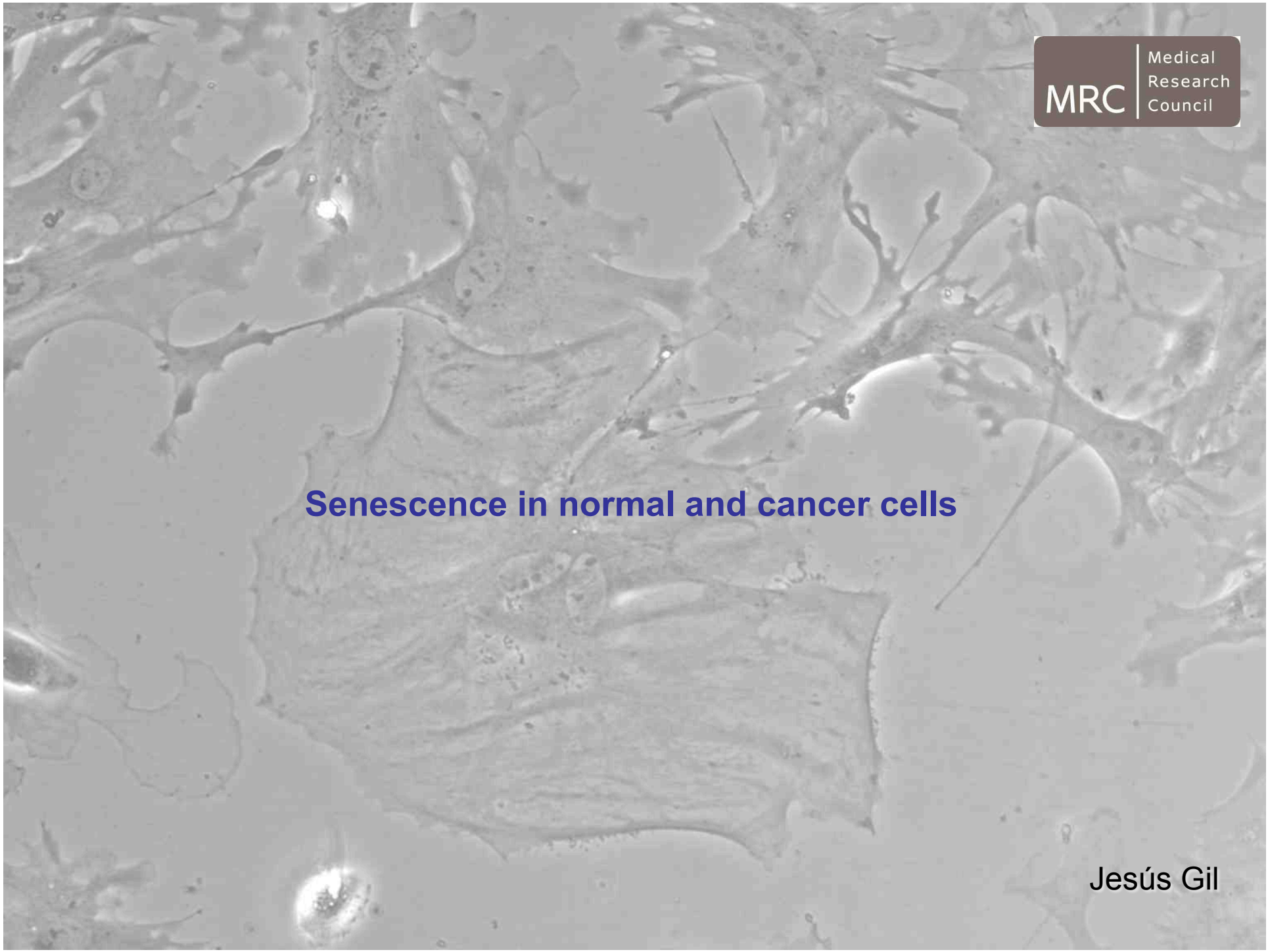
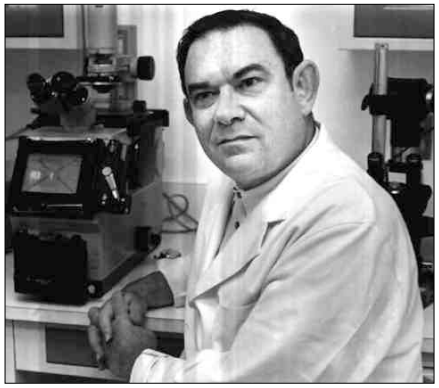



# Senescence in normal and cancer cells

Jesús Gil

# *Introduction to senescence and the molecular mechanisms controlling it.*

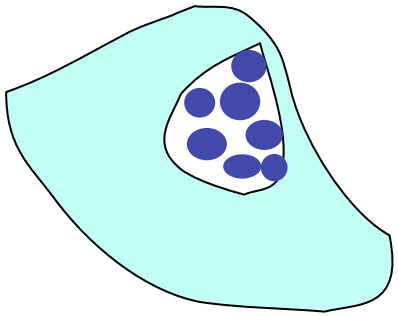
# Senescence is an irreversible growth arrest



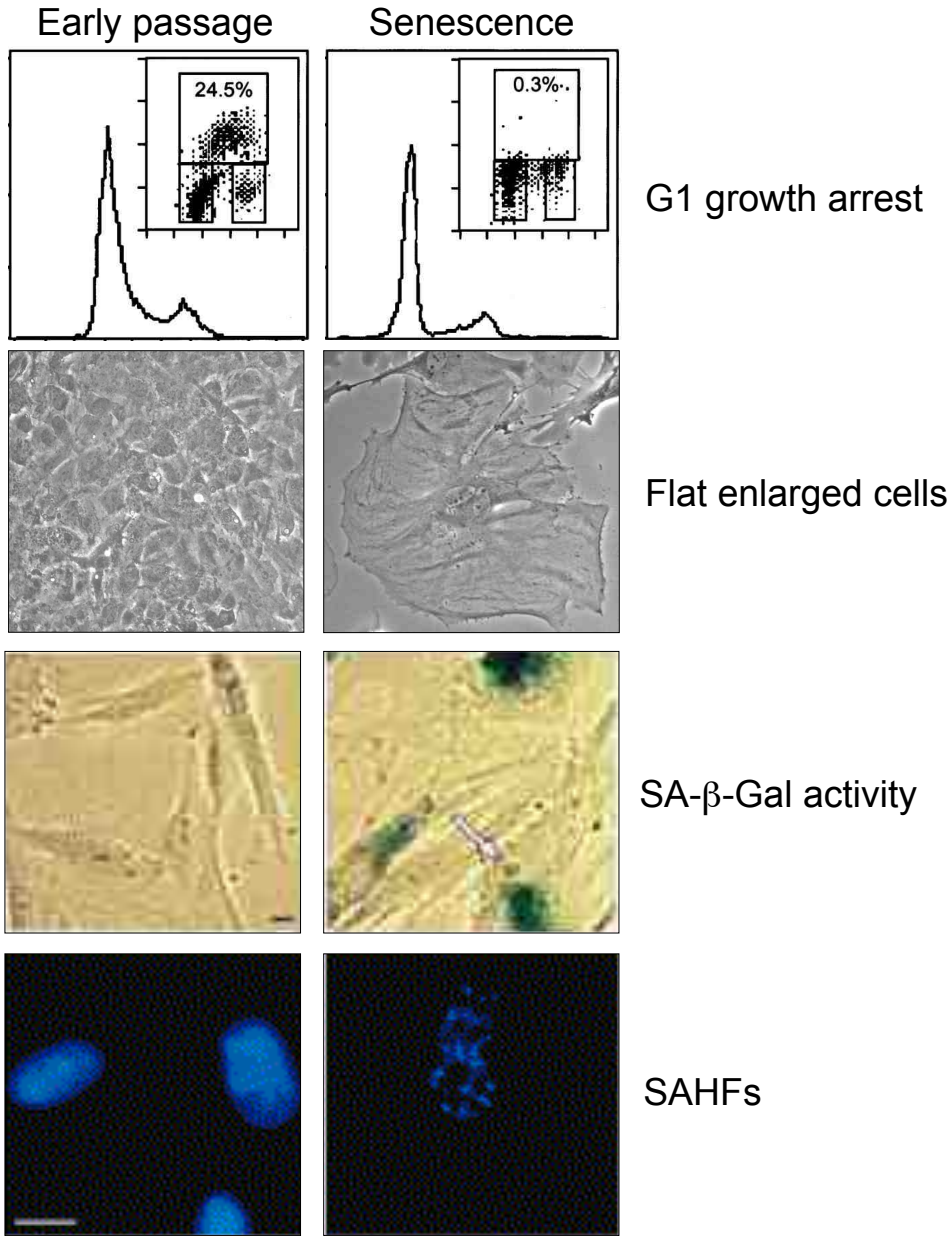
Leonard Hayflick

Primary cell

**Replicative exhaustion**



Senescent cell



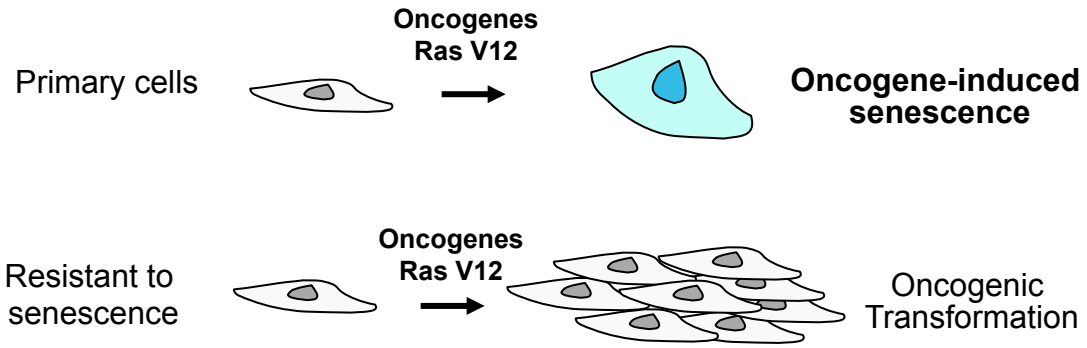
# Senescence comes in different flavours: Oncogene-Induced Senescence

Cell, Vol. 88, 593–602, March 7, 1997, Copyright ©1997 by Cell Press

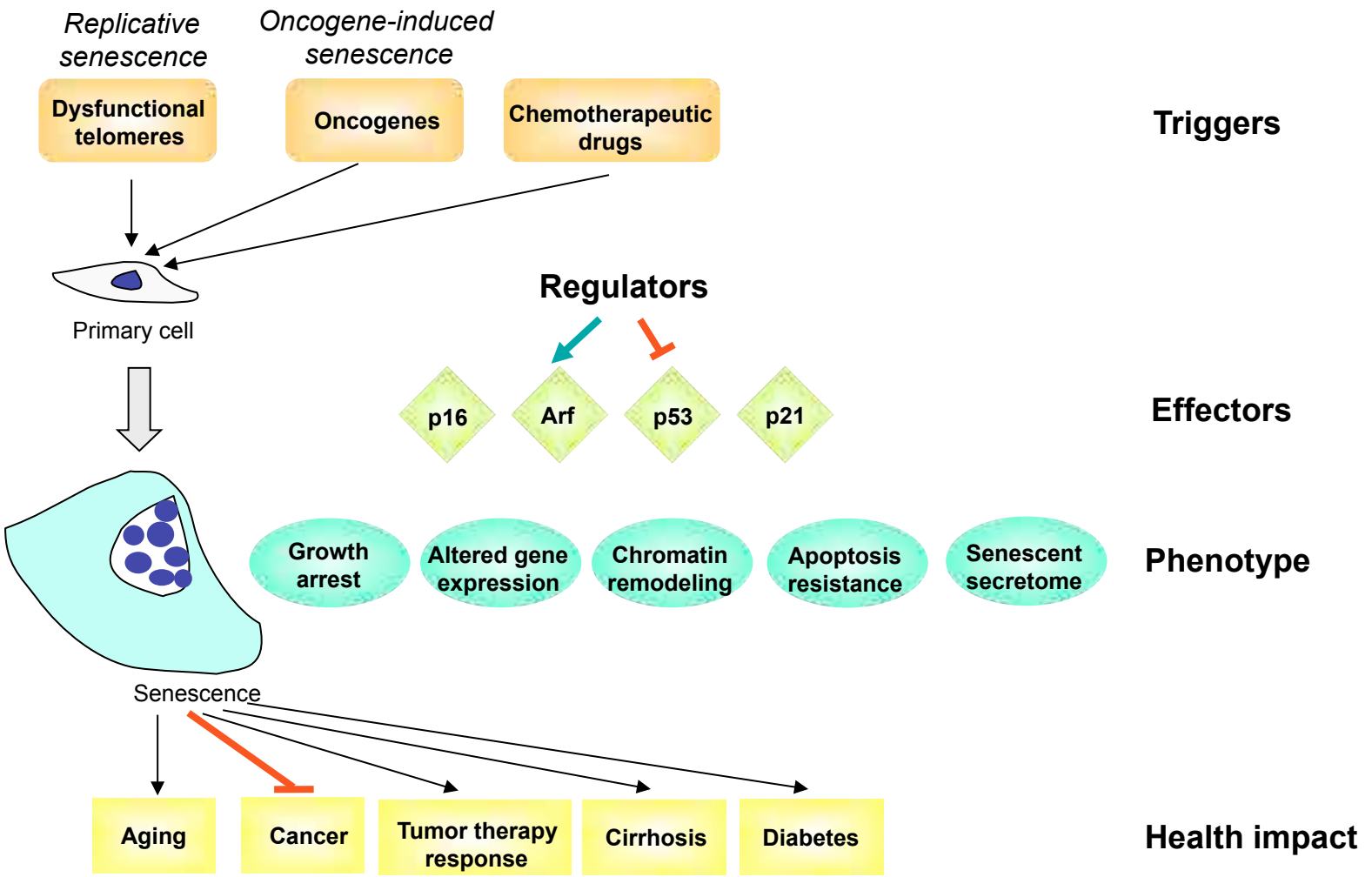
**Oncogenic *ras* Provokes Premature Cell Senescence Associated with Accumulation of p53 and $p16^{INK4a}$**

Manuel Serrano

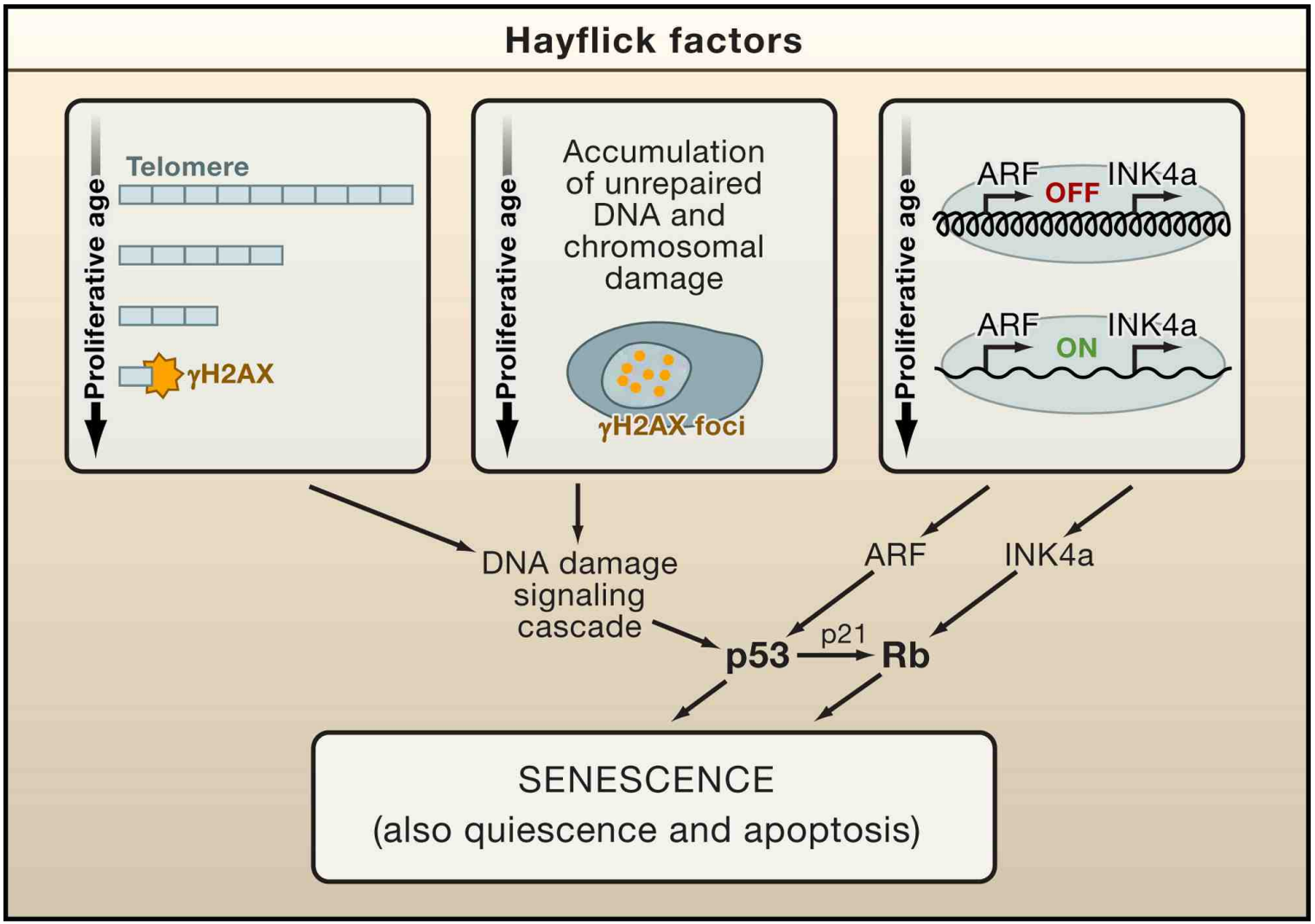
*Senescence is a potent tumor suppressor mechanism*



# Senescence and its physiological relevance

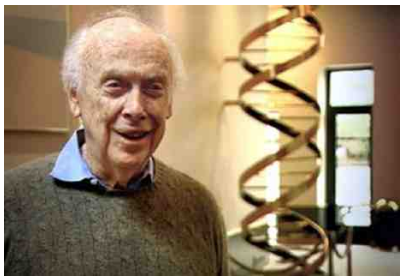


# *"HAYFLICK" factors limit cell proliferation*




Manuel Collado, María Blasco and Manuel Serrano Cell 2007

# *How do cells count their divisions?*



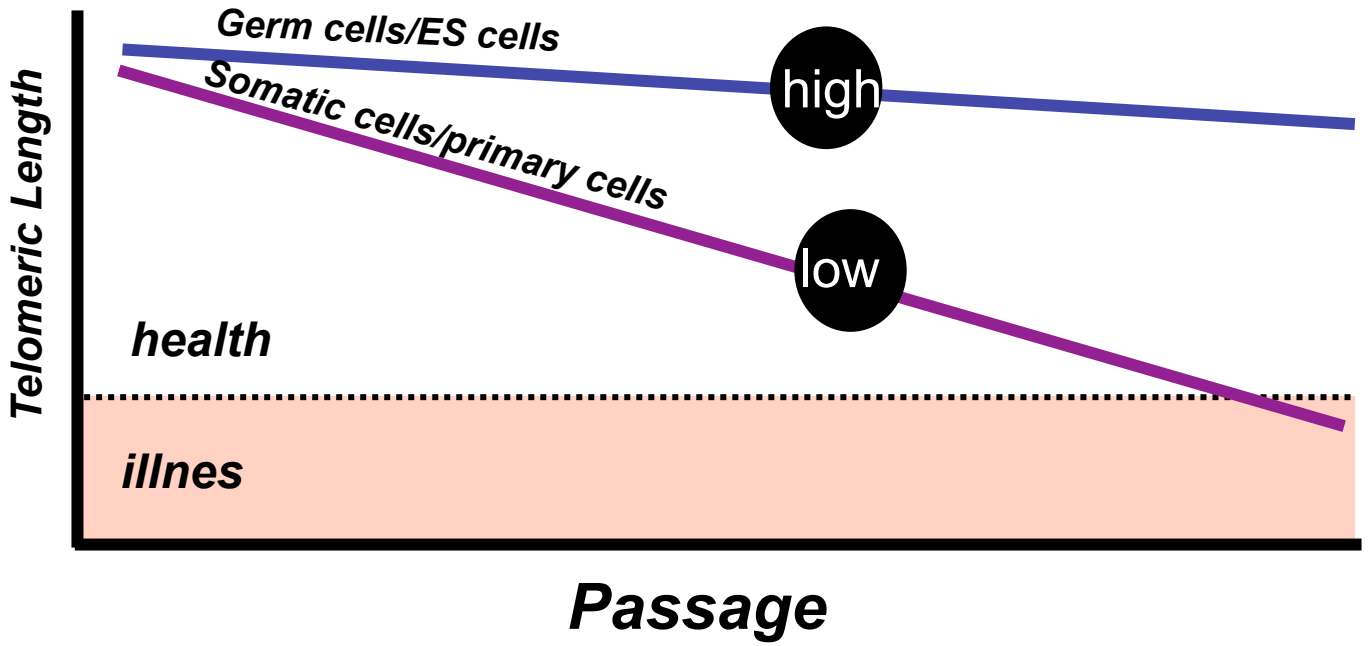
James Watson




*"End replication problem"*

Watson *Nat New Biol* 1972

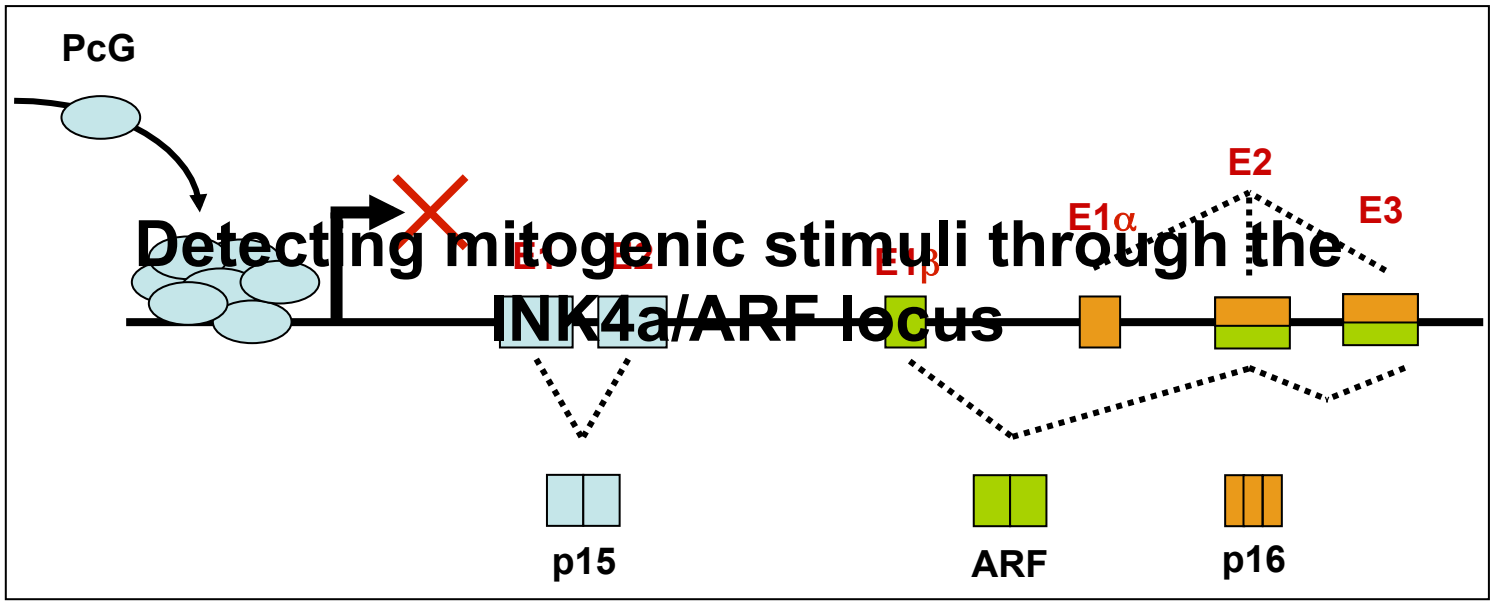
# Telomerase and senescence



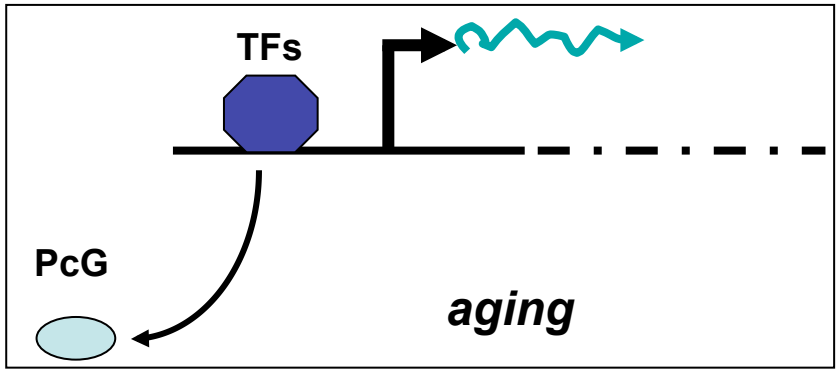

Harley, Futcher & Greider, *Nature* (1990)

# *How do cells count their divisions?*

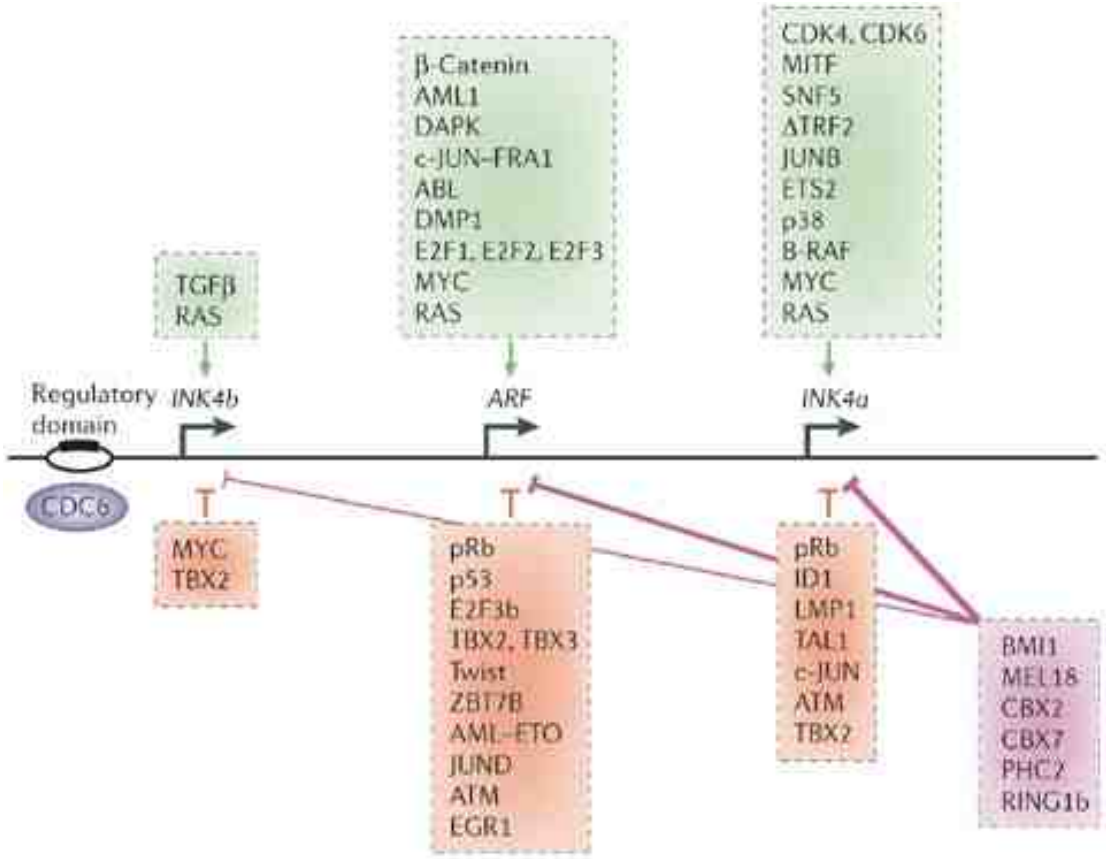
## The *INK4b-ARF-INK4a* locus



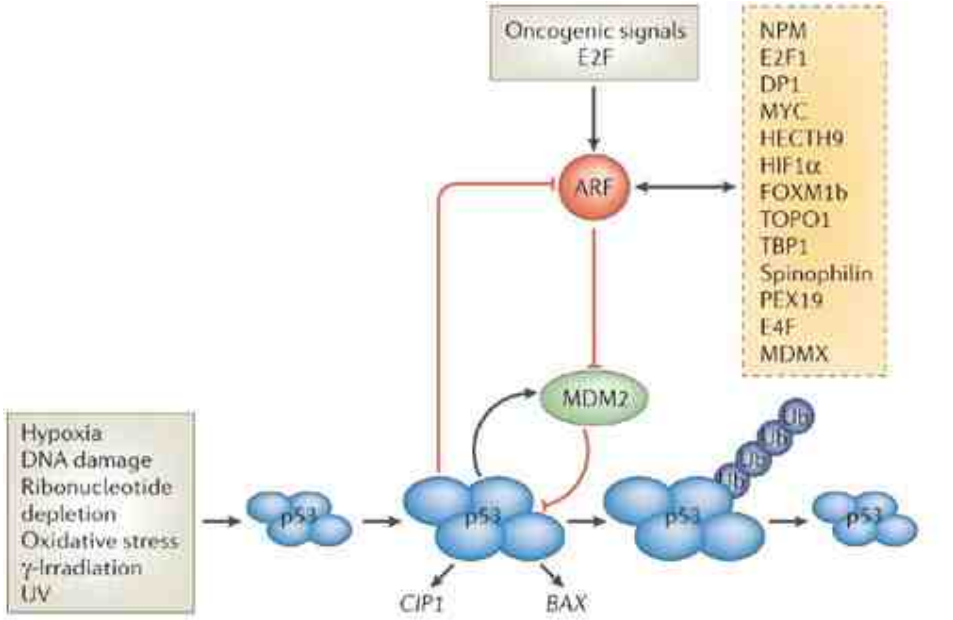

**Detecting mitogenic stimuli through the *INK4a/ARF* locus**



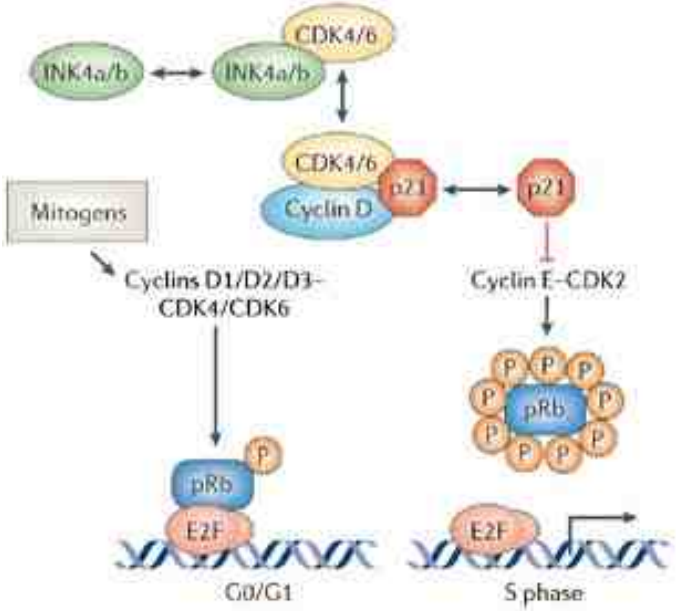
# Transcriptional control of the *INK4a/ARF* locus



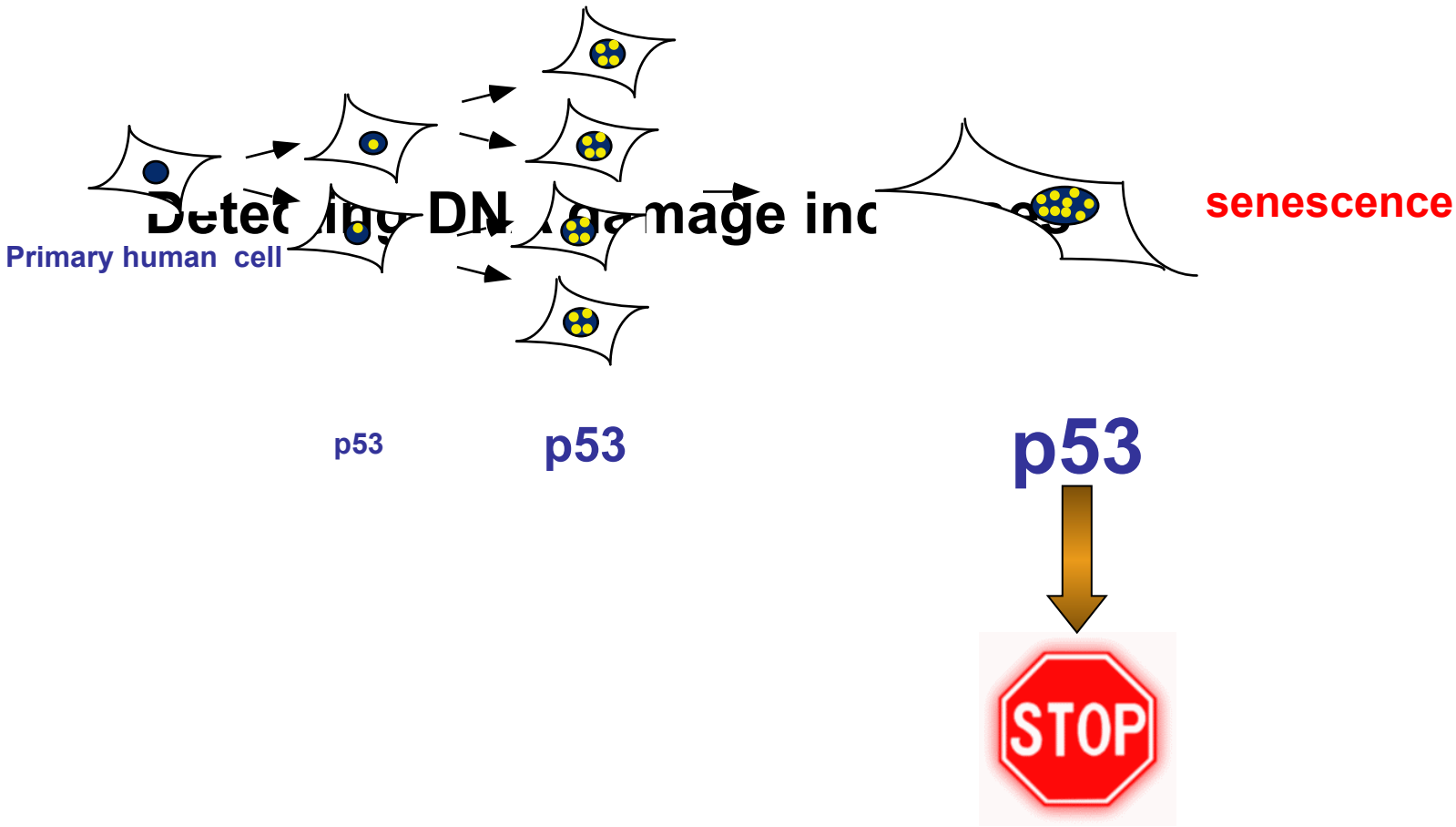
# ARF activates p53



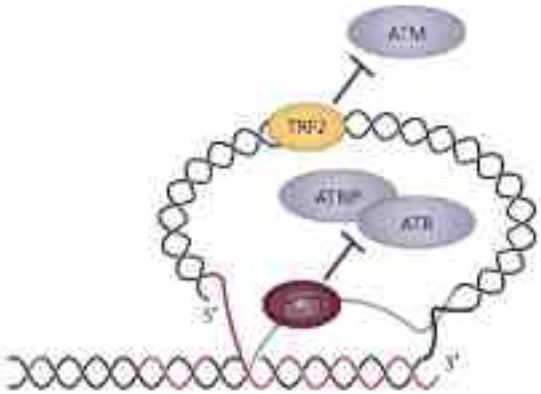
# Regulation of cell cycle by $p16^{INK4a}$

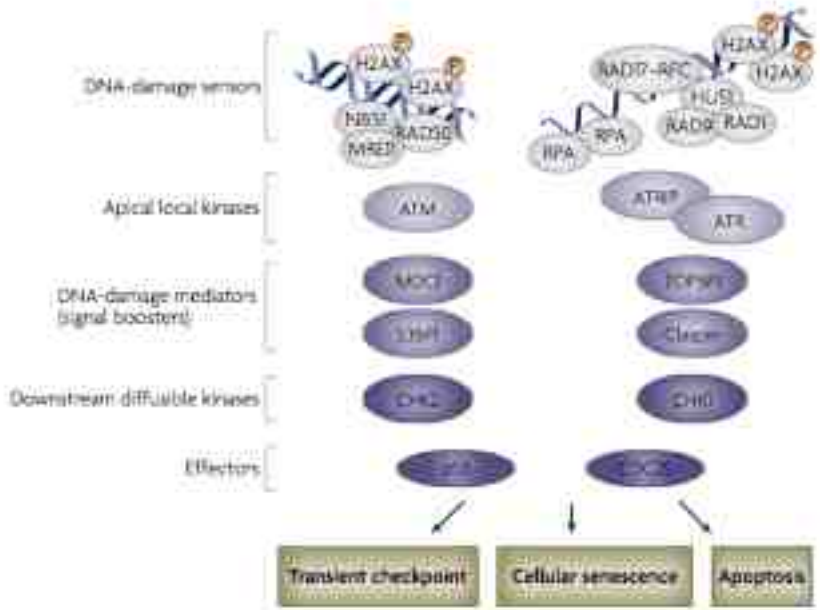


# *How do cells count their divisions?*



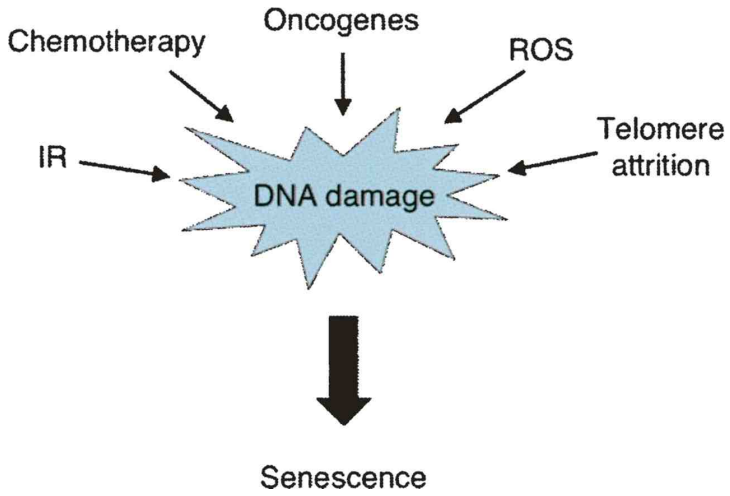
# Telomere uncapping activates the DNA damage pathway



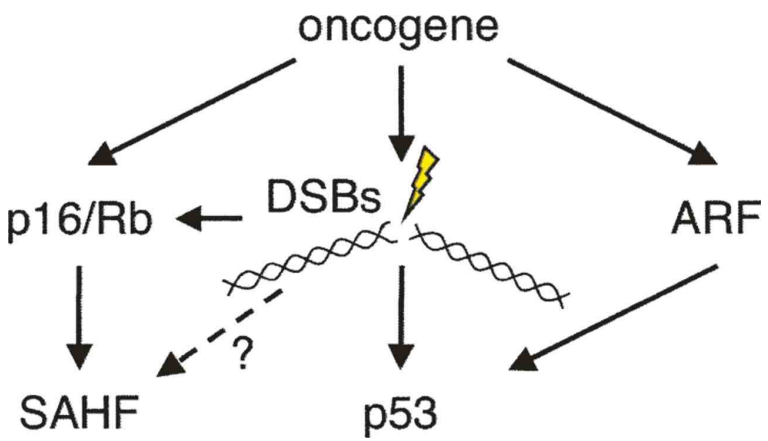



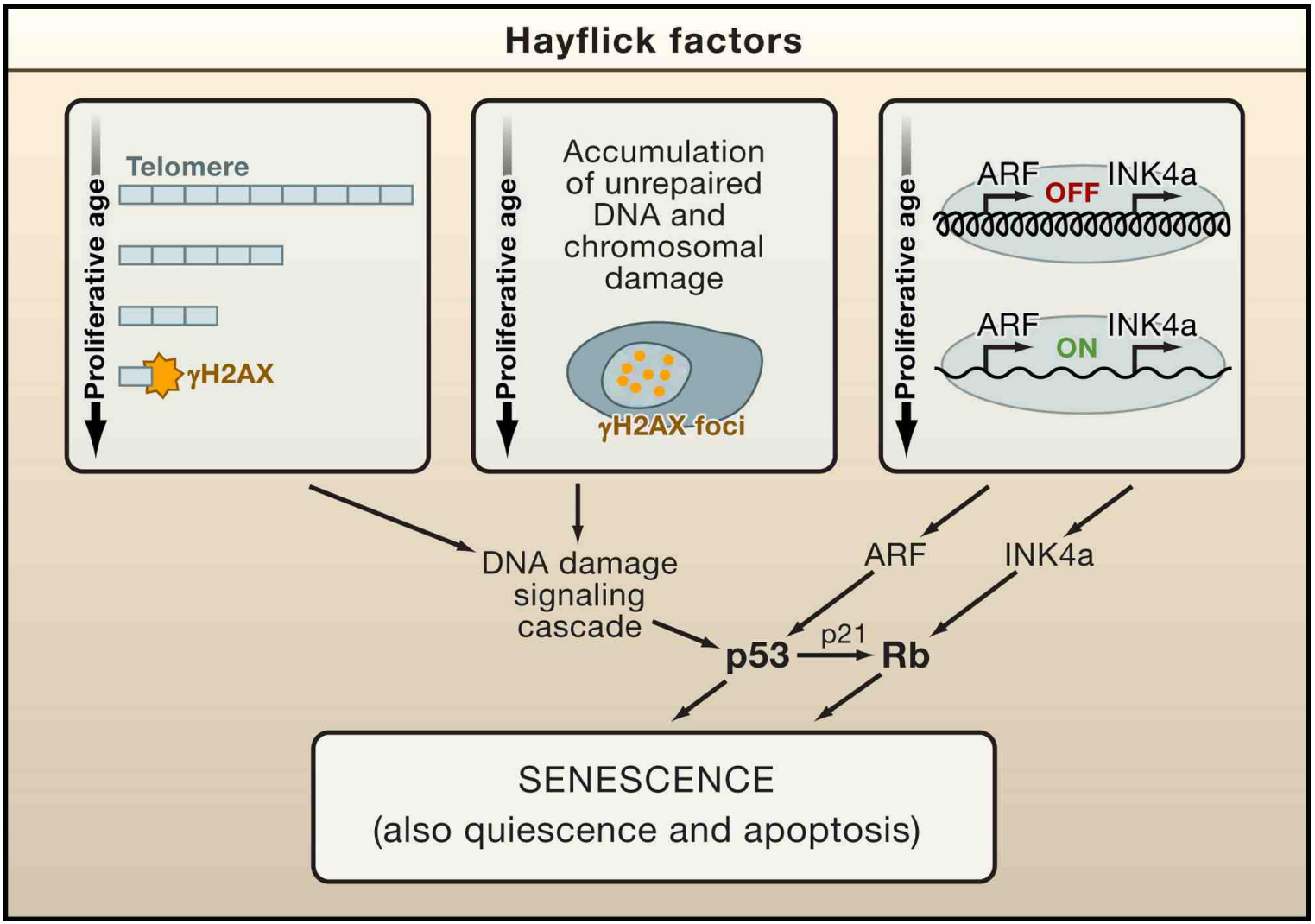
# Activation of p53 during senescence

**DNA damage as a common mediator of senescence signaling**



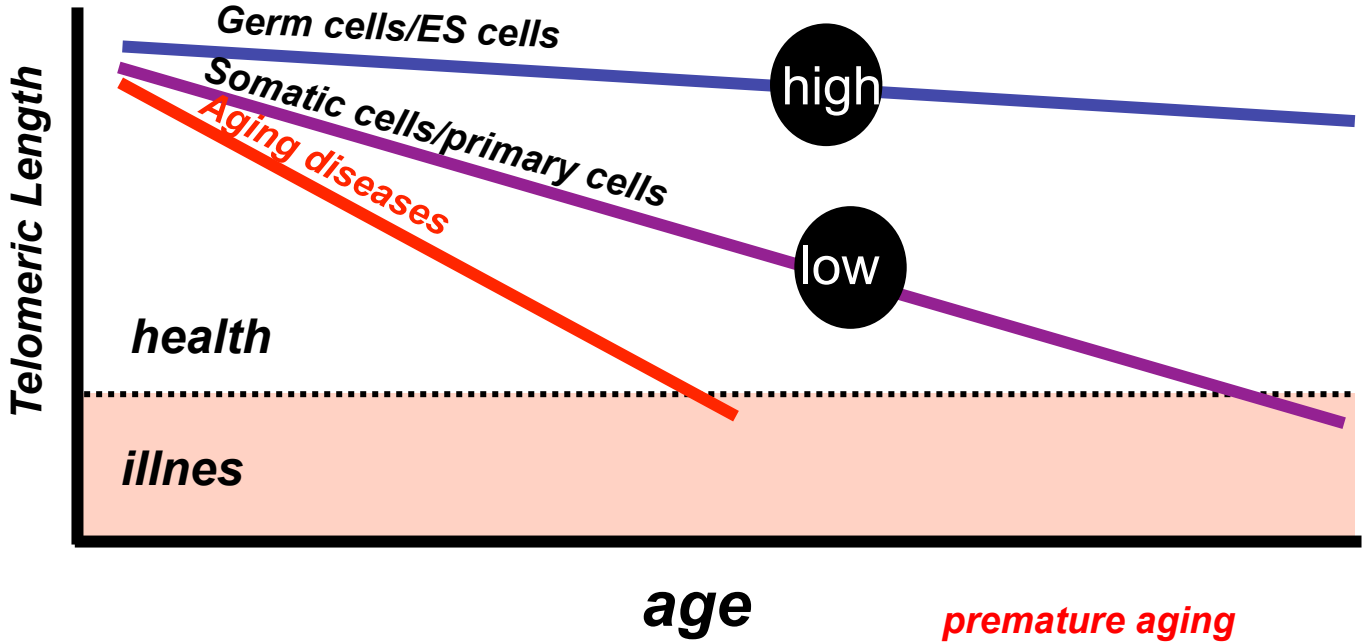

**A model for oncogene action in senescence**




Hayflick factors
Proliferative age
Telomere
γH2AX
Proliferative age
Accumulation of unrepaired DNA and chromosomal damage
γH2AX foci
Proliferative age
ARF
OFF
INK4a
ARF
ON
INK4a
DNA damage signaling cascade
ARF
INK4a
p53
p21
Rb
SENESCENCE
(also quiescence and apoptosis)

# *Relevance of senescence for cancer and aging*

# Telomerase and aging




***premature aging syndromes***

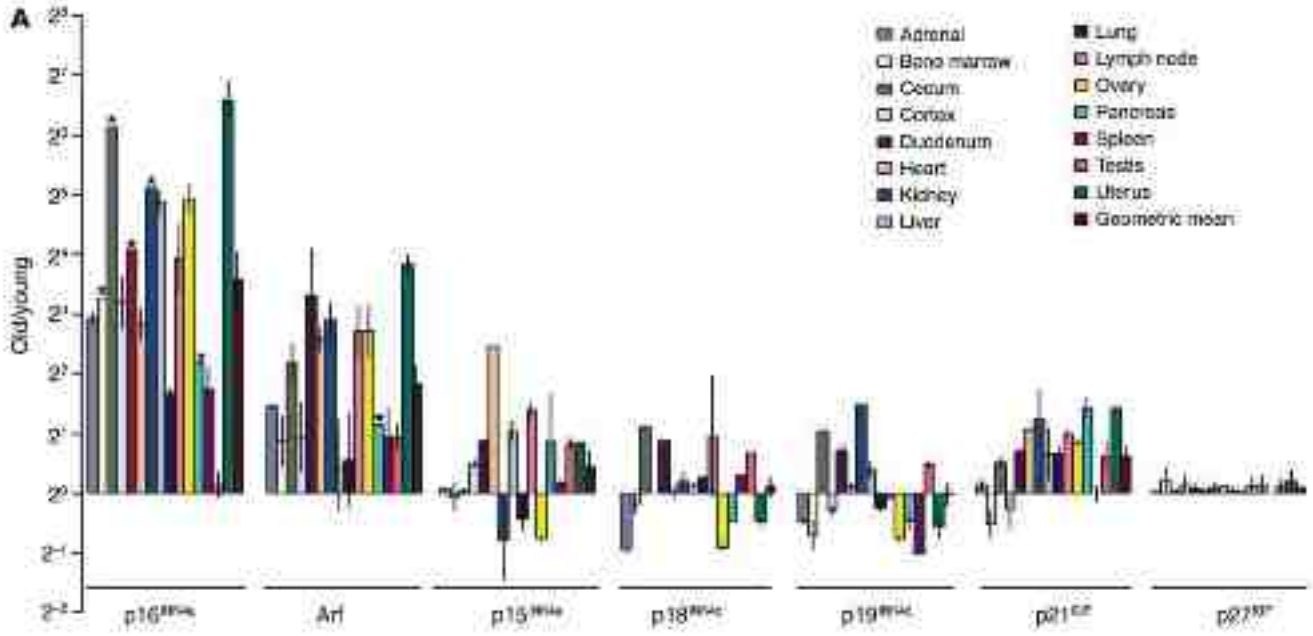
***Ataxia telangiectasia (ATM)***
***Werner syndrome (WRN)***
***Bloom syndrome (BLM)***
***Dyskeratosis congenica (DKC1, Terc)***
***Aplastic anemia (Terc, Tert)***
***Fanconi anemia (Fanc genes)***
***Nijmegen breakage syndrome (Nbs)***

***Telomerase limits life***

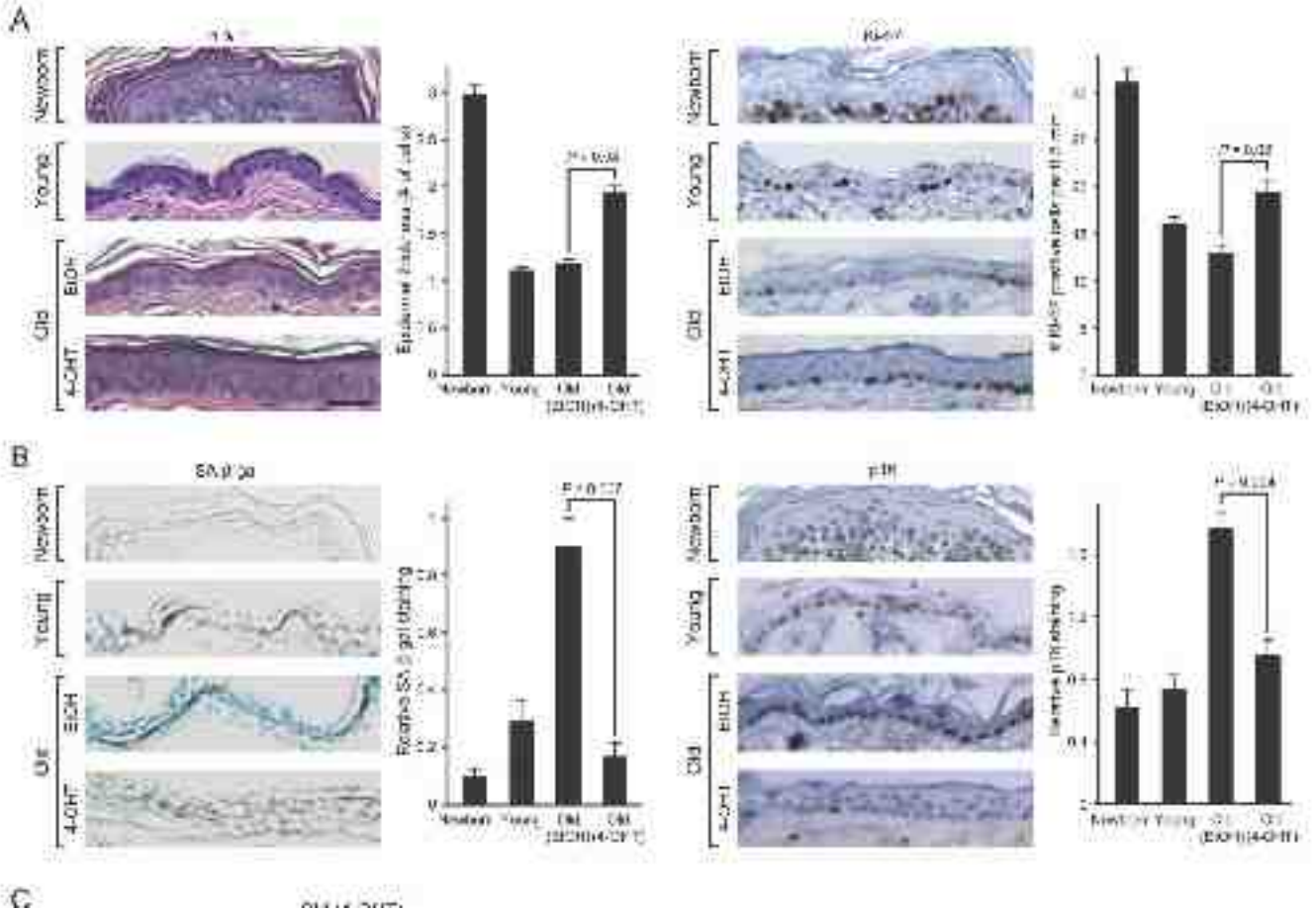
Harley, Futcher & Greider, *Nature* (1990)

# *Ink4a/Arf* expression is a biomarker of aging

Janakiraman Krishnamurthy,[1] Chad Torrice,[1] Matthew R. Ramsey,[1] Grigoriy I. Kovalev,[2]
Khalid Al-Regaiey,[3] Lishan Su,[2] and Norman E. Sharpless[1]

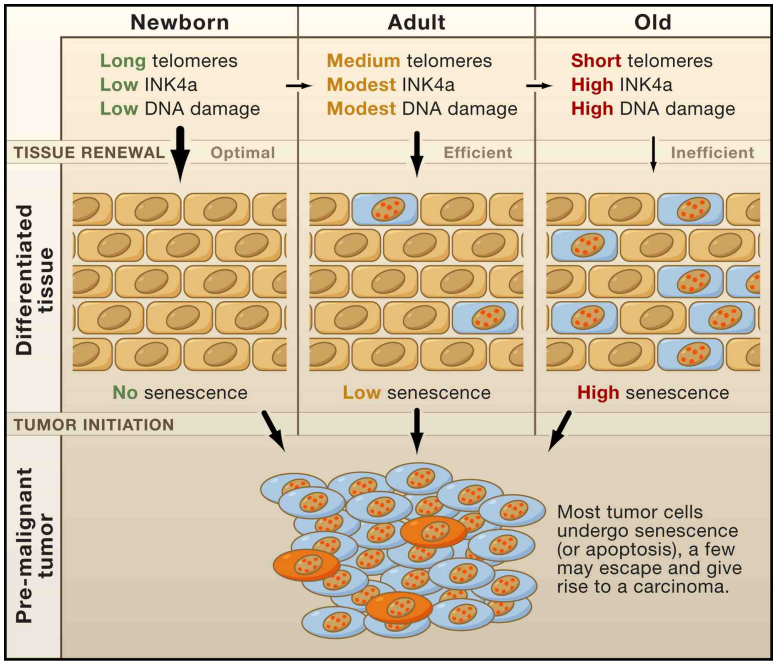


# Increase in replicative senescence during aging

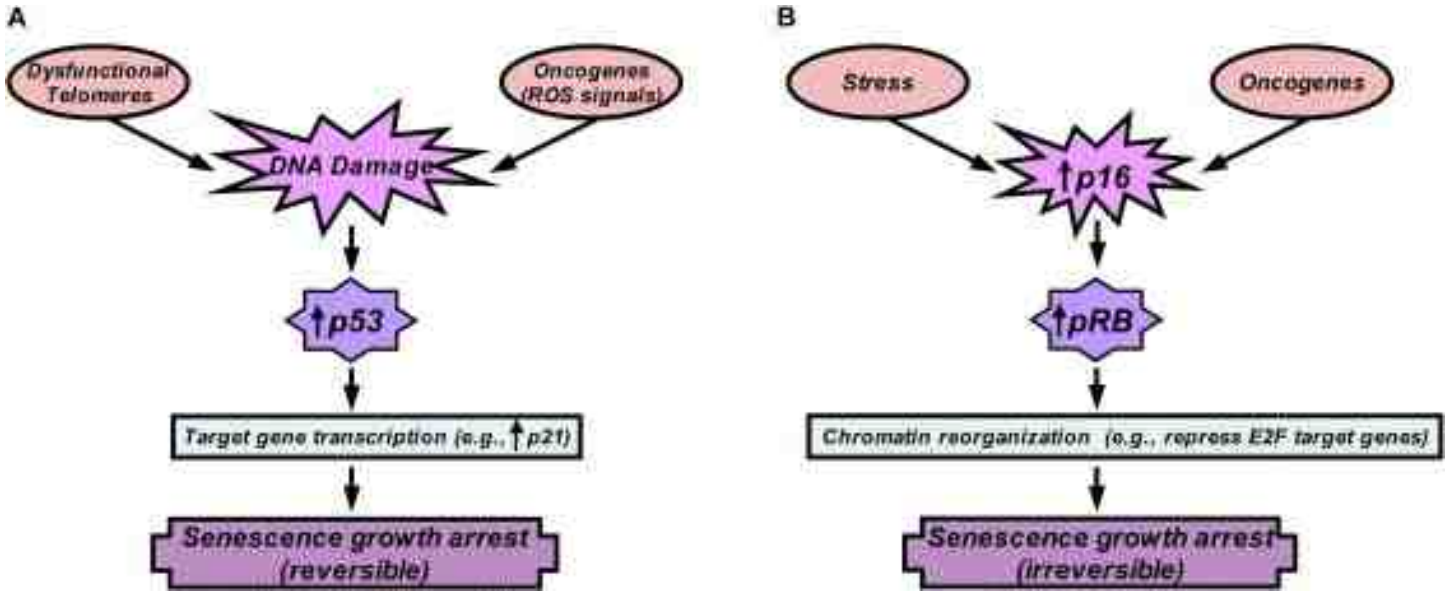


# The relation between stem cells, senescence and aging

• Bmi1 KO mice have defects in stem cell self-renewal, partially due to deregulated *Ink4a/Arf.*



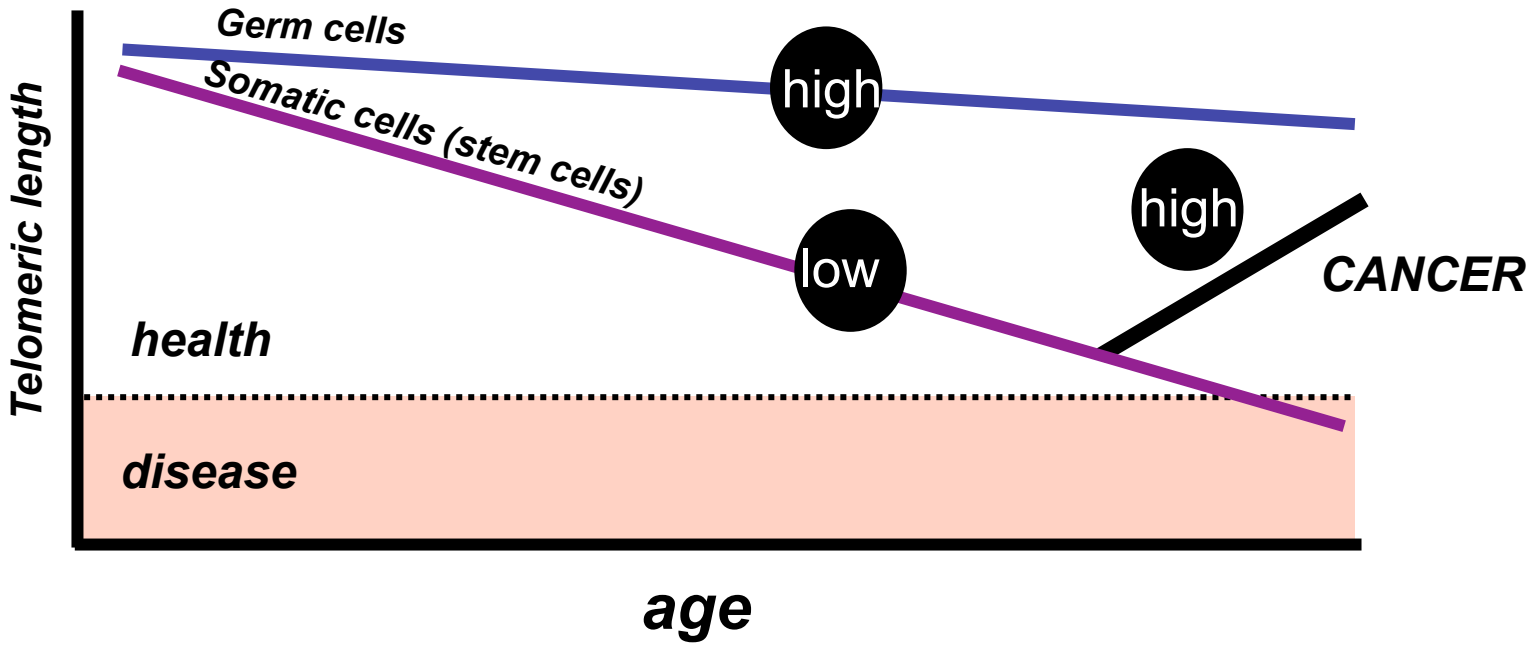
# The main tumor suppresor pathways are involved in senescence




**senescence = tumor suppression ?**

Adapted from Campisi, J. Cell Vol 120, Issue 4, 25 February 2005, Pages 513-522

# Telomerase and cancer




**senescence = tumor suppression ?**

Harley, Futcher & Greider, *Nature* (1990)

oncogene activation

Myc

RasV12

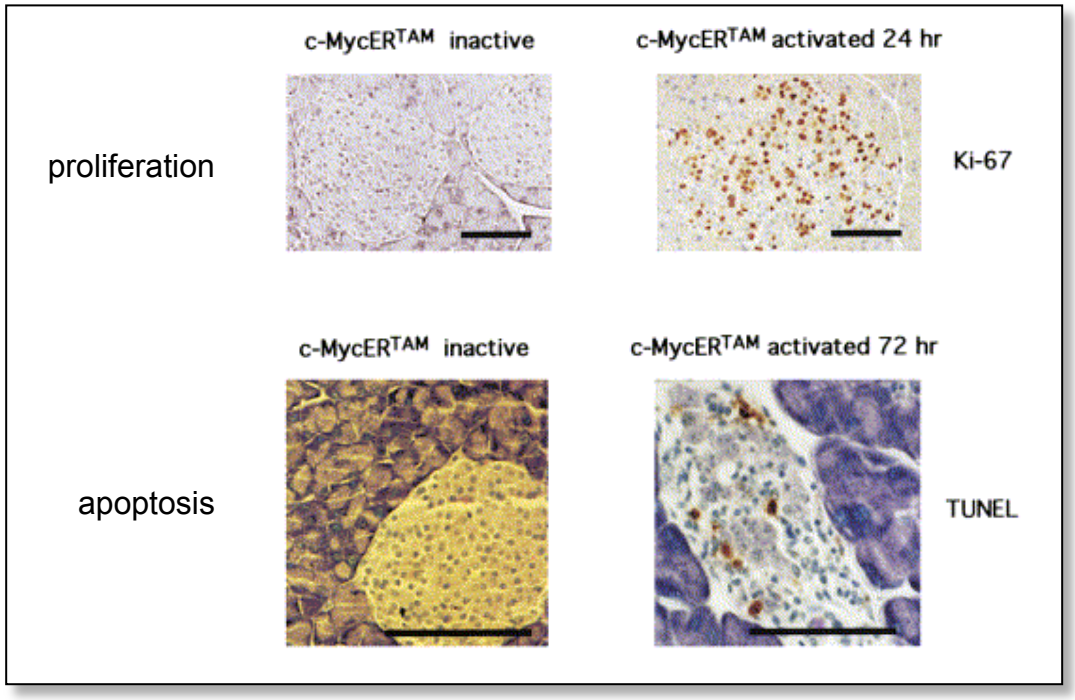
apoptosis


senescence


Tumor suppresor mechanisms

In vivo?

## “c- Myc activation in vivo induces apoptosis”

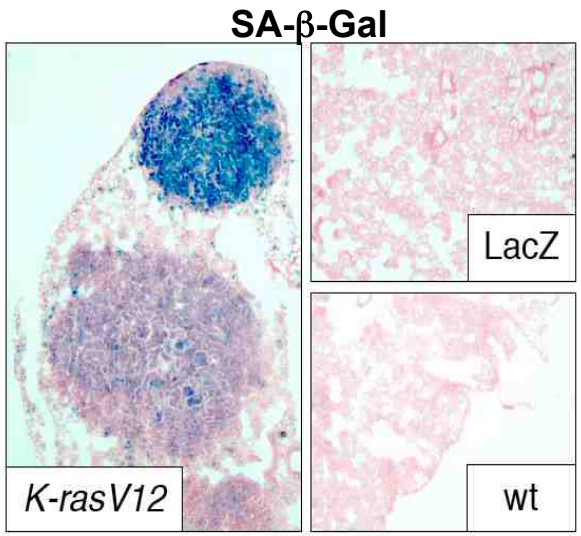



## “And apoptosis is a tumor suppressor mechanism”

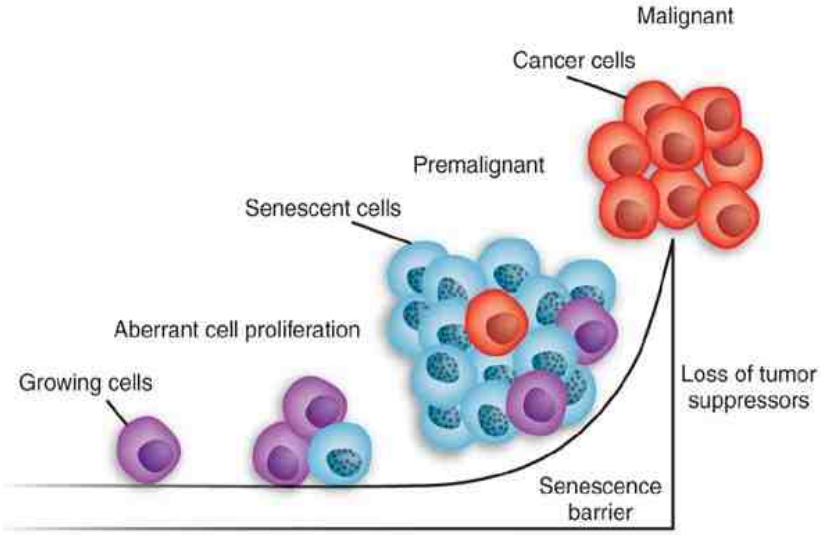
c-MycER$^{TAM}$ x BclXL-KO = ↑↑↑ tumors

Stella Pelengaris and Gerard Evan Cell 2002

# Oncogene-induced senescence is a barrier for cancer progression



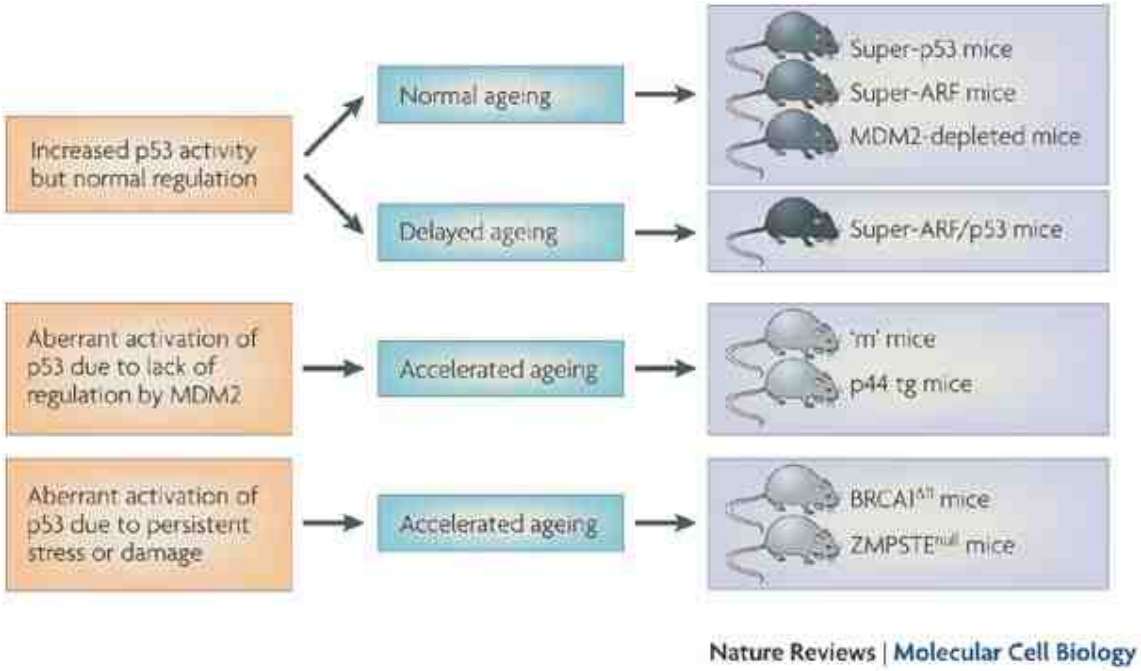

Nature (2005). **436**




Nat Med. (2005) **11**

| ***Lesion*** | ***Study*** |
|---|---|
| | |
| Human melanocytic nevi | Michaloglou et al., 2005; Gray-Schopfer et al., 2006 |
| Murine lung adenomas | Collado et al., 2005; Dankort et al., 2007 |
| Human dermal neurofibromas | Courtis-Cox et al., 2006 |
| Human and murine prostate PIN lesions | Chen et al., 2005 |
| Murine pancreatic intraductal neoplasias | Collado et al., 2005 |
| Murine papillomas | Collado et al., 2005 |
| Murine lymphomas | Braig. et al., 2005 |
| Early murine  melanomas | Ha et al., 2007 |

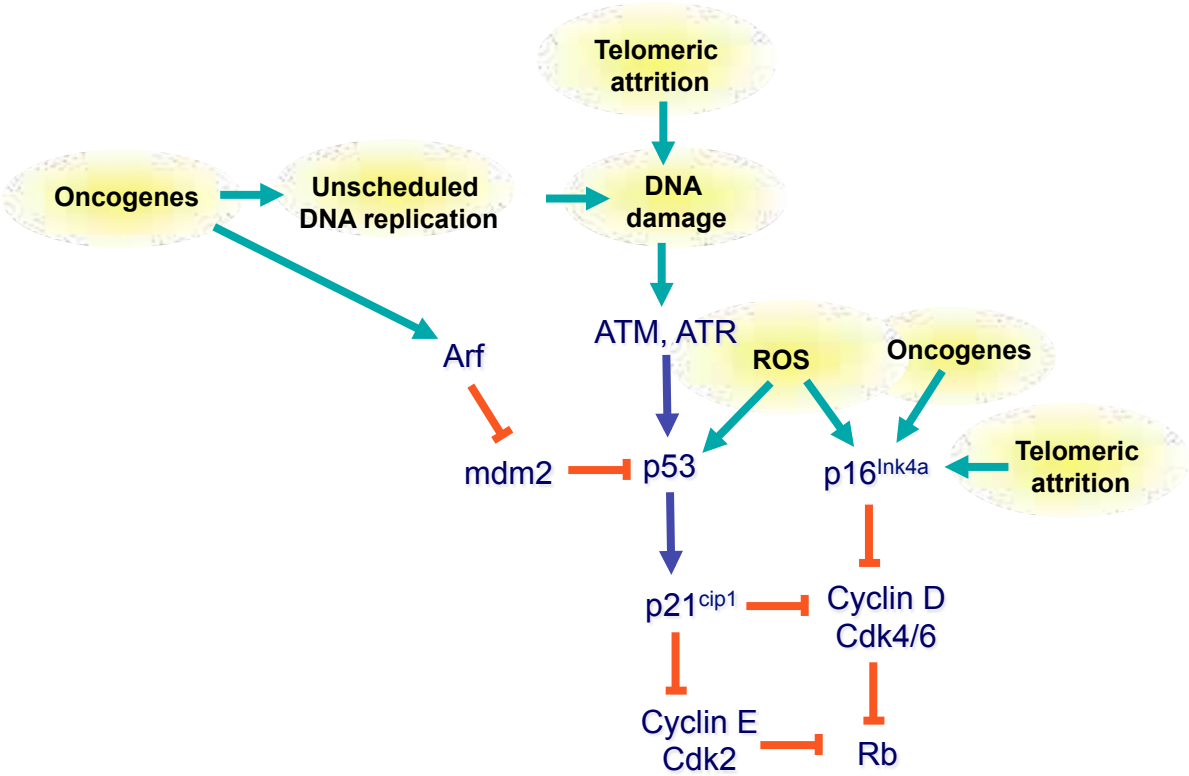
# Mouse models show a role for p53 in aging




Nature Reviews | Molecular Cell Biology

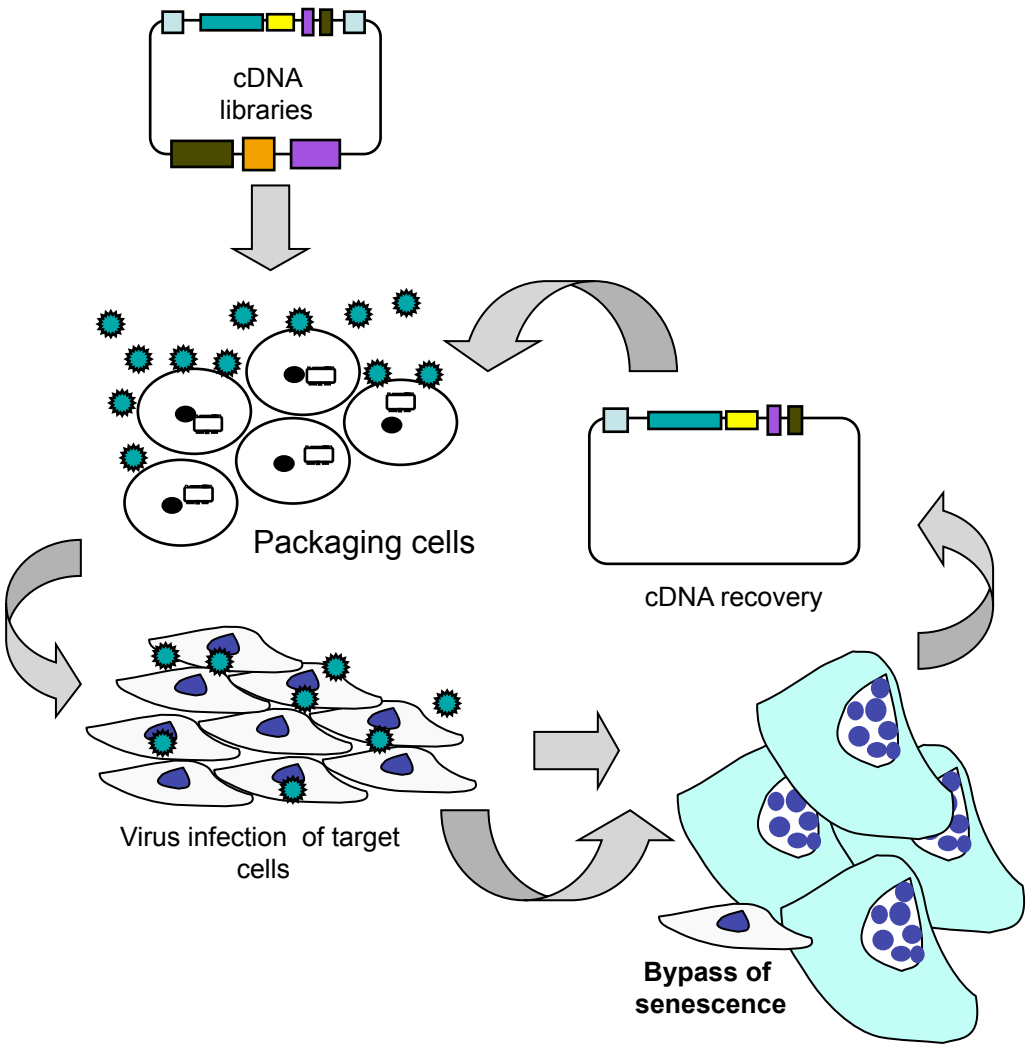
***What we do in our lab?***

*Senescence can be exploited to identify novel cancer genes in vitro*

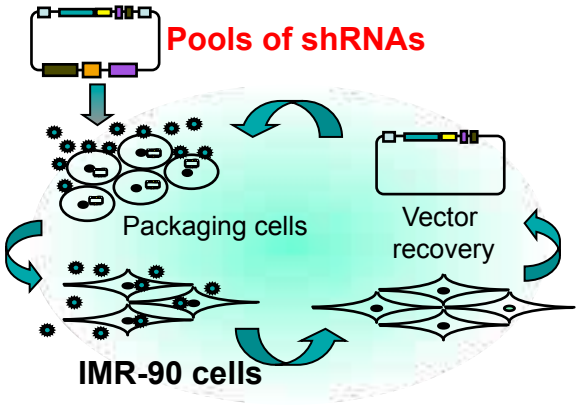


# *Unveiling novel regulators of senescence using genetic screenings*

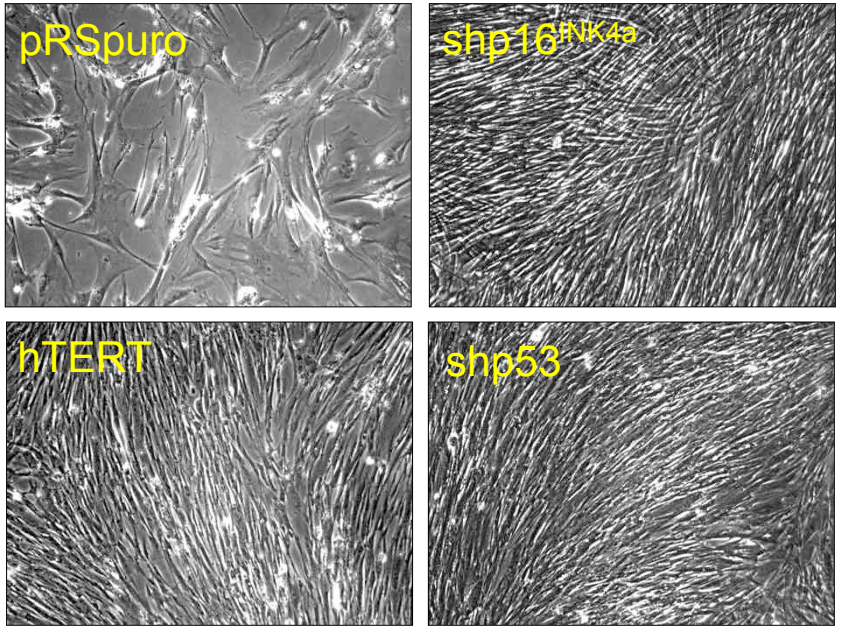
# Genetic screens for lifespan extension

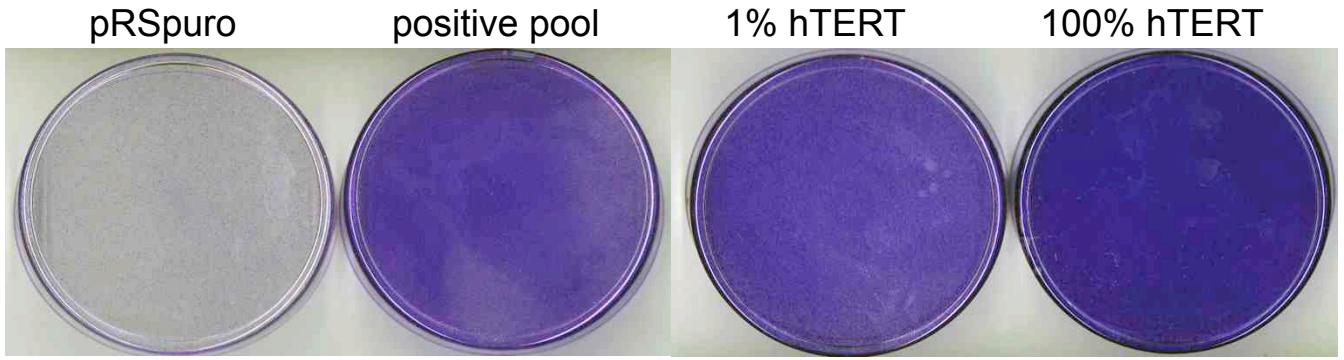


# Genetic screens for bypass of replicative senescence




- NKI pRETROSUPER library




shRNAs identified so far

p53

Rb

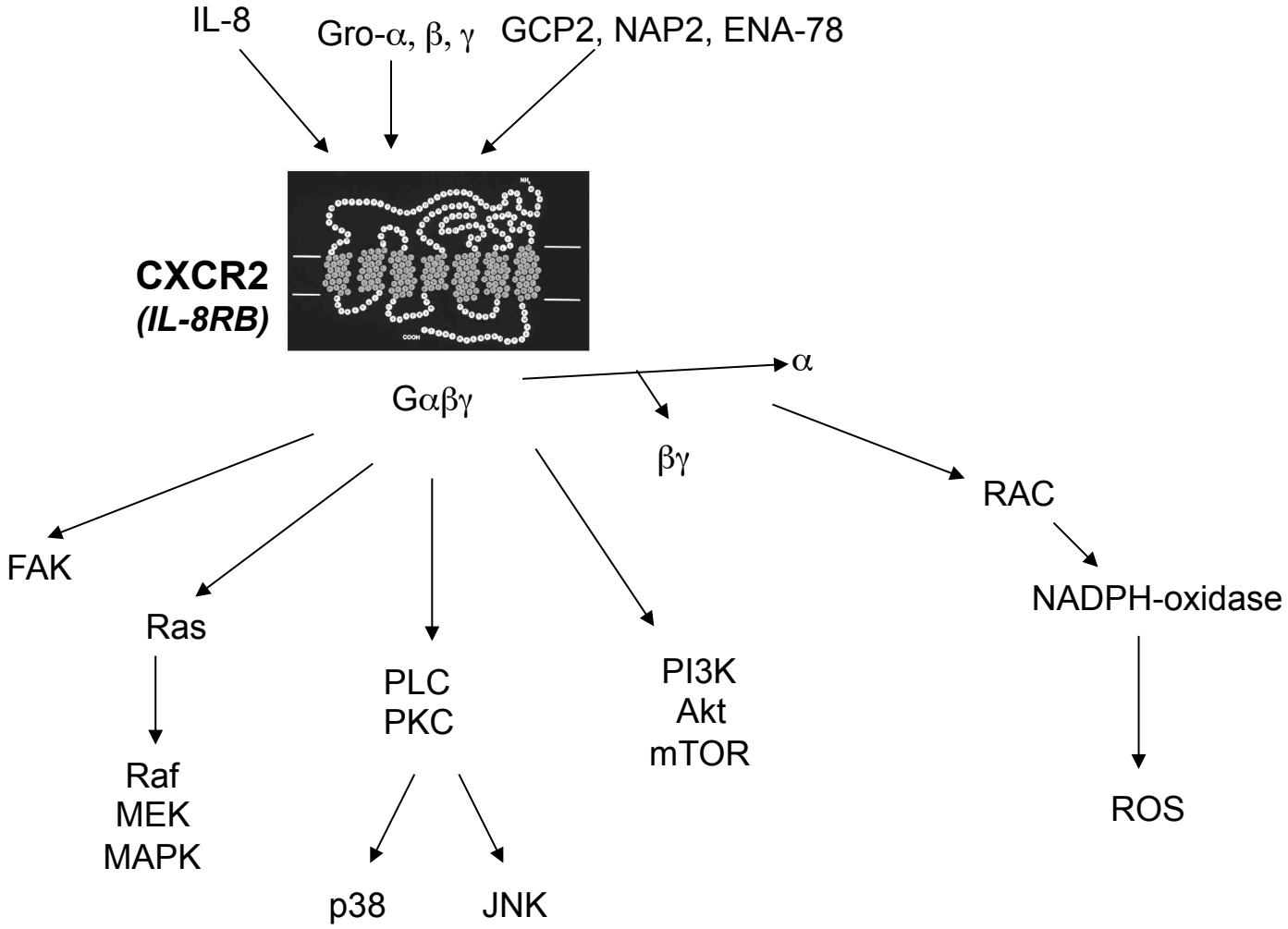
ARK5 (EMBO J, 2009 in press)

TOP1 (Cancer Res., 2009)

PLA2 (EMBO Rep, 2009)

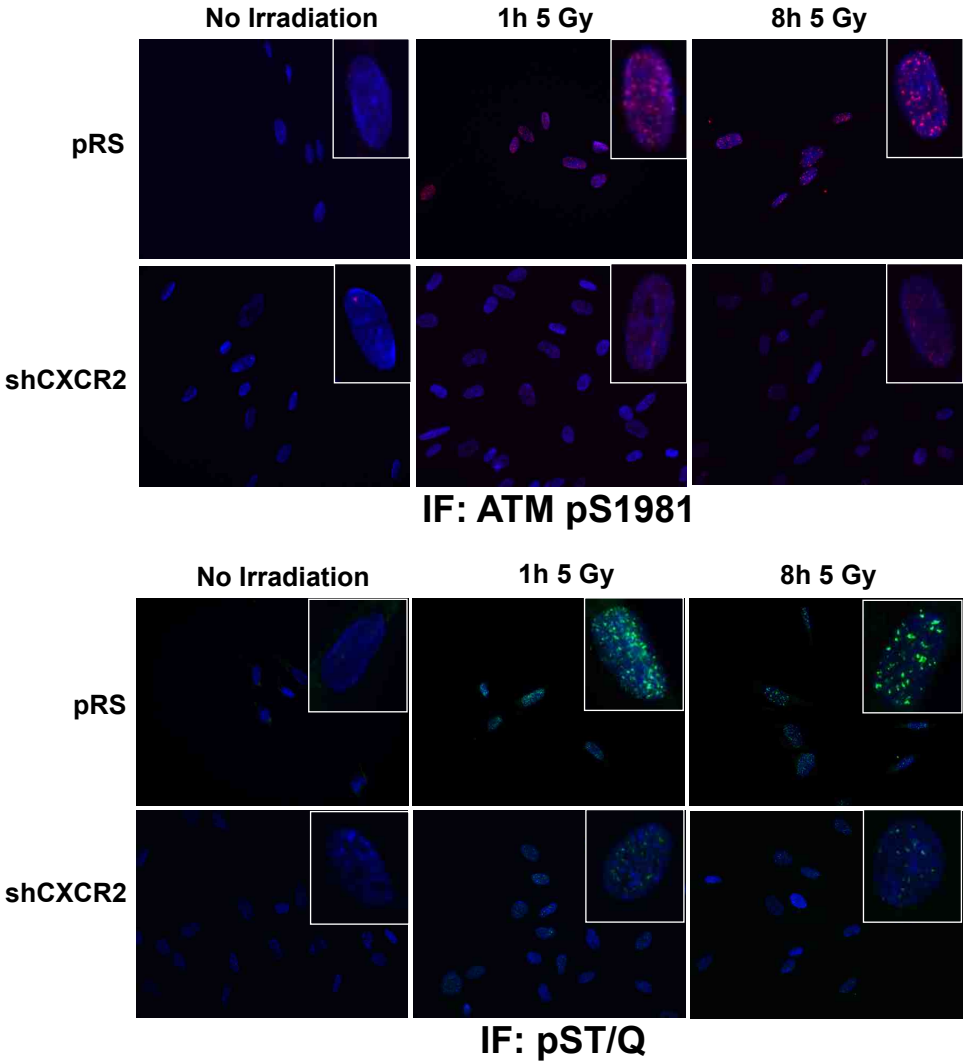
***CXCR2*** (Cell, 2008)

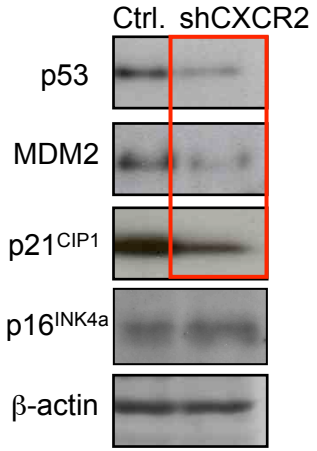
# CXCR2 signaling




Senescence ?

# CXCR2 depletion impairs the activation of the DNA damage response



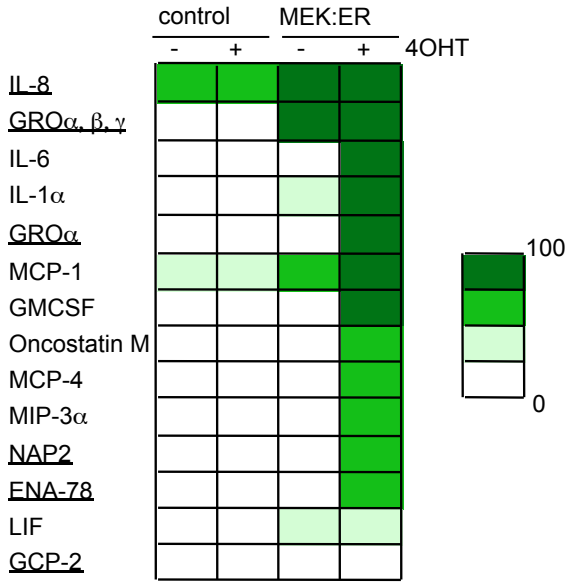




Marzia Fumigalli (d'Ada di Fagagna's lab)

Acosta et al. Cell, 2008

# Coordinated upregulation of CXCR2 and its ligands in senescence

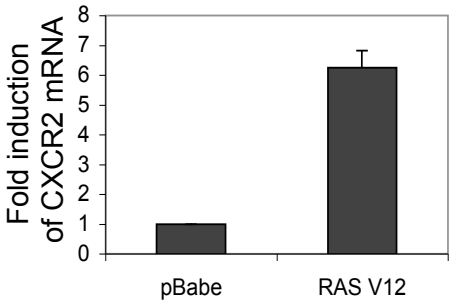
**CXCR2 ligands**

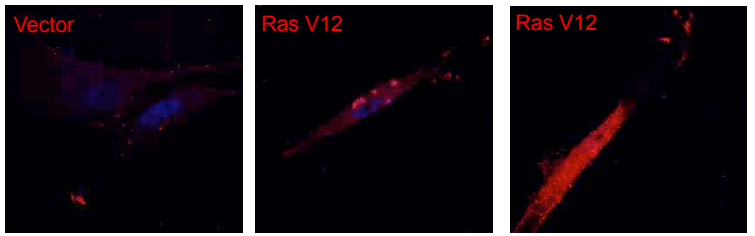



**Antibody arrays**

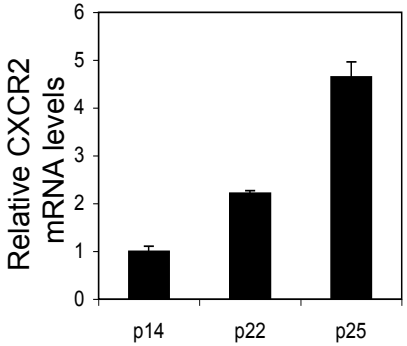
**CXCR2**

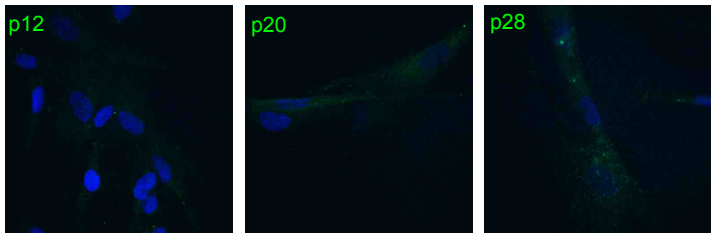
Oncogene-induced senescence



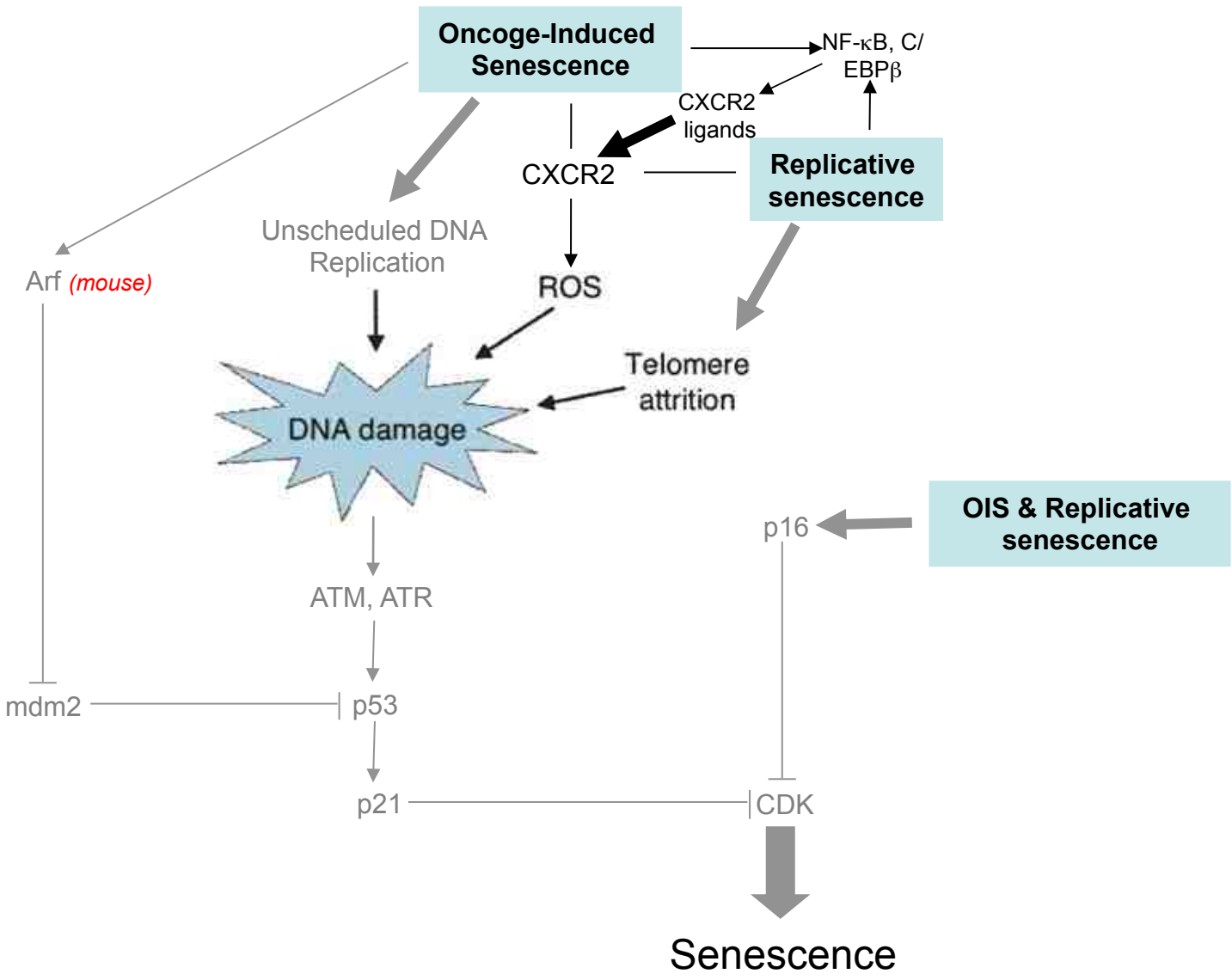




Replicative senescence



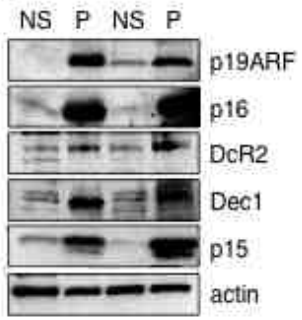



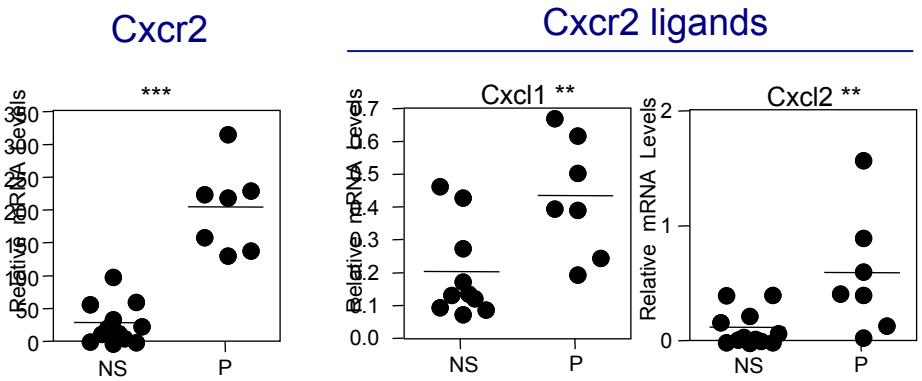

Acosta et al. Cell, 2008


Oncoge-Induced Senescence
NF-κB, C/EBPβ
CXCR2 ligands
CXCR2
Replicative senescence
Unscheduled DNA Replication
Arf (mouse)
ROS
Telomere attrition
DNA damage
OIS & Replicative senescence
p16
ATM, ATR
mdm2
p53
p21
CDK
Senescence

# Upregulation of CXCR2 in senescent premalignant lesions

## DMBA/TPA: skin papillomas

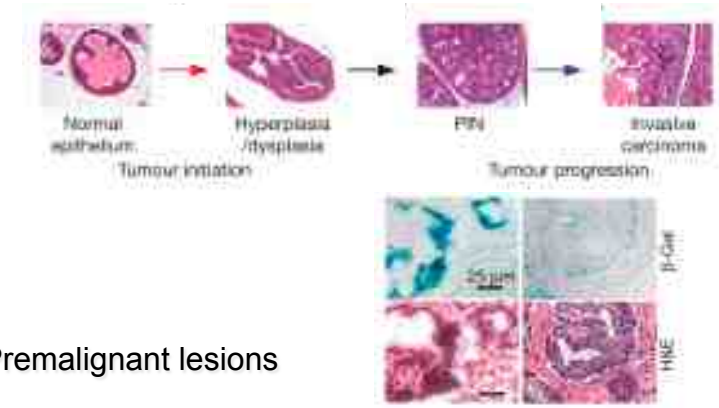



- Premalignant lesions
- 100 % Activating H-Ras mutations

Collado *et al.* Nature (2005)

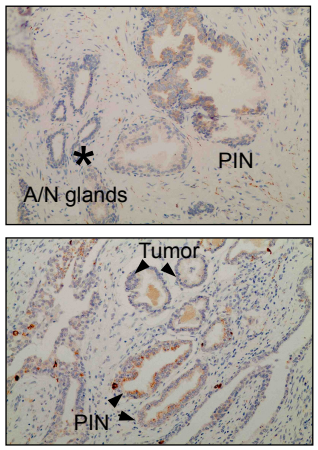
## Prostate intraepithelial neoplasia (PIN)

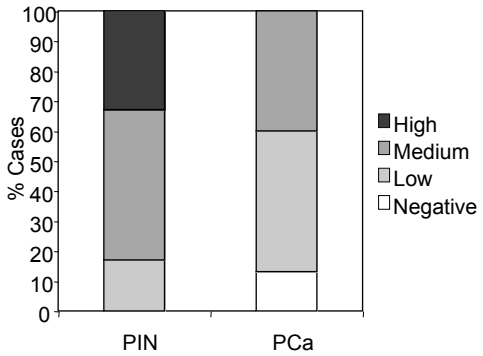



- Premalignant lesions
- Precursor of prostate cancer (Pca)

Chen *et al.* Nature (2005)

## CXCR2




Maria Guijarro (Eva Hernando's lab.)

***In which other context is senescence relevant?***

# Induced pluripotent stem cells: the Yamanaka factors

## Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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DOI 10.1016/j.cell.2006.07.024

## Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]
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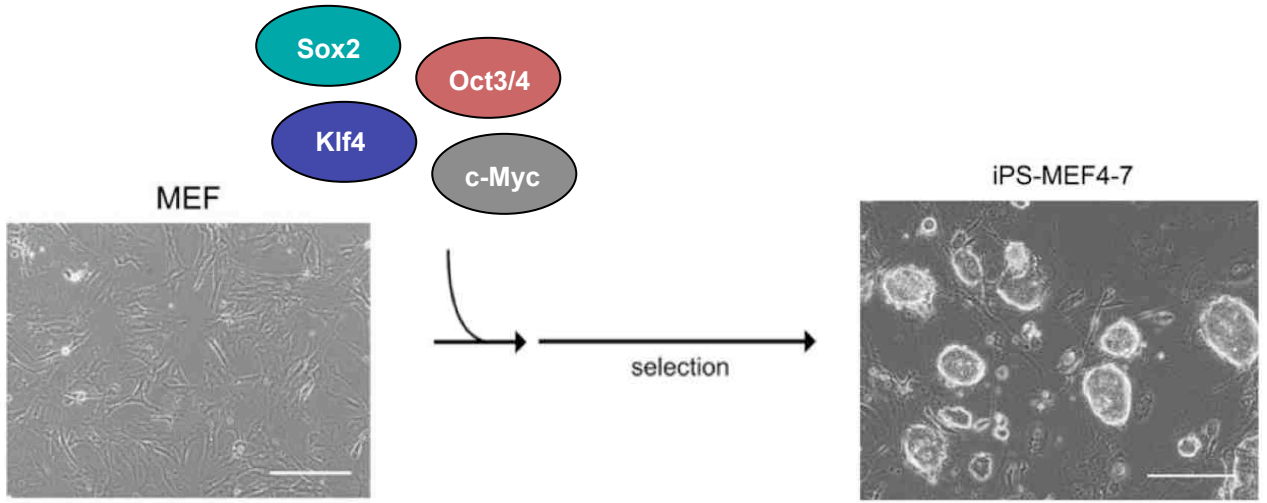
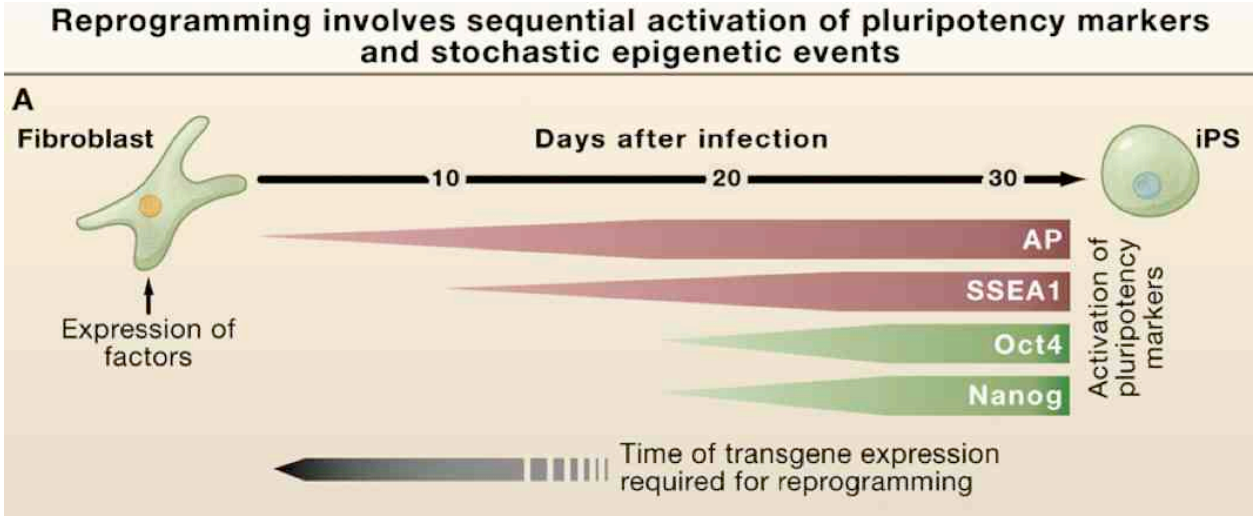
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019




**MEFs**

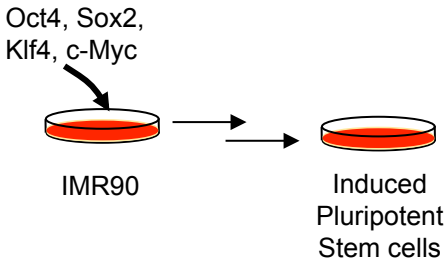
**IPS** (Induced Pluripotent Stem) Cells

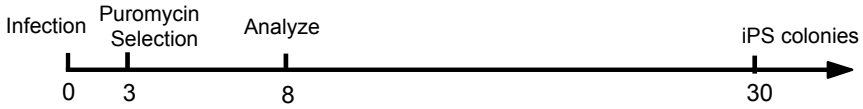
# Reprogramming: complex, slow, inefficient and dangerous



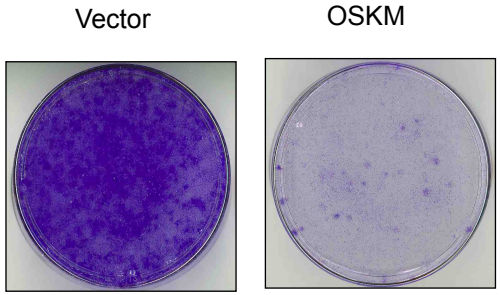

- **Complex** involves several steps, including chromatin modifications and global expression patterns alterations
- I**nefficient** and **slow,** has to overcome several barriers
- The mechanisms behind the switch to pluripontency/self-renewal remain mostly unknown

# Expression of reprogramming factors induces senescence

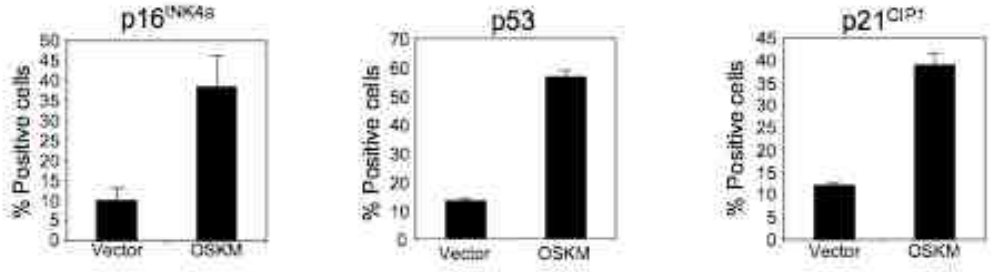



**Reprogramming factors cause growth arrest**




**Induction of effectors of senescence**

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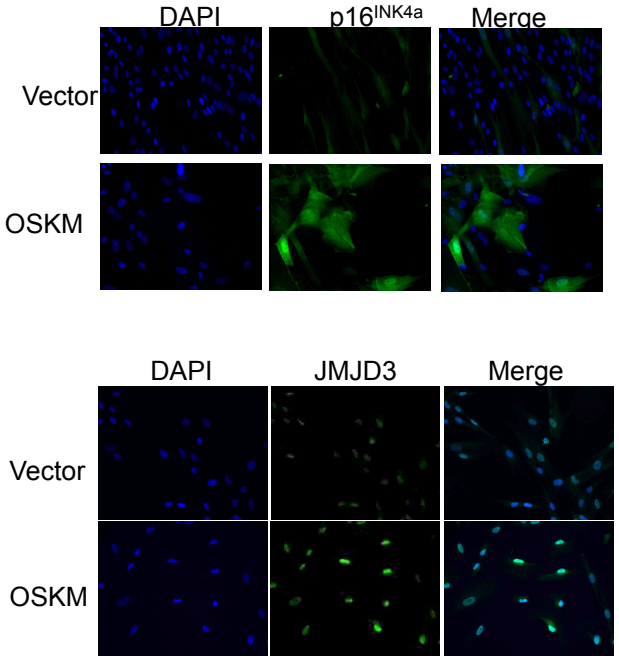
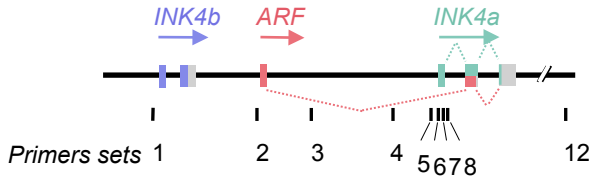
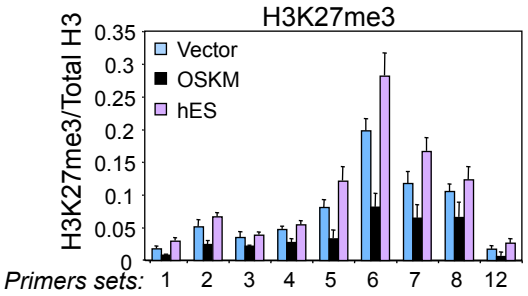
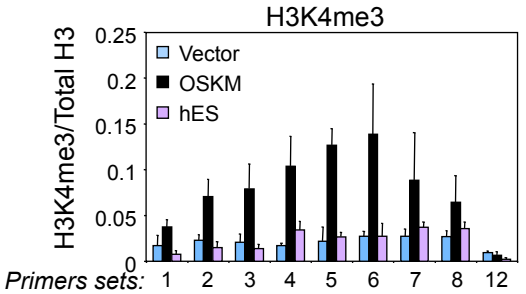
# Chromatin remodeling of the *Ink4a/Arf* locus during reprogramming

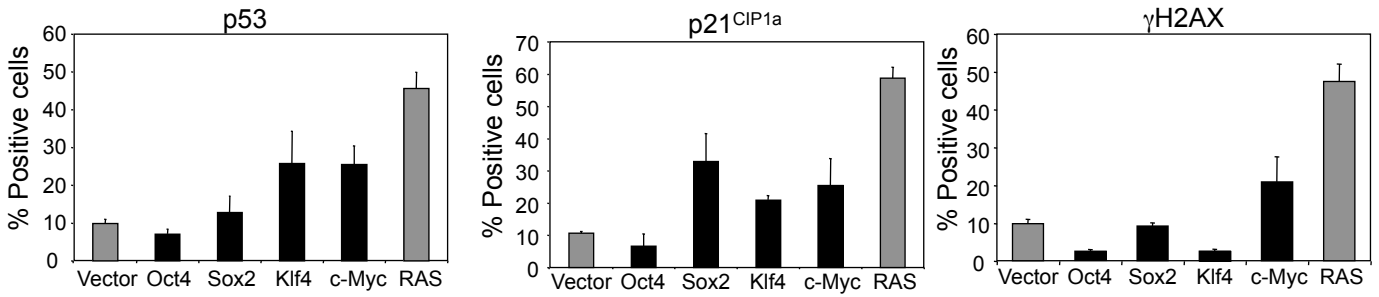


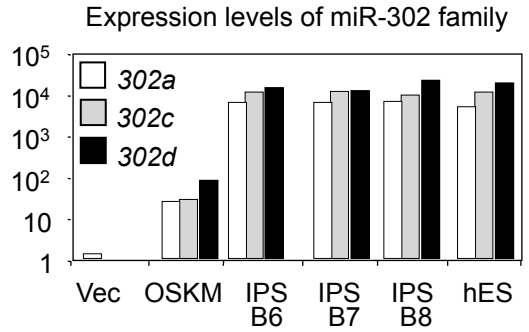



# Activation of the p53/p21 pathways at multiple points during RIS

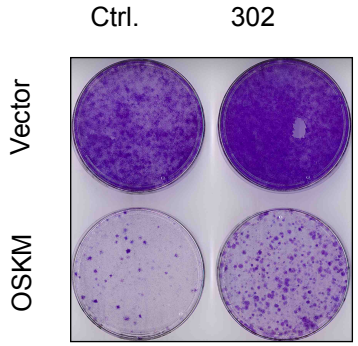


# Pluripotency-related miRNAS inhibit senescence

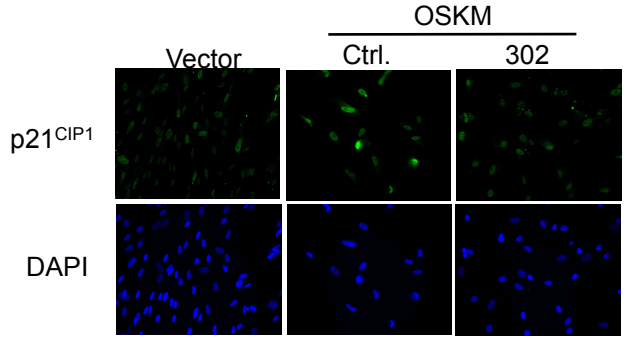
**Low expression of pluripotency-related miRNAs in cells suffering RIS**




**Partial bypass of senescence by forced expression of miR-302**




**Regulation of p21 by miR-302**

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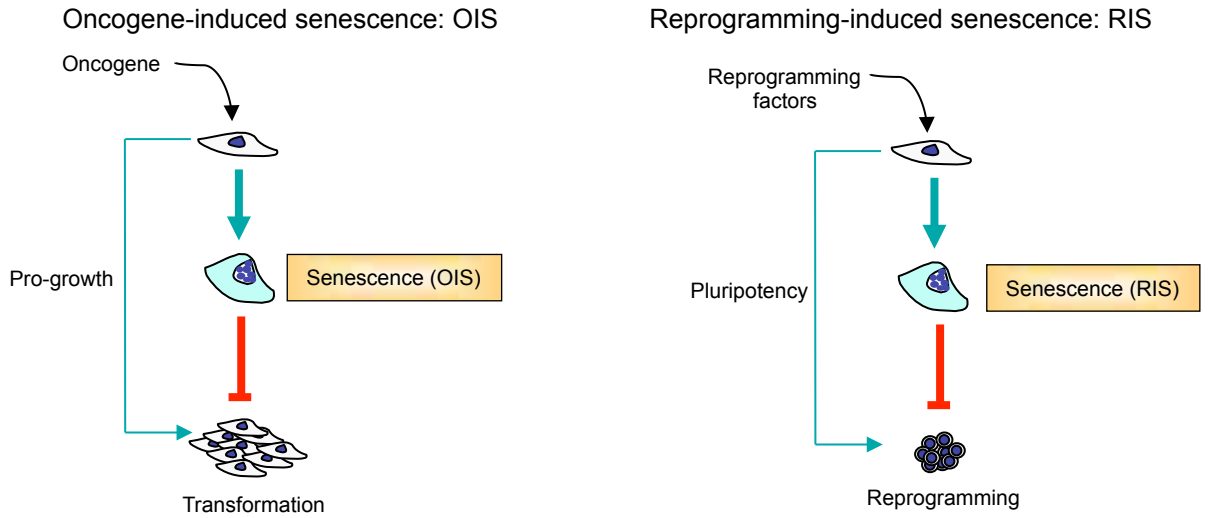
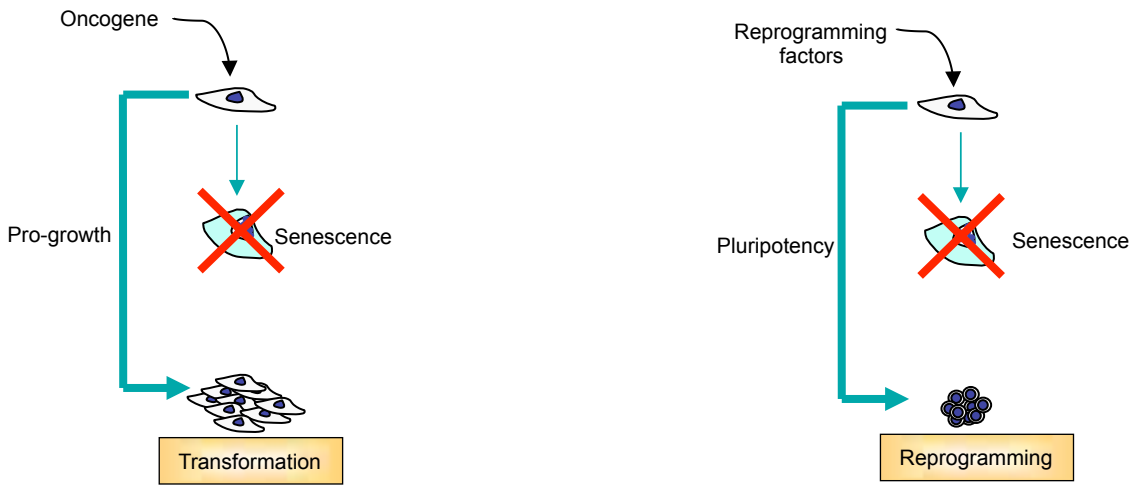
# Parallels between oncogene and reprogramming-induced senescence

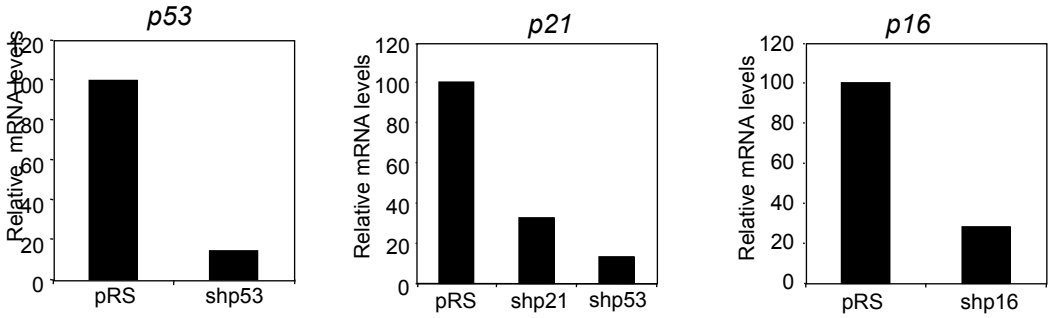



***Senescence disabled***

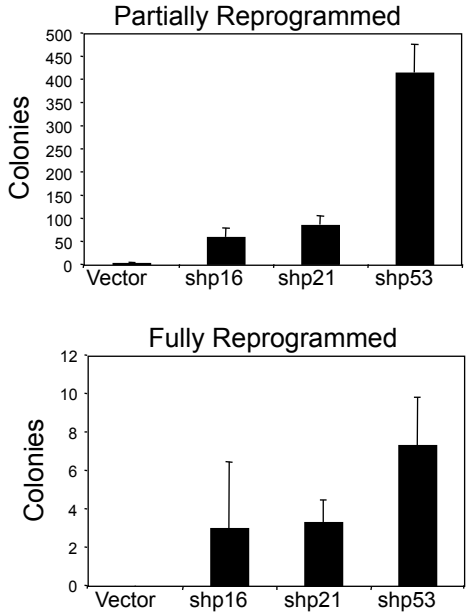


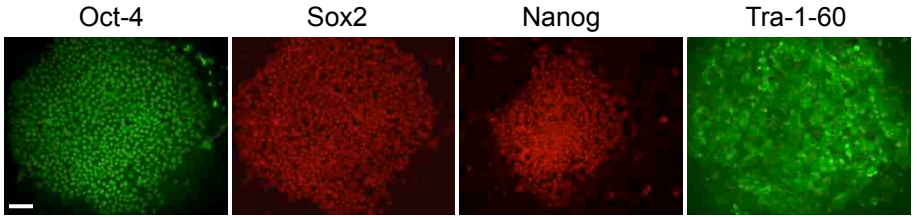
# Inhibition of senescence increases reprogramming efficiency

**Knocking down senescence effectors with shRNA**




**Increase in reprogramming efficiency**

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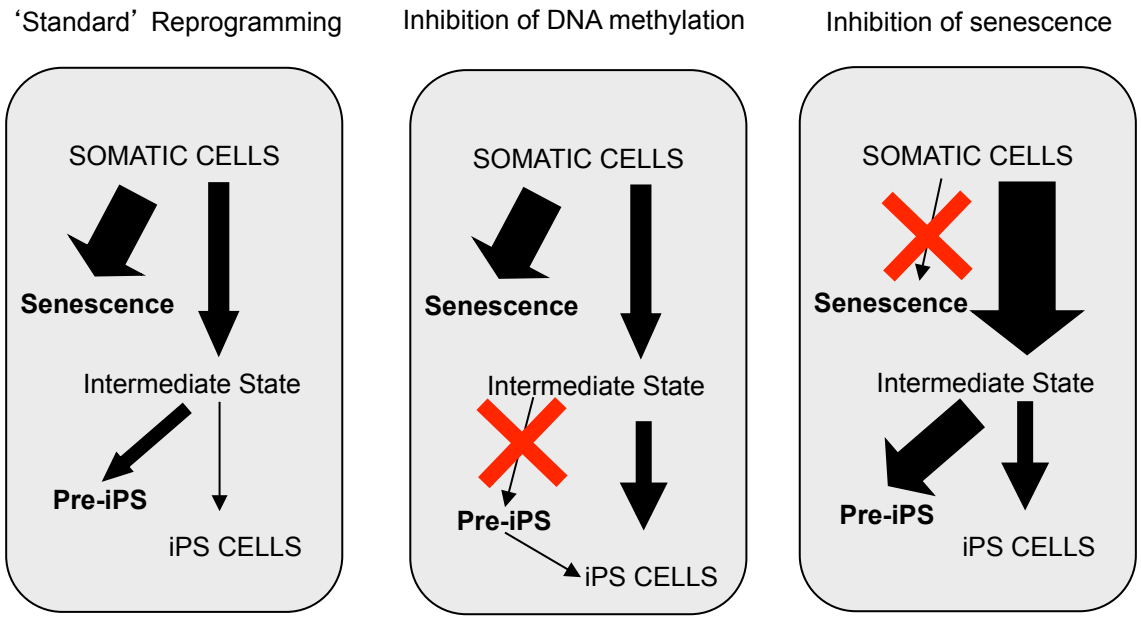
# Improving reprogramming from the technical point of view

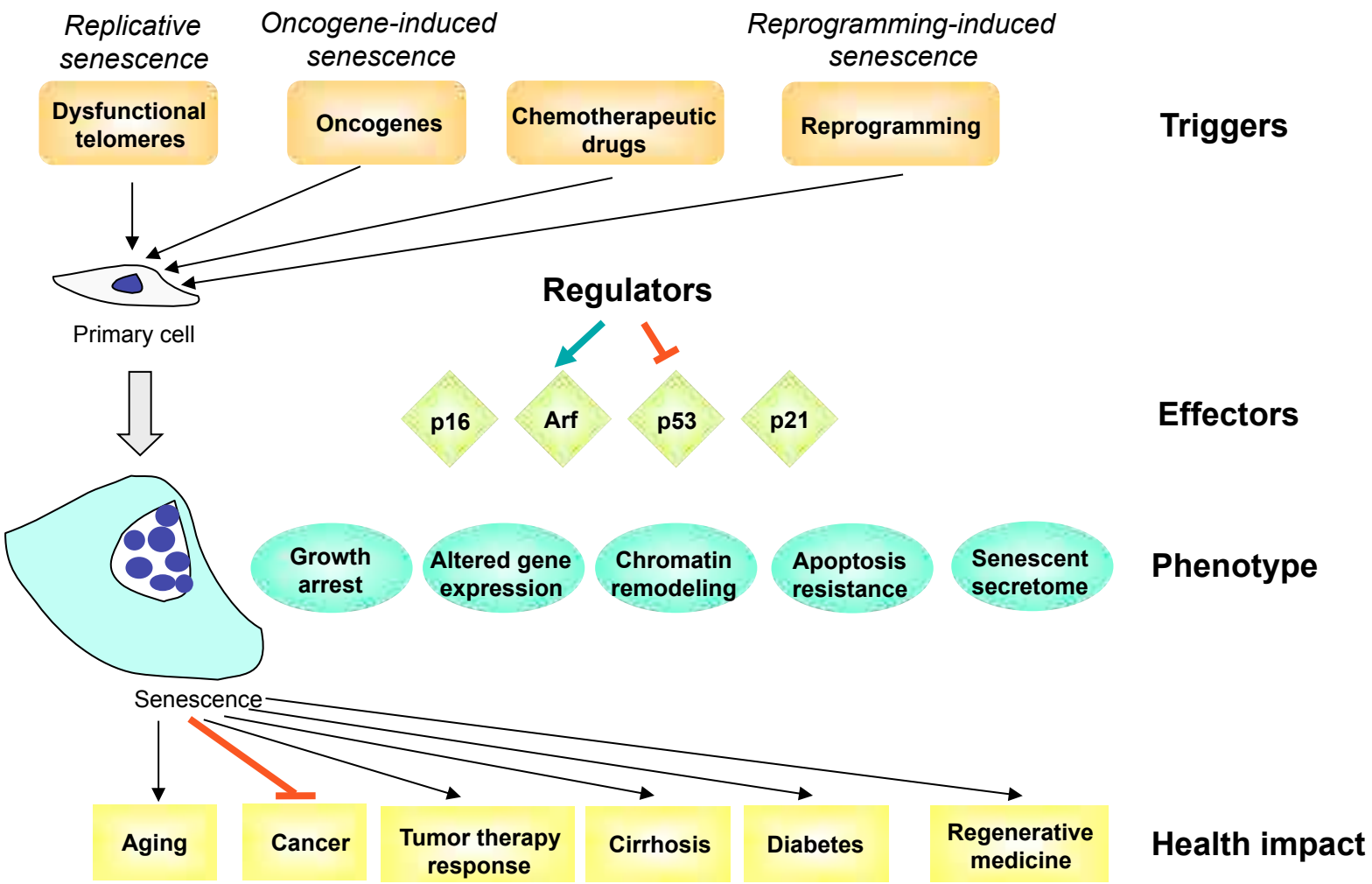



Low efficiency

**Increased proportion of Fully vs. Partial reprogrammed iPS**

**Increased numbers of both Fully and Partial reprogrammed iPS**

# Senescence and its physiological relevance



MRC Medical Research Council

MRC Clinical Sciences Centre
Cell Proliferation GROUP
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*http://cellpro.csc.mrc.ac.uk*

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