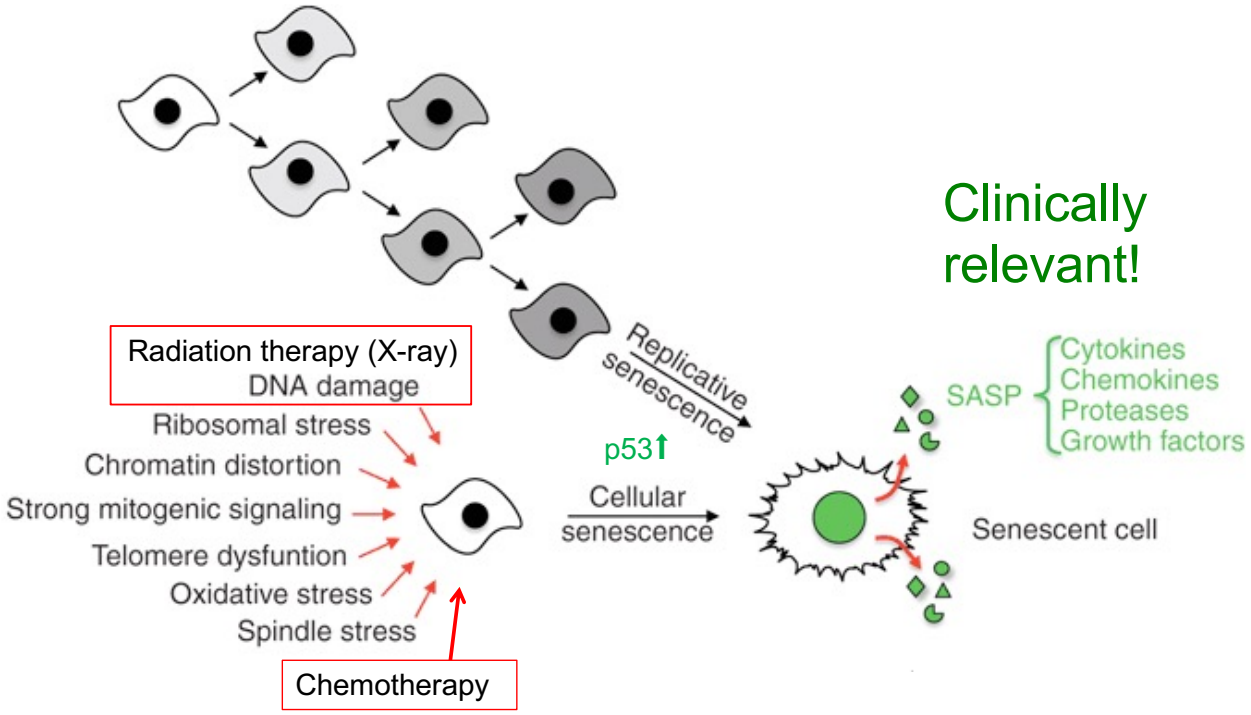
✓ Telomere uncapping engages DNA damage checkpoint: Role of p53

✓ Indefinite replication of unstable genomes in cancer cells

***Part II – Premature* cellular senescence**

- Induced by other DNA stressors, but similar outcome: SASP
  - Induction of premature senescence by oncogenes (Example: H-RasV12)
  - Senescence-associated heterochromatin foci (SAHF): Regulation by RB1
- The INK4a/ARF (CDKN2a) locus encodes two mediators of oncogene-induced premature senescence (OIS)
- Induction of premature senescence by TGFβ/SMAD signaling

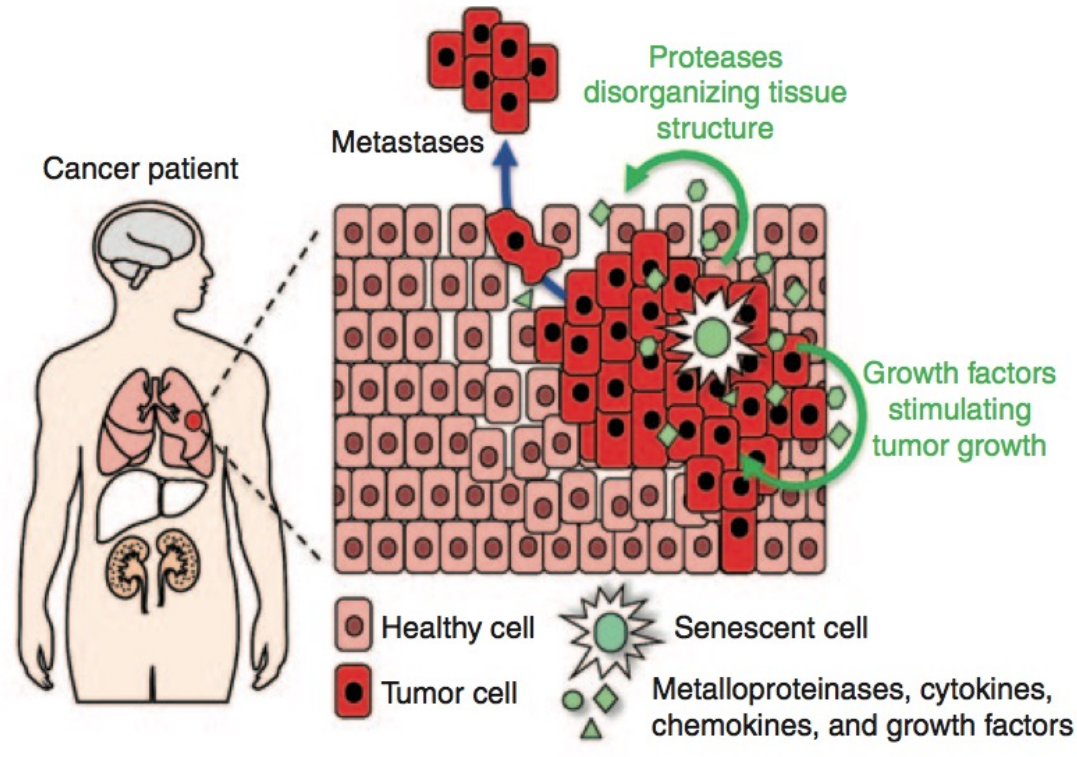
Various types of DNA stress trigger a characteristic "senescence-associated secretory phenotype" (SASP)



Clinically relevant!

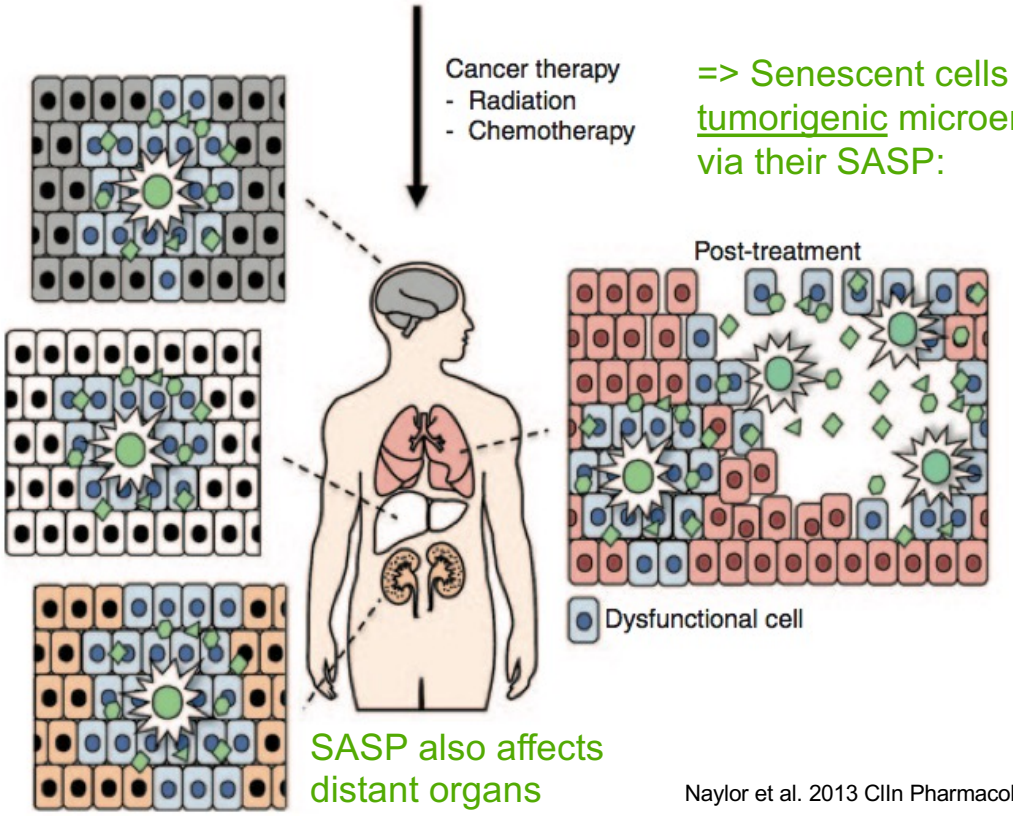
Naylor et al. 2013 Clin Pharmacol & Ther 93:105-116

Factors secreted by senescent cells influence tumor growth



Naylor et al. 2013 Clin Pharmacol & Ther 93:105-116

Risk of relapse and accelerated ageing from therapy-induced systemic senescence

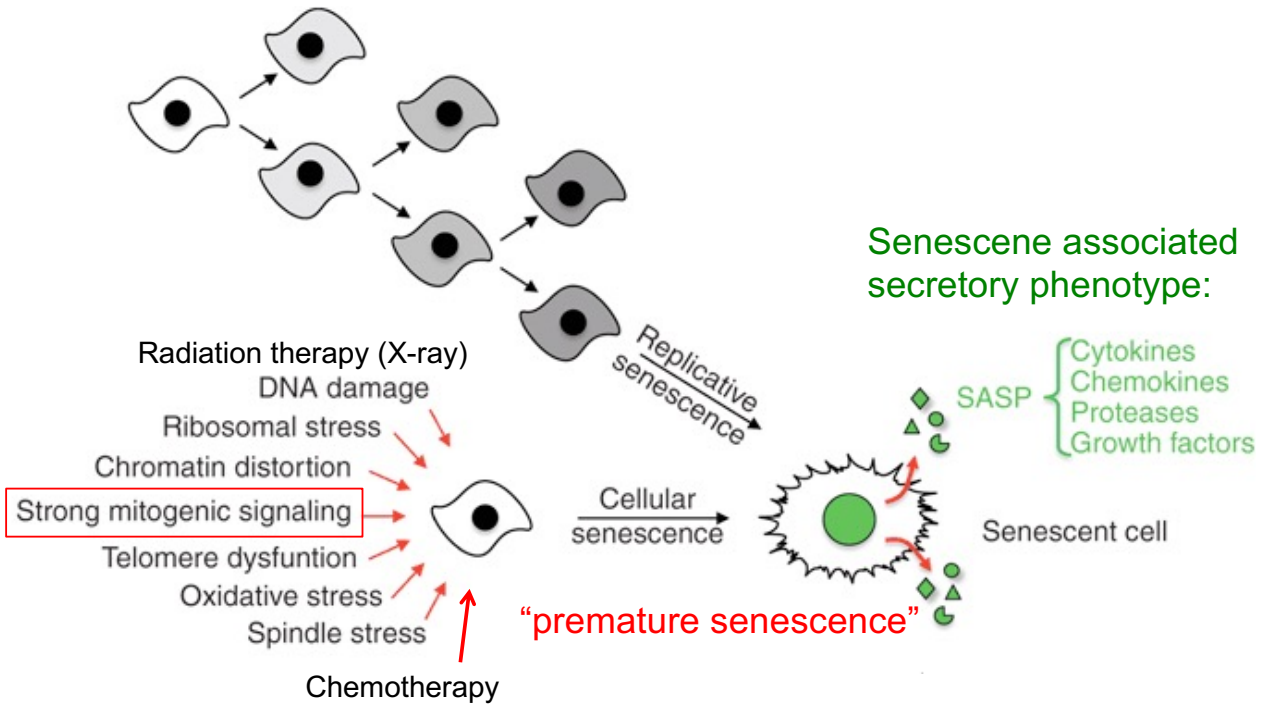


=> Senescent cells shape a tumorigenic microenvironment via their SASP:

SASP also affects distant organs

Naylor et al. 2013 Clin Pharmacol & Ther 93:105-116

Various types of DNA stress trigger premature senescence



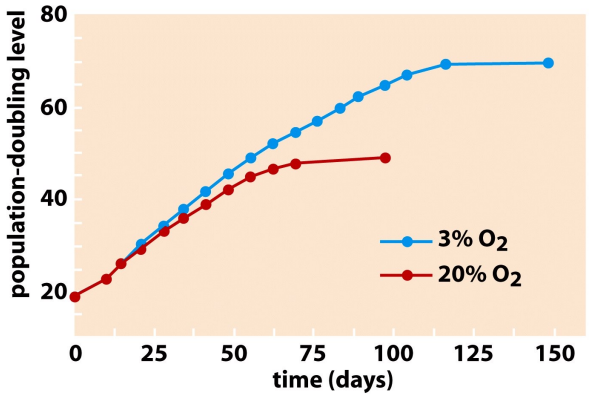
Senescene associated secretory phenotype:

Naylor et al. 2013 Clin Pharmacol & Ther 93:105-116

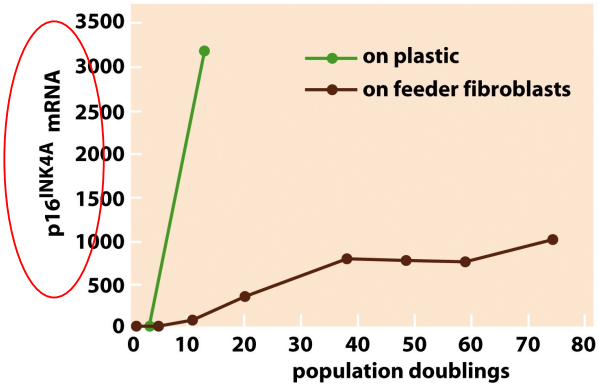
Primary *mouse* cells in culture senesce long before telomeres reach a subcritical size

⇒ Replication stress and DNA damage incurred by suboptimal tissue culture conditions can trigger "*premature* cellular senescence":

Human fibroblasts:



Human foreskin keratinocytes (epithelial):

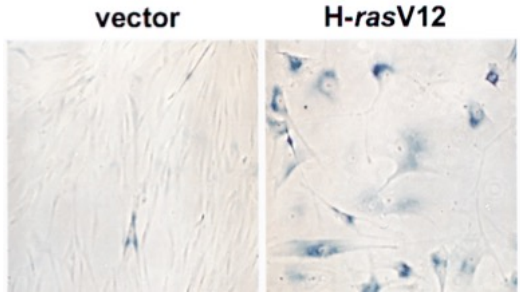


CDK4(&6) Inhibitor A, 16 kDa

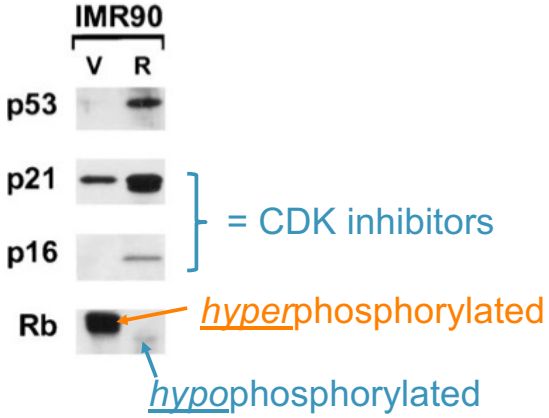
Figure 10.7 *The Biology of Cancer* (© Garland Science 2014)

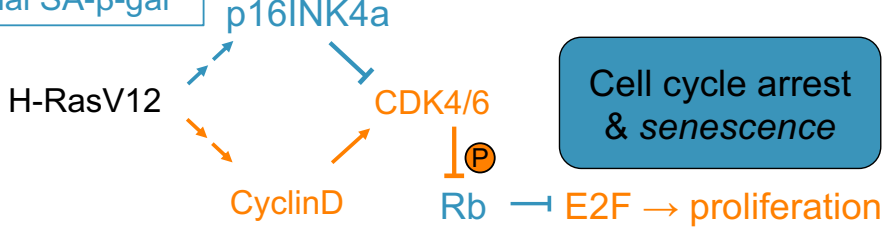
Cellular senescence is induced prematurely by oncogenes

Primary human (IMR90) fibroblasts were transduced with empty retrovirus (V) or oncogenic H-Ras^{G12V} (R):



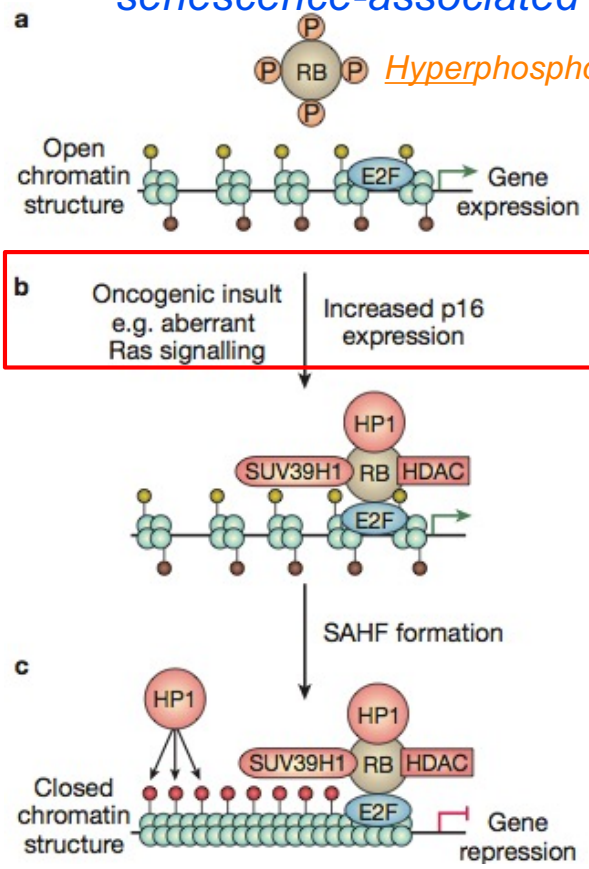
RasG12V induces "senescence-associated" lysosomal SA-β-gal





Serrano et al. 1997 Cell

Hypophosphorylated RB1 induces *senescence-associated heterochromatin foci (SAHF)*



→ cell proliferation genes

Upregulation of CKIs (p21Cip1 and p16Ink4a) inhibits RB1 phosphorylation

Hypophosphorylated RB1 now recruits histone-modifying enzymes and ***heterochromatin protein HP1*** *to E2F-regulated promoters =>*

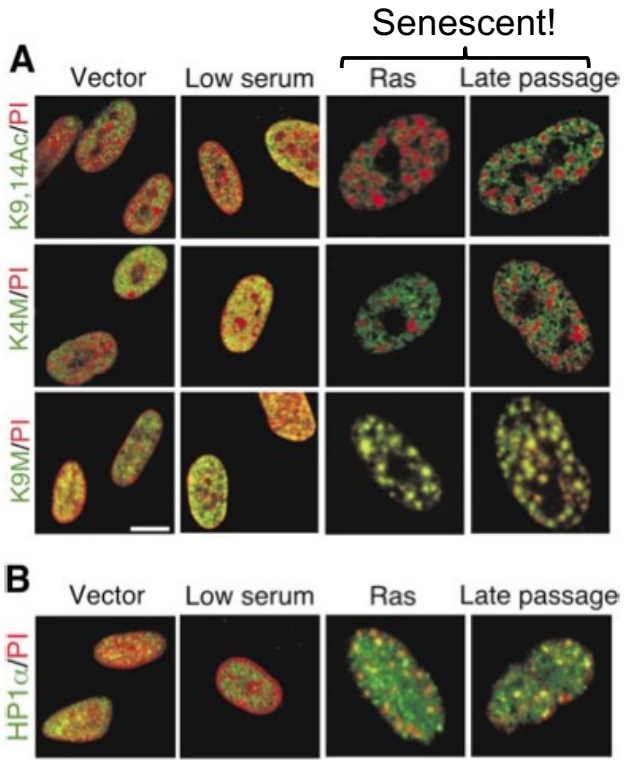
Heterochromatin induces ***permanent*** G1 arrest and a senescence-specific gene expression profile

Atkinson & Keith 2007 Expert Reviews Mol. Med.

Imaging of senescence-associated heterochromatin foci (SAHF)

Effects of oncogenic Ras in human IMR90 fibroblasts:

- DNA foci (red): ↑ in senescence
- *euchromatic* markers K9Ac-H3 and K4M-H3 (green) @ DNA foci
  Overlap (yellow): ↓ in senescence
- *repressive* histone mark K9M-H3 @ DNA foci:
  Overlap (yellow): ↑ in senescence
- HP1 staining @ DNA foci:
  Overlap (yellow): ↑ in senescence
  confirms that these foci represent heterochromatin



Narita et al. 2003 Cell

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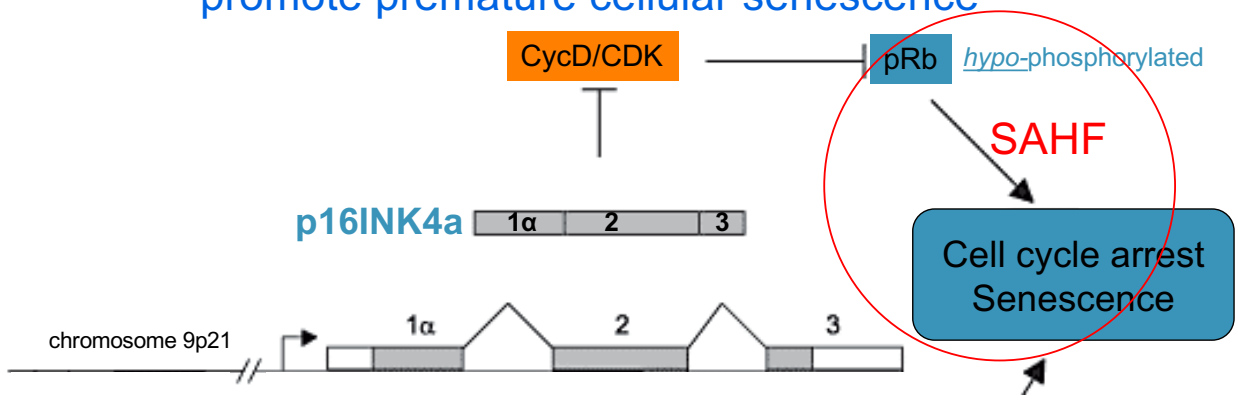
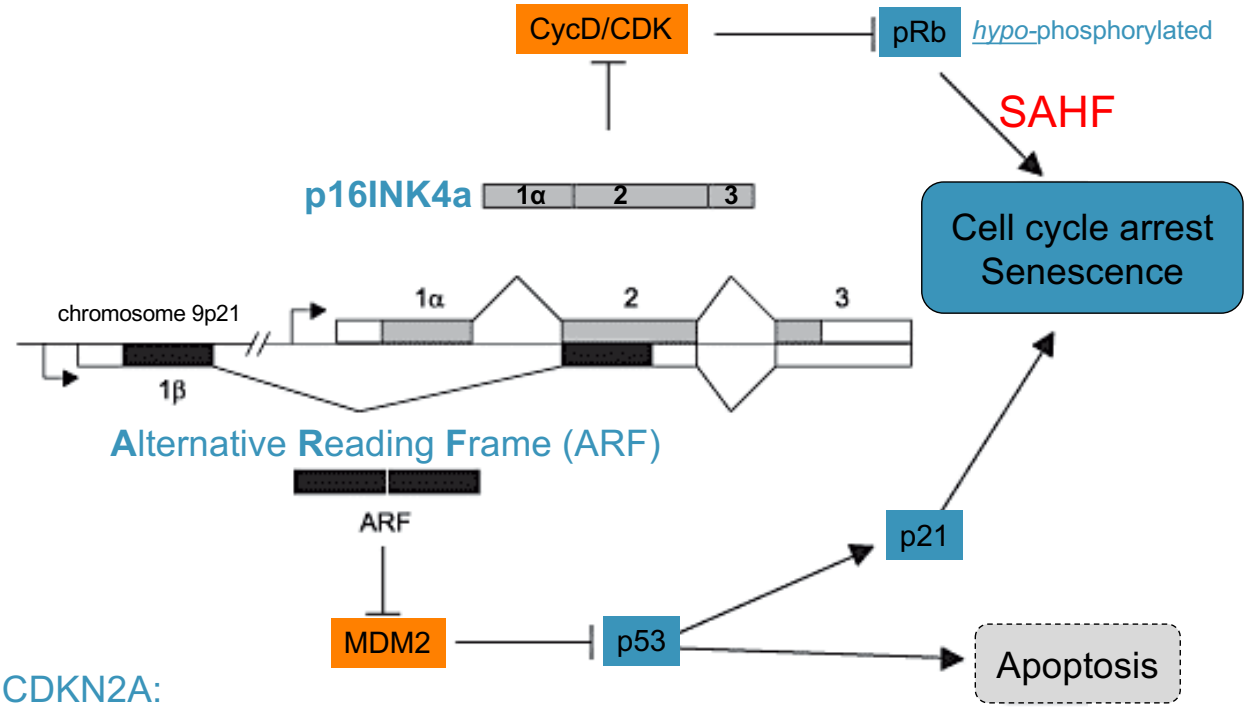
Two tumor suppressor genes encoded by *one* locus (CDKN2A) promote premature cellular senescence



- p16INK4a promotes RB1 function

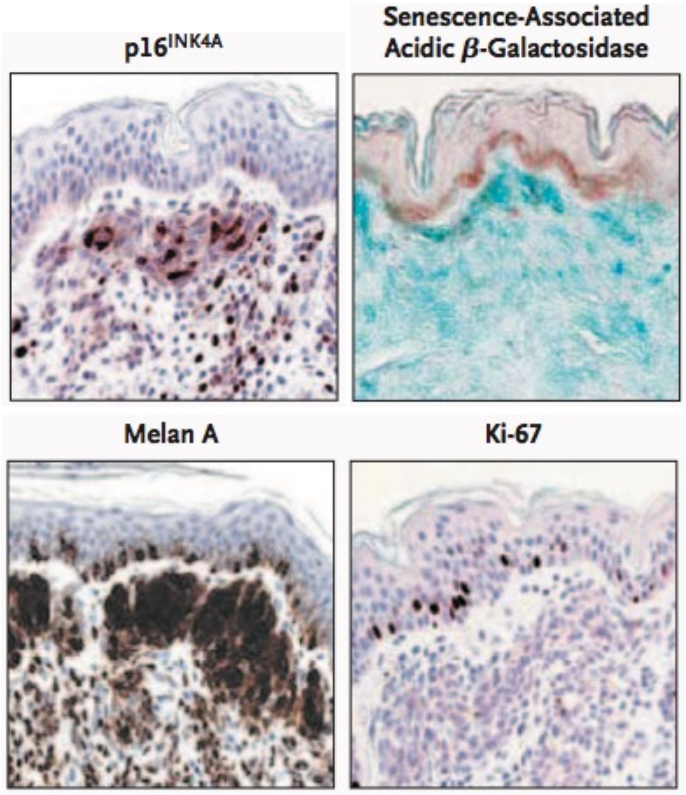
Two tumor suppressor genes encoded by *one* locus (CDKN2A) promote premature cellular senescence



CDKN2A:

- p16INK4a promotes RB1 function
- ARF binds and inhibits MDM2 to increase p53 stability

Example: Cellular senescence in benign human melanocytic nevi

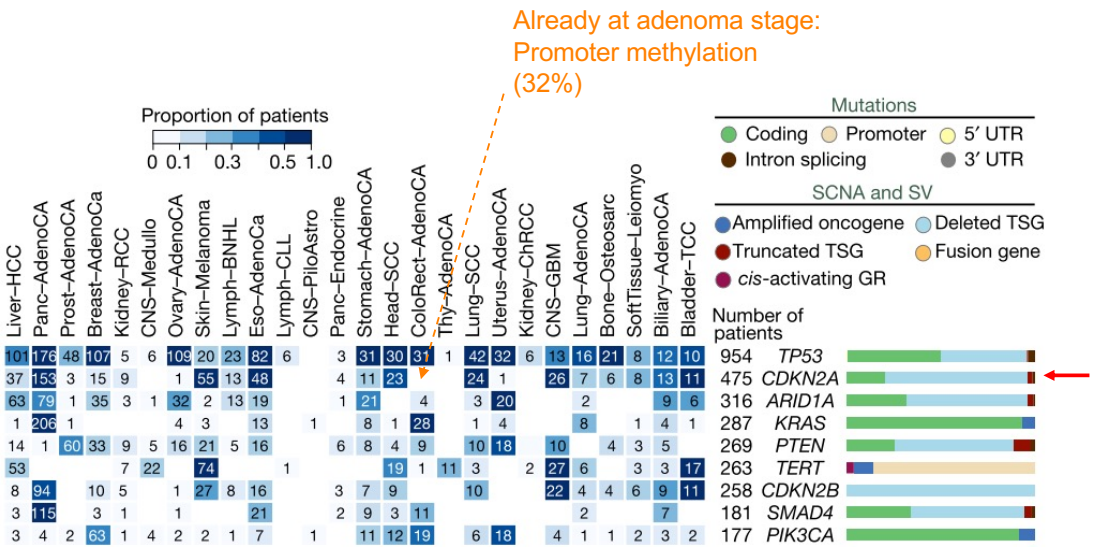


Serial sections of a human nevus

- blue areas in the dermis represent groups of nevus cells expressing SA-β-galactosidase
- p16INK4A staining marks differentiated (Melan A$^+$) melanocytes
- the proliferation marker Ki67 is absent in nevus cells and only expressed in some basal epidermal keratinocytes

Mooij & Peeper 2006 N. Engl. J. Med.

After p53, *CDKN2A* is the second-most frequently mutated or repressed tumor suppressor gene across tumor types

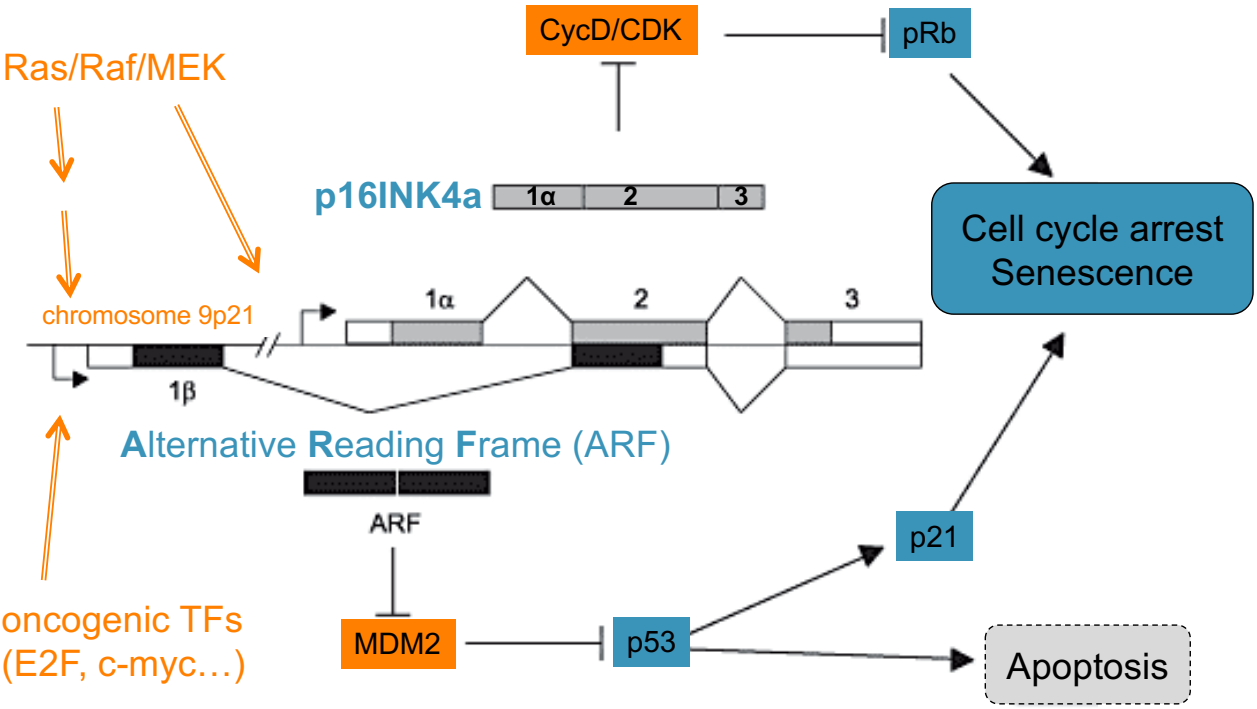


Article

Pan-cancer analysis of whole genomes

https://doi.org/10.1038/s41586-020-1969-6 The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium

Two tumor suppressor genes encoded by *one* locus (CDKN2A) promote premature cellular senescence



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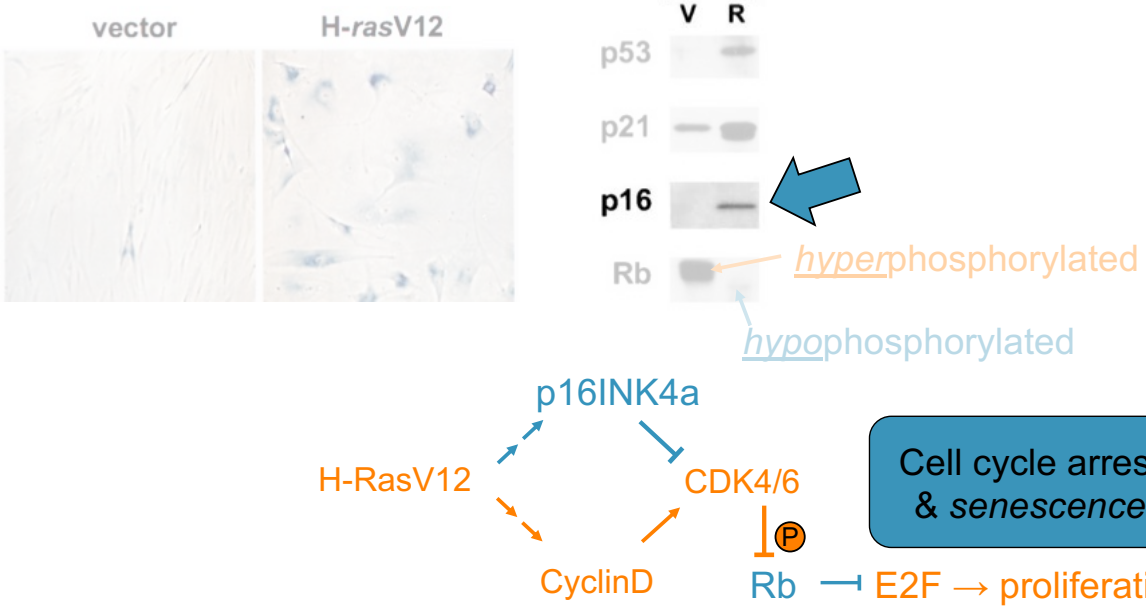
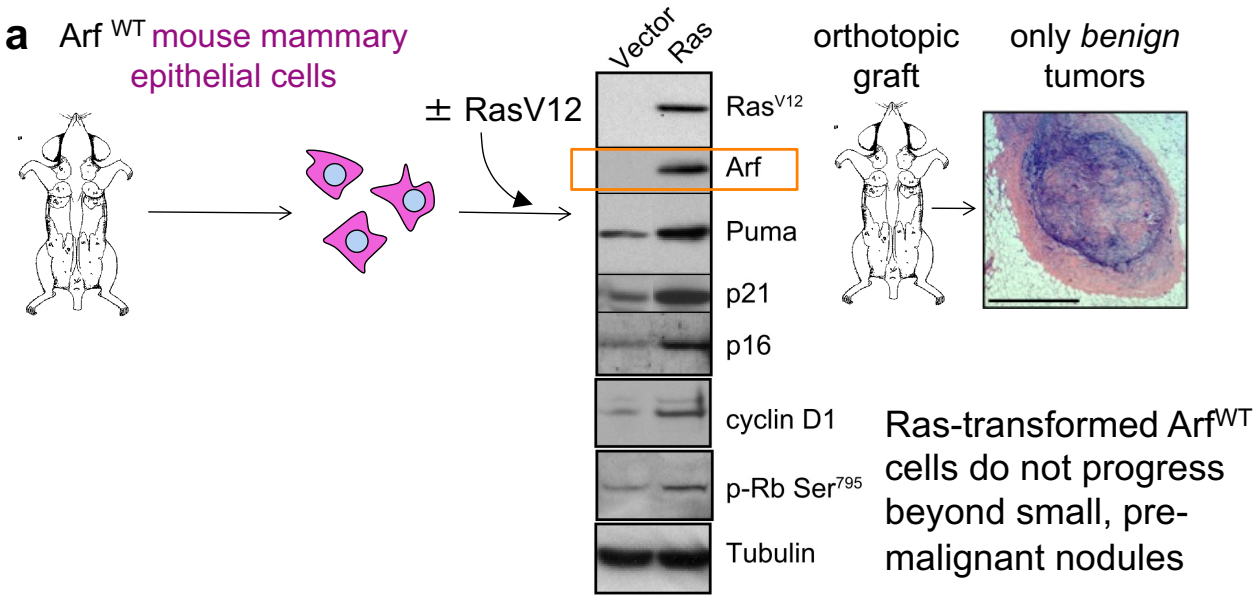


Serrano et al. 1997 Cell

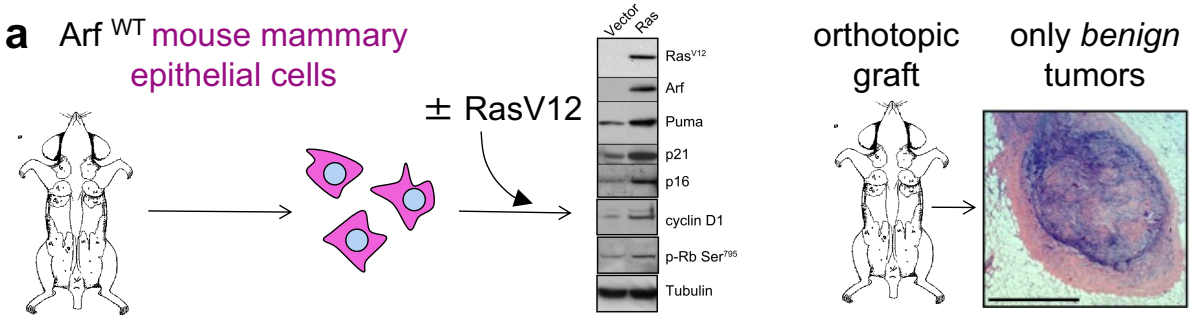
Testing the model: Induction of Arf by RasV12 in MMECs

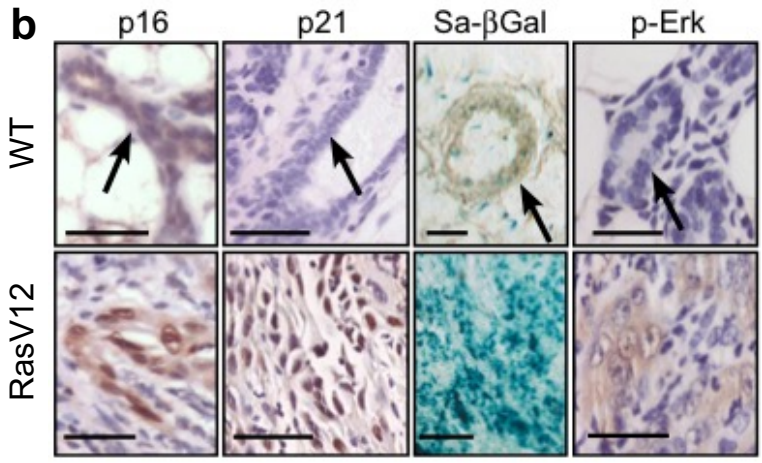


Ras-transformed Arf^{WT} cells do not progress beyond small, pre-malignant nodules

Swarbrick et al. 2008 PNAS

RasV12-transformed Arf^{WT} tumors remain benign





Swarbrick et al. 2008 PNAS

Ras-transformed Arf^{WT} cells do not progress beyond small, pre-malignant nodules.

The benign lesions show signs of cellular senescence.

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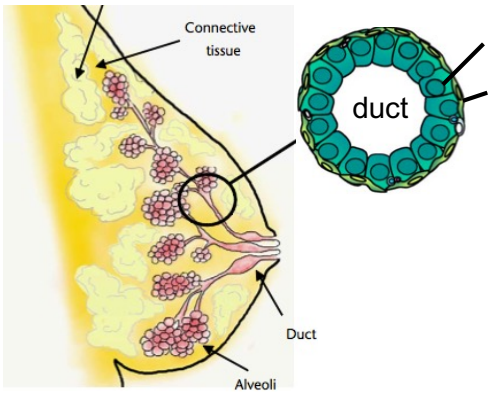
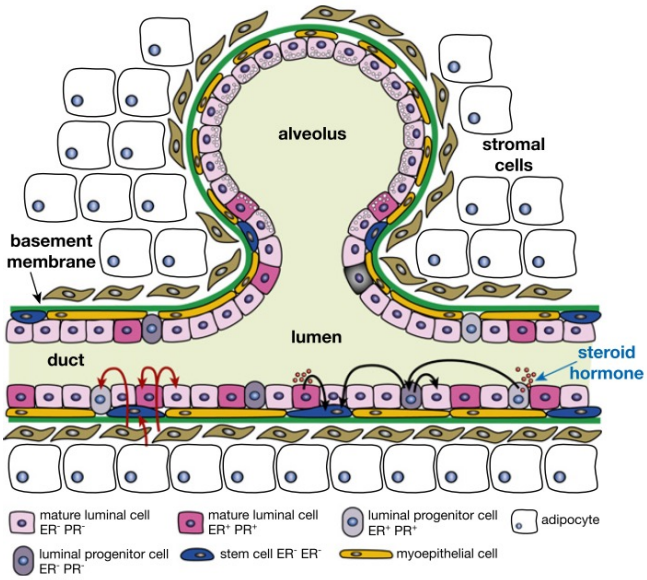
Cytostatic TGF-β signaling
Example last week: The mammary gland



luminal

basal (myoepithelial)

- Heterogenous stem and progenitor cell populations
- Proliferation is regulated by endocrine hormones and paracrine growth factors



Cytostatic TGF-β signaling
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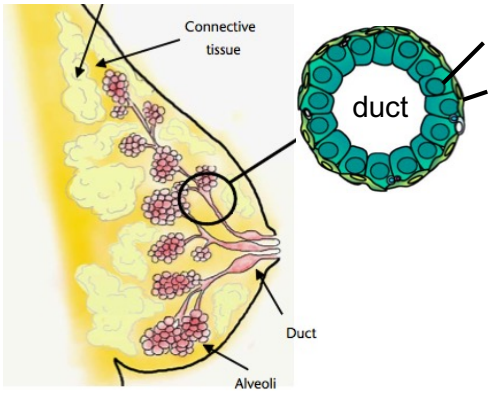
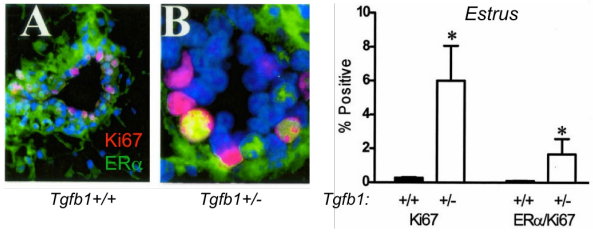
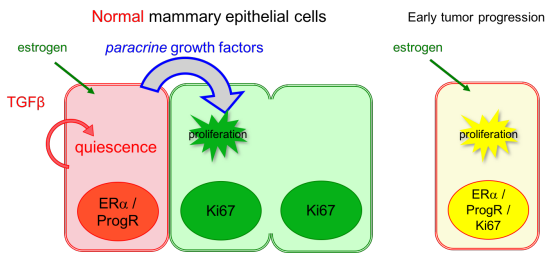


luminal

basal (myoepithelial)

yellow = red + green
blue: Nuclei



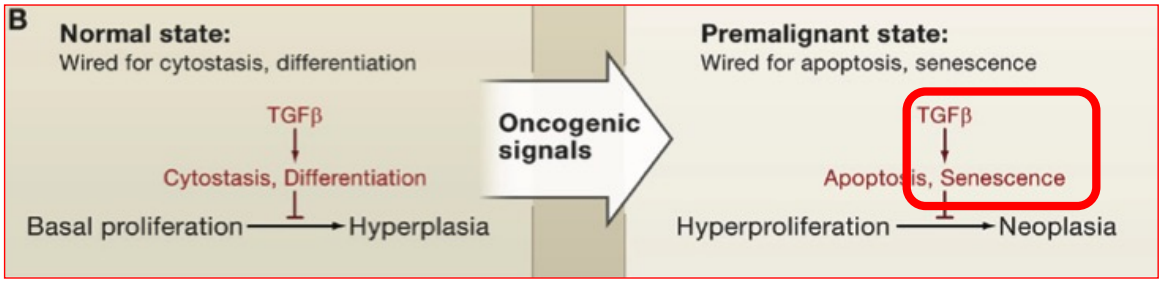


Last week's exercise:

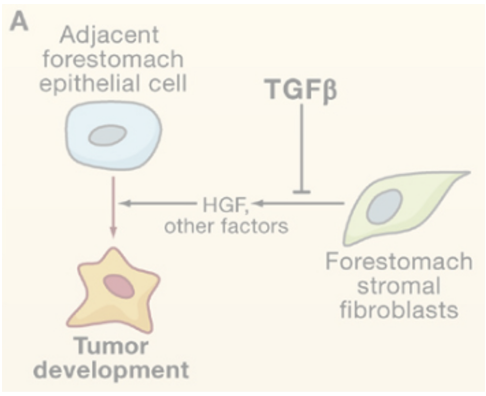
TGF-β1 inhibits proliferation of ERα⁺ cells

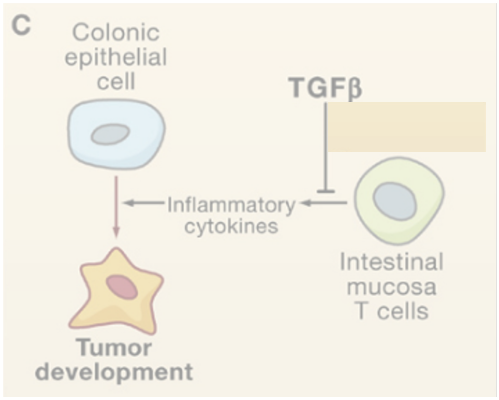
Tumor suppressive functions of TGF-β

- In epithelial cells: Cell cycle arrest, senescence, or even death:



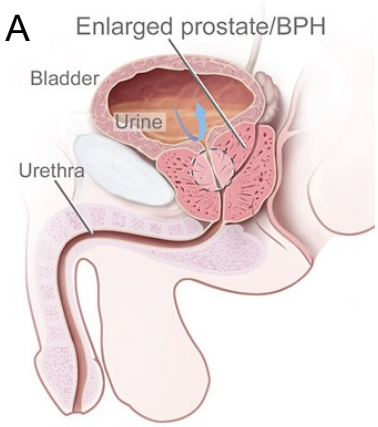
- In stromal cells: Inhibition of secreted oncogenic factors



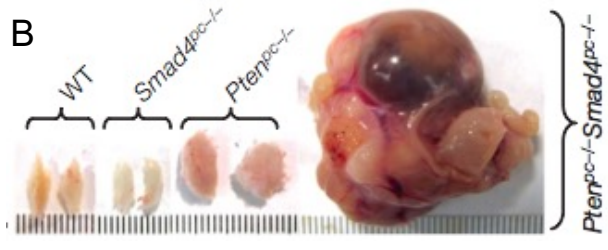


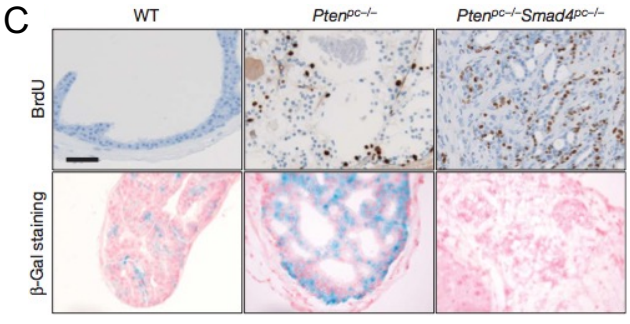
Massagué 2008 Cell 134:215-230

Smad4 mediates oncogene-induced senescence in prostate adenoma of PTEN$^{pc-/-}$ mice



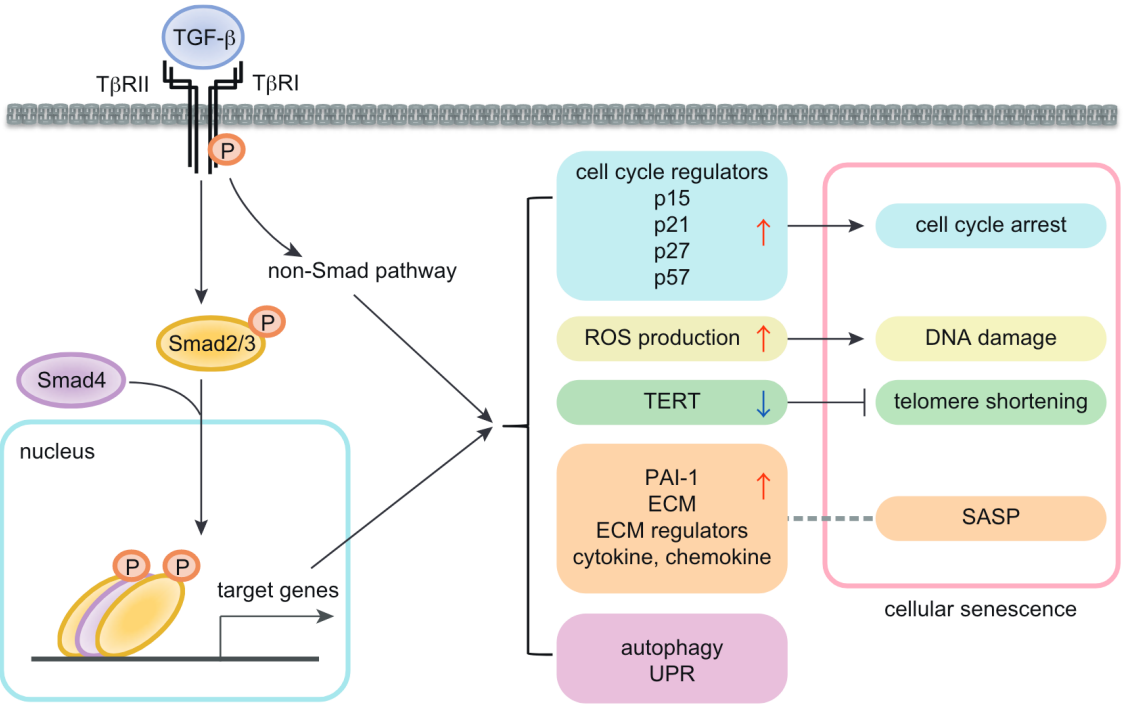
- Prostate gland adjusts (alkaline) pH of seminal fluid and secretes prostate-specific antigent (PSA), a diagnostic marker of prostate adenocarcinoma
- PTEN$^{pc-/-}$ mice develop prostate adenoma
- Smad4 induces senescence (C) and suppresses progression to invasion & metastasis (B)





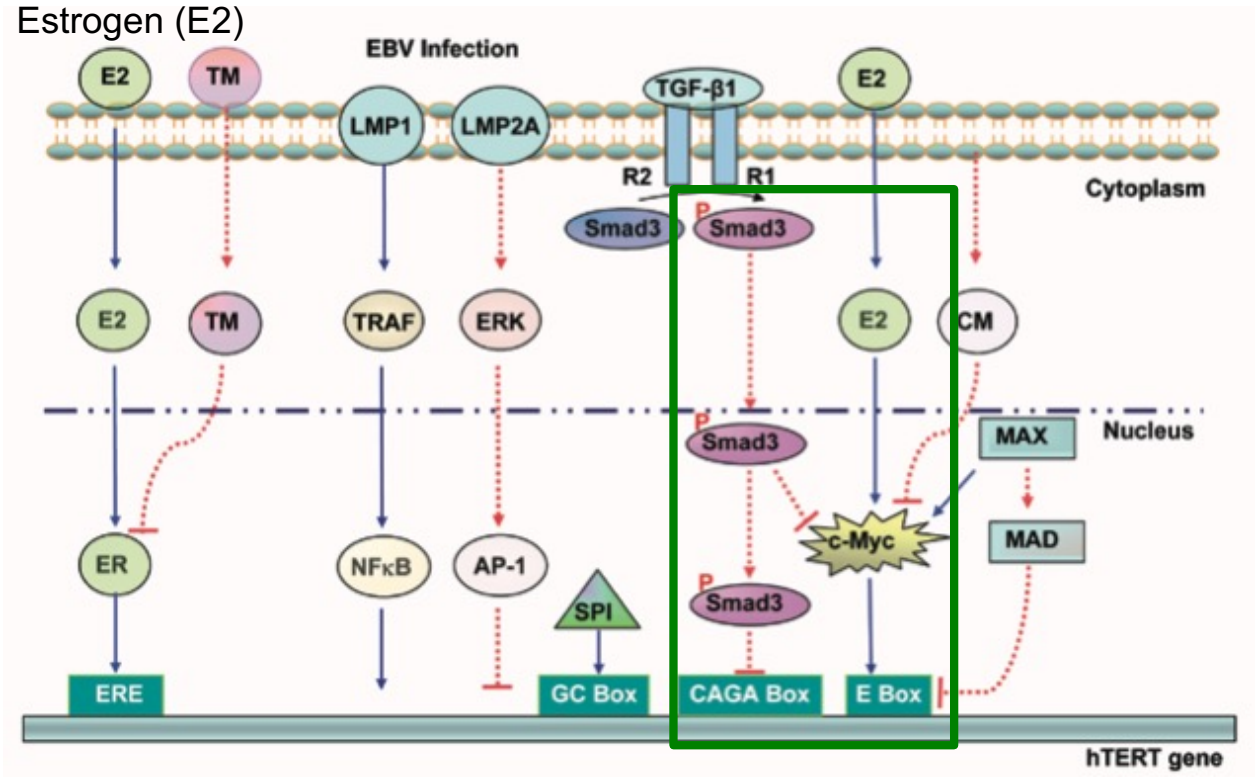
Ding et al. 2011 Nature 470:269-273

Stimulation of cell senescence by TGF-β signaling



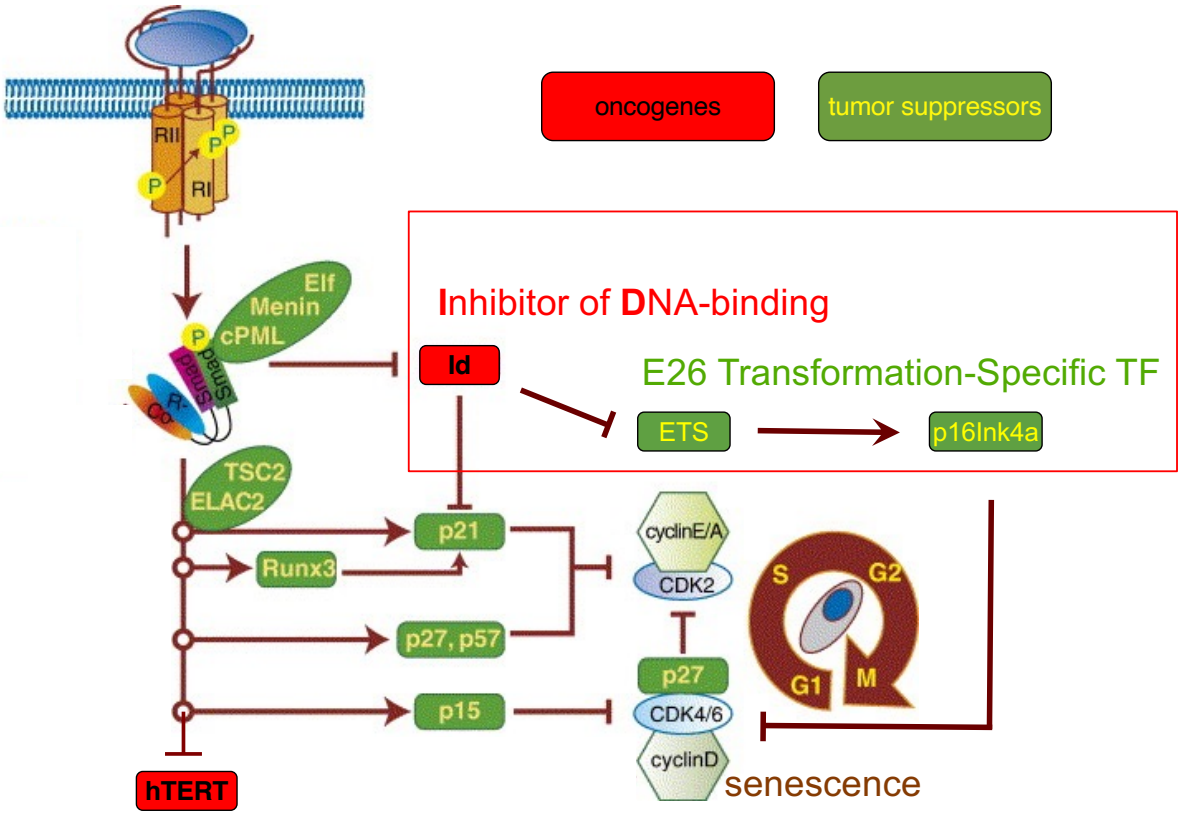
Suzuki et al. 2019, Int. J. Mol. Sci. 20:5002

TGF-β can promote replicative senescence by repressing TERT



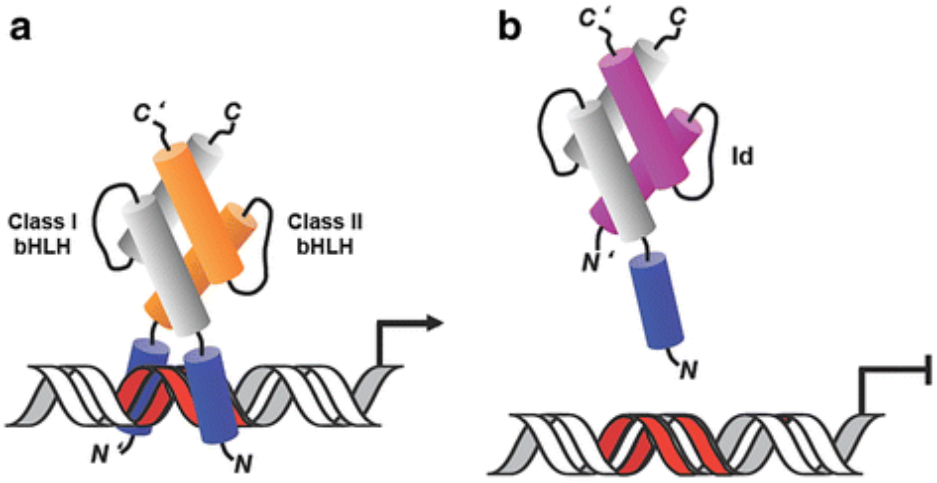
Liu et al. 2006 Cell Res. 16:809-817

In premalignant epithelial cells, TGF-β also represses *Id1*



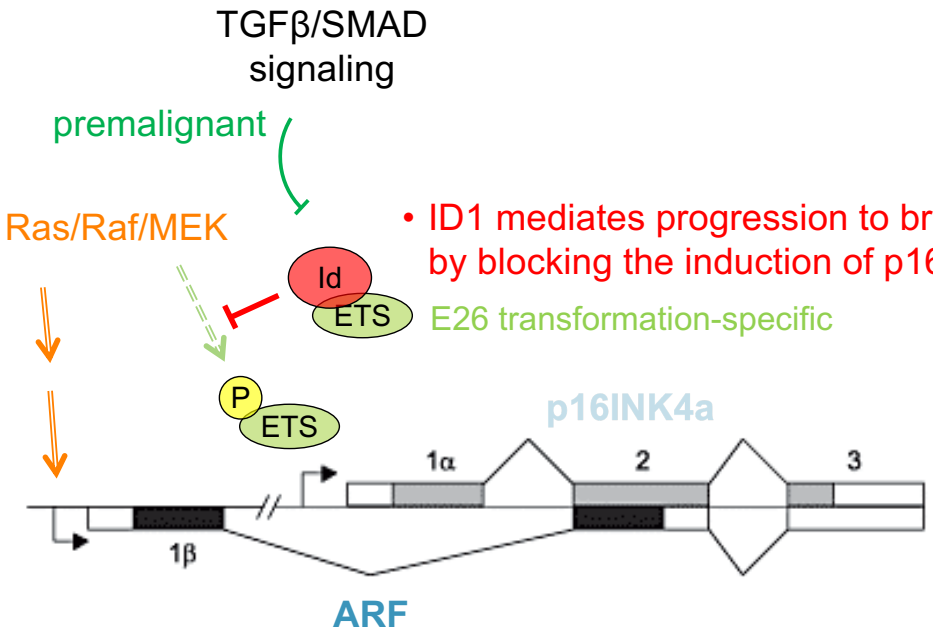
after Pardali & Moustakas, 2007

Inhibitors of DNA-binding (Id)



Roschger & Cabrele, 2017, Cell Comm. Signal.

TGF-β *inhibits* Id1 expression in *pre*malignant epithelial cells



- ID1 mediates progression to breast carcinoma by blocking the induction of p16:

E26 transformation-specific

Premature cellular senescence - Summary

- Cellular senescence can be induced prematurely (i.e. before telomere erosion) by oncogene-induced replication stress and DNA damage, and *systemically* by cancer treatments (chemotherapy, X-ray)
- *Replicative* and *premature* cellular senescence have similar outcomes: Permanent G1 arrest; cell shapes & size; altered gene expression (SASP, which in turn can unfavorably alter the tumor microenvironment)
- Multiple senescence-inducing signals (p53, p16Ink4a/Arf, TGFβ) converge on RB1 (and its ability to assemble SAHFs)
- The high frequency of mutations in these tumor suppressors and in the hTERT promoter underscores the relevance of senescence as a key defence mechanism against cancer.

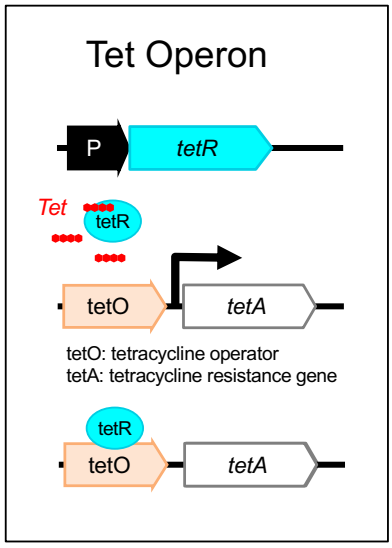
Exercise question Q5:

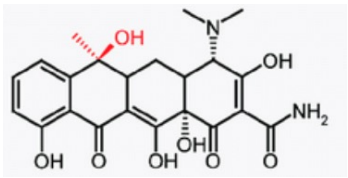
Introduction:

Tetracyclin-regulated transgenes

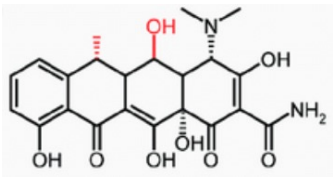
Tet-OFF system to control the expression of transgenes

tetR: tetracycline repressor (*E.coli*)





Tetracycline

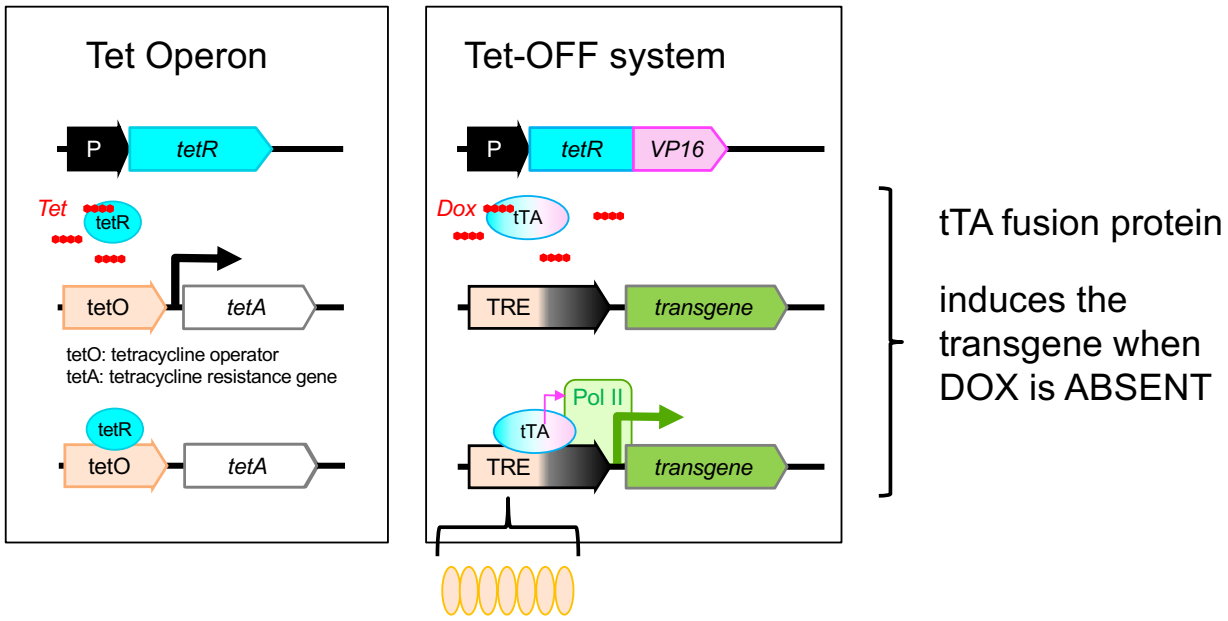


Doxycycline
(better absorbed)

Tet-OFF system to control the expression of transgenes

tetR: tetracycline repressor (*E.coli*)

tTA: tet Trans-Activator (tetR DBD + VP16 AD)



tTA fusion protein

induces the transgene when DOX is ABSENT

7x tetO fused to a minimal promoter
= Tet-responsive element (TRE)