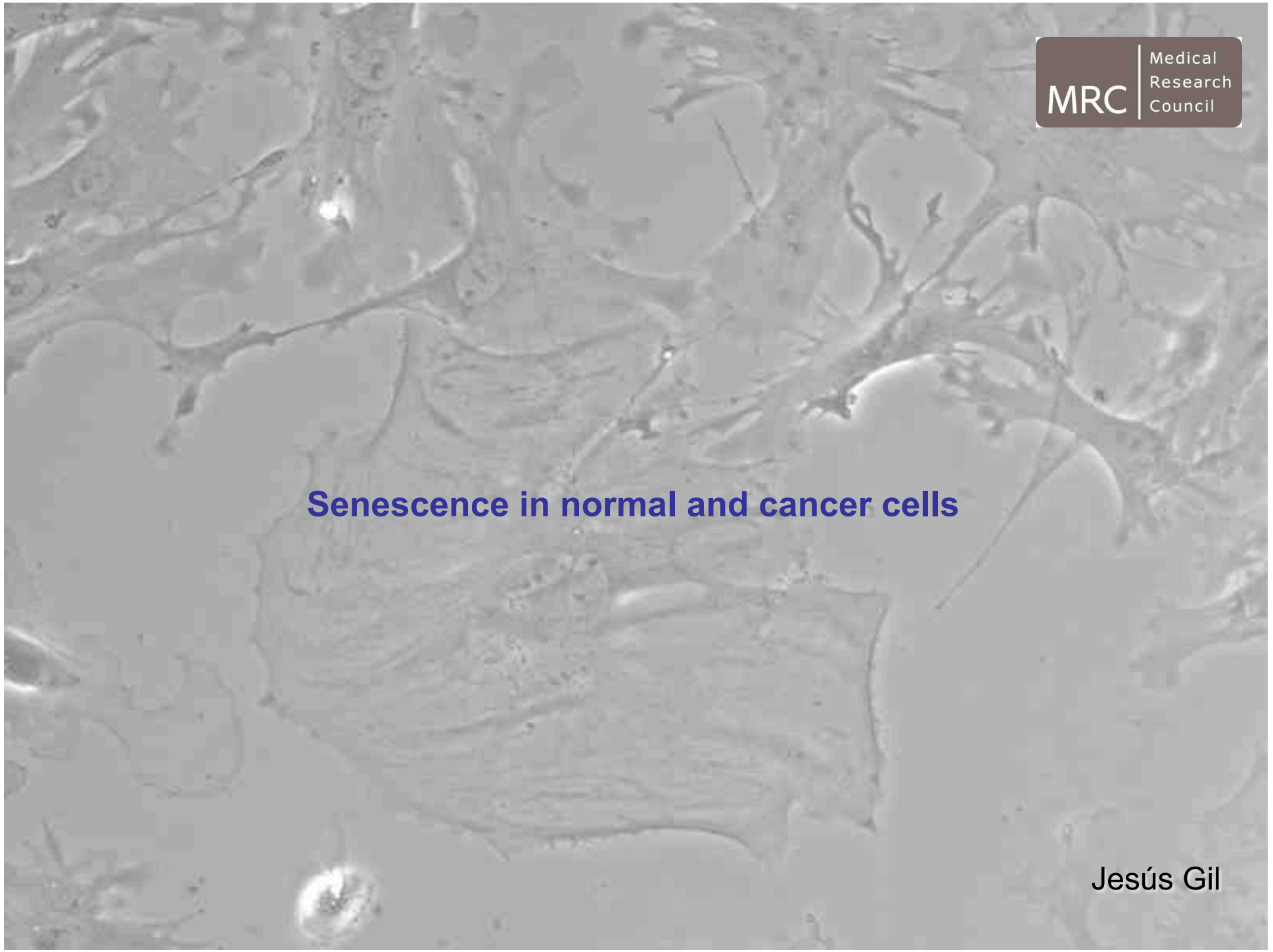
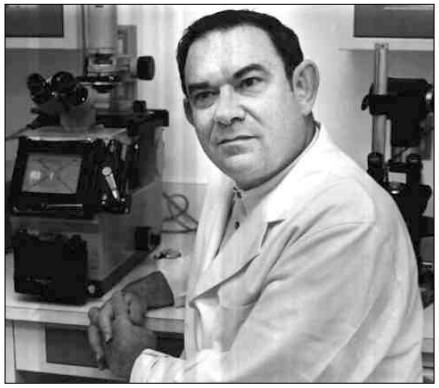

MRC
Medical
Research
Council
Senescence in normal and cancer cells
Jesús Gil

Introduction to senescence and the molecular mechanisms controlling it.

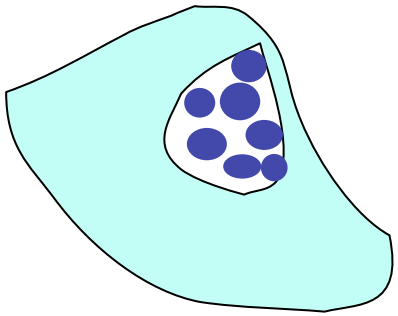
Senescence is an irreversible growth arrest



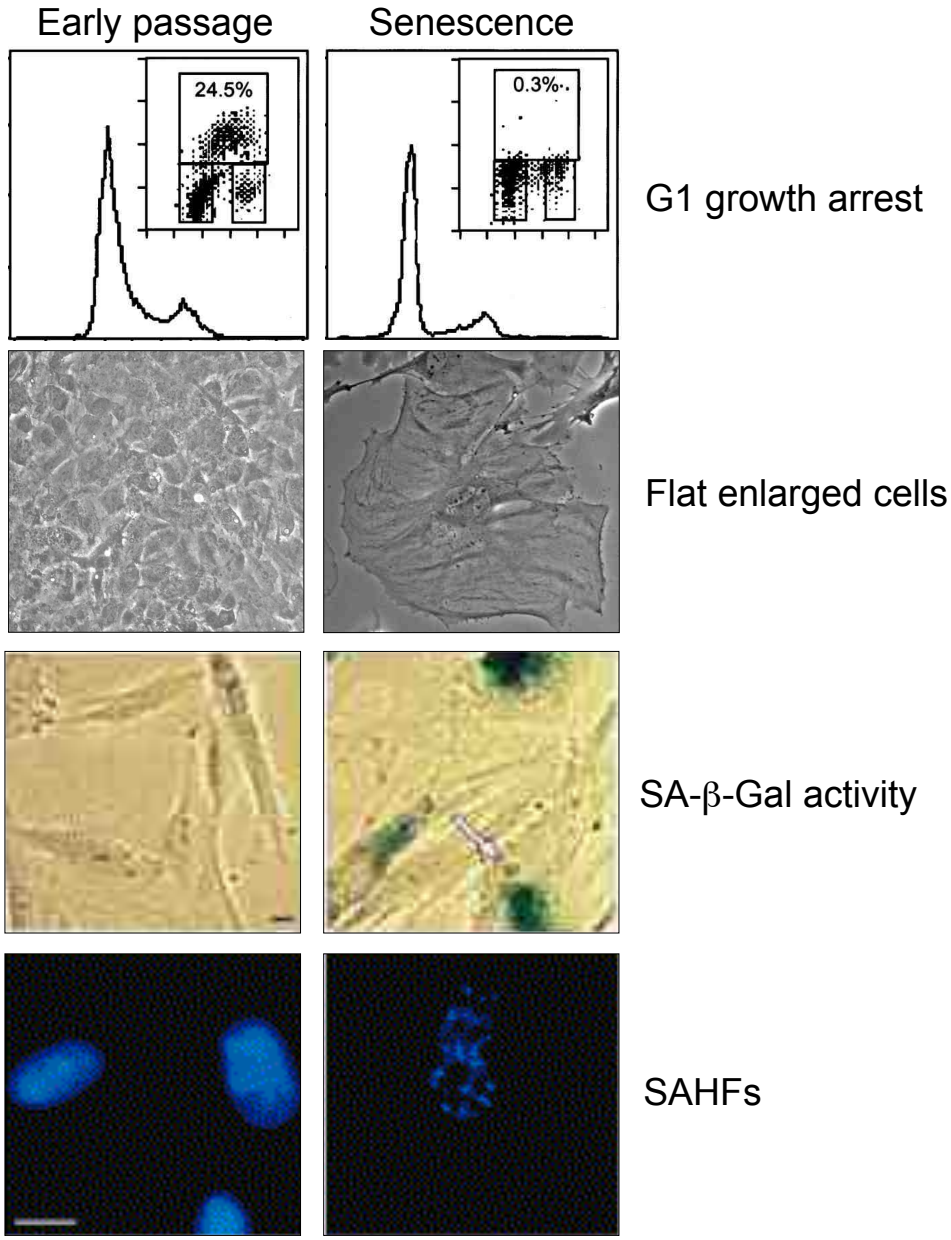
Leonard Hayflick

Primary cell

Replicative exhaustion



Senescent cell



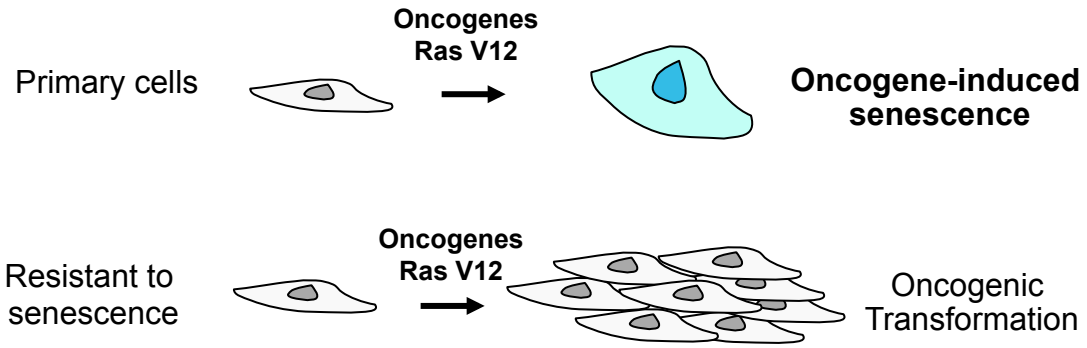
Senescence comes in different flavours: Oncogene-Induced Senescence

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

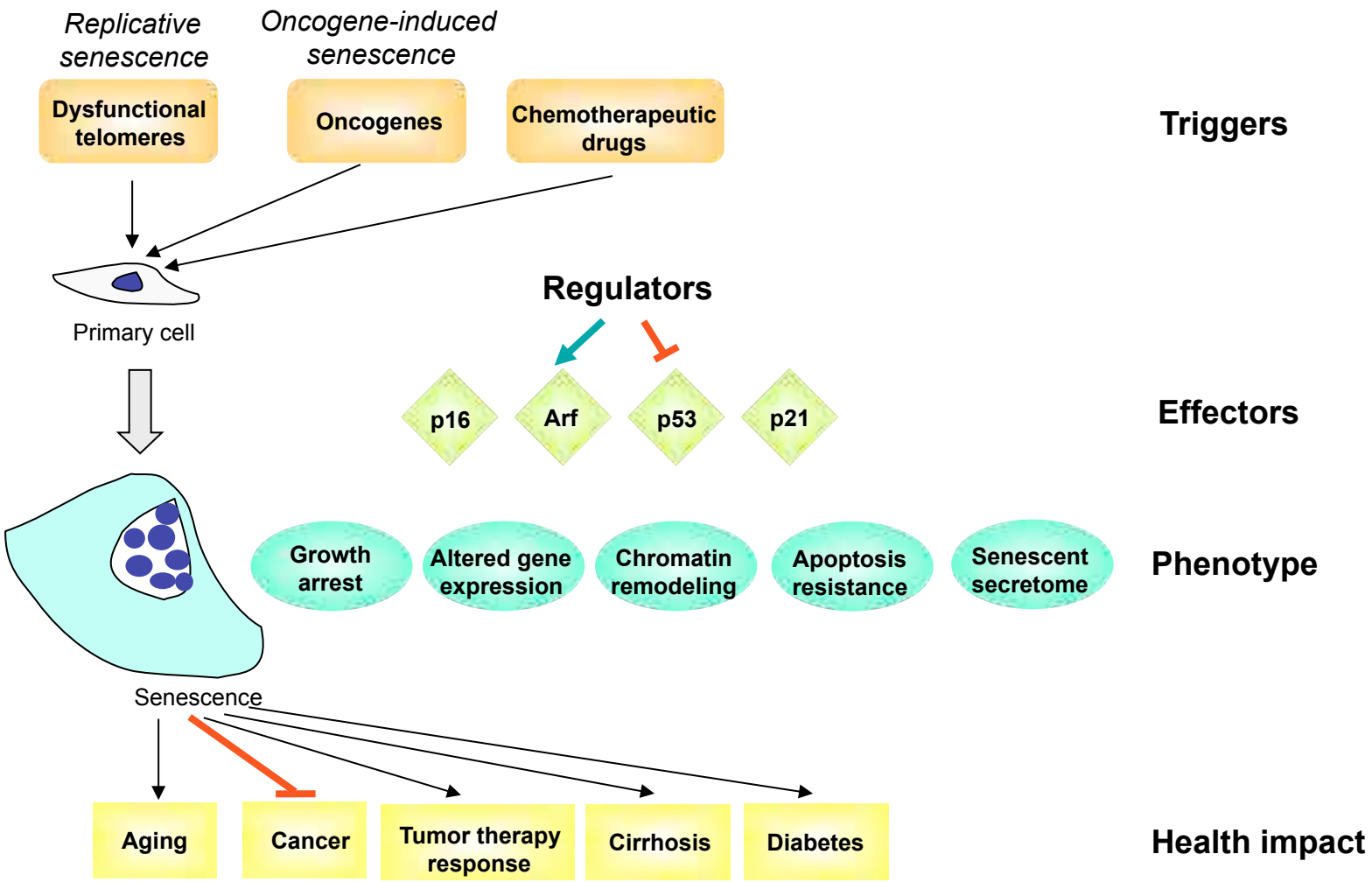
Oncogenic *ras* Provokes Premature Cell Senescence Associated with Accumulation of p53 and $p16^{INK4a}$

Manuel Serrano

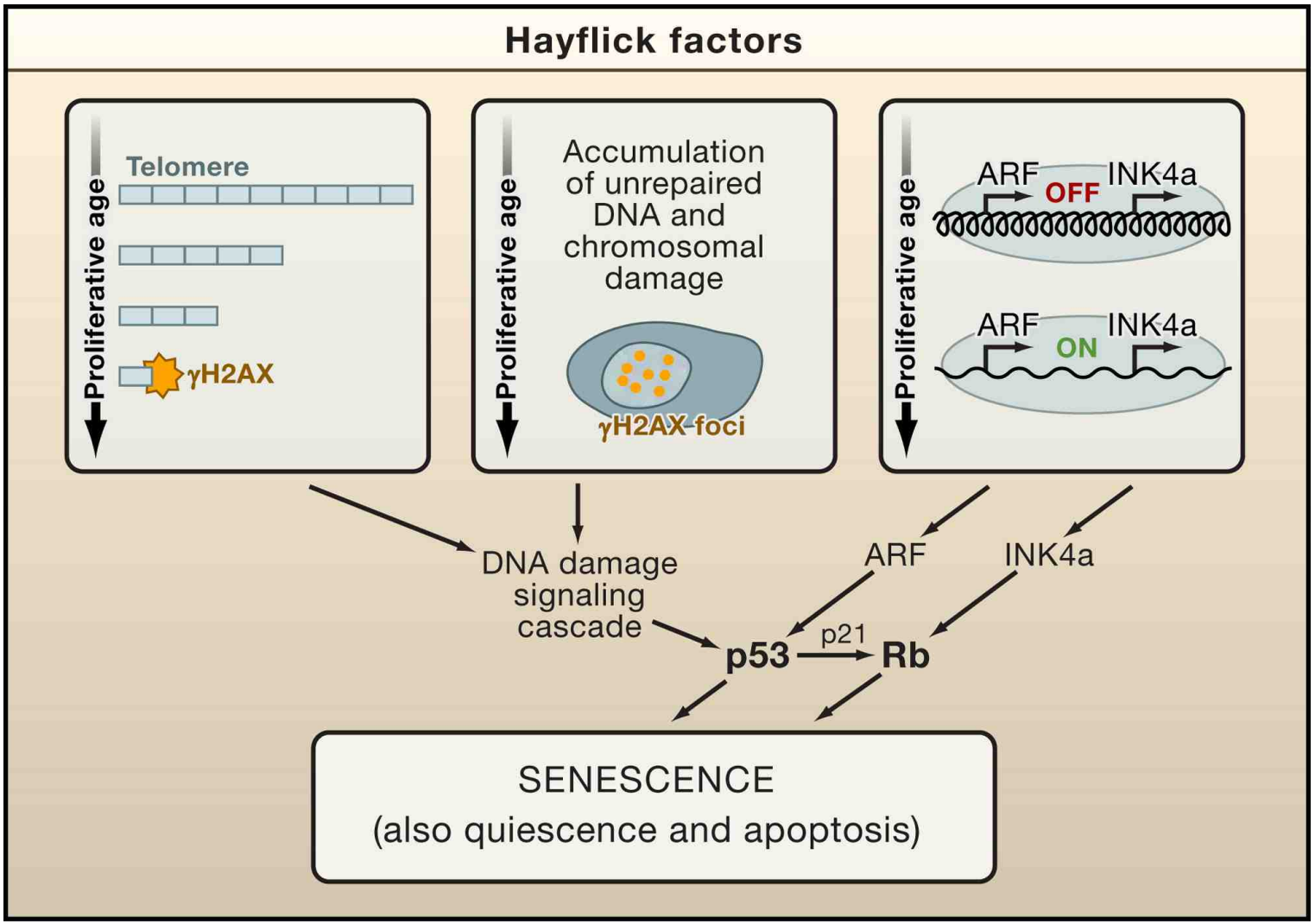
Senescence is a potent tumor suppressor mechanism



Senescence and its physiological relevance

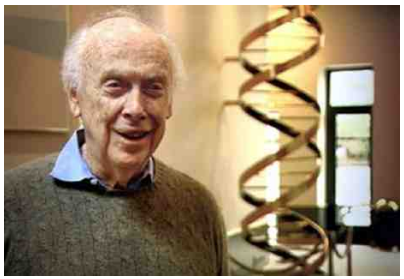


"HAYFLICK" factors limit cell proliferation



Manuel Collado, María Blasco and Manuel Serrano Cell 2007

How do cells count their divisions?

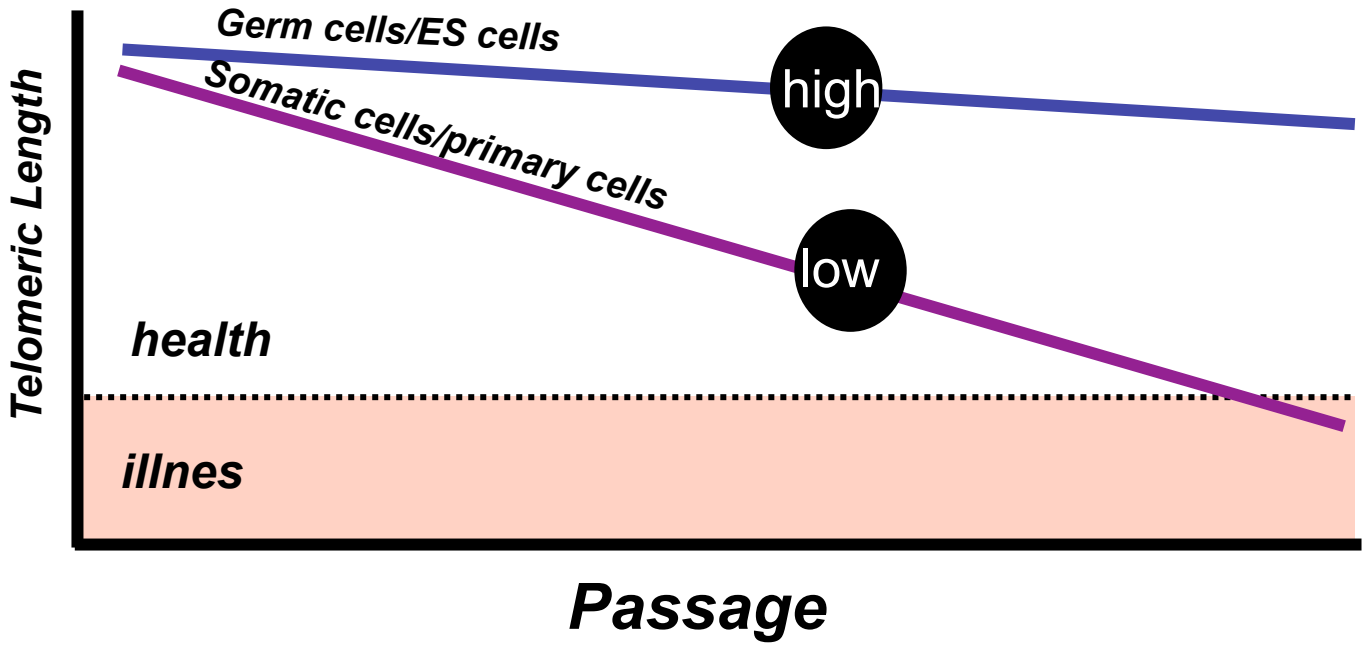


James Watson

"End replication problem"

Watson *Nat New Biol* 1972

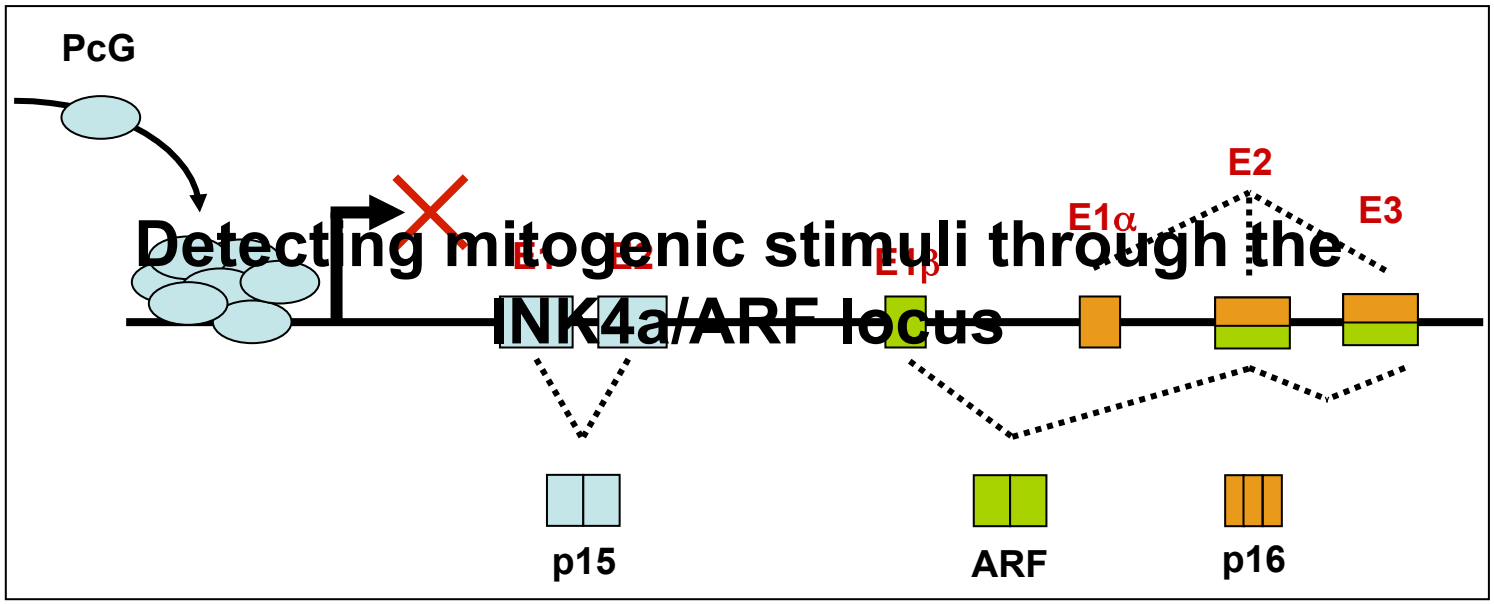
Telomerase and senescence



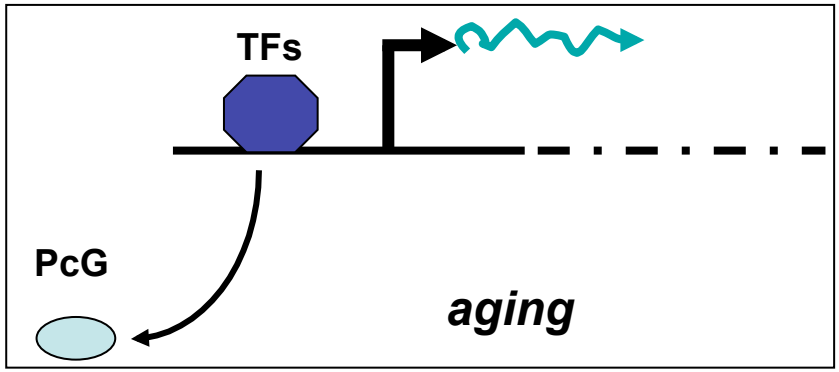
Harley, Futcher & Greider, *Nature* (1990)

How do cells count their divisions?

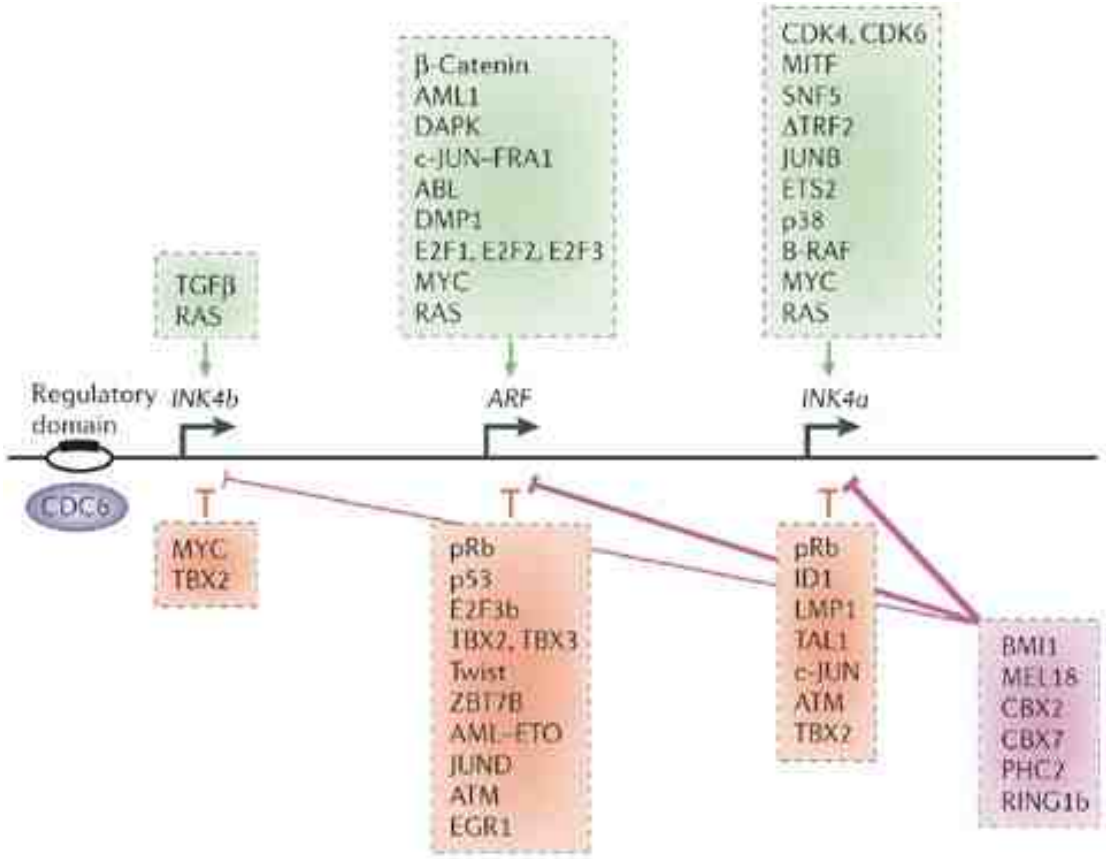
The *INK4b-ARF-INK4a* locus



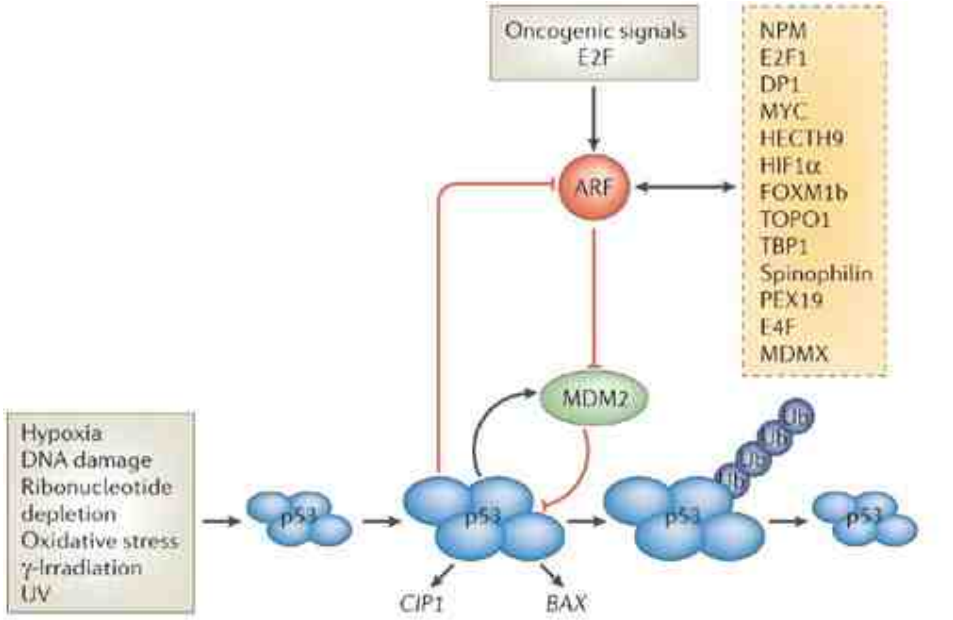
Detecting mitogenic stimuli through the INK4a/ARF locus



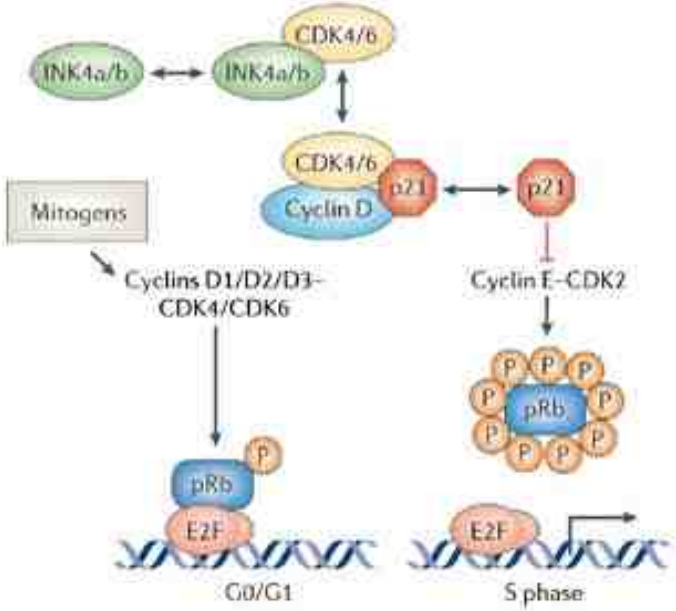
Transcriptional control of the *INK4a/ARF* locus



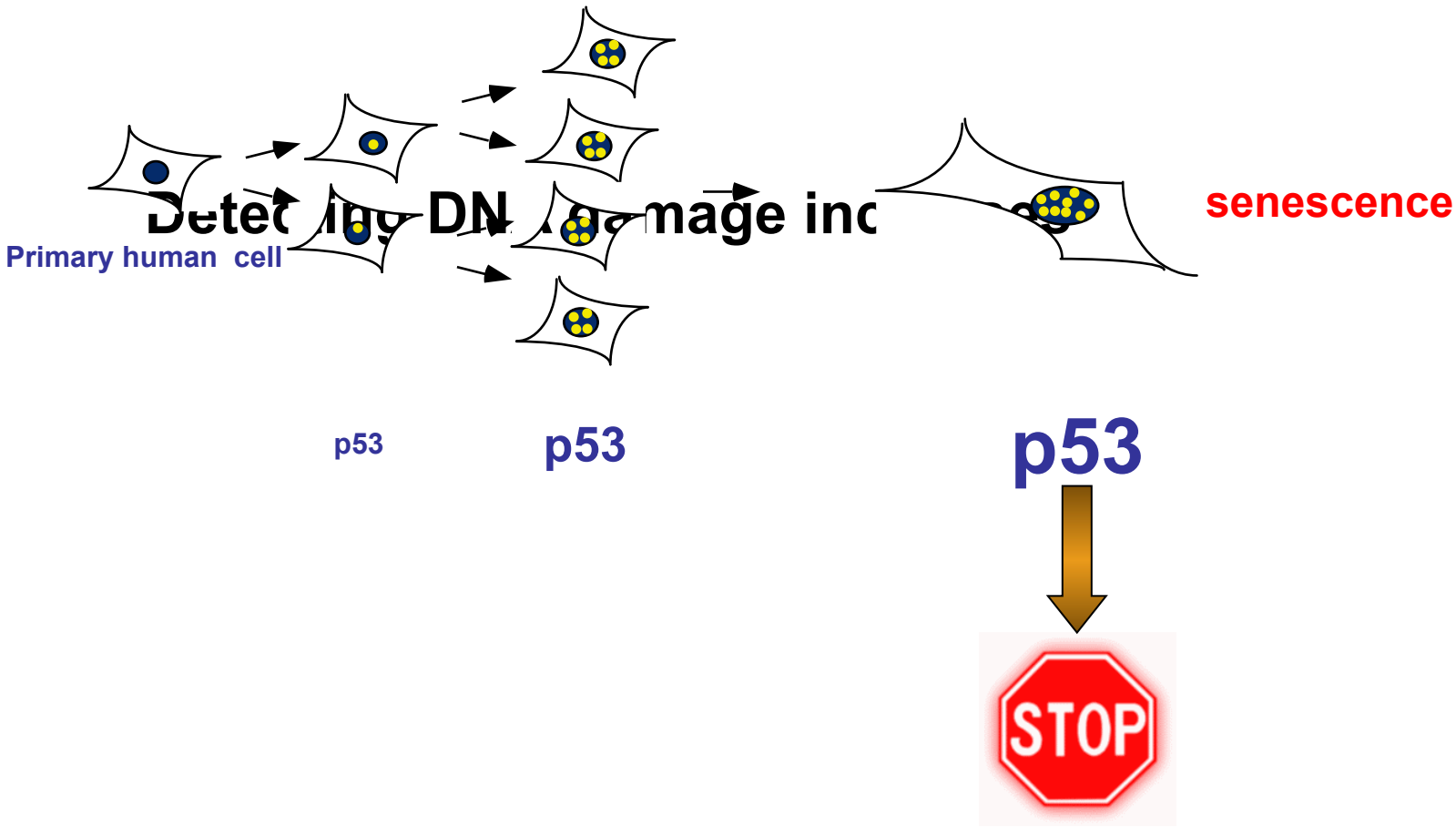
ARF activates p53



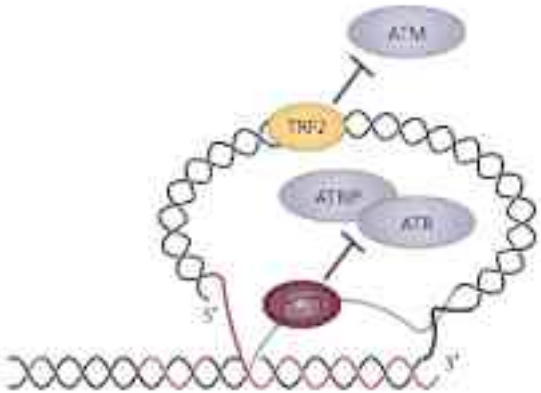
Regulation of cell cycle by $p16^{INK4a}$

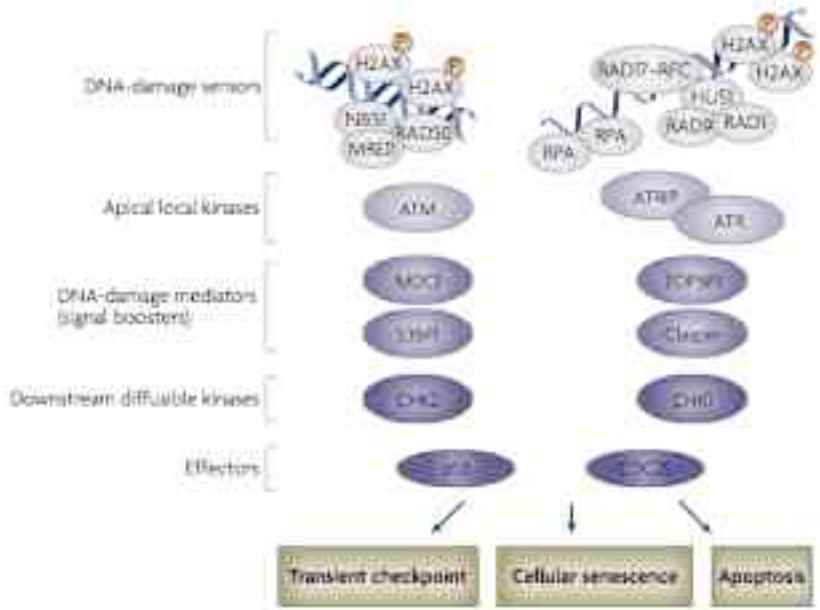


How do cells count their divisions?



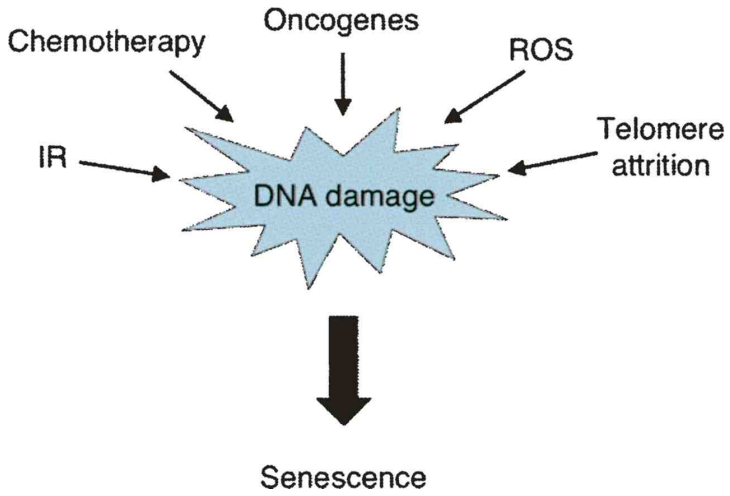
Telomere uncapping activates the DNA damage pathway



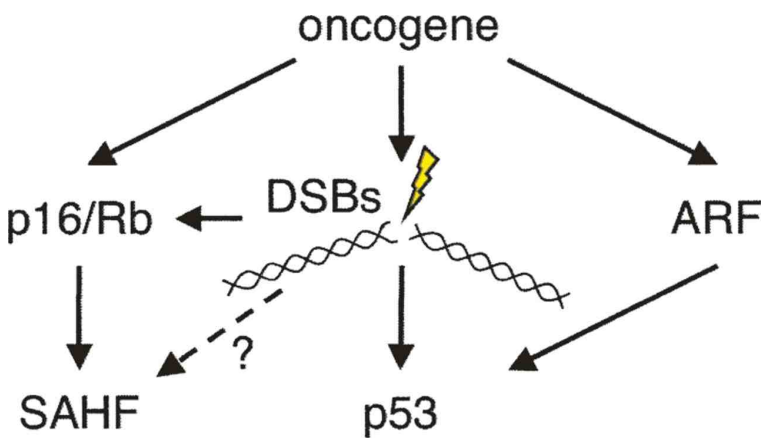


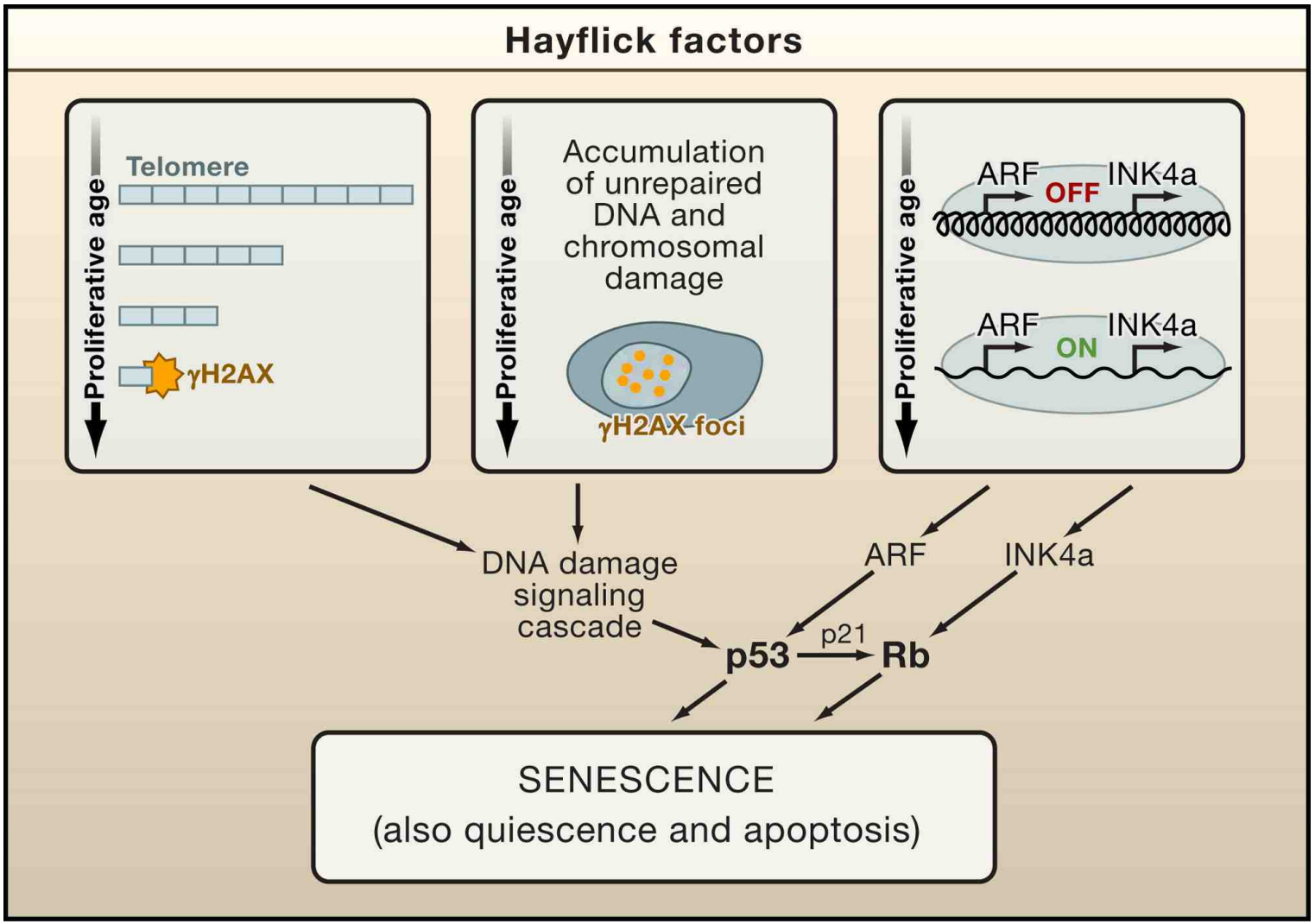
Activation of p53 during senescence

DNA damage as a common mediator of senescence signaling



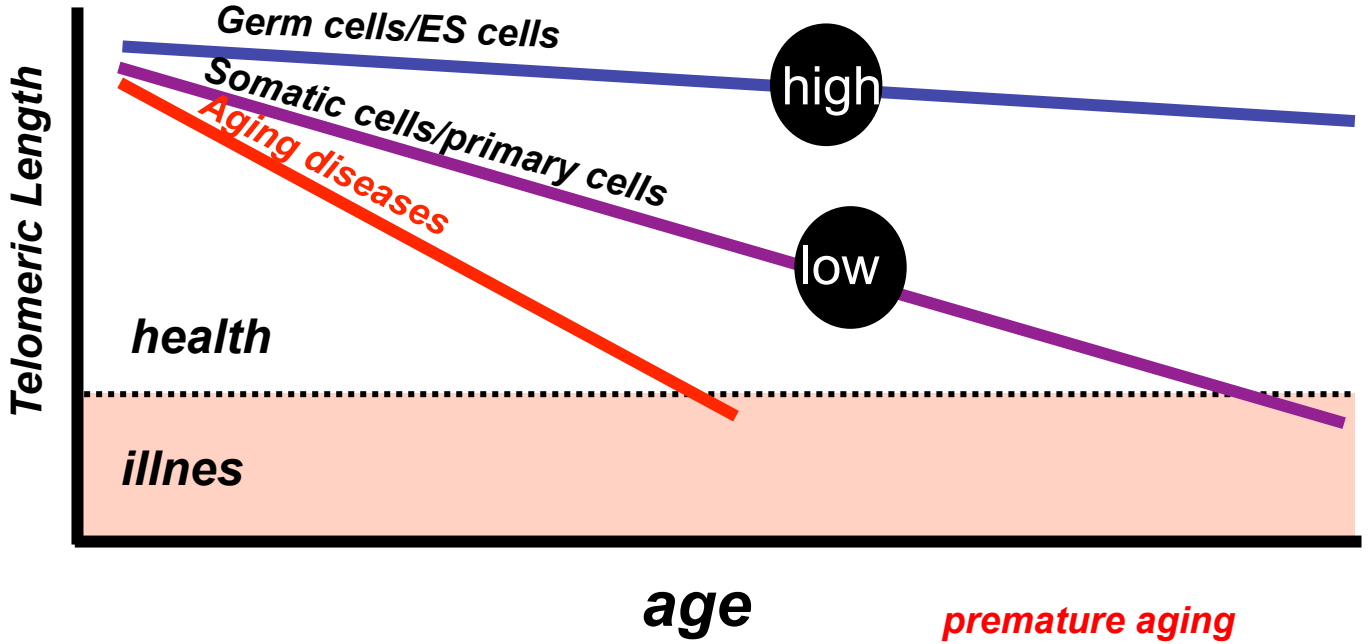
A model for oncogene action in senescence




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

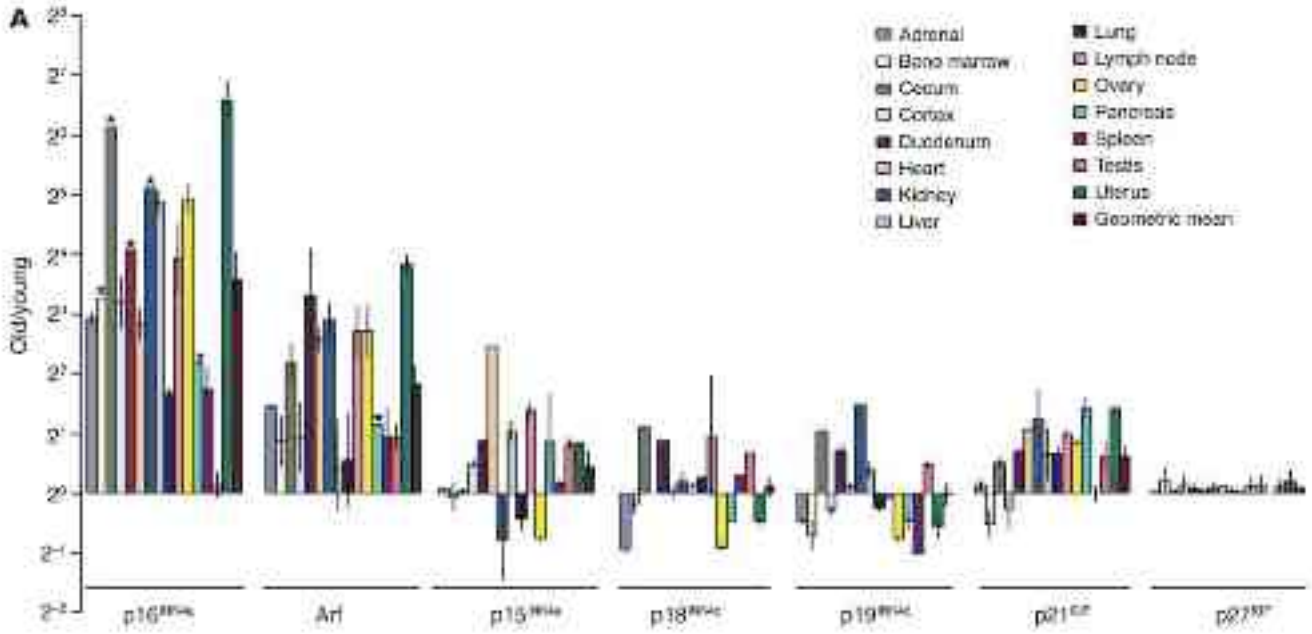
Ataxia telangiectasia (ATM)
Werner syndrome (WRN)
Bloom syndrome (BLM)
Dyskeratosis congenica (DKC1, Terc)
Aplastic anemia (Terc, Tert)
Fanconi anemia (Fanc genes)
Nijmegen breakage syndrome (Nbs)

Telomerase limits life

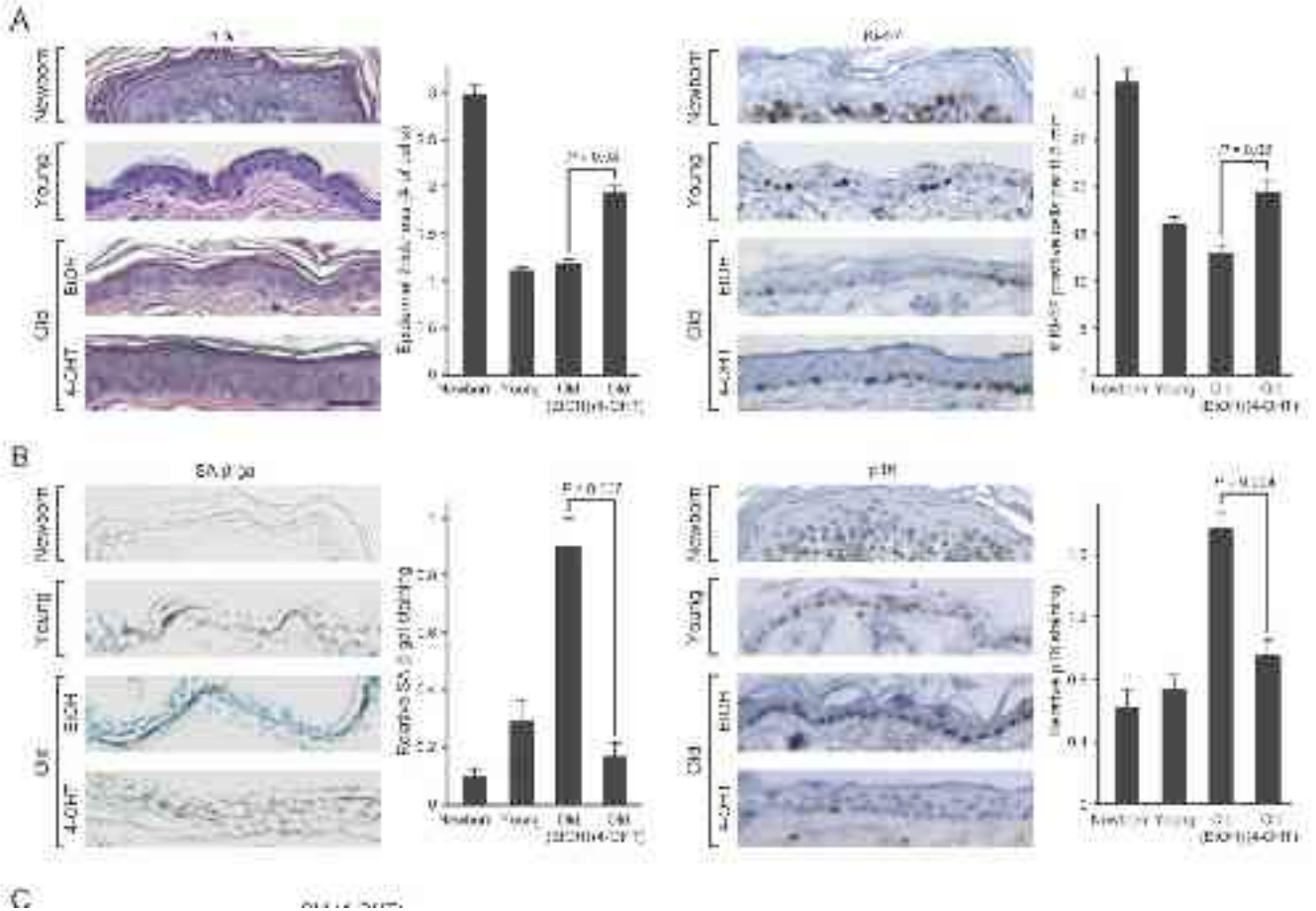
Harley, Futcher & Greider, *Nature* (1990)

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]

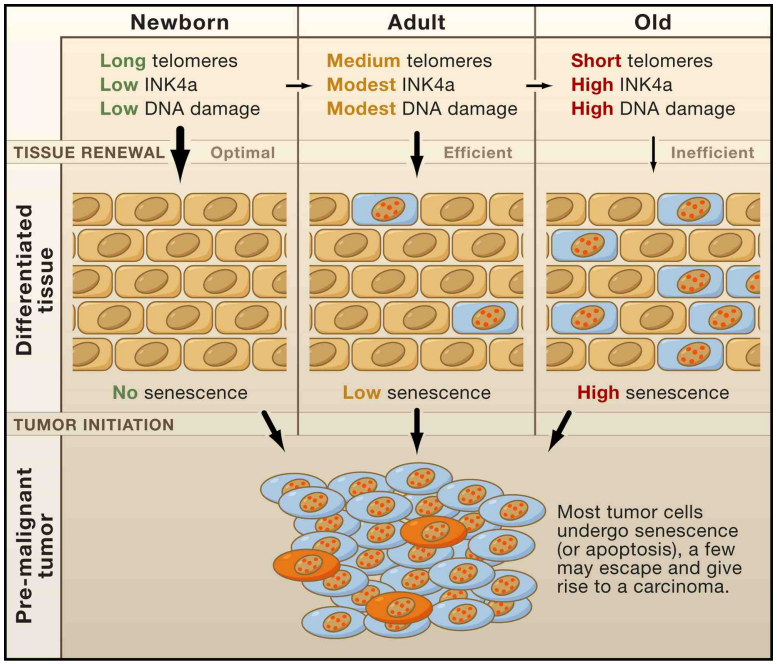


Increase in replicative senescence during aging

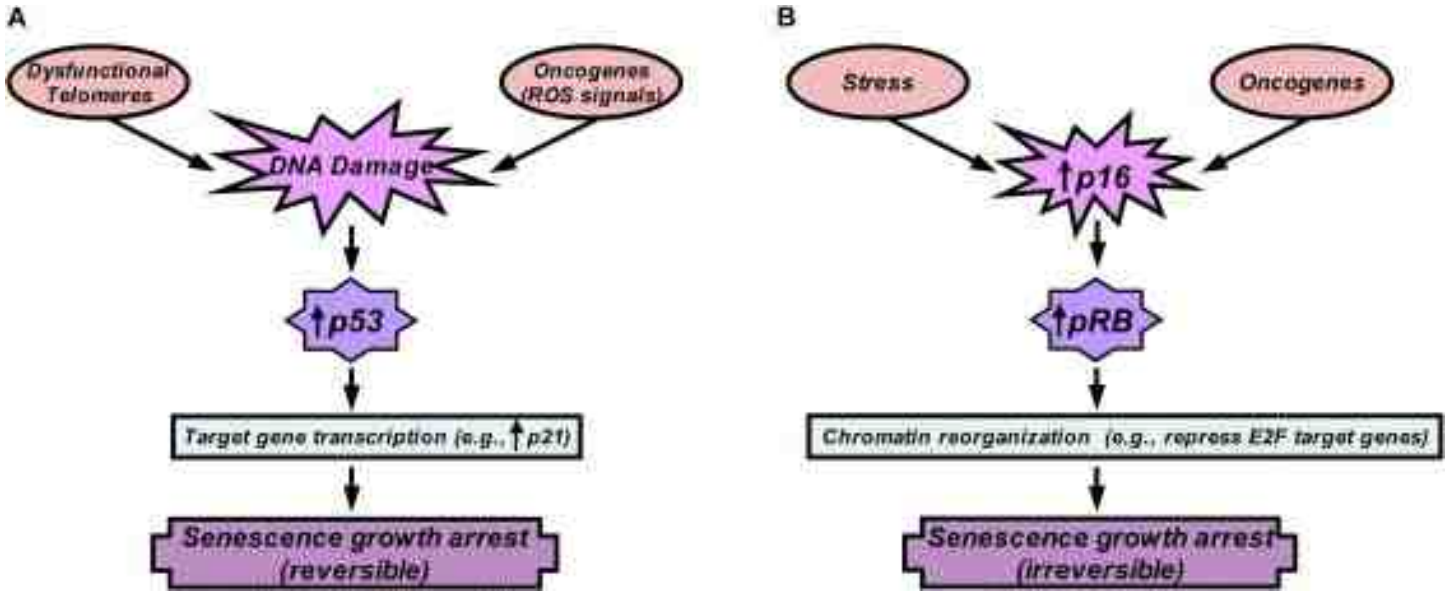


The relation between stem cells, senescence and aging

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



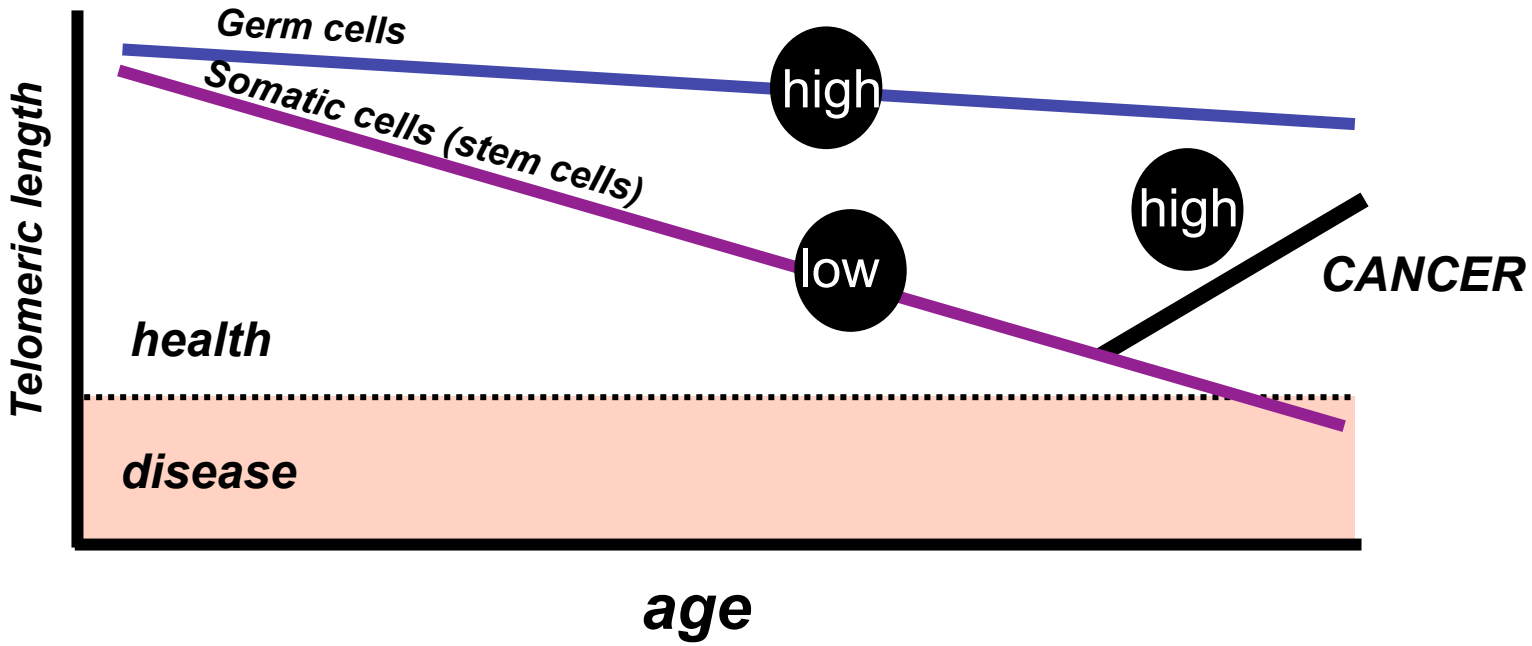
The main tumor suppresor pathways are involved in senescence



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

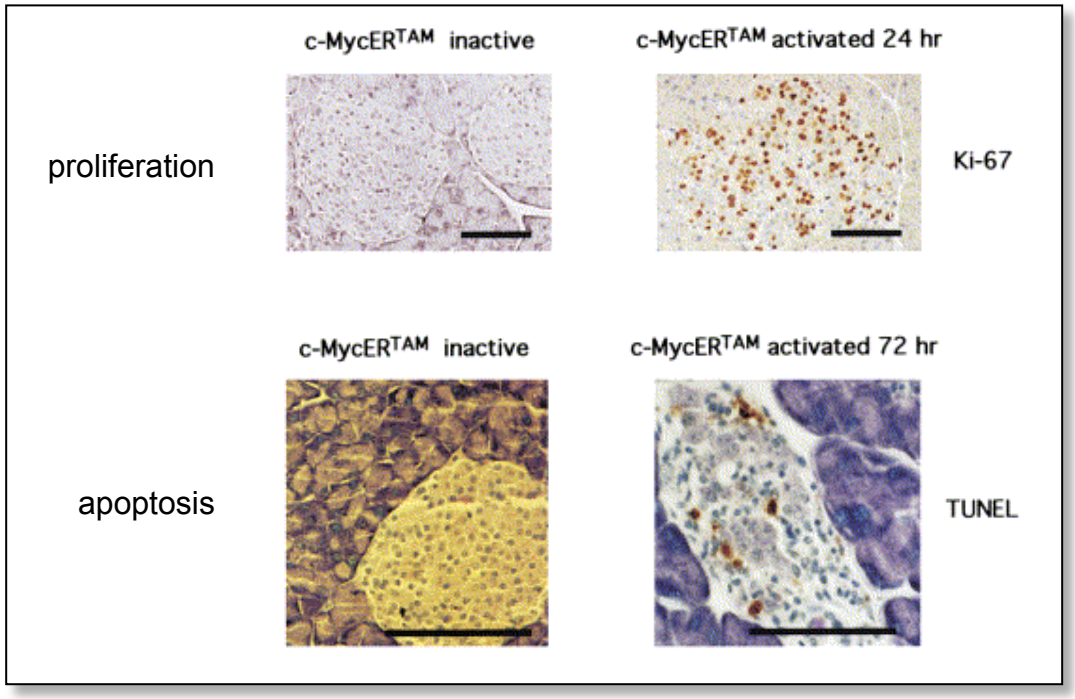
apoptosis

senescence

Tumor suppresor mechanisms

In vivo?

“c- Myc activation in vivo induces apoptosis”

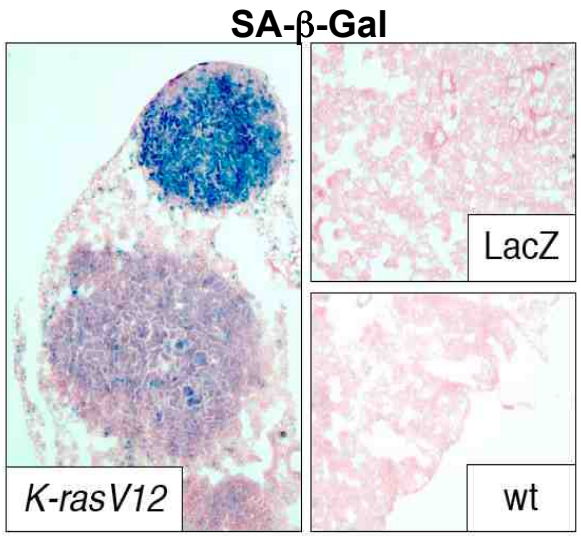


“And apoptosis is a tumor suppressor mechanism”

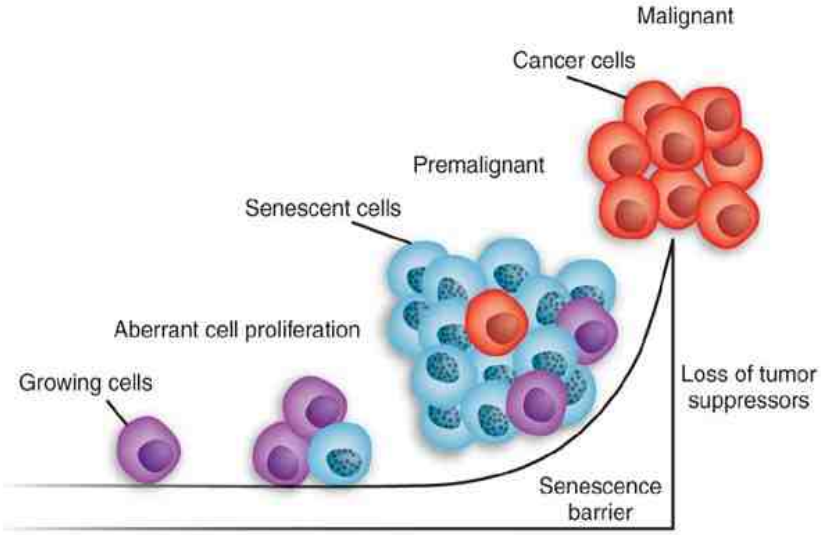
c-MycER^{TAM} x BclXL-KO = ↑↑↑ tumors

Stella Pelengaris and Gerard Evan Cell 2002

Oncogene-induced senescence is a barrier for cancer progression



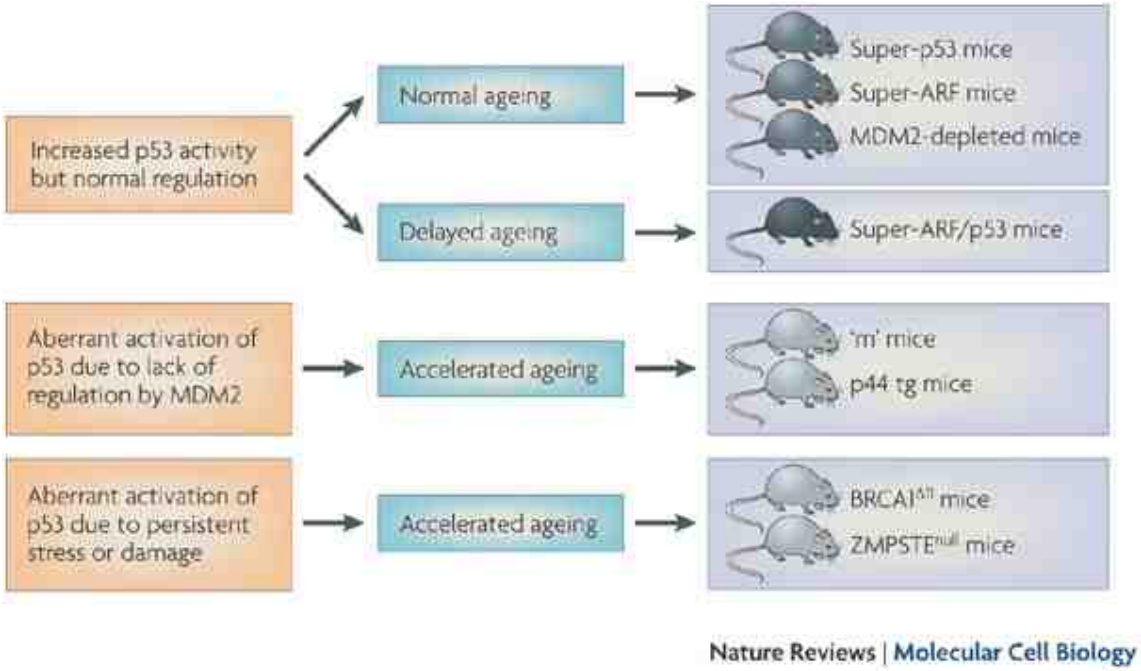
Nature (2005). **436**



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

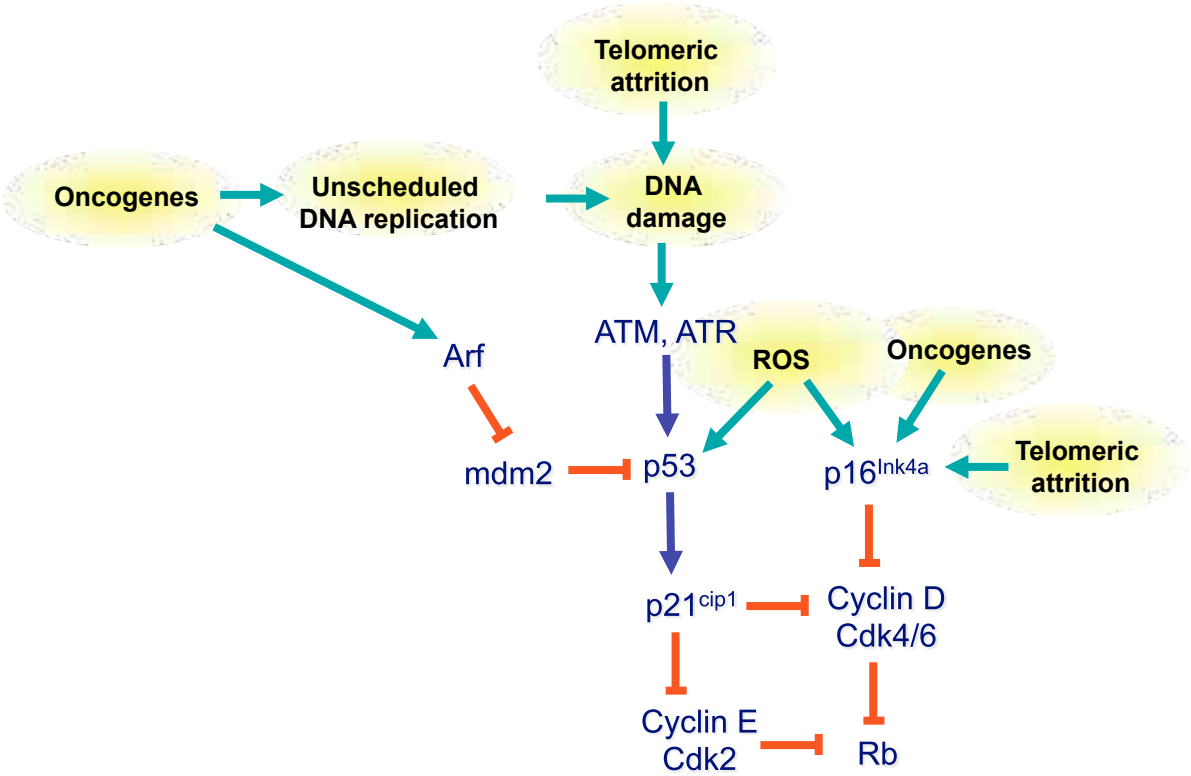
Mouse models show a role for p53 in aging



Nature Reviews | Molecular Cell Biology

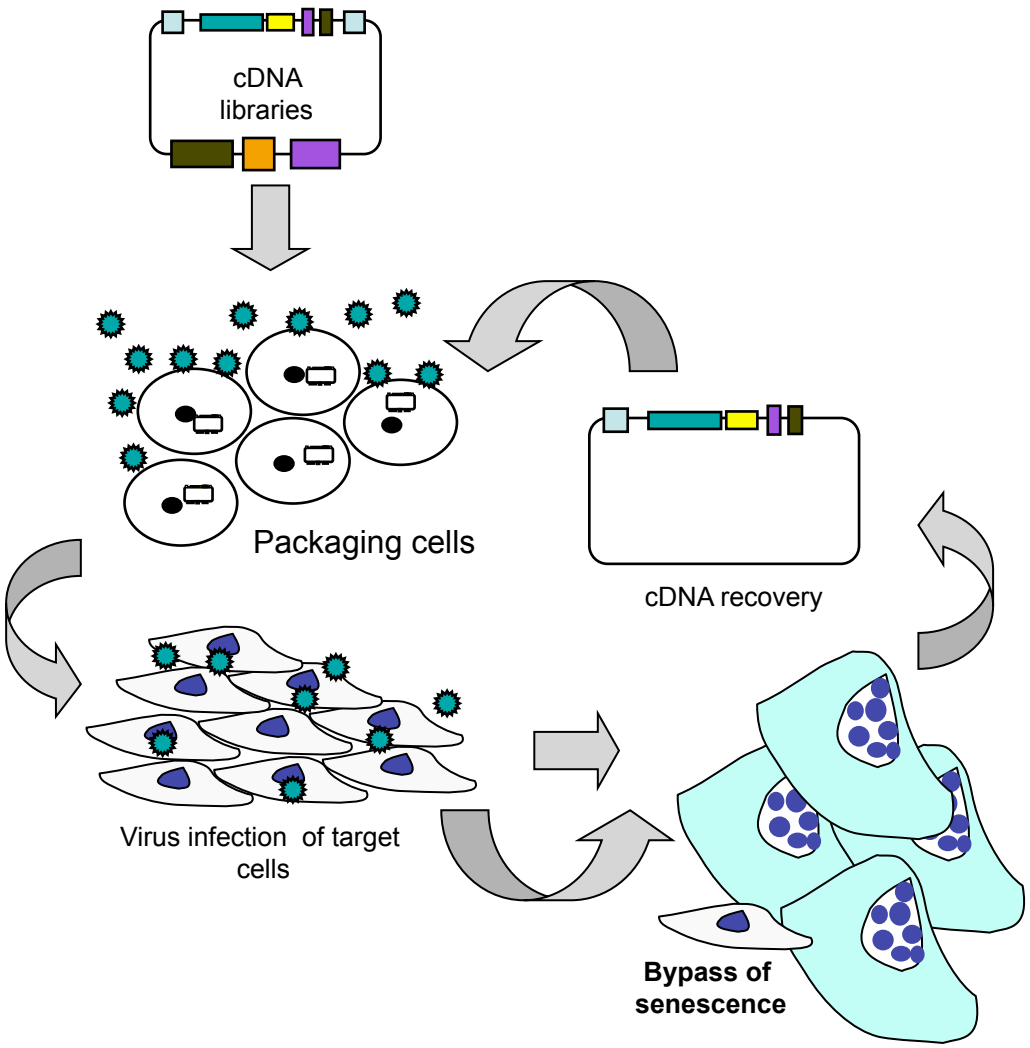
What we do in our lab?

Senescence can be exploited to identify novel cancer genes in vitro

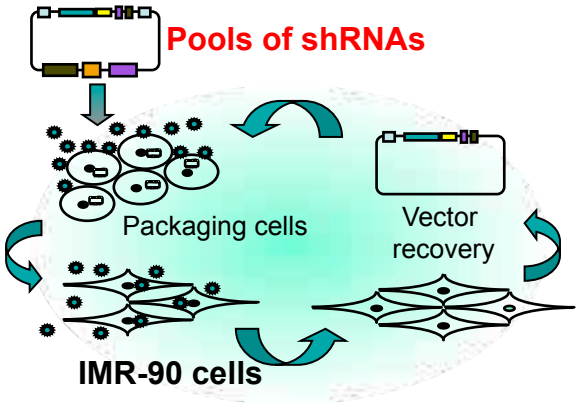


Unveiling novel regulators of senescence using genetic screenings

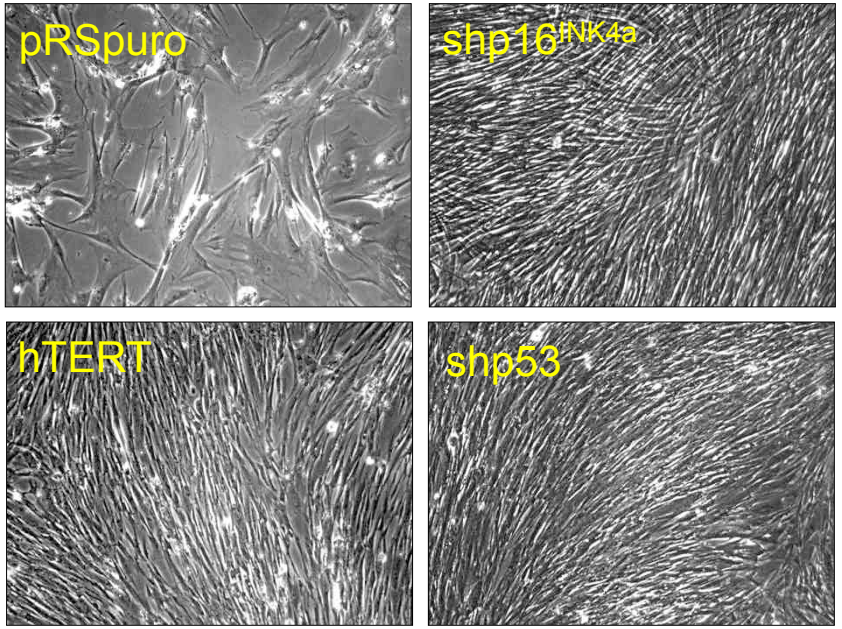
Genetic screens for lifespan extension



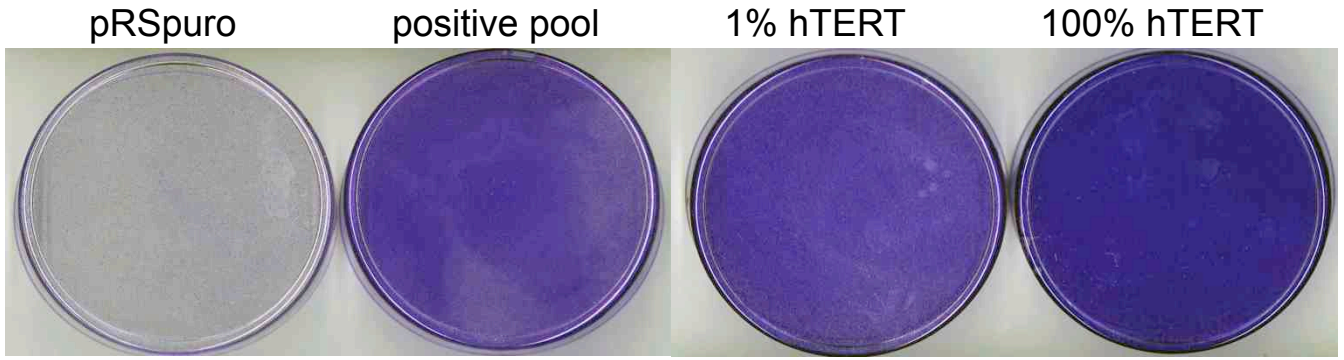
Genetic screens for bypass of replicative senescence



- NKI pRETROSUPER library



shRNAs identified so far



p53

Rb

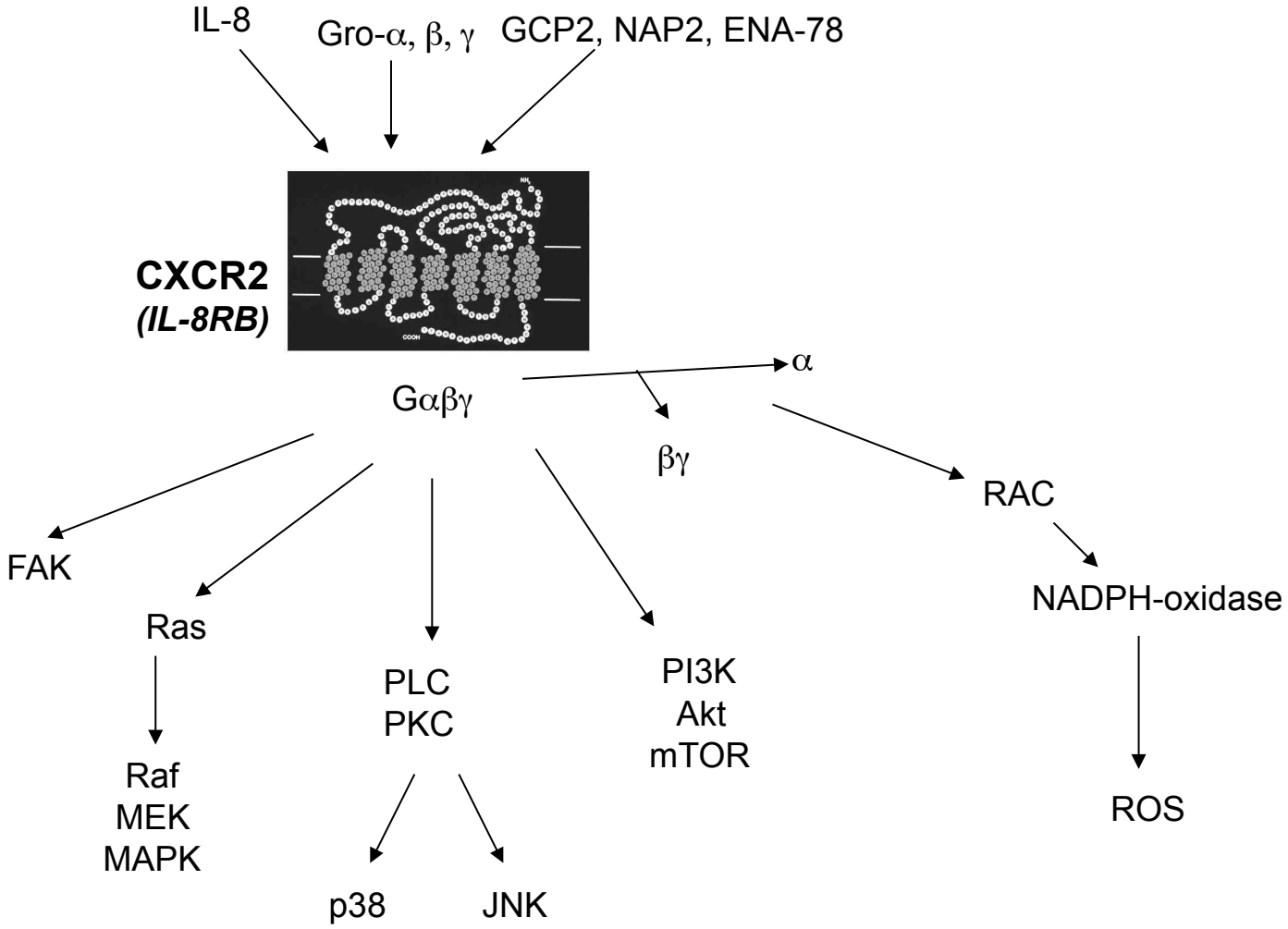
ARK5 (EMBO J, 2009 in press)

TOP1 (Cancer Res., 2009)

PLA2 (EMBO Rep, 2009)

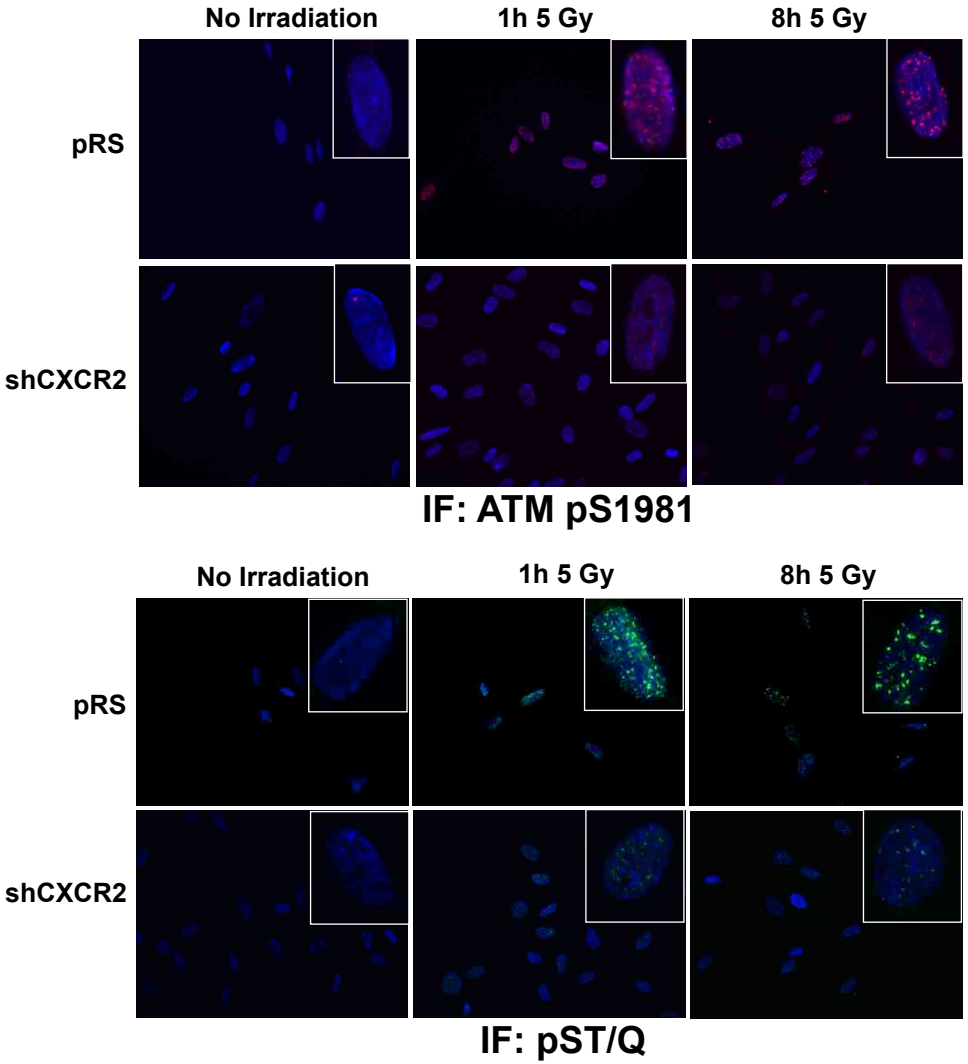
CXCR2 (Cell, 2008)

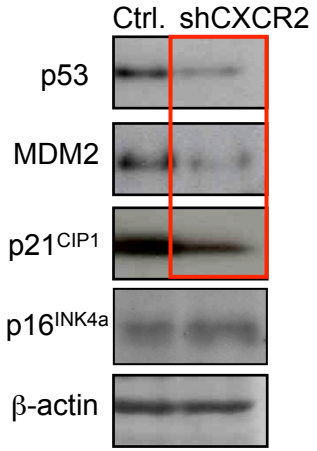
CXCR2 signaling



Senescence ?

CXCR2 depletion impairs the activation of the DNA damage response



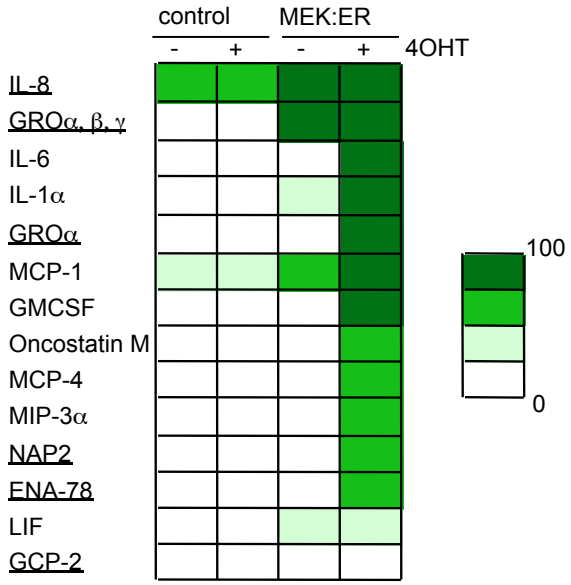


Marzia Fumigalli (d'Ada di Fagagna's lab)

Acosta et al. Cell, 2008

Coordinated upregulation of CXCR2 and its ligands in senescence

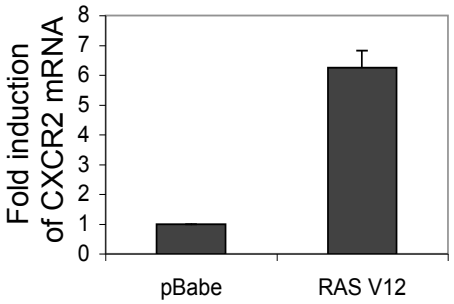
CXCR2 ligands

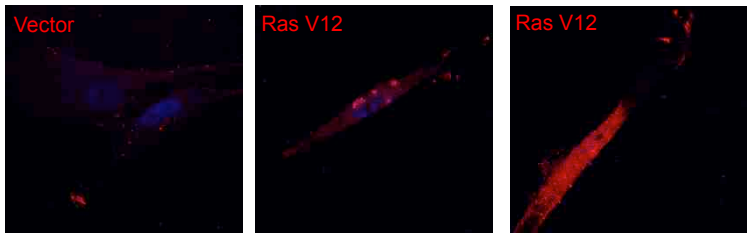


Antibody arrays

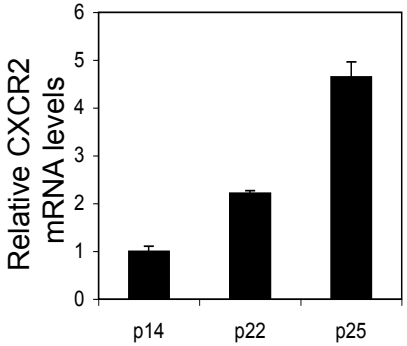
CXCR2

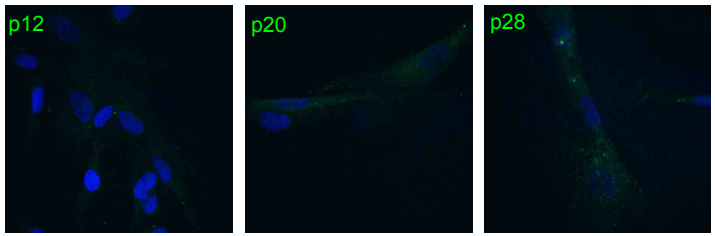
Oncogene-induced senescence



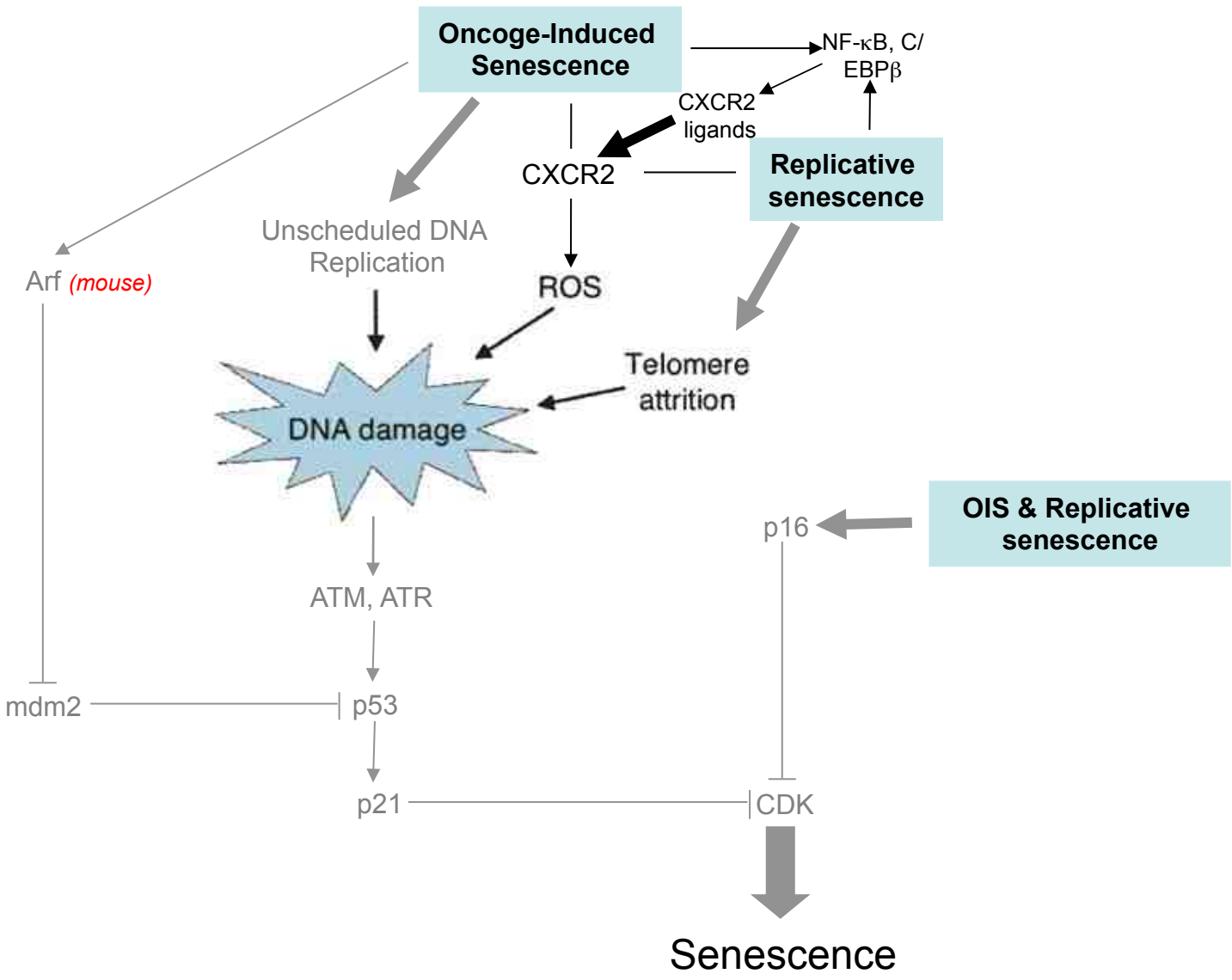


Replicative senescence



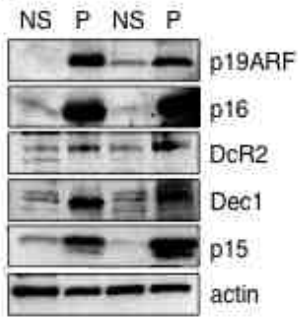


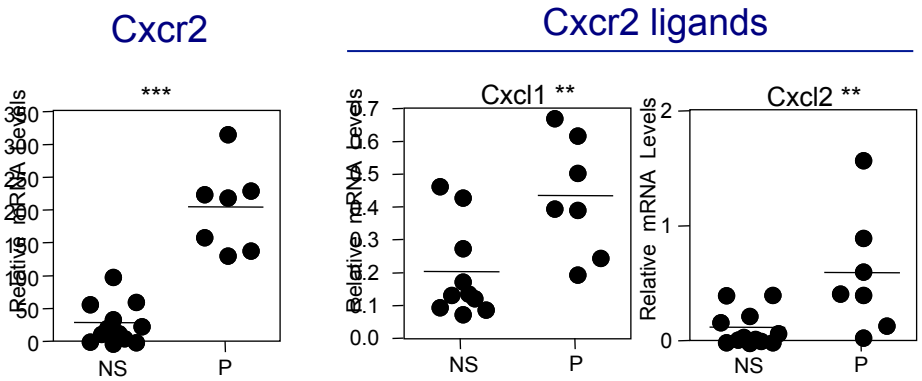
Acosta et al. Cell, 2008


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

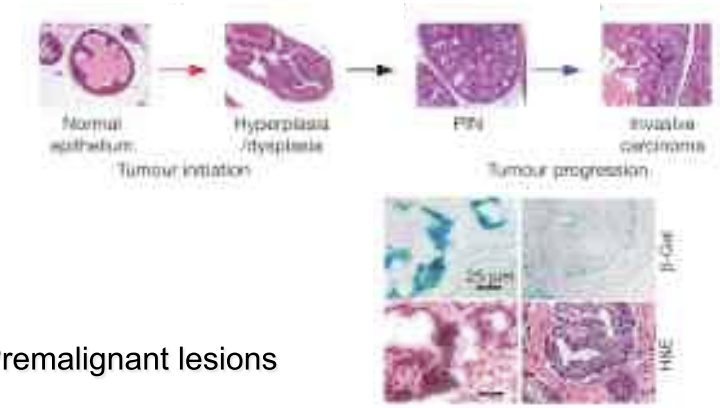




- Premalignant lesions
- 100 % Activating H-Ras mutations

Collado *et al.* Nature (2005)

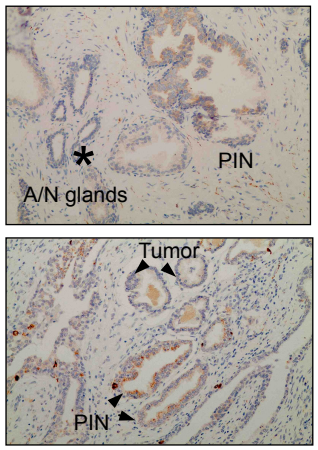
Prostate intraepithelial neoplasia (PIN)

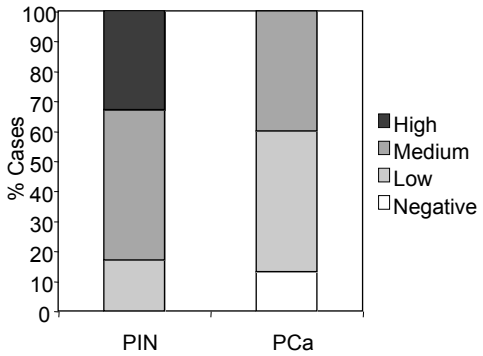


- Premalignant lesions
- Precursor of prostate cancer (Pca)

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

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
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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,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3] Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[4] Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
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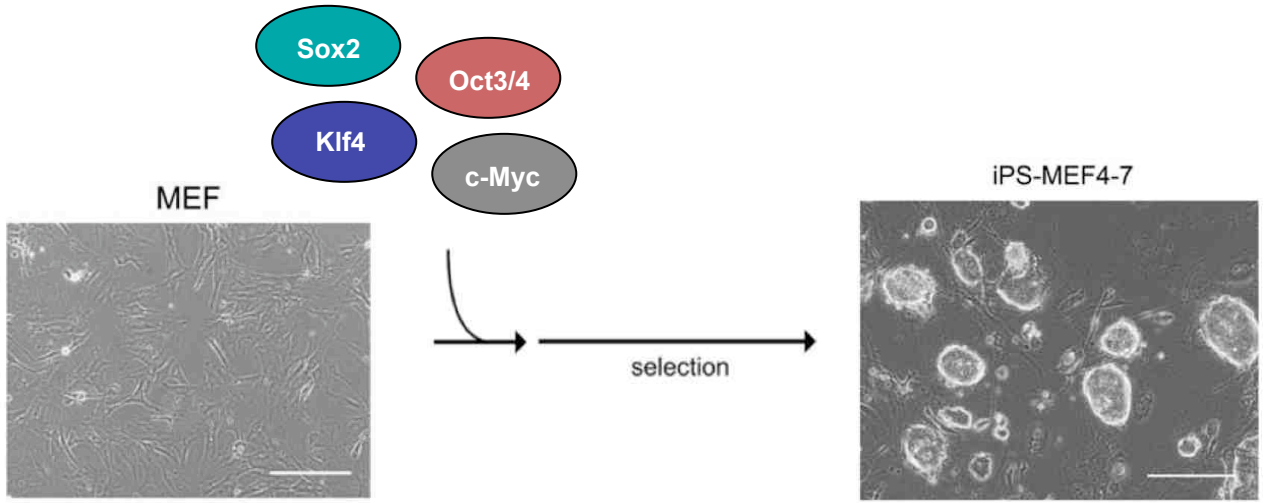
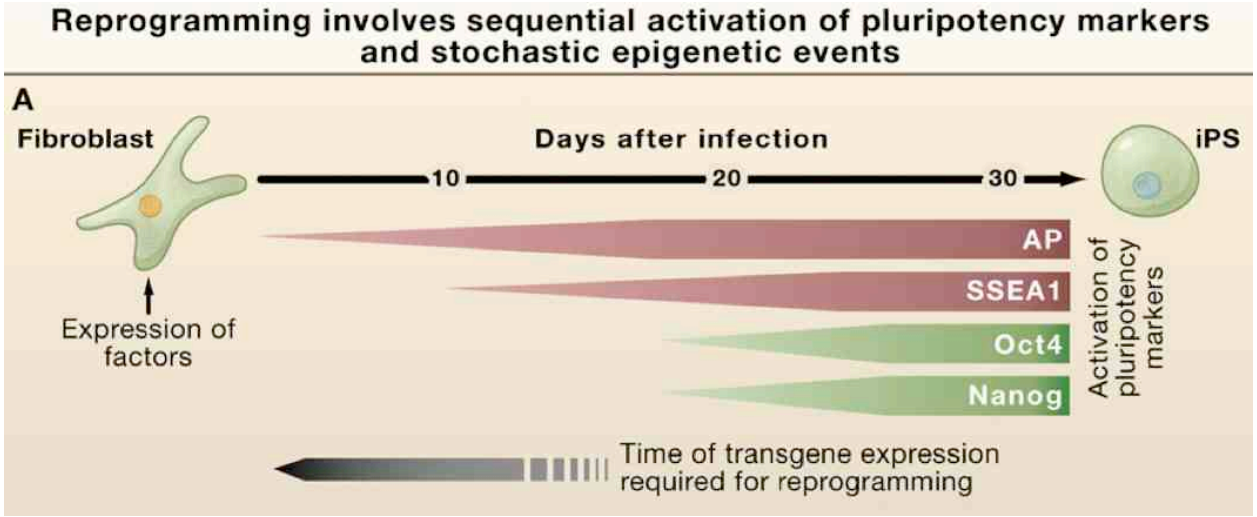


MEFs

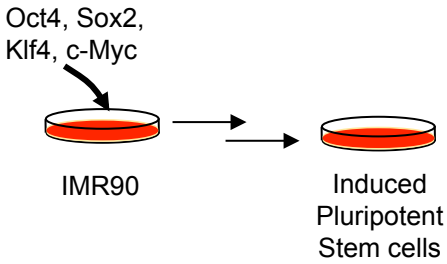
IPS (Induced Pluripotent Stem) Cells

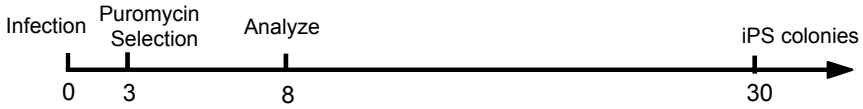
Reprogramming: complex, slow, inefficient and dangerous



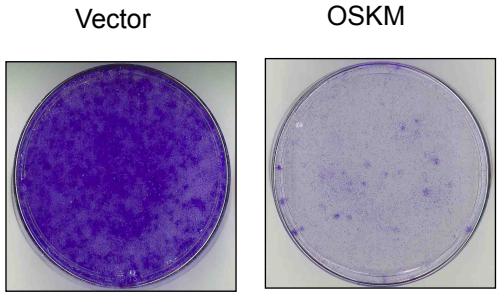
- **Complex** involves several steps, including chromatin modifications and global expression patterns alterations
- I**nefficient** and **slow,** has to overcome several barriers
- The mechanisms behind the switch to pluripontency/self-renewal remain mostly unknown

Expression of reprogramming factors induces senescence

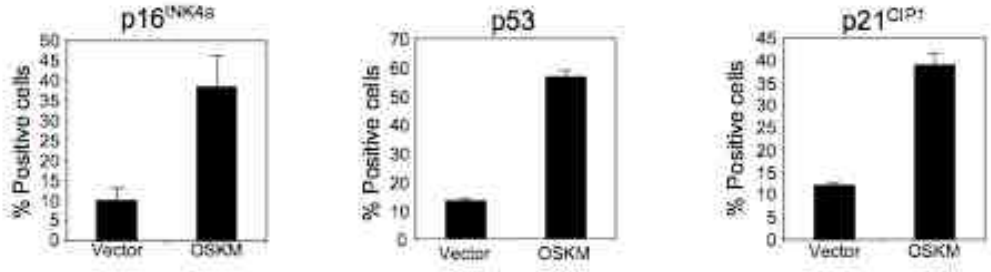




Reprogramming factors cause growth arrest



Induction of effectors of senescence



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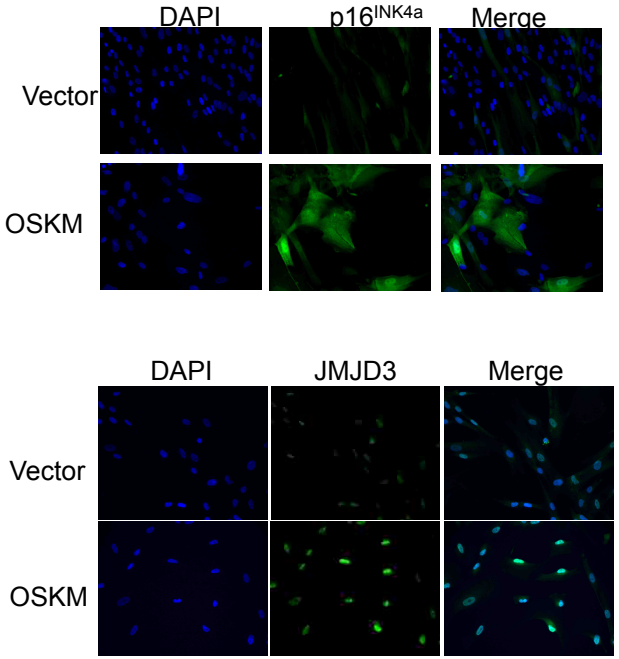
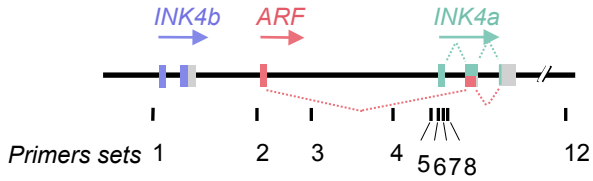
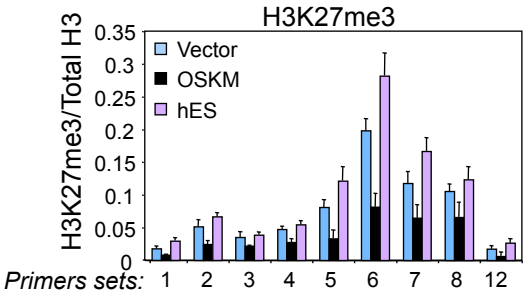
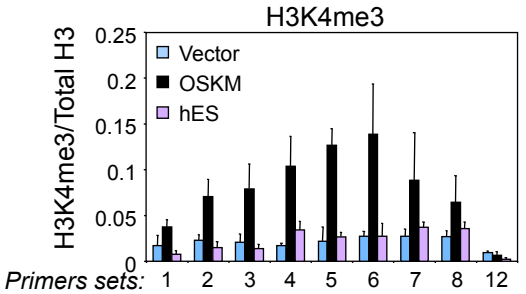
Chromatin remodeling of the *Ink4a/Arf* locus during reprogramming

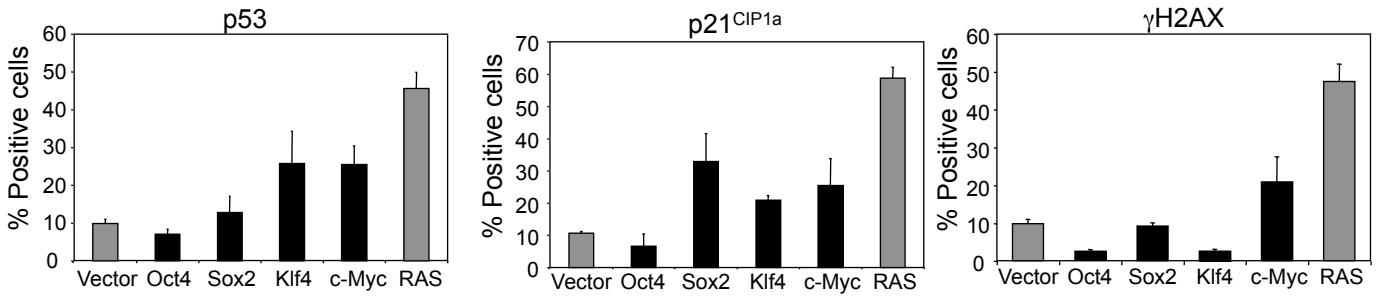






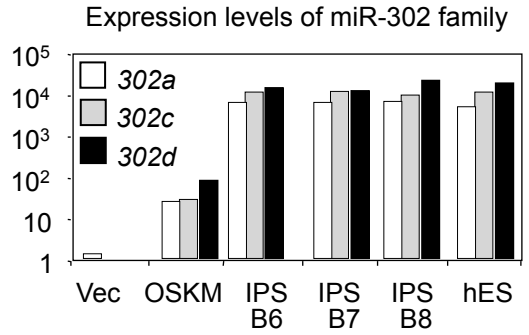


Activation of the p53/p21 pathways at multiple points during RIS

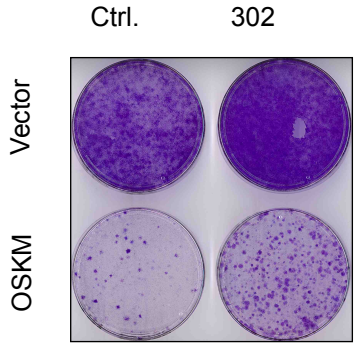


Pluripotency-related miRNAS inhibit senescence

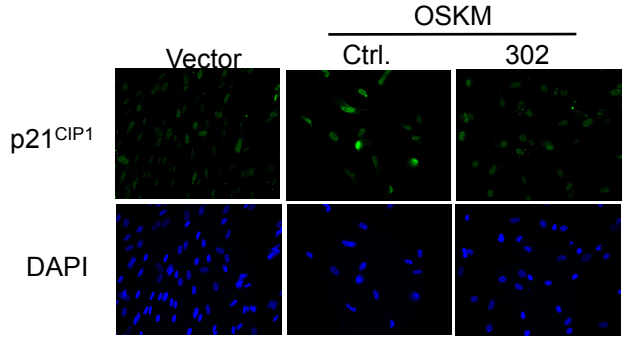
Low expression of pluripotency-related miRNAs in cells suffering RIS



Partial bypass of senescence by forced expression of miR-302



Regulation of p21 by miR-302



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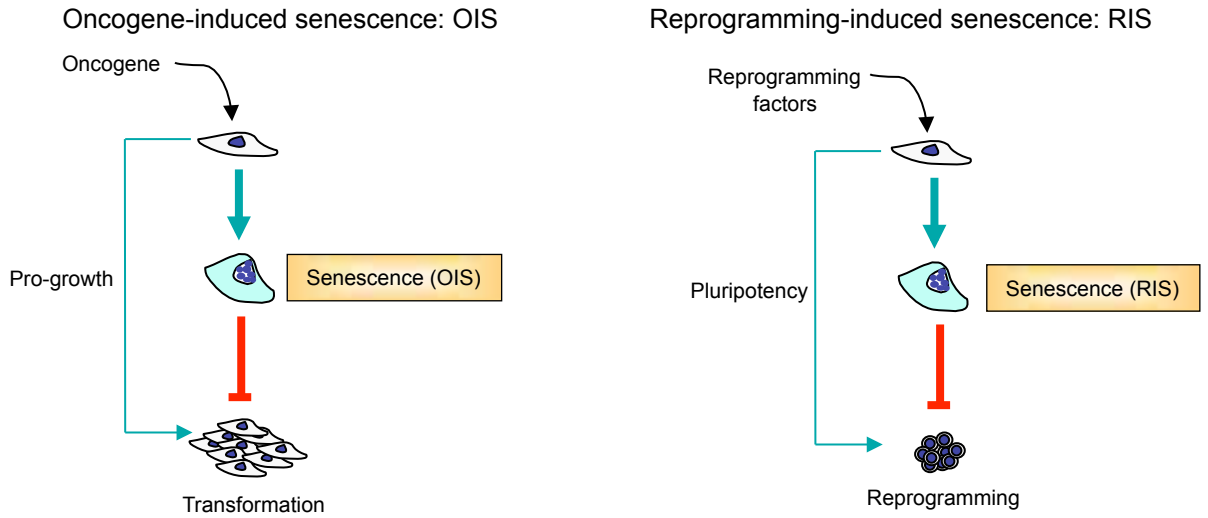
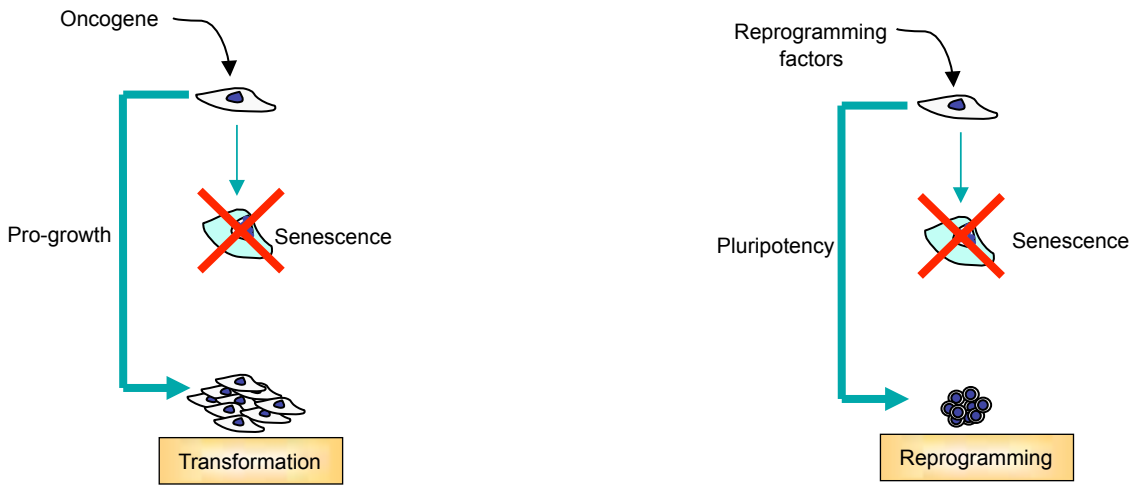
Parallels between oncogene and reprogramming-induced senescence

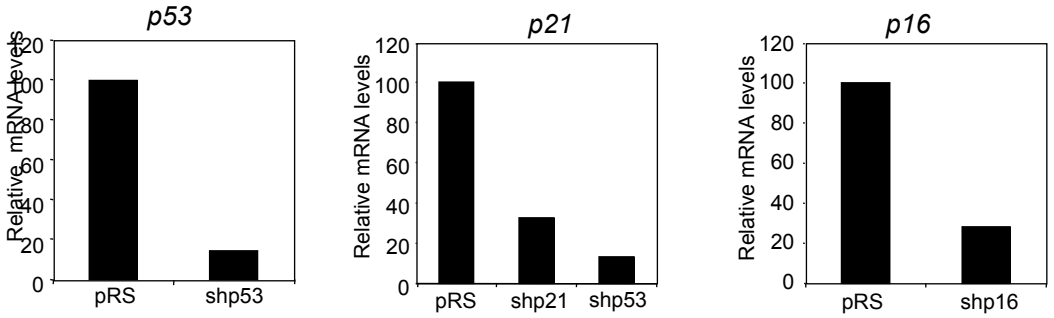


Senescence disabled

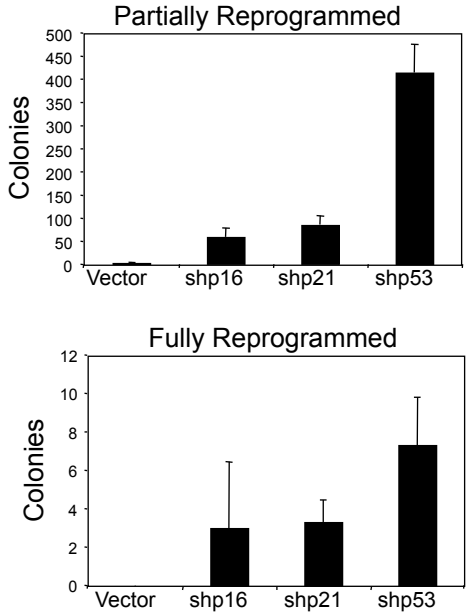


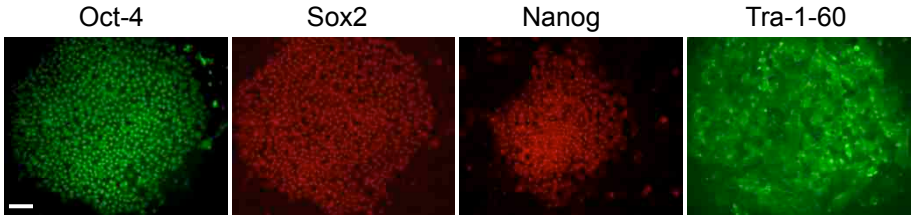
Inhibition of senescence increases reprogramming efficiency

Knocking down senescence effectors with shRNA



Increase in reprogramming efficiency





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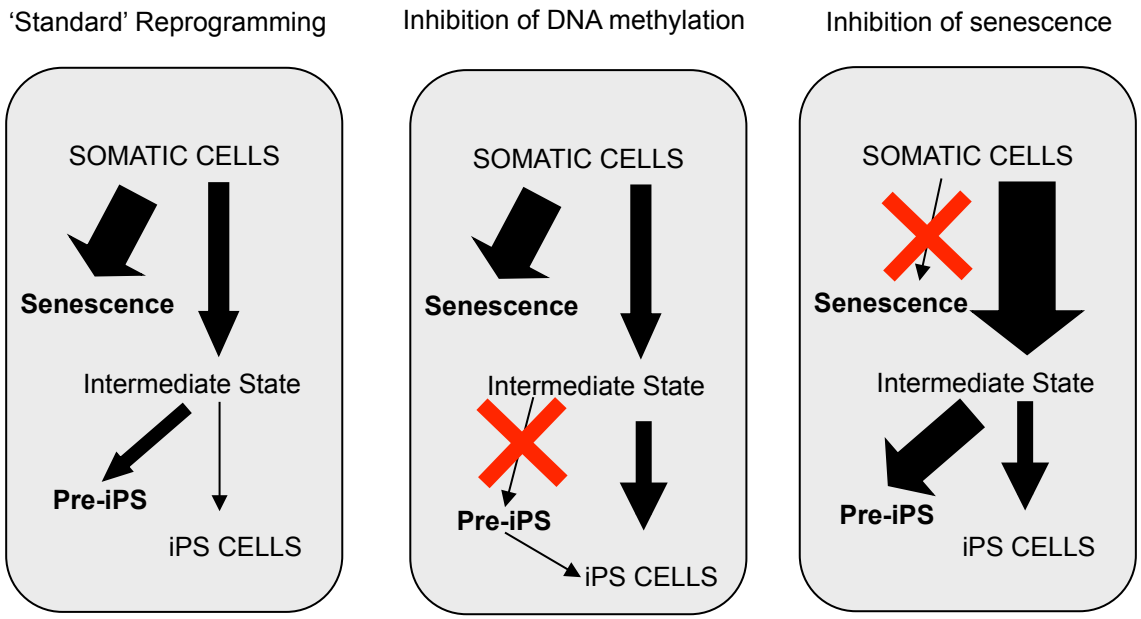
Improving reprogramming from the technical point of view

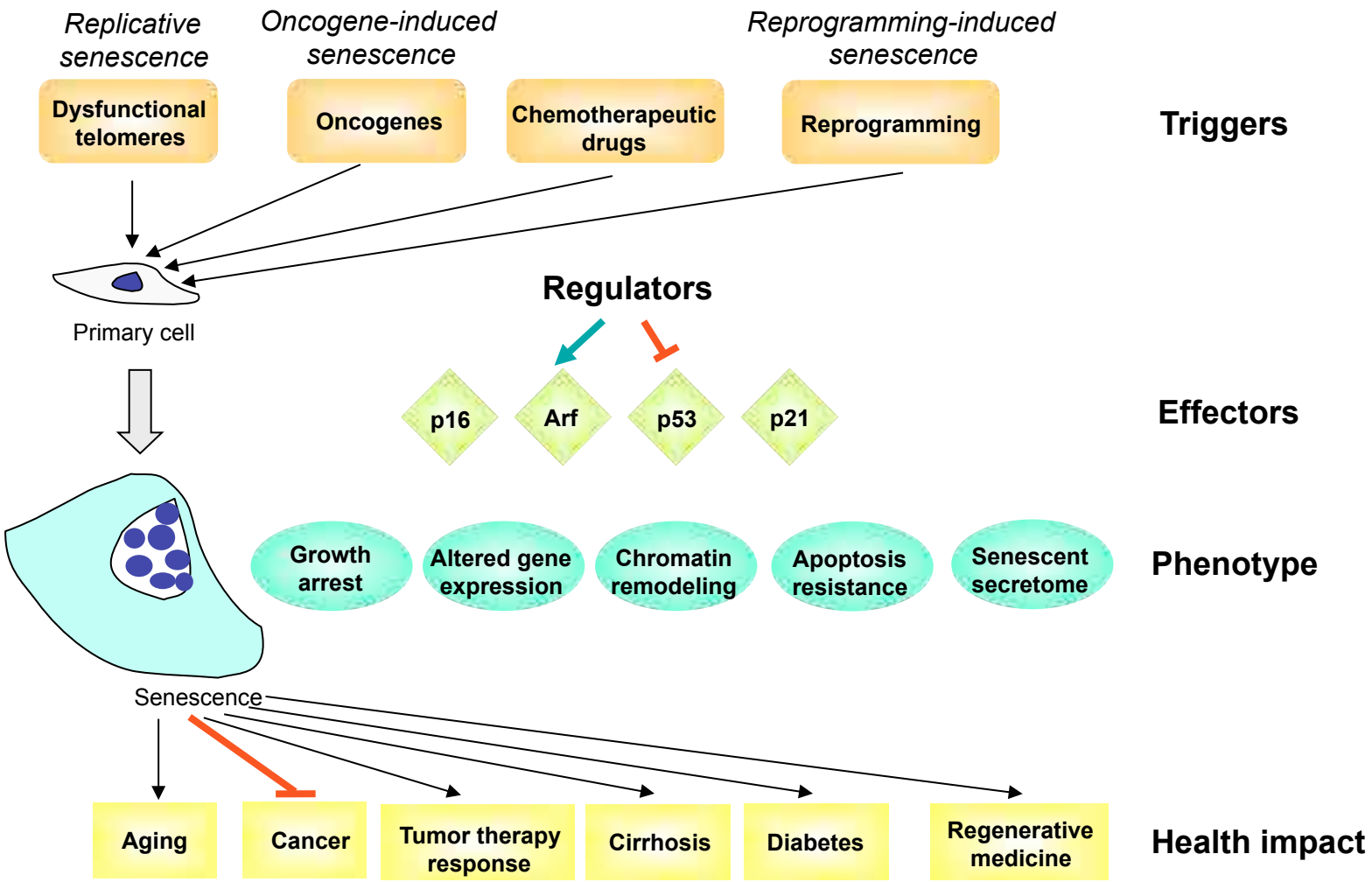


Low efficiency

Increased proportion of Fully vs. Partial reprogrammed iPS

Increased numbers of both Fully and Partial reprogrammed iPS

Senescence and its physiological relevance



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Cell Proliferation GROUP
Jesús Gil

http://cellpro.csc.mrc.ac.uk

Juan Carlos Acosta

Ana Banito

Ana O'Loghlen

Nikolay Popov

Selina Raguz

Nadine Martin

Marta Barradas

Rita Franca

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Collaborators

David Bernard CNRS, Lille

Gordon Peters lab (Emma Anderton) LRI London

Martin Walsh lab (SiDe Li) Mount Sinai, NY

Eva Hernando/J. Melamed NYU

Fabrizio d'Adda di Fagagna (M Fumagalli) IFOM, Milan

Mariano Barbacid/Carmen Guerra CNIO, Madrid