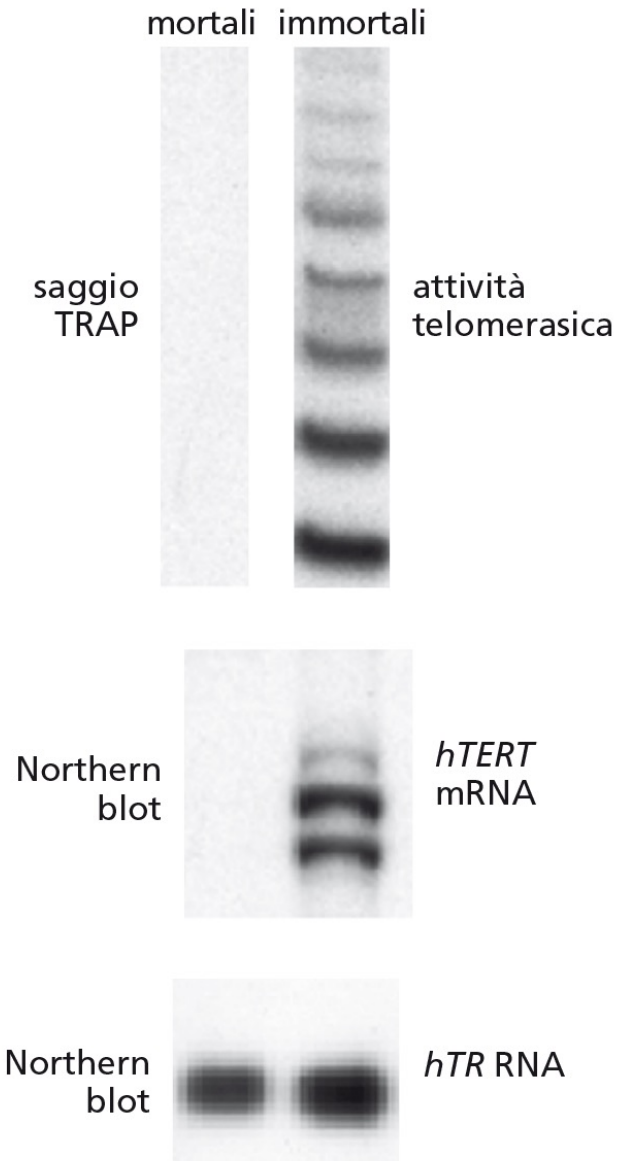

L'ACQUISIZIONE
DELL'ATTIVITÀ
TELOMERASICA È
LA CAUSA DELLA
FUGA DALLA CRISI?

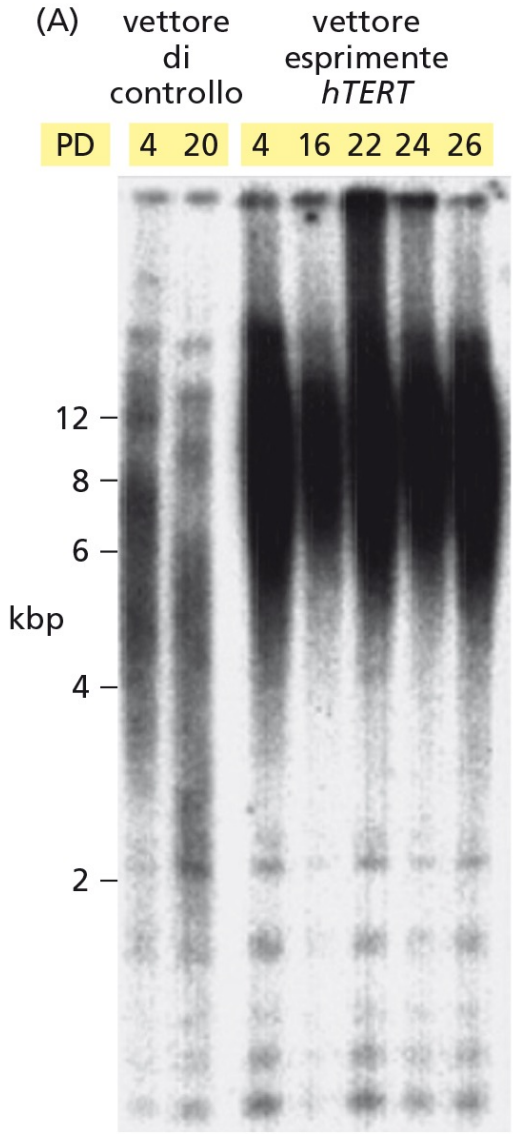
Telomerasi e immortalizzazione cellulare



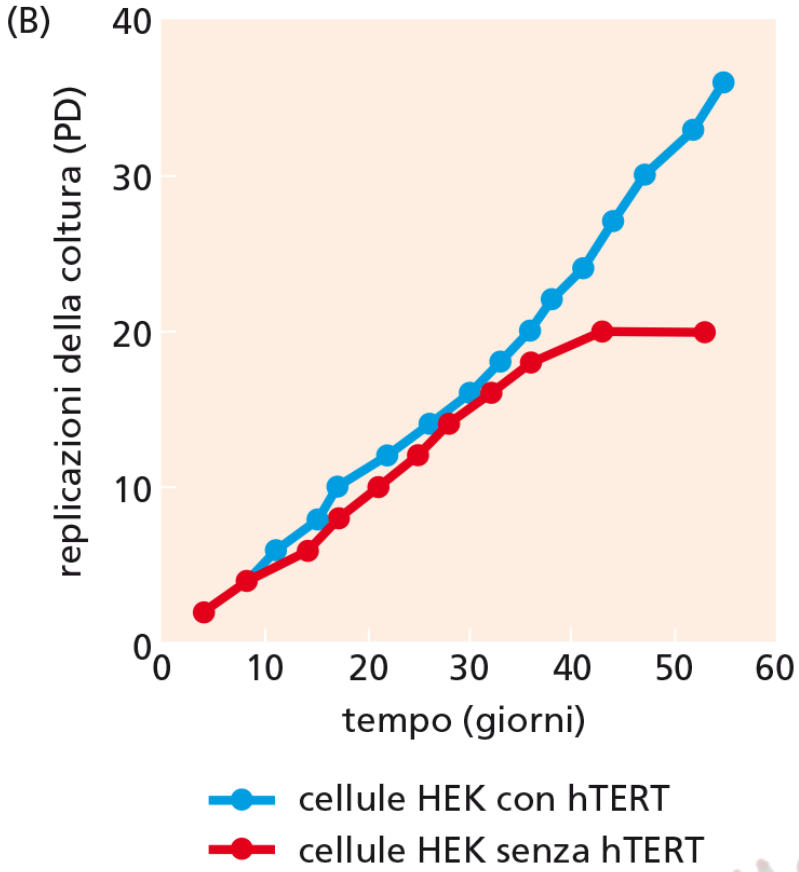
C'è solo una correlazione tra attività telomerasica e immortalizzazione o o la telomerasi causa immortalizzazione?

Weinberg, la biologia del cancro

Espressione ectopica *hTERT*



Cell HEK in coltura + cDNA hTERT

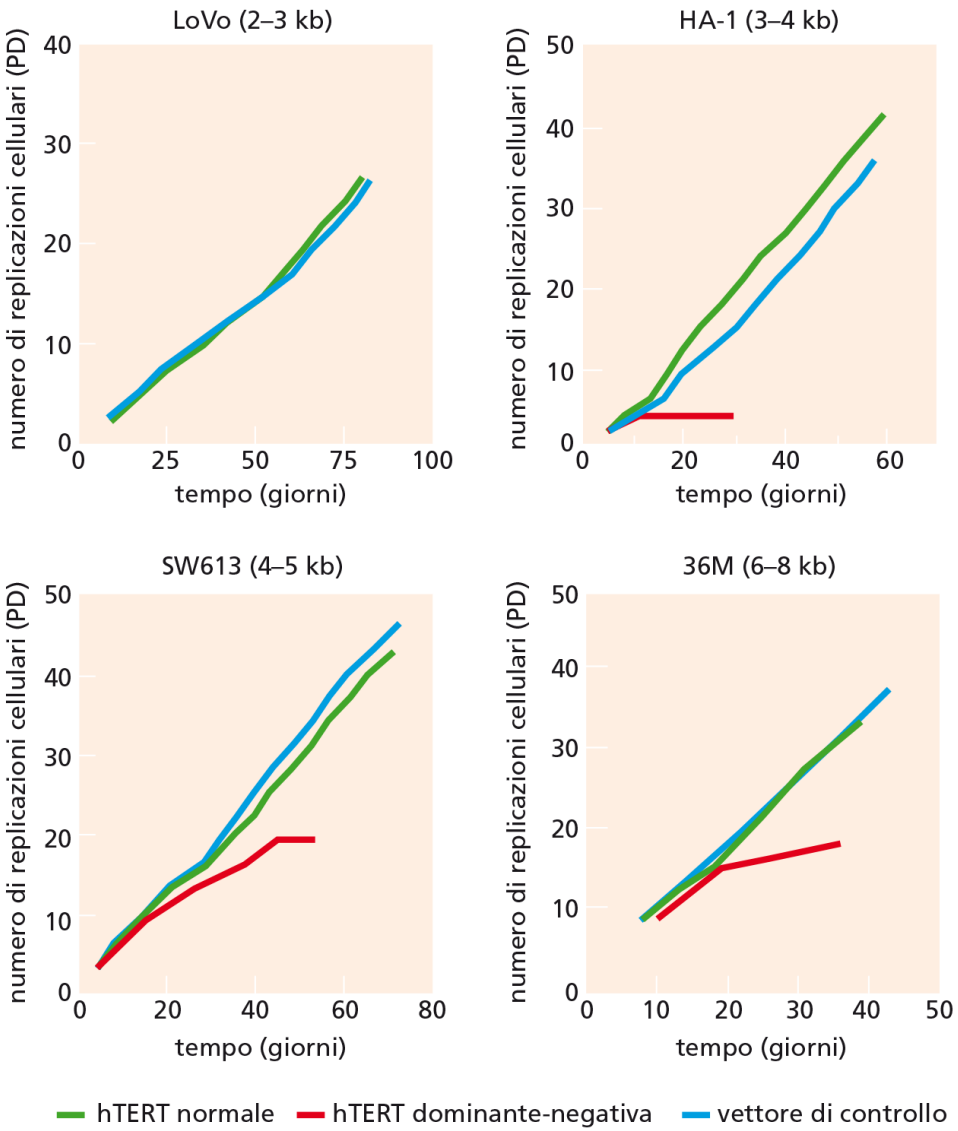


Weinberg, la biologia del cancro

Inibizione telomerasi e proliferazione

- Sh *hTR* riduce la proliferazione di cellule HeLa dopo 23-26 giorni dal trattamento

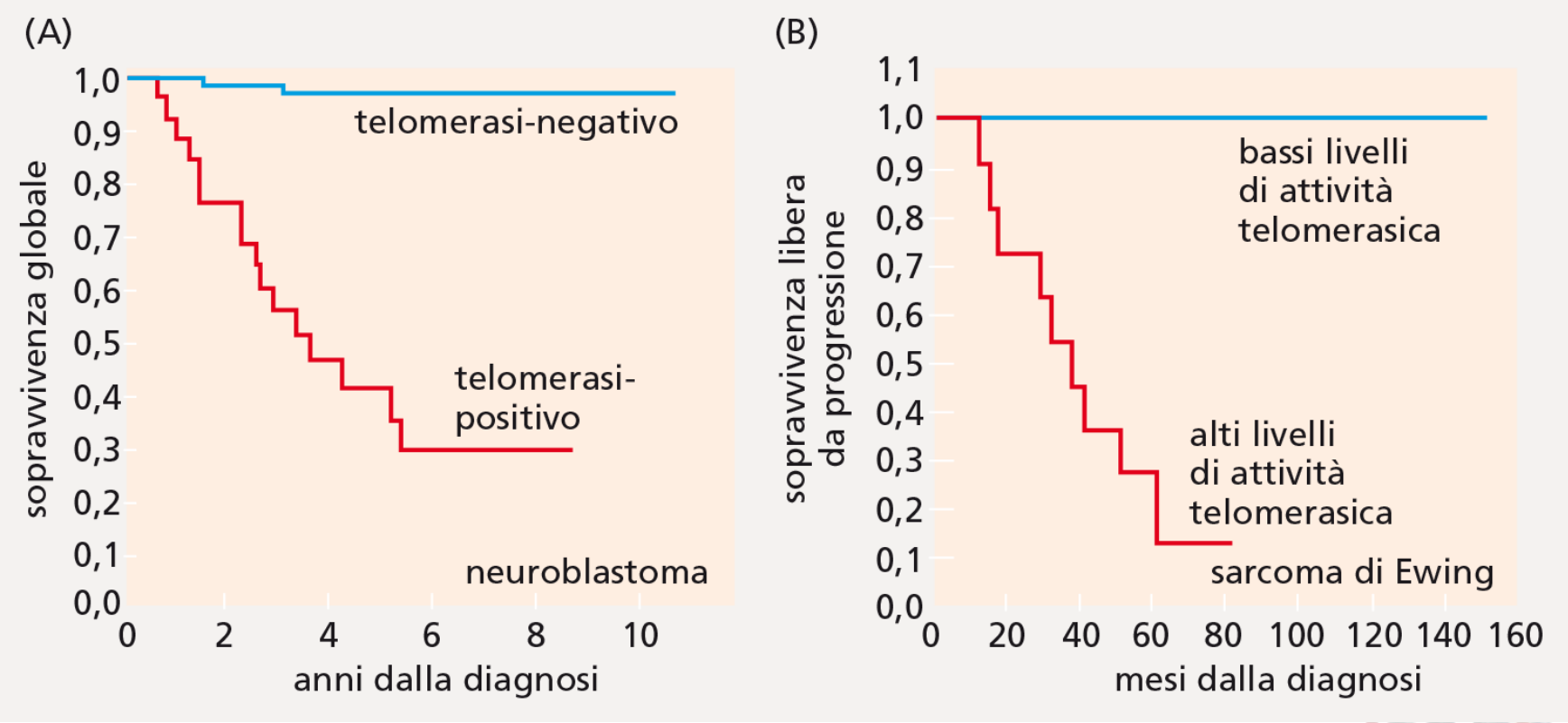
- Mutante dominante negativo di *hTERT*: *dn-hTERT:*
  - tempo per entrare in crisi dipende da lunghezza telomero
  -crisi associata a perdita attività telomerasica



Weinberg, la biologia del cancro

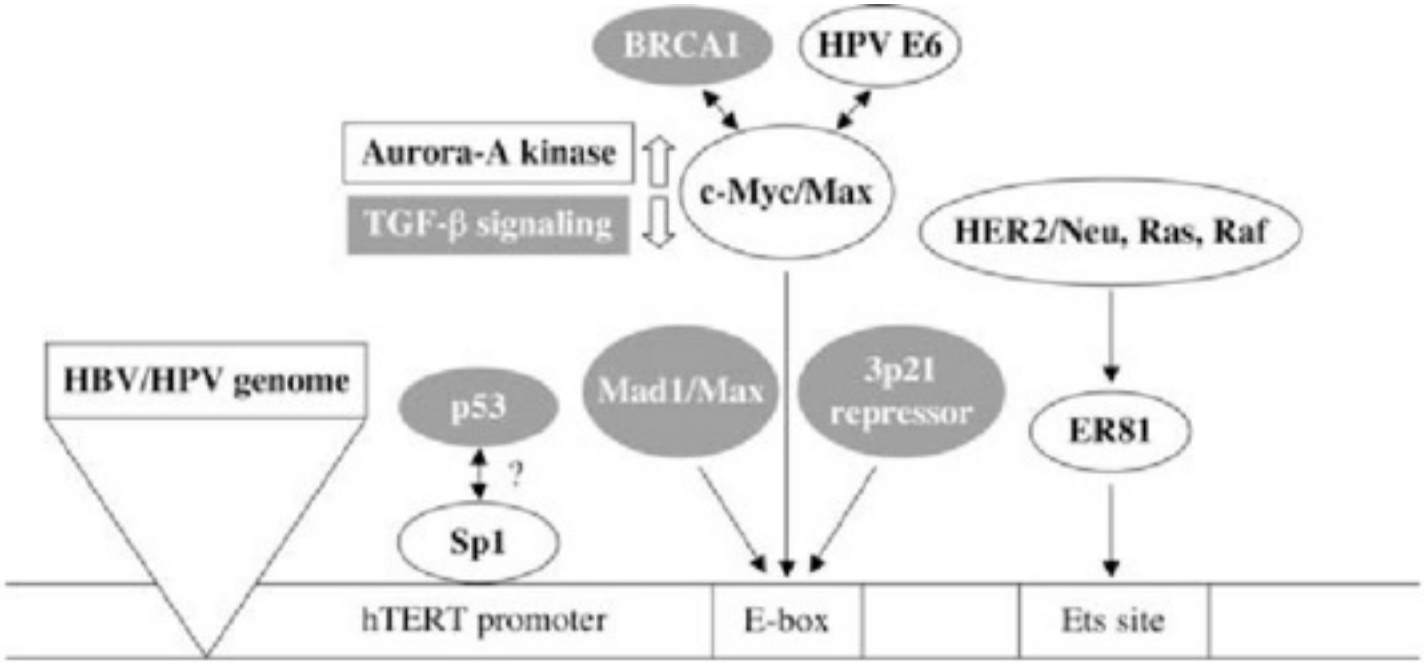
Telomerasi nei tumori pediatrici

- Attività telomerasica e prognosi nei tumori pediatrici



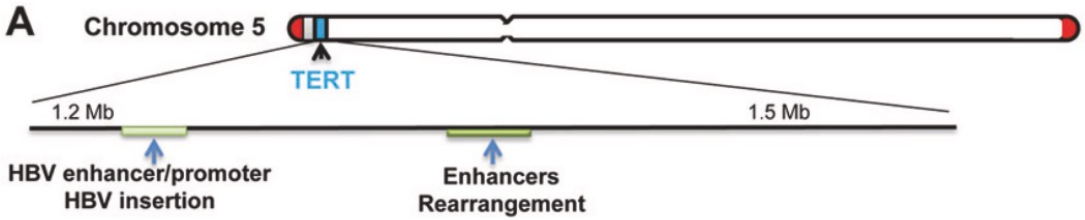
Weinberg, la biologia del cancro

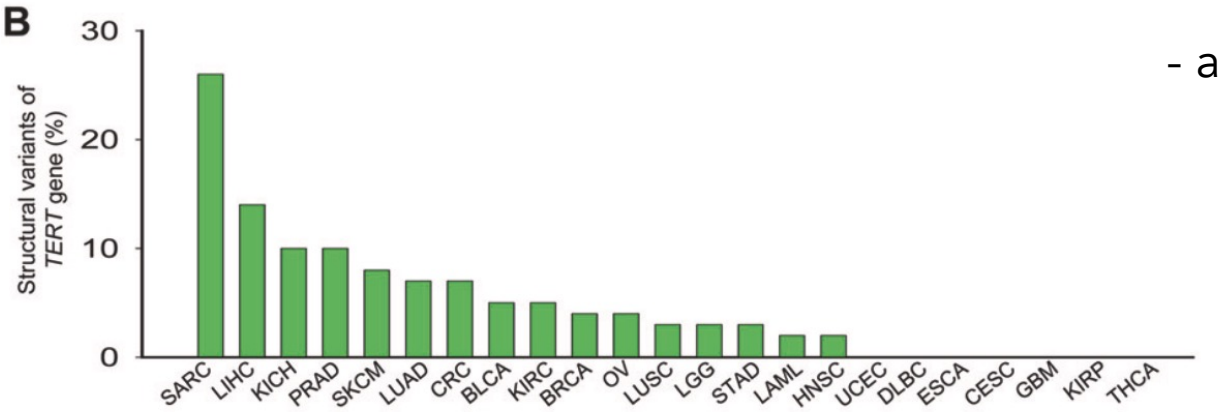
Come si riattiva la telomerasi?

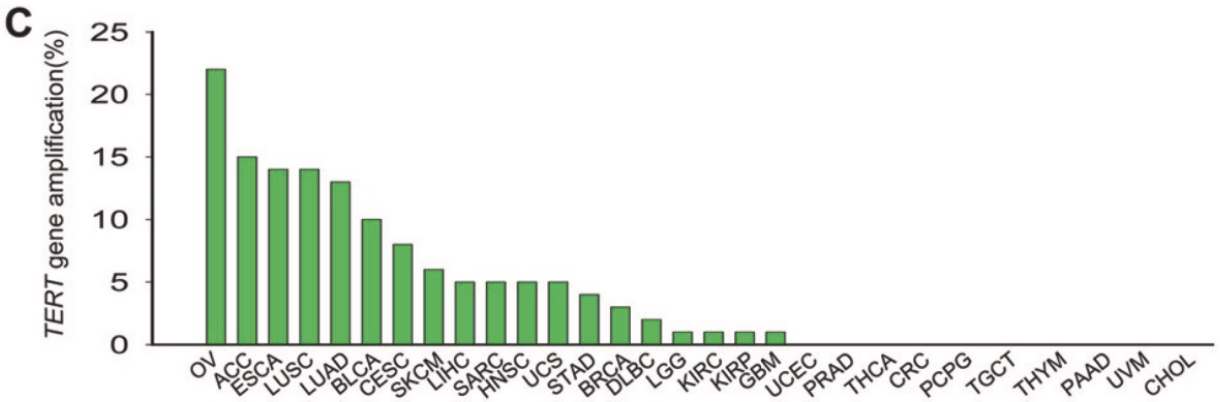


Horikawa et al., 2004

Come si riattiva la telomerasi?







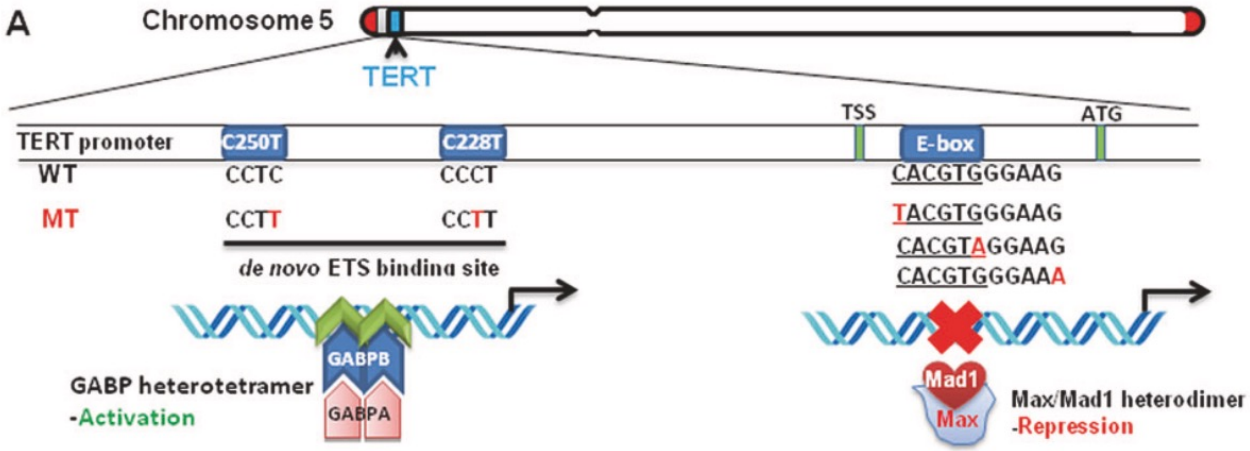
Variazioni strutturali al locus *hTERT:*

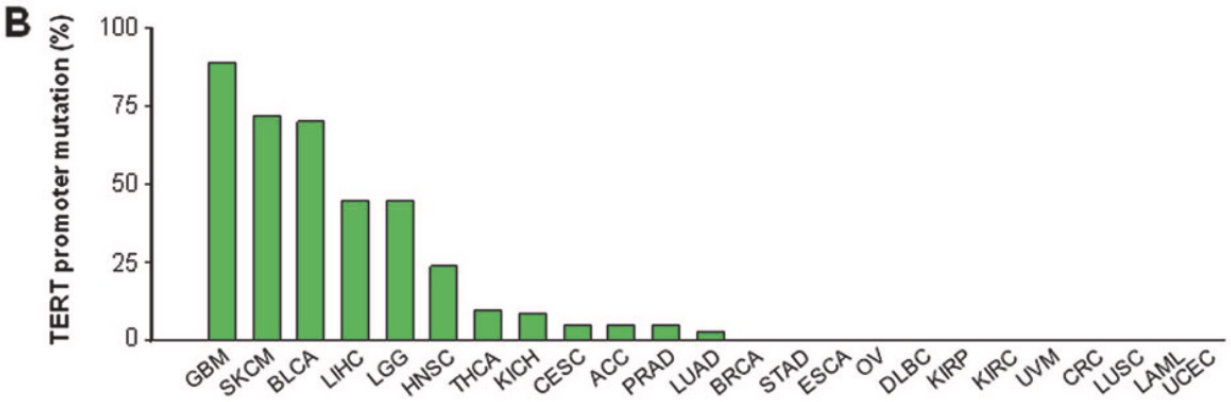
-introduzione enhancers, traslocazioni

- amplificazione genica

Yuan et al., 2019

Come si riattiva la telomerasi?





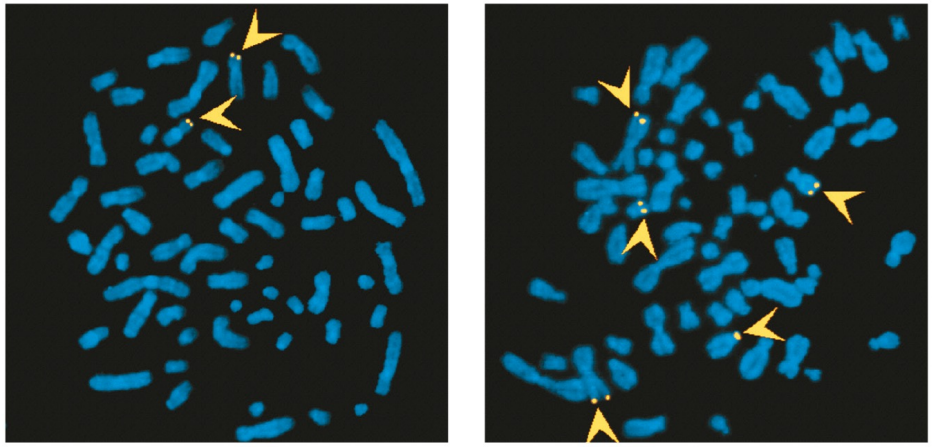
Mutazioni nel promotore di *hTERT:* nuovi siti di legame per fattori trascrizionali (ETS)

Come fanno a continuare a dividersi quel 10-15% di tumori che non attivano la telomerasi?

Yuan et al., 2019

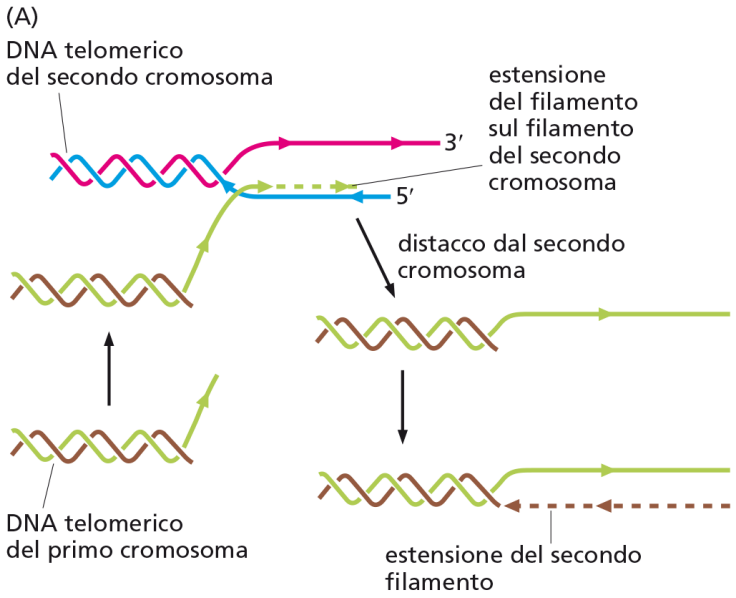
Alternative Lengthening of Telomeres (ALT)

FISH per un marcatore genetico di uno specifico telomero

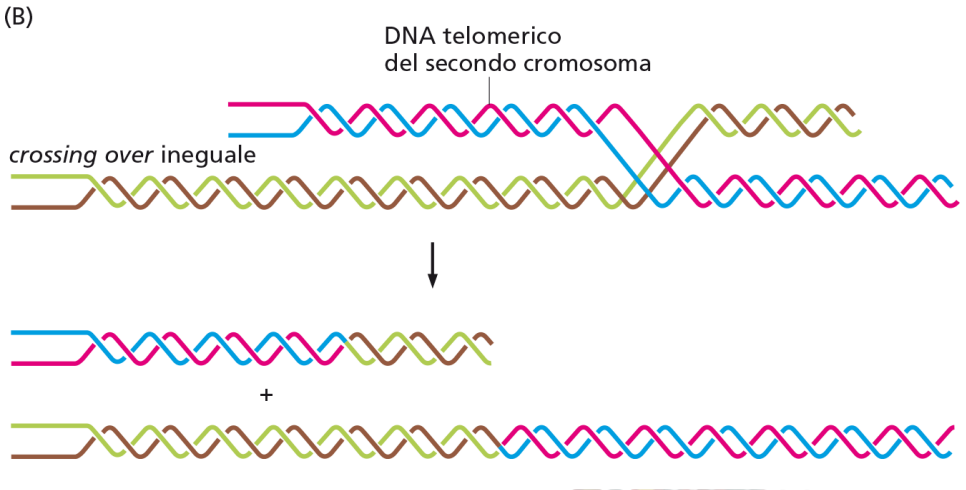


passaggio cellulare iniziale

dopo 40 replicazioni cellulari

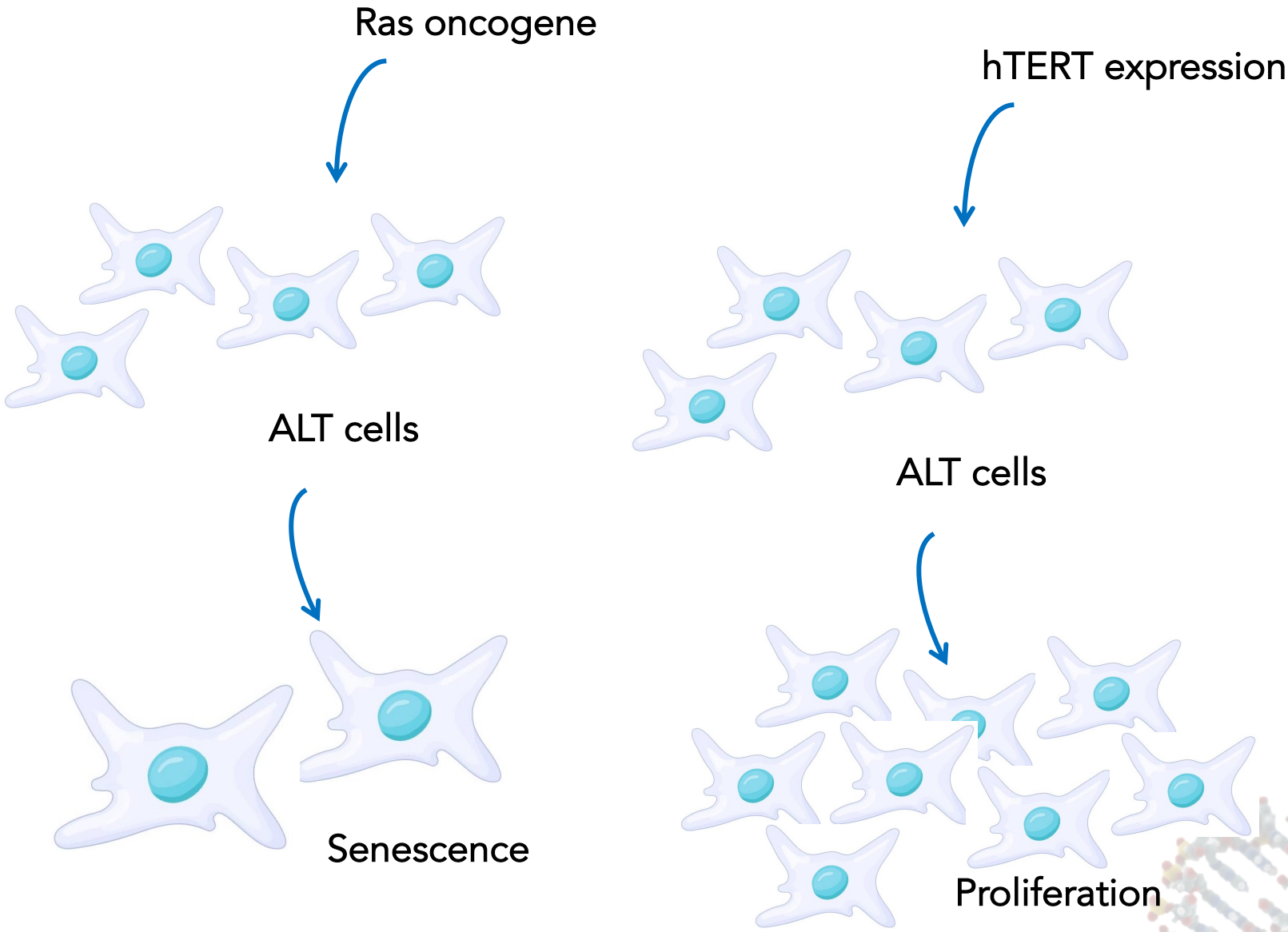


2 meccanismi proposti per ALT



Horikawa et al., 2004

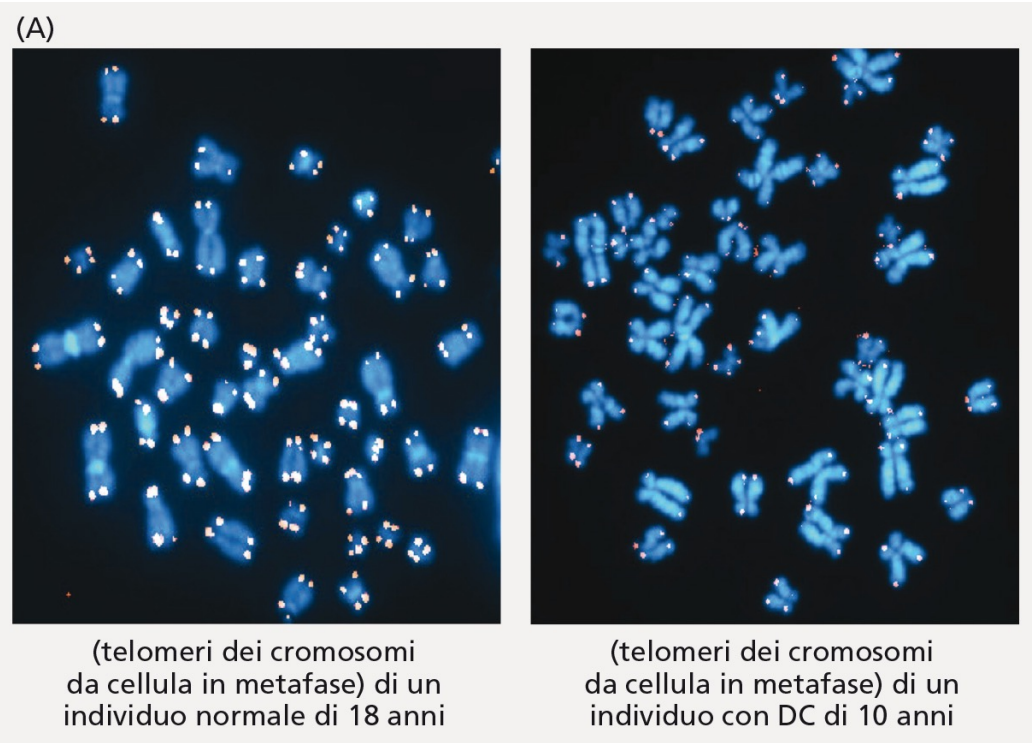
Alternative Lengthening of Telomeres (ALT)



TELOMERASI IN VIVO

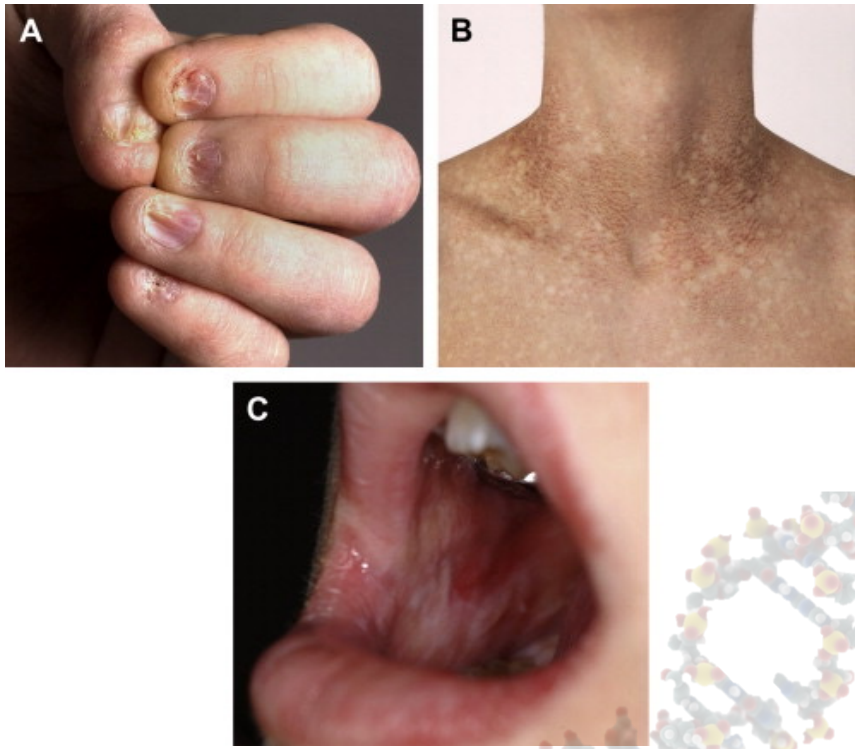
Discheratosi Congenita (DC)

Displasia ectodermica rara: alterazioni della pigmentazione cutanea e leucoplachia orale, rischio elevato di insufficienza del midollo osseo e di neoplasie, invecchiamento precoce.
Forma autosomica dominante: mutazioni nei
geni TERC (3q26.2), TERT (5p15.33), TINF2(14q12), RTEL1 (20q13.3), PARN (16p13.12),
e ACD (16q22.1)



(telomeri dei cromosomi da cellula in metafase) di un individuo normale di 18 anni

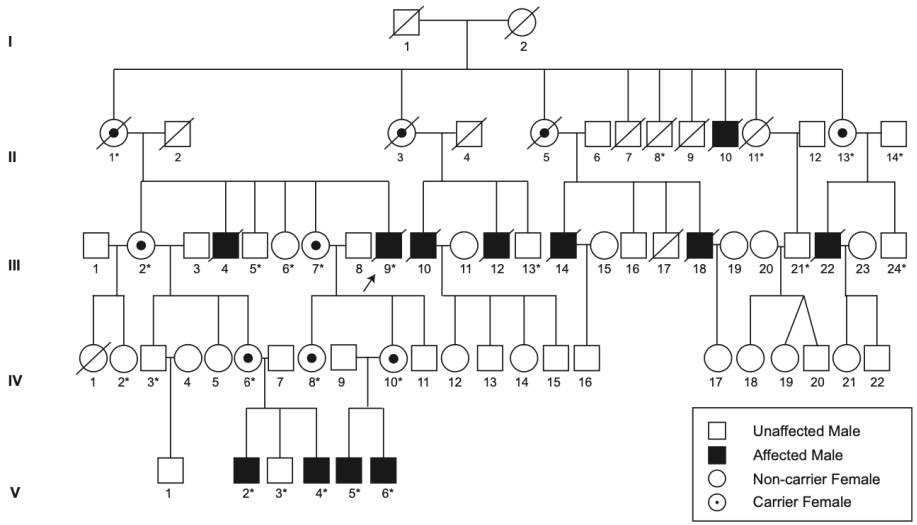
(telomeri dei cromosomi da cellula in metafase) di un individuo con DC di 10 anni

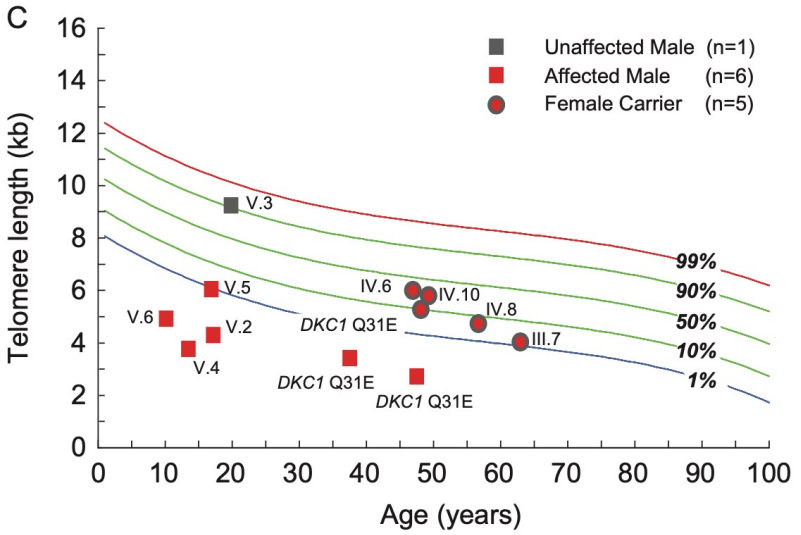


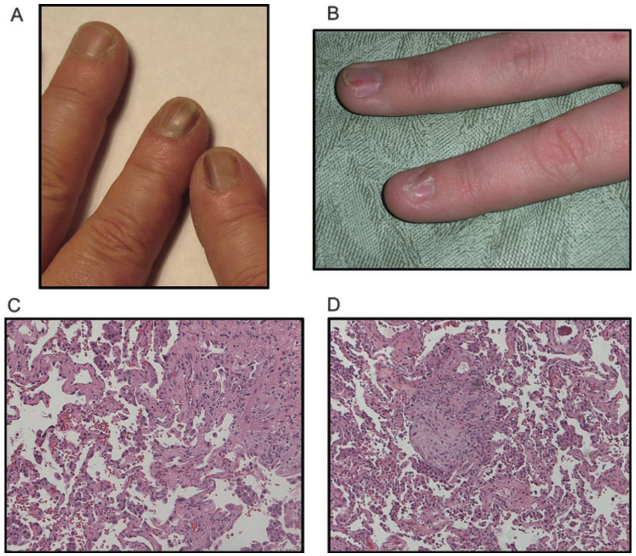
Weinberg, la biologia del cancro; Savage and Alter, 2009

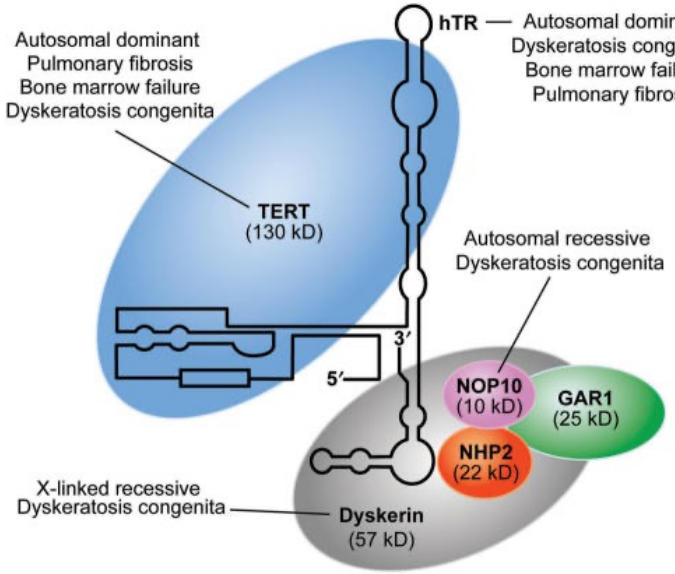
Discheratosi Congenita (DC)

Forma legata a X e Discherina





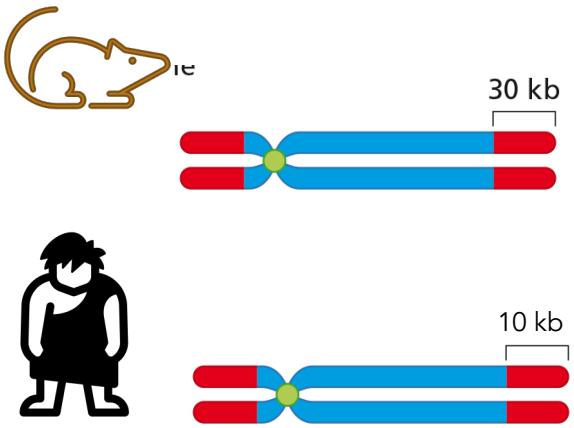


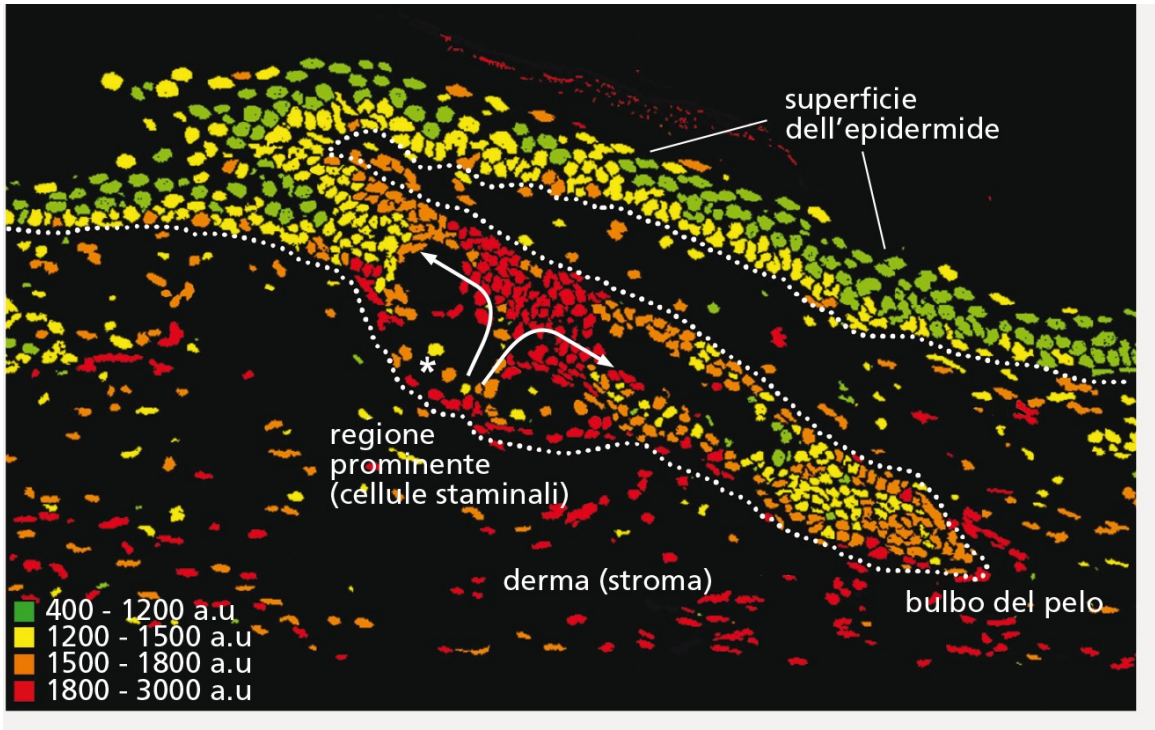


Parry et al., 2011; Garcia et al., 2007

Telomeri e telomerasi nel topo

Studio della funzione della telomerasi in vivo: modello murino

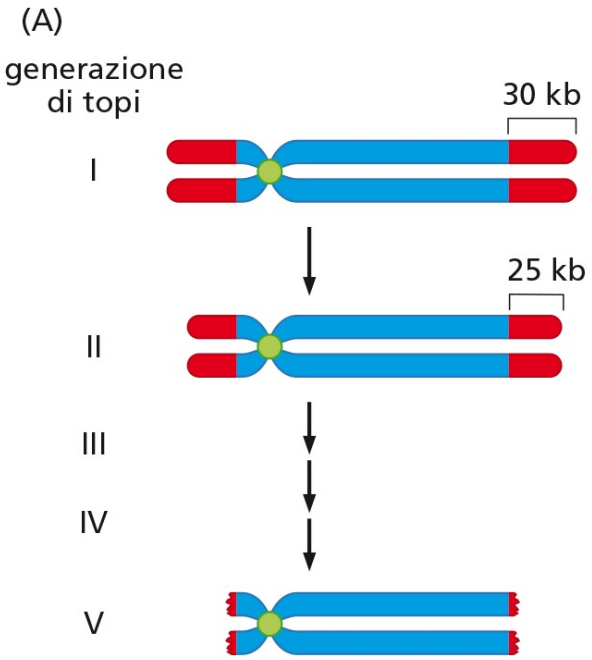


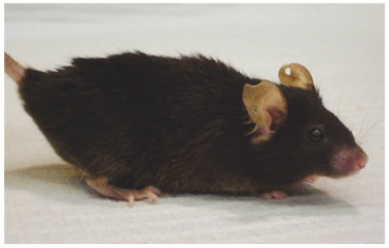


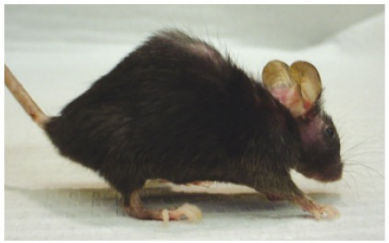
Weinberg, la biologia del cancro

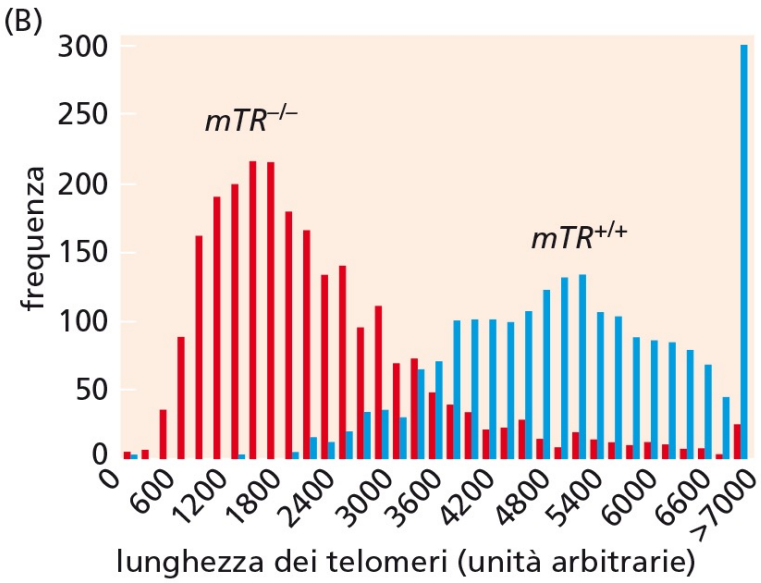
Topo telomerasi-

Prima generazione









Weinberg, la biologia del cancro

Telomerasi e suscettibilità ai tumori

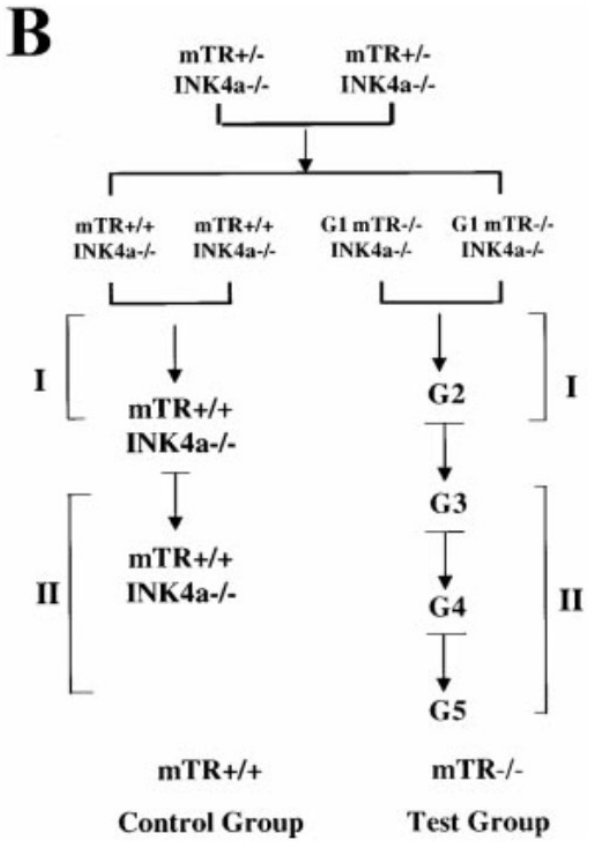
- Topi da laboratorio suscettibili a tumore spontanei (leucemie e linfomi)
- Attivazione proto-oncogeni o inattivazione oncogeni nella linea germinale aumenta la suscettibilità a tumori
- Effetto del telomero in topi *mTR*$^{-/-}$ su suscettibilità ai tumori nelle generazioni? Ridotta suscettibilità ai tumori in topi vecchi?

Cell, Vol. 97, 515–525, May 14, 1999, Copyright ©1999 by Cell Press

Short Dysfunctional Telomeres Impair Tumorigenesis in the INK4a$^{\Delta 2/3}$ Cancer-Prone Mouse

Roger A. Greenberg,[4] Lynda Chin,[1,3]
Andrea Femino,[5] Kee-Ho Lee,[1]
Geoffrey J. Gottlieb,[6] Robert H. Singer,[5]
Carol W. Greider,[7] and Ronald A. DePinho[1,2,8]

known as mortality stage 1 [M1]), suggesting that th
tumor suppressor pathways are critical mediators
this telomere length checkpoint response (reviewe
Wright and Shay, 1995). Cellular proliferation bey



Greenberg et al., 1999

Topo telomerasi

A

Genotype	mTR+/+ INK4a-/-	mTR+/- INK4a-/-	G1 mTR-/- INK4a-/-	G2 mTR-/- INK4a-/-
# Mice Analyzed	14	16	13	19
Tumor Incidence (%)	6 (43%)	8 (50%)	7 (54%)	9 (47%)
Mean Latency (wks)	10	11	11	10

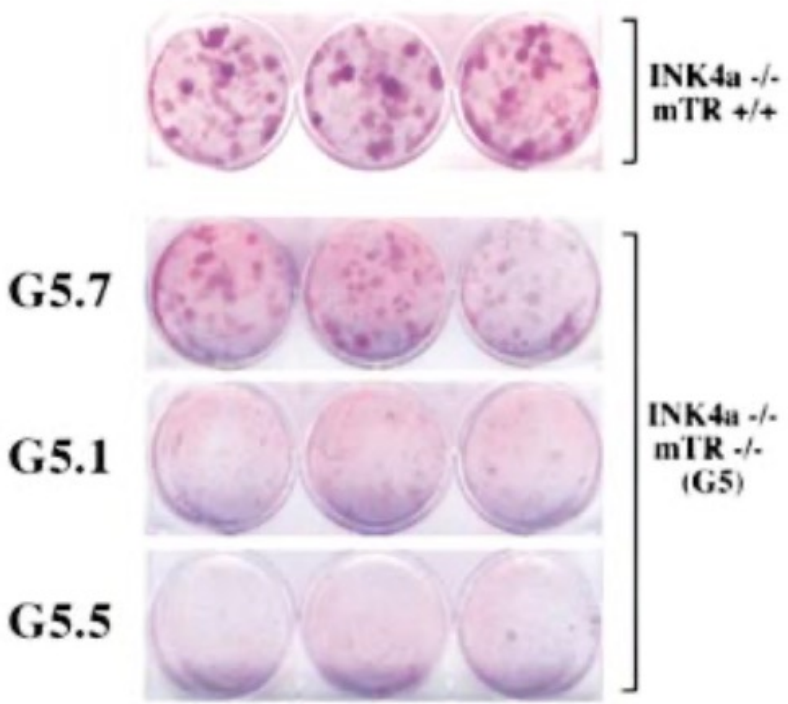
B

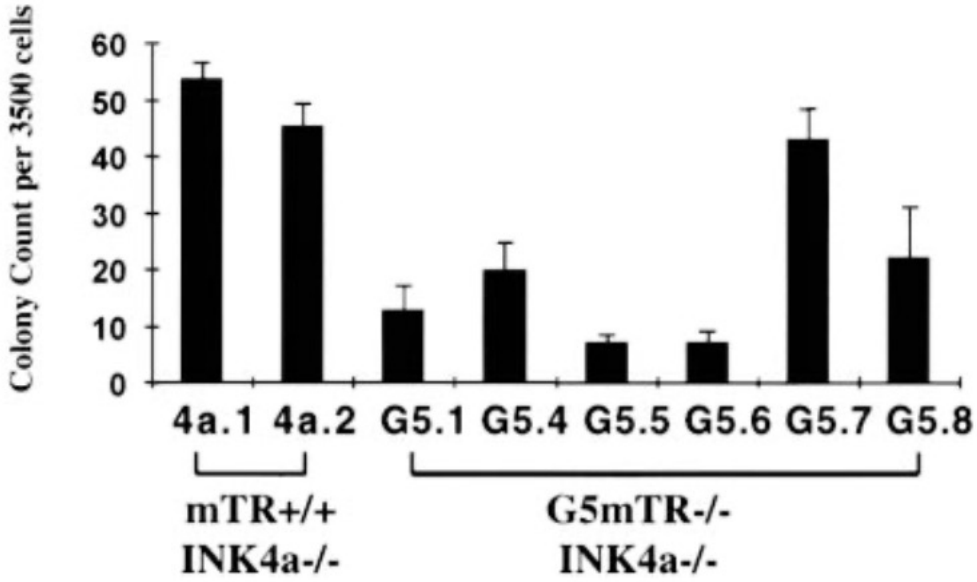
Genotype	mTR+/+ INK4a-/-	G3 mTR-/- INK4a-/-	G4 mTR-/- INK4a -/-	G5 mTR-/- INK4a-/-
# Mice Analyzed	25	12	16	26
Tumor Incidence (%)	16 (64%)	6 (50%)	5 (31%)	8 (31%)
Mean Latency (wks)	9.5	11	13	10
# Survived (%)	3 (12%)	4 (33%)	7 (44%)	14 (54%)

Greenberg et al., 1999

Topo telomerasi

Colture primarie di MEFs





Greenberg et al., 1999

Topo telomerasi

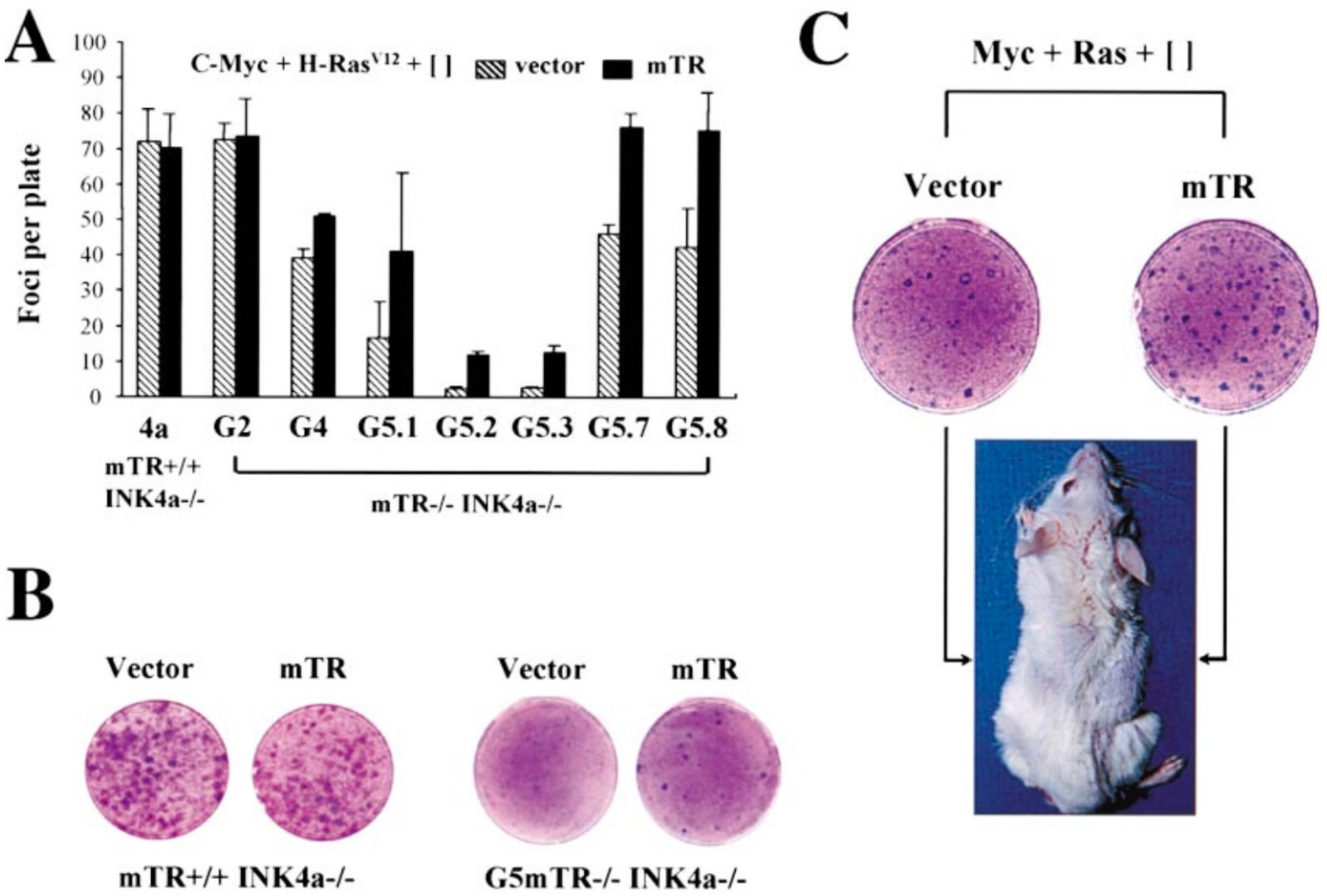


Figure 4. mTR Partially Rescues Myc/RAS Transformation of G5 *mTR*$^{-/-}$ *INK4a*$^{-/-}$ MEFs

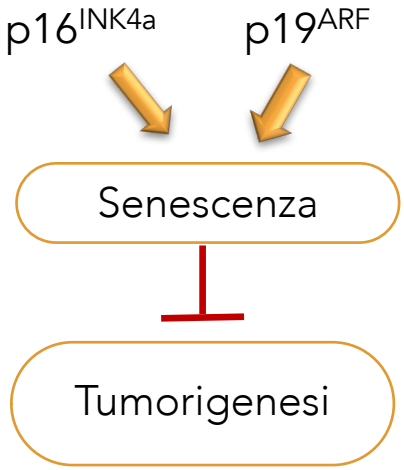
(A) Graphic illustration of the number of foci per 10 cm plate 9 days following transfection of oncogenes Myc + RAS and either empty vector (hatched) or mTR (black).

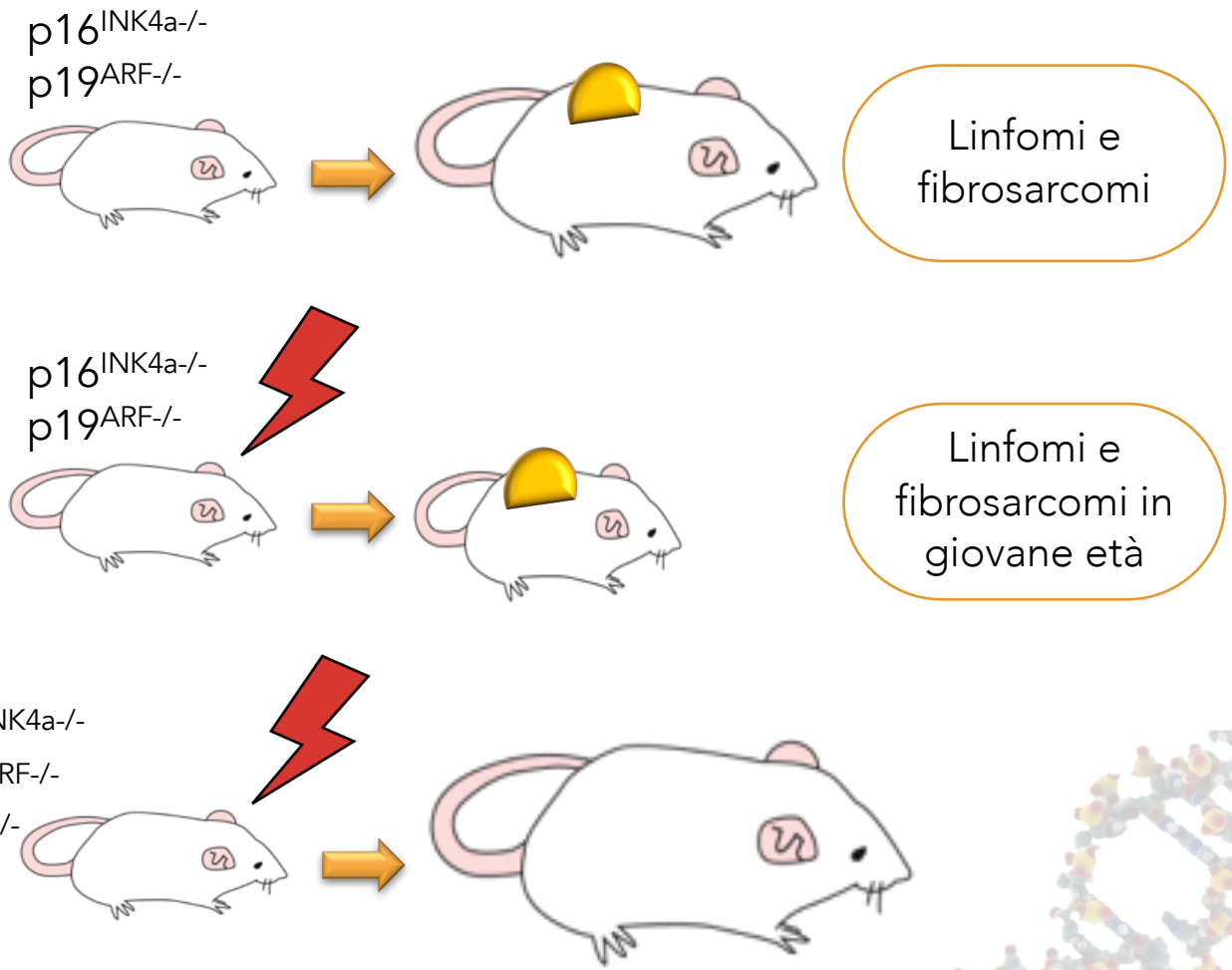
(B) Representative plates of the same MEF culture transfected with Myc + RAS + empty vector or mTR. Plates were stained with 0.1% crystal violet.

(C) Growth of transformed cells in SCID mice. Entire plates transformed with Myc + RAS + vector (left) or mTR (right) were trypsinized and 5×10^5 cells were injected subcutaneously into SCID mice. Pictures were taken 12 days following injection.

Greenberg et al., 1999

Topo telomerasi-





E' sempre così?

Topo telomerasi

	G6 mTR-/- p53+/+ Cultures			G6 mTR-/- p53-/- Cultures		
	448.5	448.7	Total	448.1	448.8	Total
# metaphases	18	8	**26**	17	14	**31**
% aneuploid	44%	75%	**54%**	94%	79%	**87%**
# fusion per metaphase	0.44	0.375	**0.42**	1.24	2.14	**1.65**

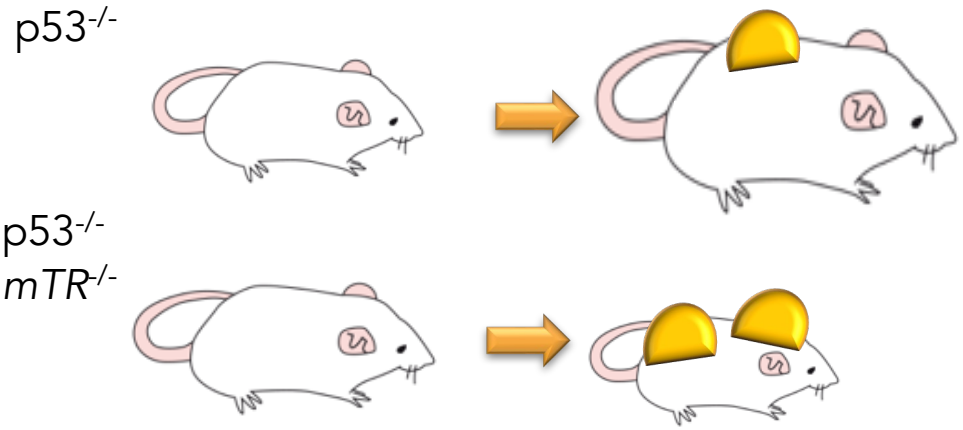
p = 0.012

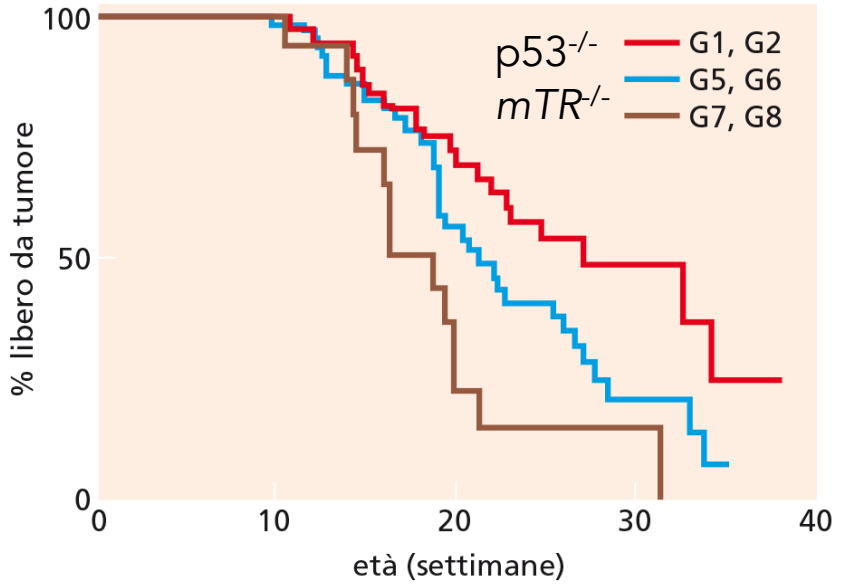
Cell, Vol. 97, 527–538, May 14, 1999, Copyright ©1999 by Cell Press

p53 Deficiency Rescues the Adverse Effects of Telomere Loss and Cooperates with Telomere Dysfunction to Accelerate Carcinogenesis

Lynda Chin,[1,3,7] Steven E. Artandi,[1,7]
Qiong Shen,[1] Alice Tam,[1]
Shwu-Luan Lee,[1] Geoffrey J. Gottlieb,[4]
Carol W. Greider,[5] and Ronald A. DePinho[1,2,6]

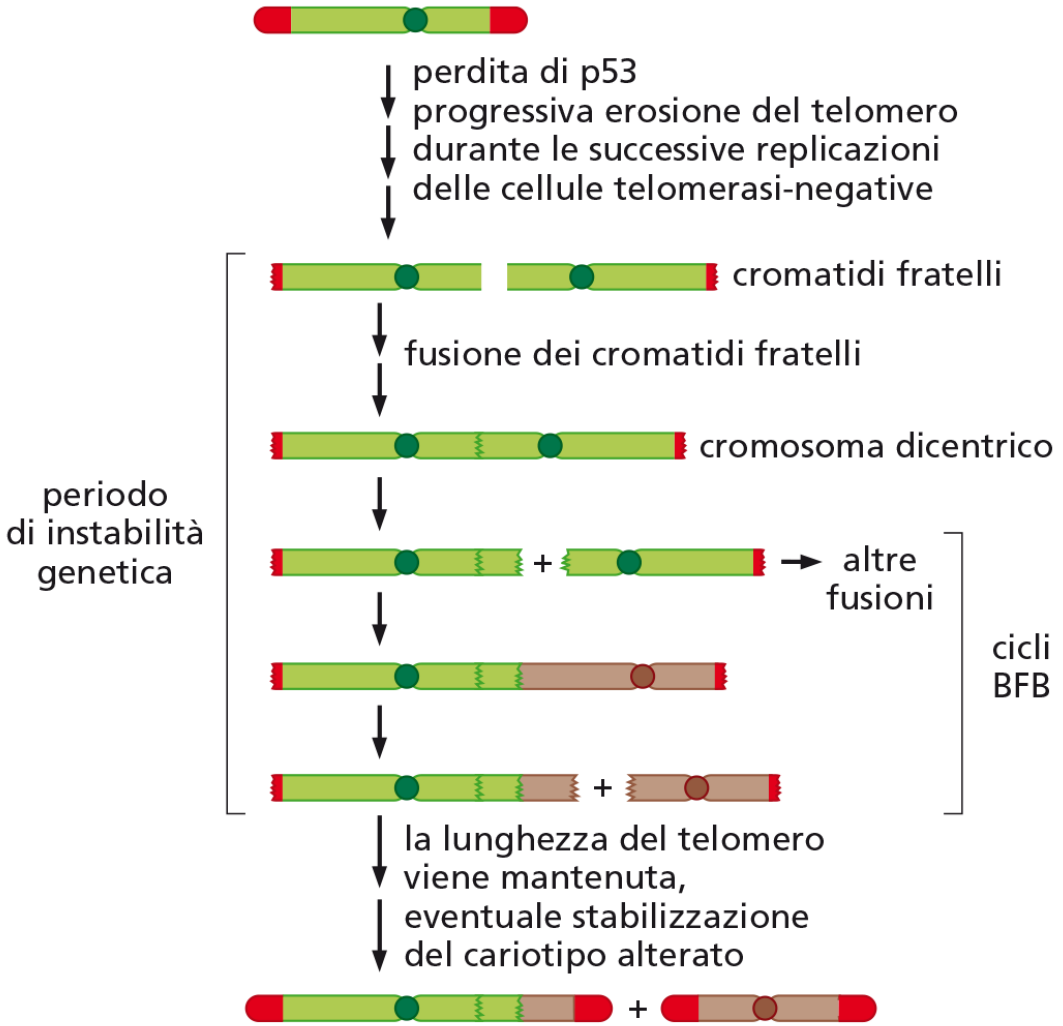
Most somatic human tissues and primary
sess low or undetectable telomerase activity,
meres shorten with each cell division in vivo ar
In human cells, a critical telomere length is





Weinberg, la biologia del cancro; Chin et al., 1999

Telomeri e progressione tumorale

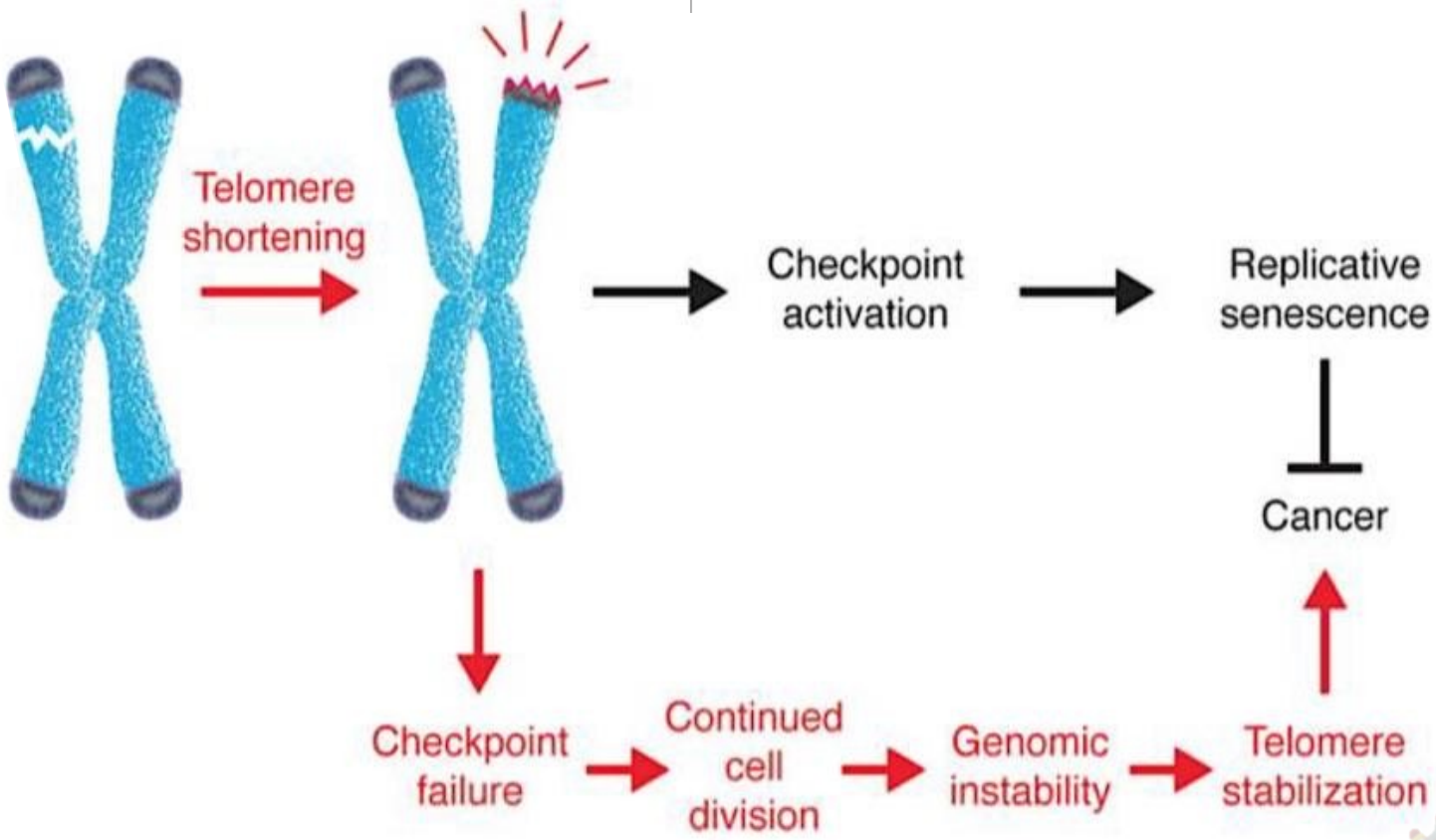


Weinberg, la biologia del cancro

Telomeri e progressione tumorale

Telomeres in cancer: tumour suppression and genome instability

John Maciejowski and Titia de Lange



Maciejowski and de Lange, 2017

Telomeri e progressione tumorale

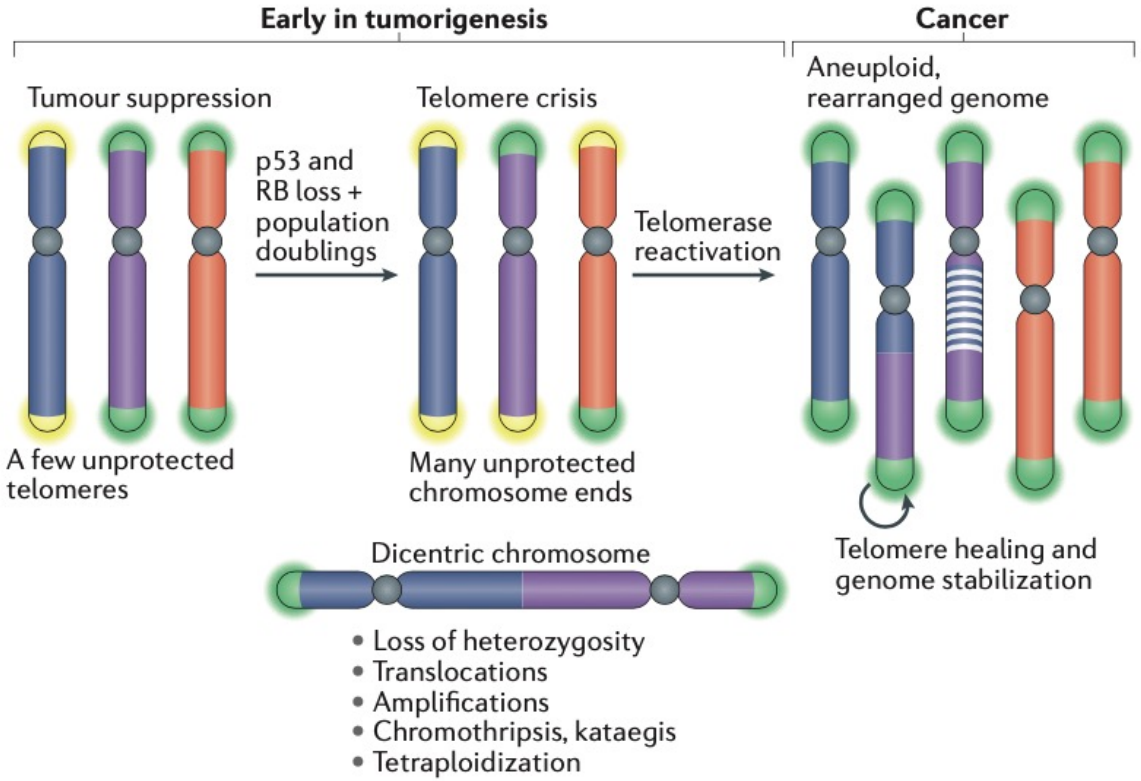


Figure 3 | **Telomere crisis.** Loss of the RB and p53 tumour suppressor pathways disables the ability of cells to respond with cell cycle arrest to ATR and ATM signalling. As the cells continue to divide, their telomeres continue to shorten. Once many telomeres become too short to function, the unprotected chromosome ends generate end-to-end fusions and dicentric chromosomes, leading to many forms of genome instability. Ultimately, telomerase reactivation provides a route out of telomere crisis by healing critically shortened telomeres and improving genomic stability, thereby increasing cell viability. The resulting tumour will have active telomerase and a heavily rearranged genome.

Maciejowski and de Lange, 2017

Telomeri e progressione tumorale

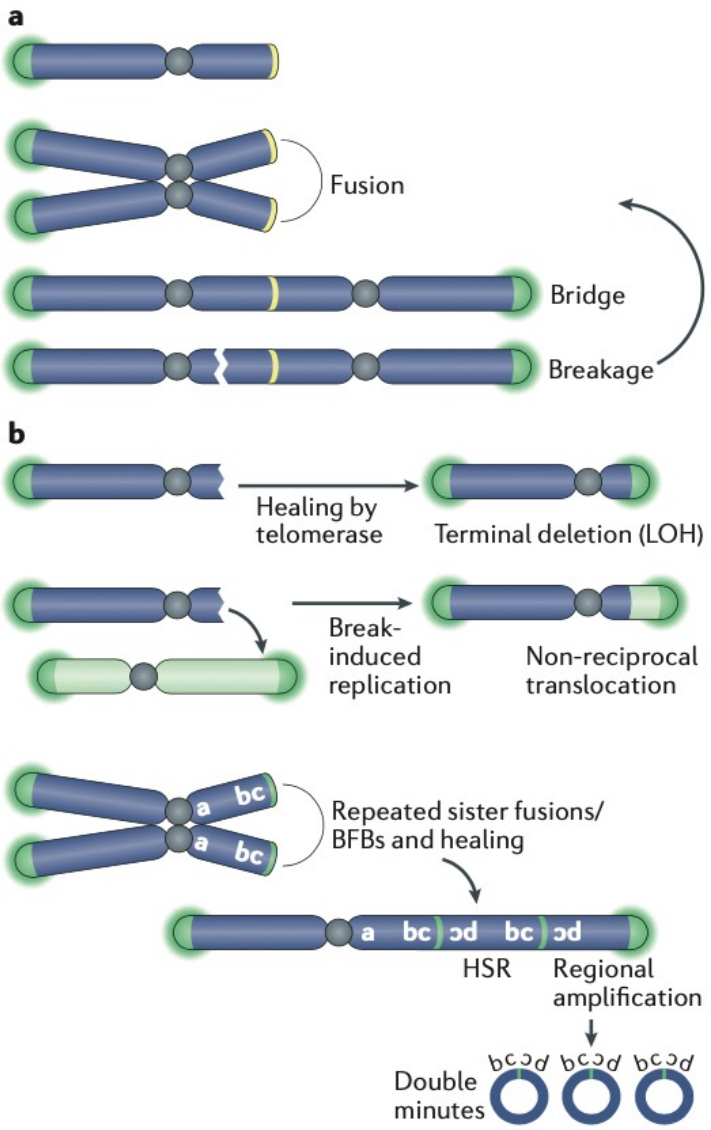


Figure 4 | **BFB cycles and chromosomal rearrangements during telomere crisis. a** | Breakage–fusion–bridge (BFB) cycles can occur when telomere fusion generates a dicentric chromosome. During anaphase, the mitotic spindle pulls this dicentric chromosome towards opposite spindle poles, thereby generating the widely observed anaphase bridges. During cell division, the dicentric chromosome undergoes breakage and the broken ends fuse again, giving rise to another dicentric chromosome. **b** | BFB cycles can be interrupted by telomerase-mediated telomere healing. If this process occurs following breakage, it can result in the formation of a terminal chromosome deletion and loss of heterozygosity (LOH). Alternatively, broken chromosomes can be repaired by break-induced replication, yielding a non-reciprocal translocation. Repeated cycles of BFB that occur between sister chromatids can result in regional amplification and the generation of a homogeneously staining region (HSR) following chromosome staining. This HSR consists of multiple amplicons of inverted repeats. Excision of the amplified sequences out of the chromosome will generate circular double-minute chromosomes.

Maciejowski and de Lange, 2017

Telomeri e progressione tumorale

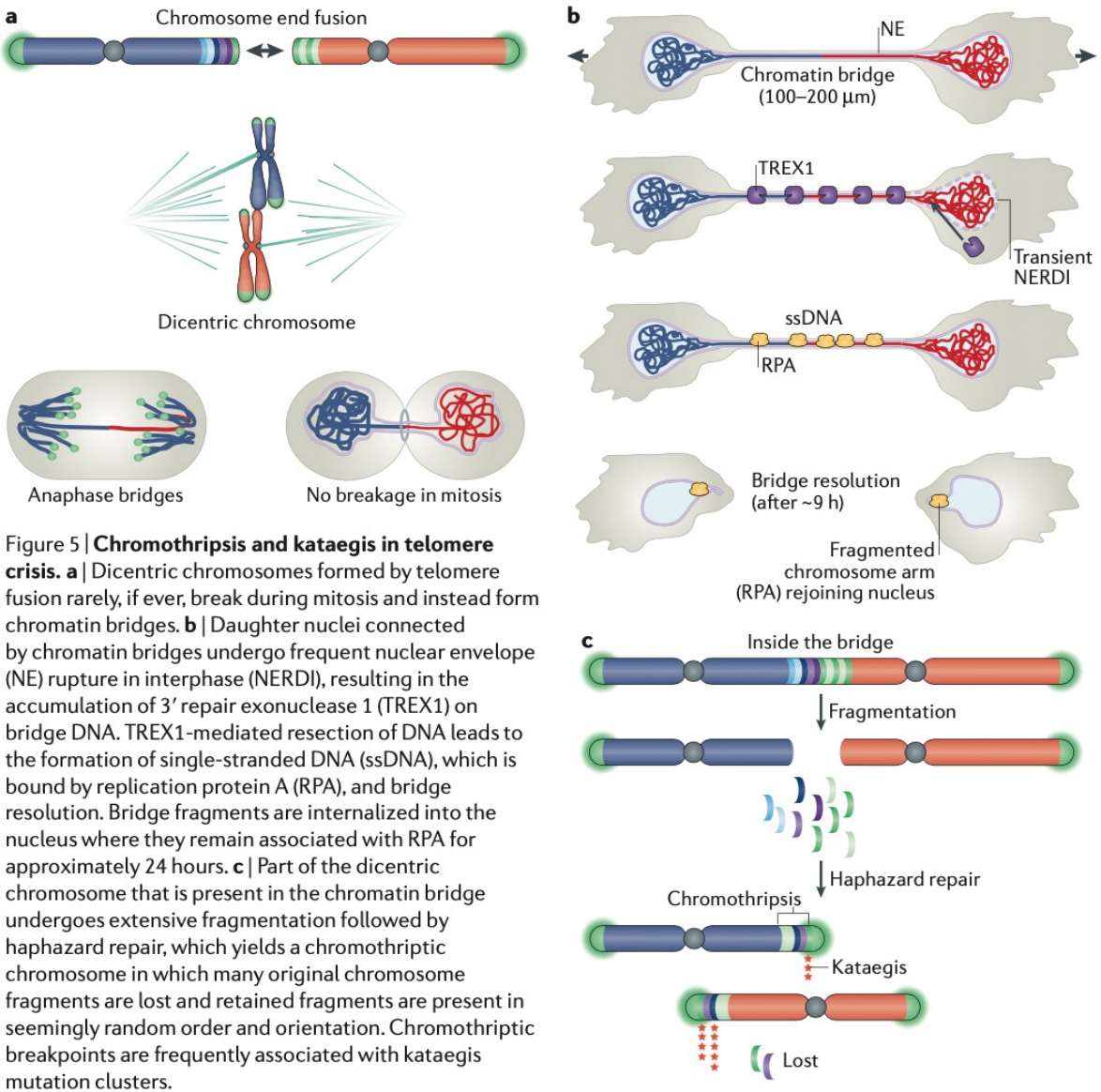


Figure 5 | **Chromothripsis and kataegis in telomere crisis. a** | Dicentric chromosomes formed by telomere fusion rarely, if ever, break during mitosis and instead form chromatin bridges. **b** | Daughter nuclei connected by chromatin bridges undergo frequent nuclear envelope (NE) rupture in interphase (NERDI), resulting in the accumulation of 3′ repair exonuclease 1 (TREX1) on bridge DNA. TREX1-mediated resection of DNA leads to the formation of single-stranded DNA (ssDNA), which is bound by replication protein A (RPA), and bridge resolution. Bridge fragments are internalized into the nucleus where they remain associated with RPA for approximately 24 hours. **c** | Part of the dicentric chromosome that is present in the chromatin bridge undergoes extensive fragmentation followed by haphazard repair, which yields a chromothriptic chromosome in which many original chromosome fragments are lost and retained fragments are present in seemingly random order and orientation. Chromothriptic breakpoints are frequently associated with kataegis mutation clusters.

Maciejowski and de Lange, 2017

Telomeri e progressione tumorale

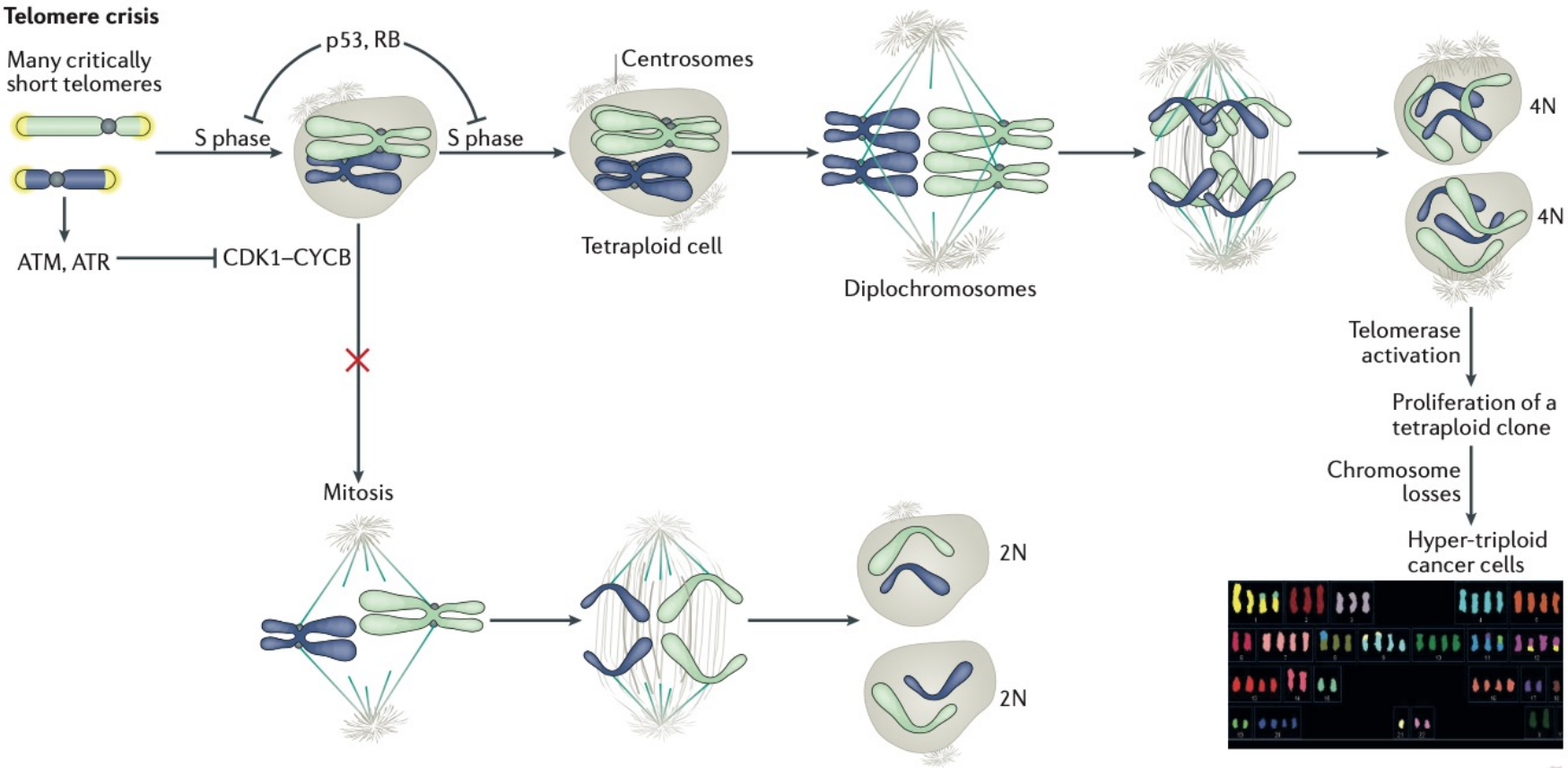
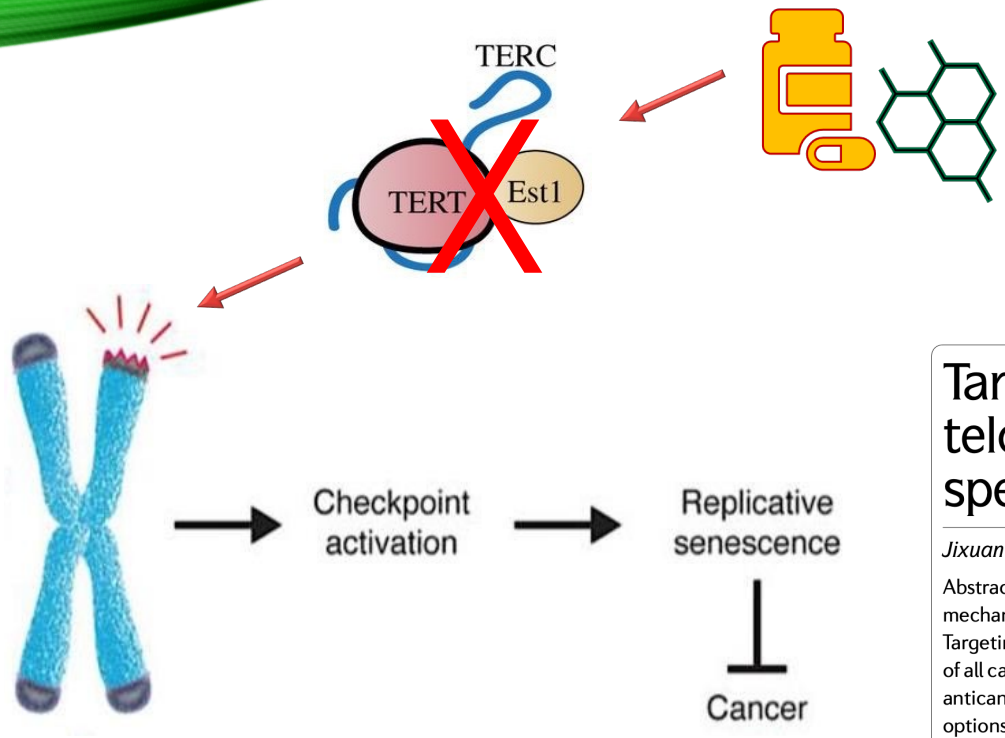


Figure 6 | **Tetraploidization during telomere crisis.** Telomere crisis can lead to persistent DNA damage signalling when repair fails to join all the unprotected ends and dysfunctional telomeres persist. The persistent ATM and ATR signalling and activation of their downstream effector kinases checkpoint kinase 2 (CHK2) and CHK1, respectively, results in prolonged inhibition of cyclin-dependent kinase 1 (CDK1)–cyclin B (CYCB), thus blocking entry into mitosis. Eventually, cells bypass mitosis, enter a G1-like state and then undergo a second S phase. The resulting tetraploid cells have diplochromosomes in the first mitosis following endoreduplication. Subsequently, the cells undergo frequent chromosome losses, leading to the hyper-triploid cells that are frequently observed in cancer. The example karyotype shown is from Capan-2, a hyper-triploid pancreatic cancer cell line (http://www.pawefish.path.cam.ac.uk/PancCellLineDescriptions/Capan-2.html), courtesy of Vorapan Sirivatanauksorn and Paul Edwards.

Maciejowski and de Lange, 2017

Targeting telomeres in cancer



Targeting telomeres: advances in telomere maintenance mechanism-specific cancer therapies

Jixuan Gao and Hilda A. Pickett ✉

Abstract | Cancer cells establish replicative immortality by activating a telomere-maintenance mechanism (TMM), be it telomerase or the alternative lengthening of telomeres (ALT) pathway. Targeting telomere maintenance represents an intriguing opportunity to treat the vast majority of all cancer types. Whilst telomerase inhibitors have historically been heralded as promising anticancer agents, the reality has been more challenging, and there are currently no therapeutic options for cancer types that use ALT despite their aggressive nature and poor prognosis. In this Review, we discuss the mechanistic differences between telomere maintenance by telomerase and ALT, the current methods used to detect each mechanism, the utility of these tests for clinical diagnosis, and recent developments in the therapeutic strategies being employed to target both telomerase and ALT. We present notable developments in repurposing established therapeutic agents and new avenues that are emerging to target cancer types according to which TMM they employ. These opportunities extend beyond inhibition of telomere maintenance, by finding and exploiting inherent weaknesses in the telomeres themselves to trigger rapid cellular effects that lead to cell death.

Gao & Pickett, 2022