LA TELOMERASI

The end-replication problem

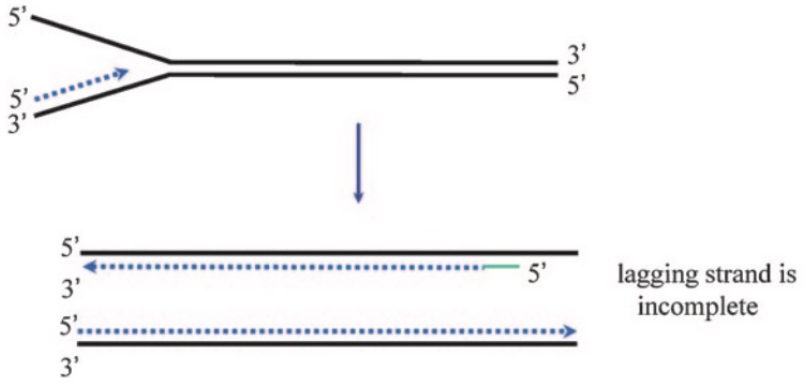
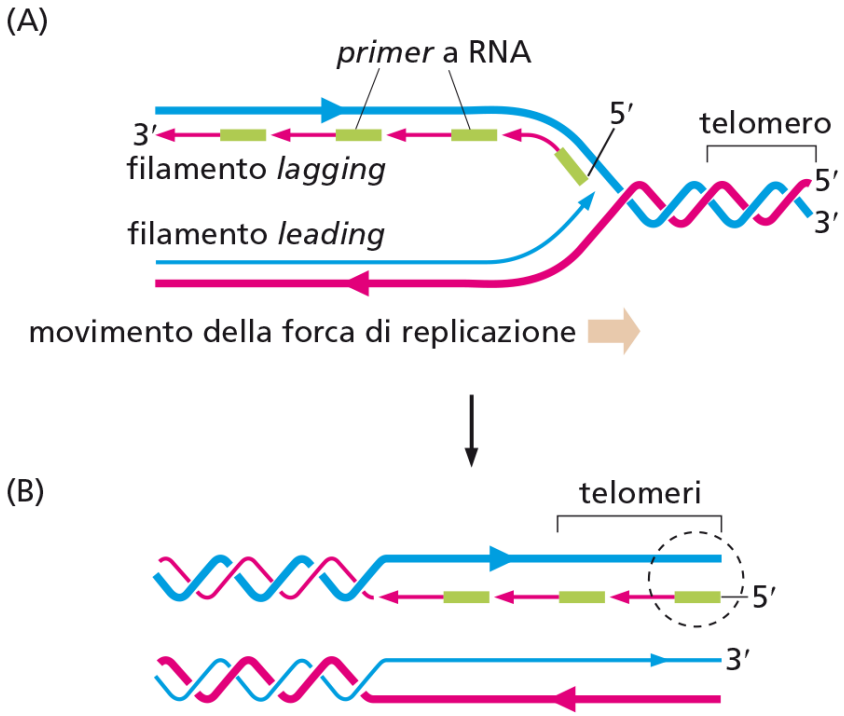


Figure 2. The end-replication problem as posed by Watson[18] and by Olovnikov.[19] When a replication fork reaches the end of a chromosome, the lagging strand will necessarily be incomplete as a result of the removal and potentially internal location of the last primer generated by primase.



Weinberg, la biologia del cancro

The Nobel Prize in Physiology or Medicine 2009

© The Nobel Foundation. Photo: U. Montan

Elizabeth H. Blackburn

Prize share: 1/3

© The Nobel Foundation. Photo: U. Montan

Carol W. Greider

Prize share: 1/3

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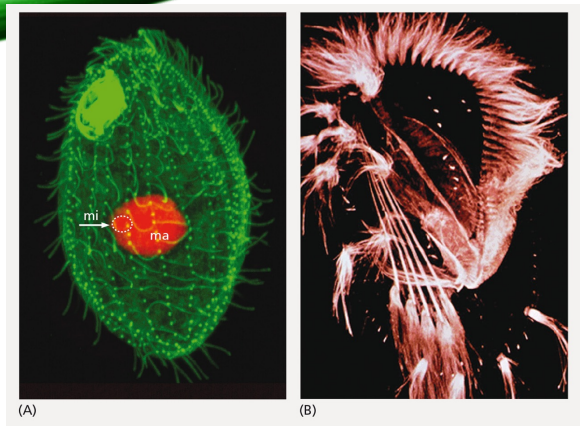
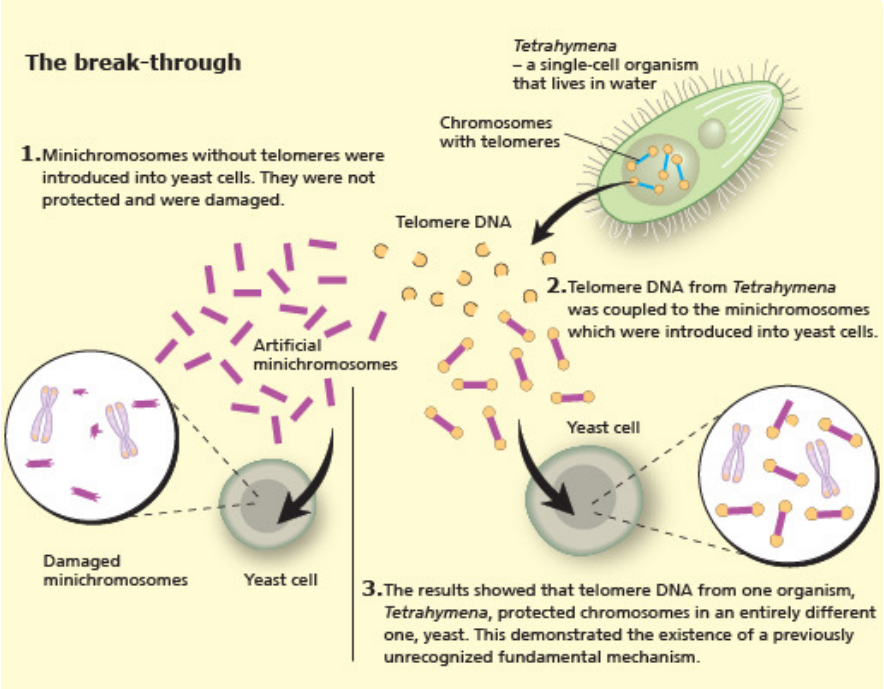
Jack W. Szostak

Prize share: 1/3

Telomeri e telomerasi



Elizabeth Blackburn studied the single-cell organism *Tetrahymena thermophila* and had found that the ends of chromosomes contain a short DNA sequence repeated many times.



Jack Szostak studied yeast cells and observed that linear artificial minichromosomes were rapidly degraded. Together they decided to test if telomere DNA from Tetrahymena could protect minichromosomes in yeast.

Carol Greider and Elizabeth Blackburn asked if an enzyme might synthesize telomeres.

Szostak et al., 2010

Telomeri e telomerasi

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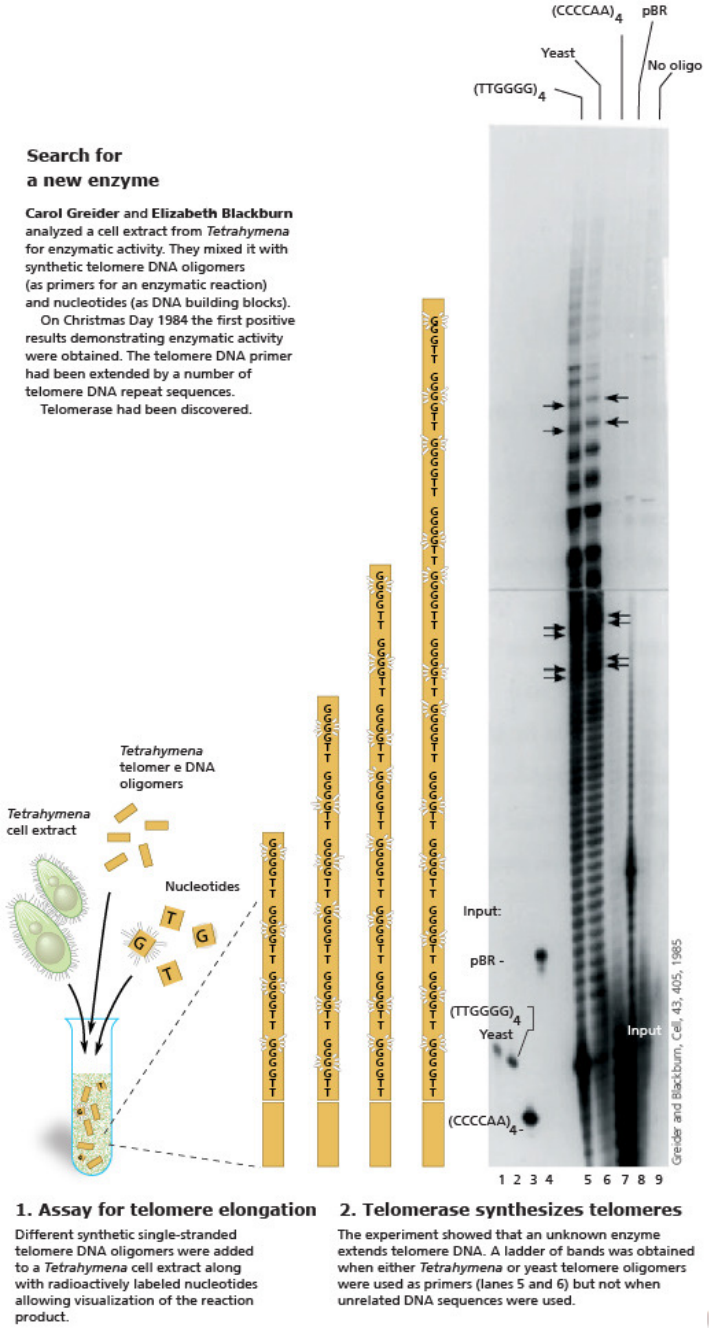


Szostak et al., 2010

TRAP assay

Supplementary Sidebar 10.1 The use of the TRAP assay and adaptations thereof permits the rapid and quantitative assessment of the levels of the catalytic activity of telomerase enzyme in eukaryotic cells In adult human cells, with the exception of the hTERT catalytic subunit, the remaining multiple subunits of the telomerase holoenzyme appear to be present at levels that are consistently adequate for robust telomerase activity. Consequently, enzyme activity is governed by changes in the levels of the hTERT catalytic subunit, which may vary dramatically from one cell type to another (Figure S10.1).

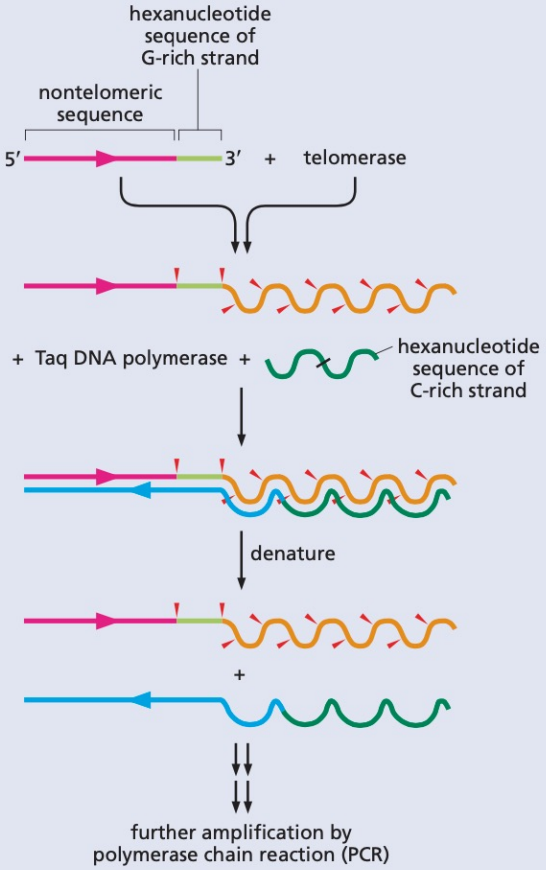
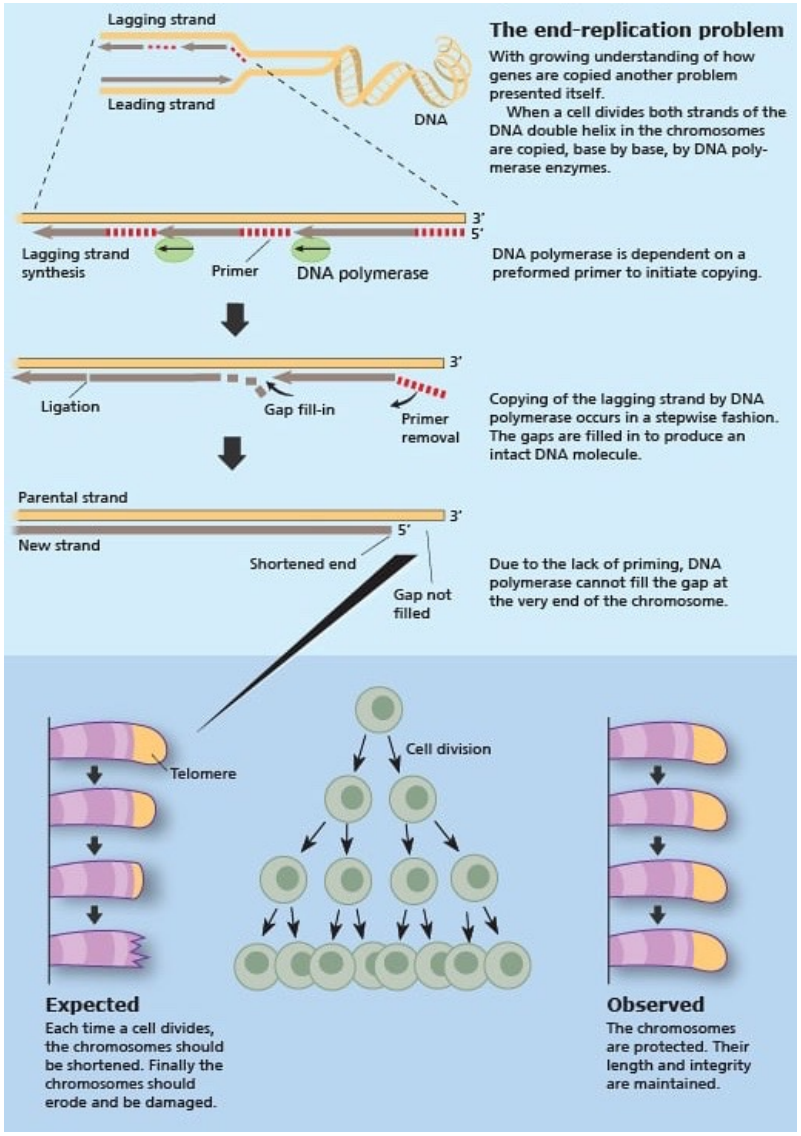


Figure S10.1 Detecting telomerase activity The telomeric repeat amplification protocol (TRAP) assay permits detection of minute levels of telomerase activity in cell lysates by relying on the polymerase chain reaction (PCR) to amplify the products of the telomerase enzyme. A primer consisting of nontelomeric sequences (*dark pink*) and telomeric hexanucleotide sequences (from the G-rich strand; *light green*) is added to a cell lysate in the presence of deoxyribonucleotide triphosphates. This primer is extended (*light orange*) by any telomerase that may be present in a cell lysate. The thermostable Taq polymerase is then added together with a primer from the C-rich strand (*dark green*), and the second strand is elongated (*light blue*). These two DNA strands are then denatured and recopied repeatedly by the PCR in the presence of appropriate primers.

Telomeri e telomerasi



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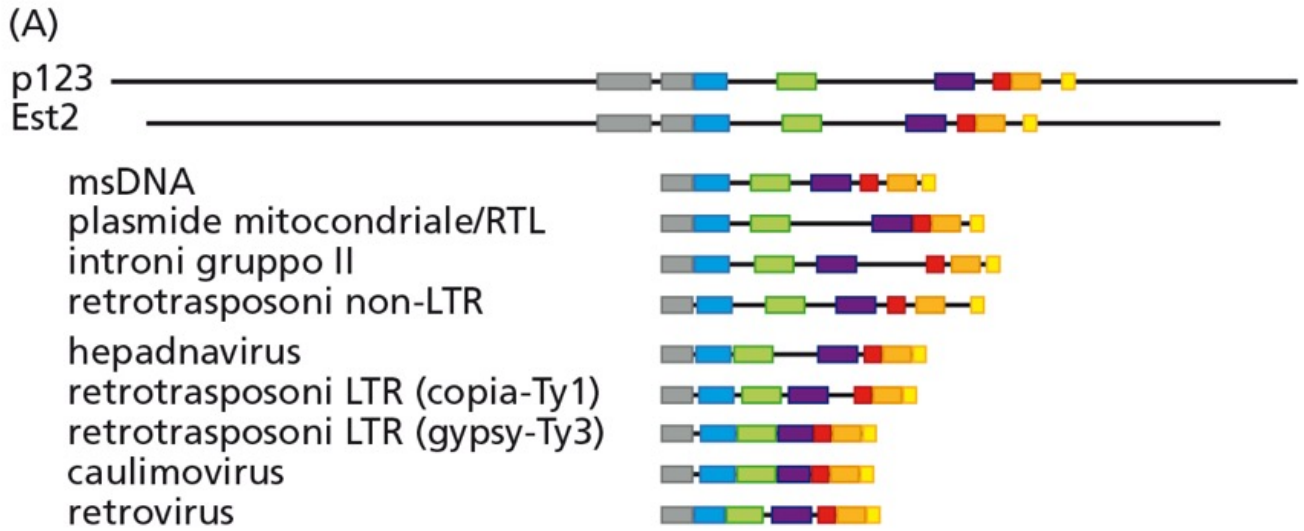
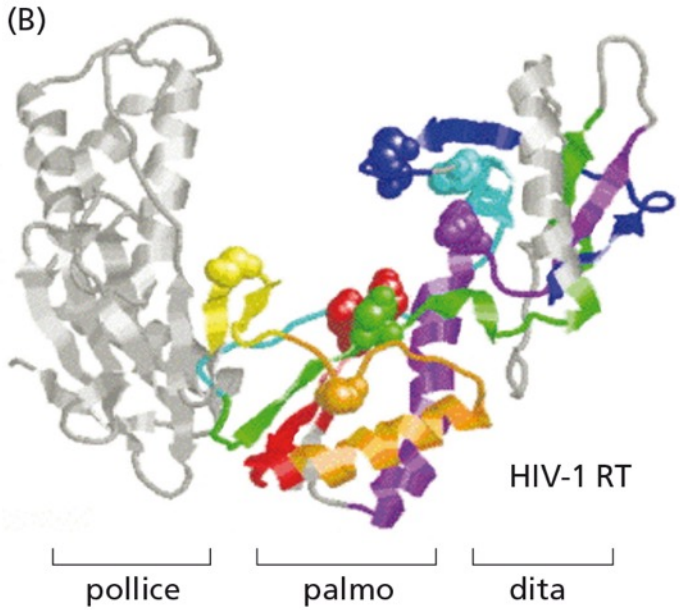
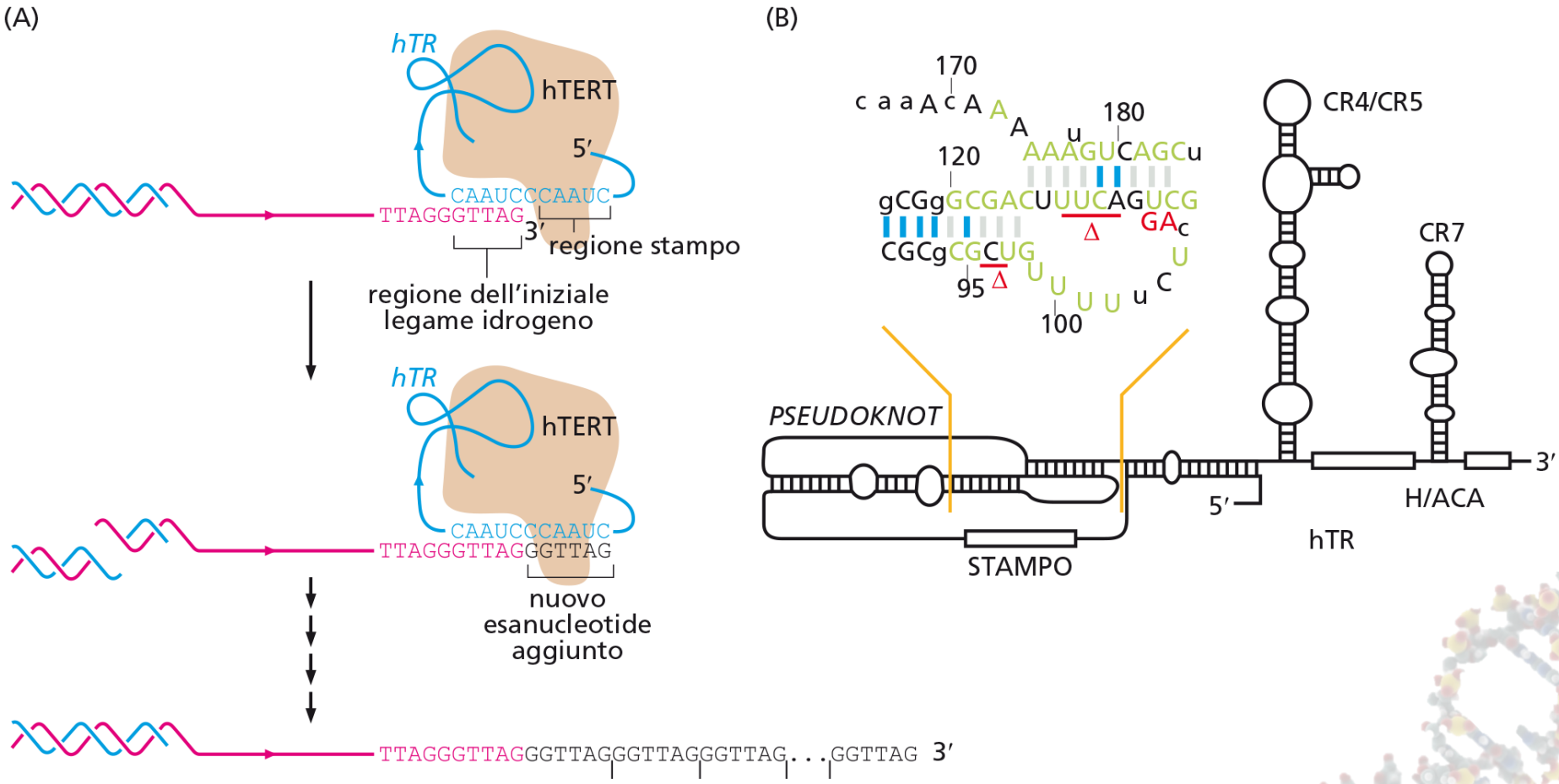
La telomerasi





Weinberg, la biologia del cancro

La telomerasi è una trascrittasi inversa



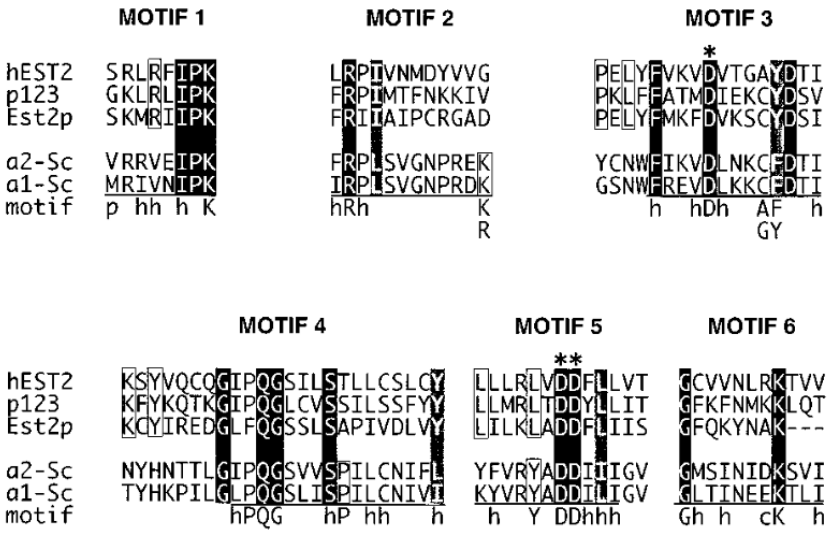
Weinberg, la biologia del cancro

La telomerasi

Cell, Vol. 90, 785–795, August 22, 1997, Copyright ©1997 by Cell Press

hEST2, the Putative Human Telomerase Catalytic Subunit Gene, Is Up-Regulated in Tumor Cells and during Immortalization

Matthew Meyerson,*†# Christopher M. Counter,*#
Elinor Ng Eaton,* Leif W. Ellisen,‡
Philipp Steiner,* Stephanie Dickinson Caddle,*
Liuda Ziaugra,* Roderick L. Beijersbergen,*
Michael J. Davidoff,§ Qingyun Liu,§
Silvia Bacchetti,‖ Daniel A. Haber,‡
and Robert A. Weinberg*

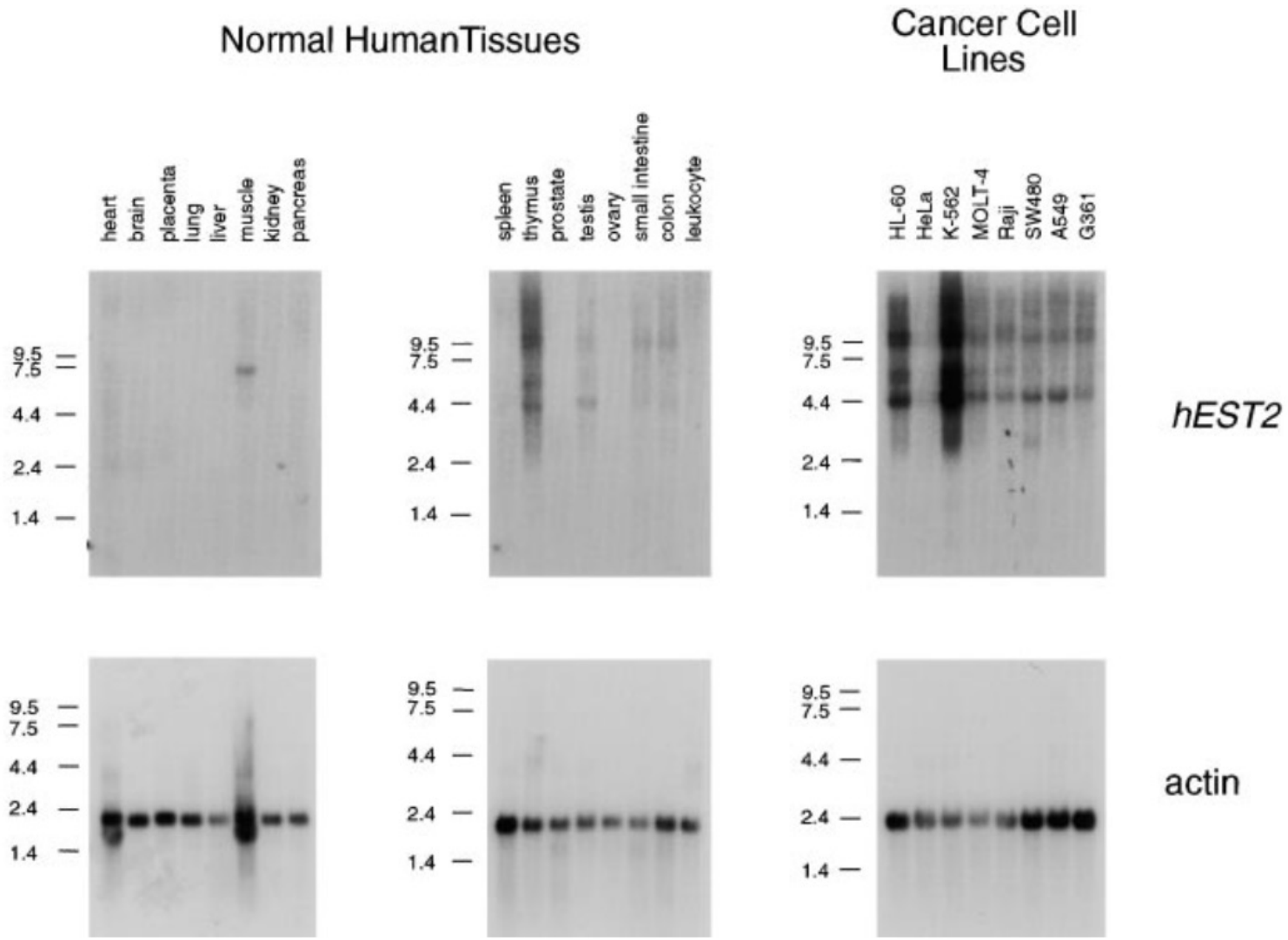


Ricerca del gene per la telomerasi umana per omologia con quella di lievito e di Euplotes

Meyerson et al., 1997

La telomerasi

Expression of hEST2 in normal and cancer cells



Meyerson et al., 1997

La telomerasi

Expression of hEST2 in primary tumors

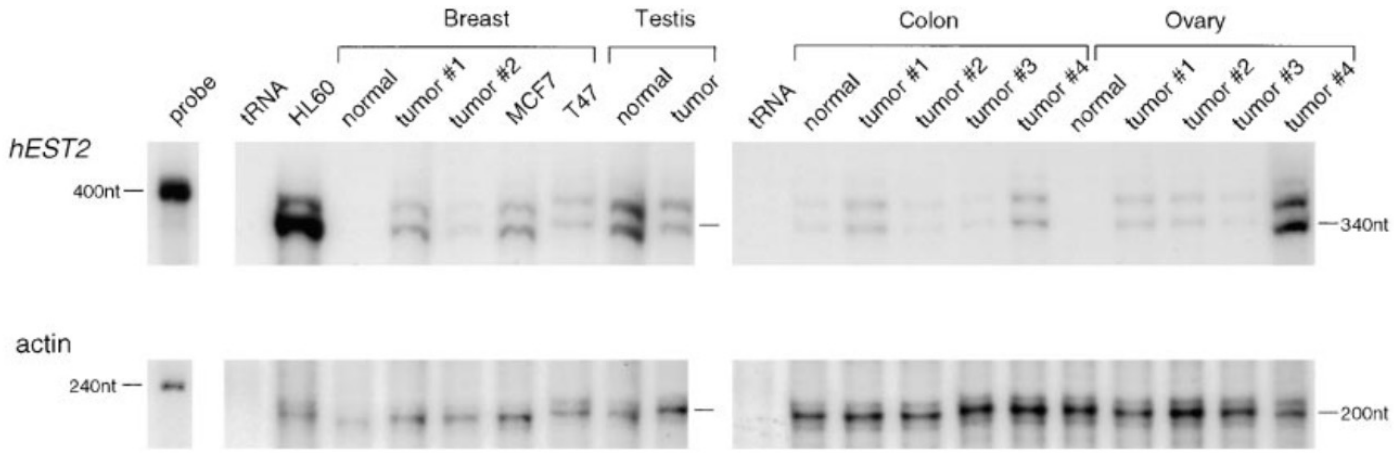


Figure 5. *hEST2* Expression in Primary Human Tumors

RNase protection assays are shown for *hEST2* and β-actin controls. Sizes of the full-length and protected bands are indicated. Shown are the HL-60 leukemia cell line (control), normal breast tissue, two primary breast tumors, the MCF7 and T47D breast cancer cell lines, and normal and primary tumor tissues from the testis, colon, and ovary. The doublet seen protected by the *hEST2* probe is invariant and may be a result of probe secondary structure.

85-90% dei tumori umani sono positivi alla telomerasi

Hanno telomeri lunghi?

Meyerson et al., 1997

La telomerasi

Expression of h*EST2* and telomerase activity

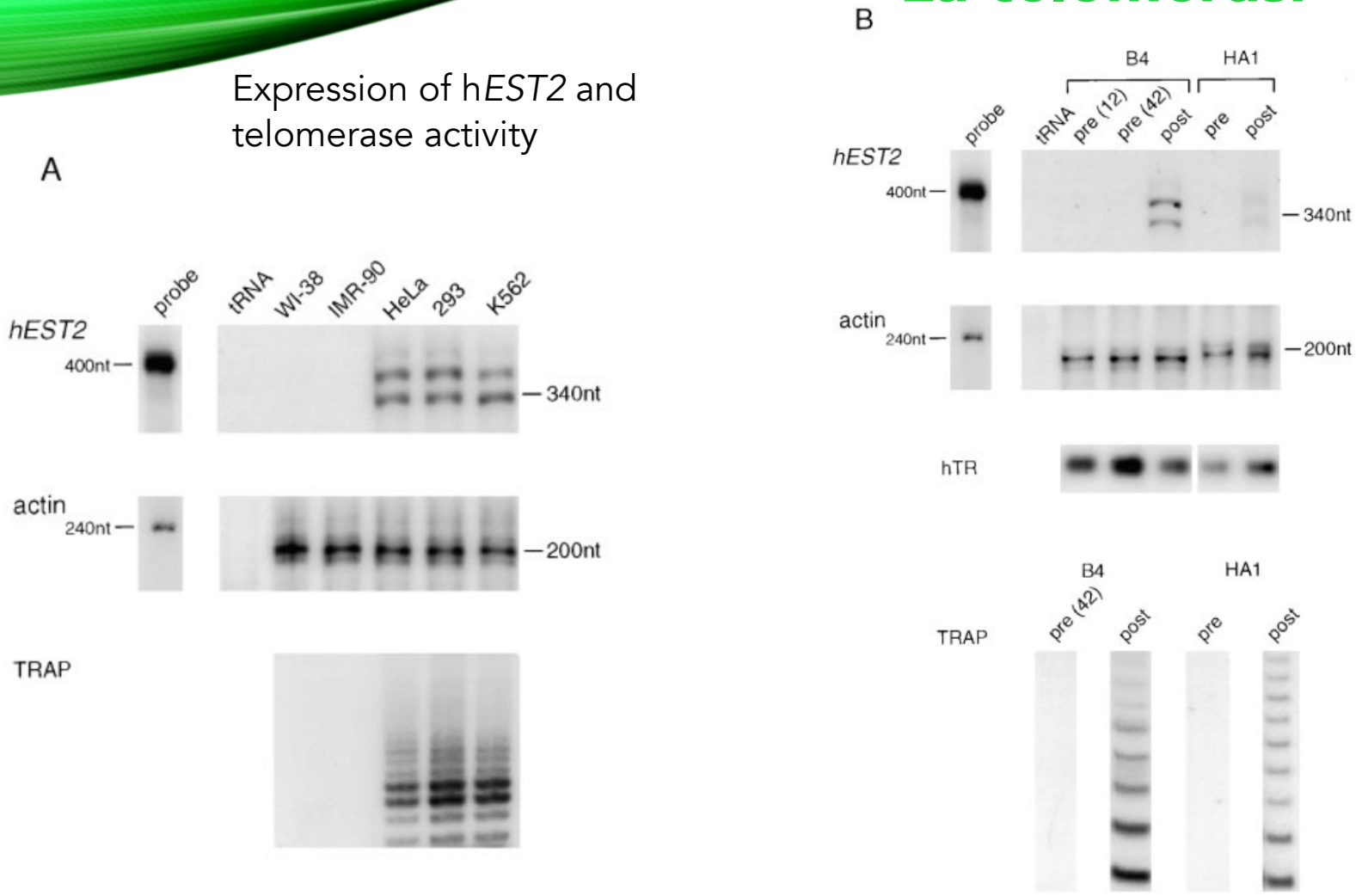


Figure 6. Correlation in Cultured Cells of *hEST2* RNA with Telomerase Activity and Immortalization Status

(A) Comparison of normal and immortalized cells. *hEST2* and β-actin control RNA levels were determined by RNase protection analysis using 40 μg of total RNA from each cell type. Sizes of the full-length probes and protected fragments are indicated. Telomerase was assayed with the TRAP protocol on 100 ng of cell lysate (bottom panel).

(B) Comparison of EBV-transformed B cells (B4) and SV40 T antigen–transformed kidney cells (HA1) precrisis and postcrisis. Cell line passage numbers are indicated in parentheses. The top two panels show RNase protection of *hEST2* and β-actin as a control. The third panel shows Northern blot analysis of the *hTR* transcript. The bottom panel shows TRAP telomerase assays on 1 μg of cell lysate.

Meyerson et al., 1997

La telomerasi

Expression of h*EST2* during cell differentiation

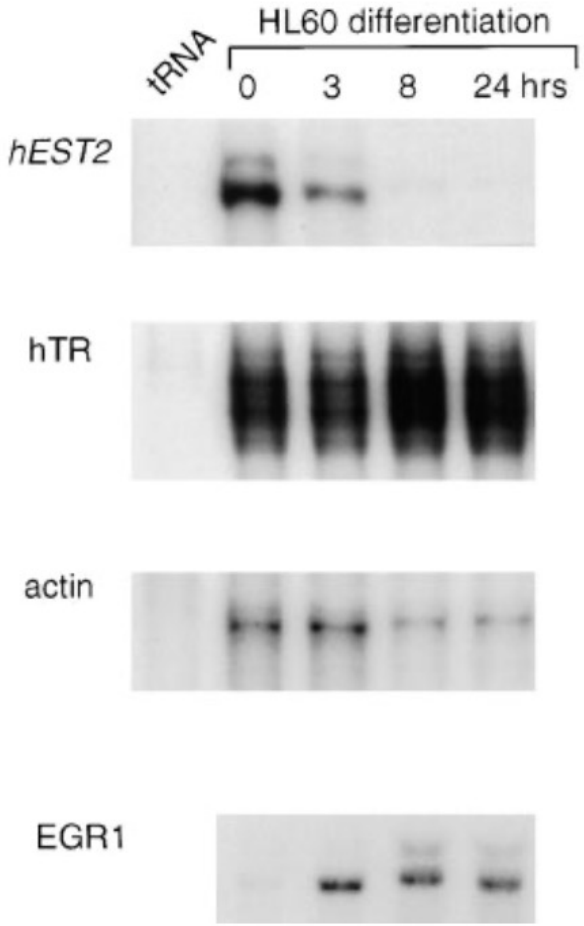
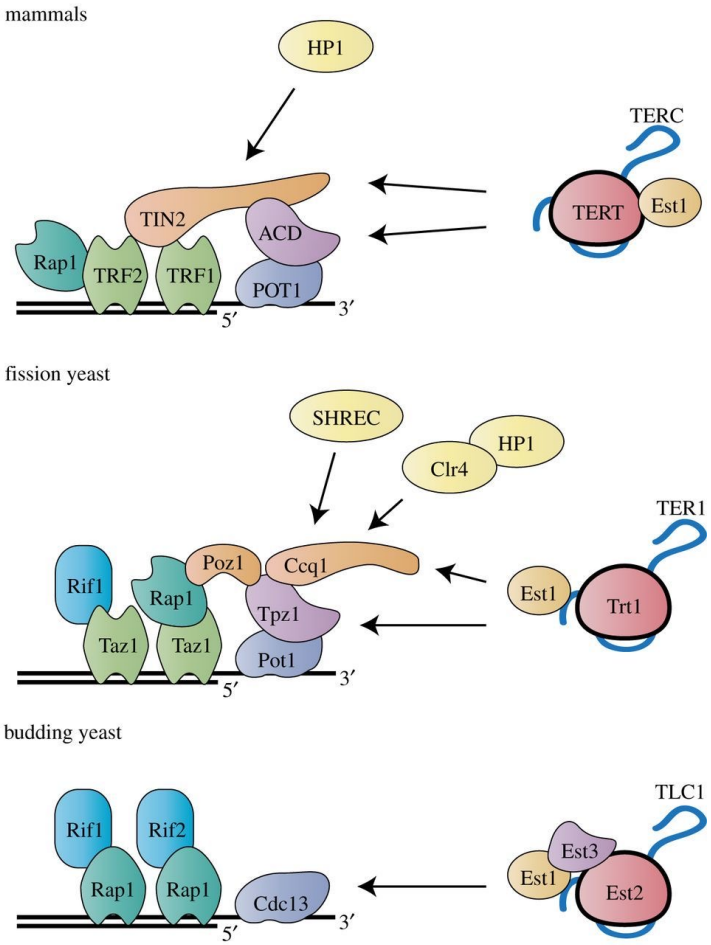


Figure 7. Changes in *hEST2* RNA Expression during Induced Differentiation of HL-60 Cells

Differentiation of the human promyelocytic leukemia cell line HL-60 was induced with all-*trans* retinoic acid as described in Experimental Procedures, and cells were collected for analysis at the indicated times. Total cellular RNA (20 μg) was analyzed for each time point. The first three panels show RNase protection analysis of the *hEST2*, β-actin control, and *hTR* transcripts, respectively. Analysis of the human β-actin transcript was performed in the same reaction tube as that for *hEST2*. The fourth panel shows Northern blot analysis of the early growth response gene *EGR-1*, whose expression is rapidly induced during HL-60 differentiation (Nguyen et al., 1993); this is used as a control to verify cell differentiation. RNA quantity and integrity were assessed by ethidium bromide staining (data not shown).

Meyerson et al., 1997

Inattivazione della telomerasi e senescenza



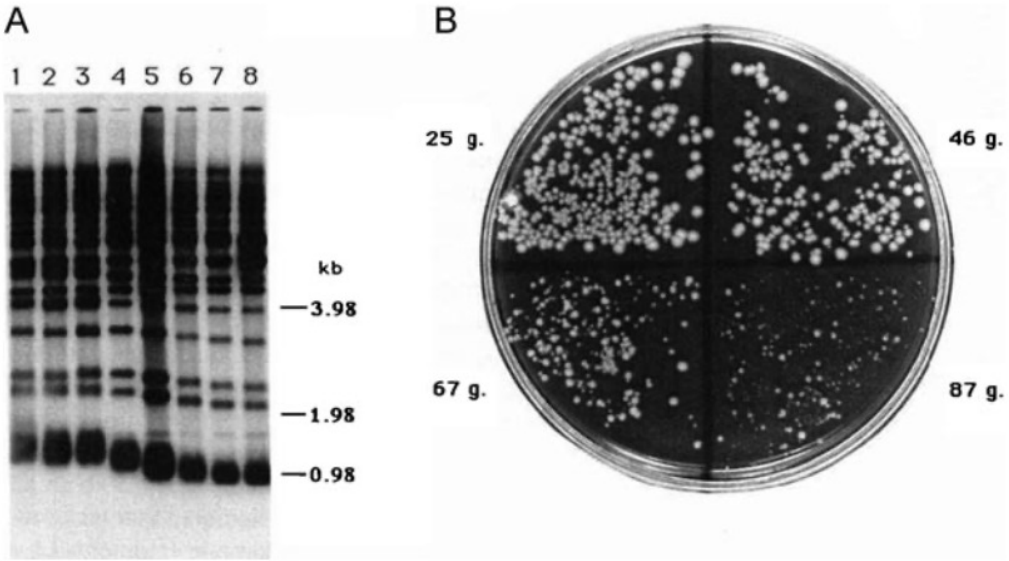
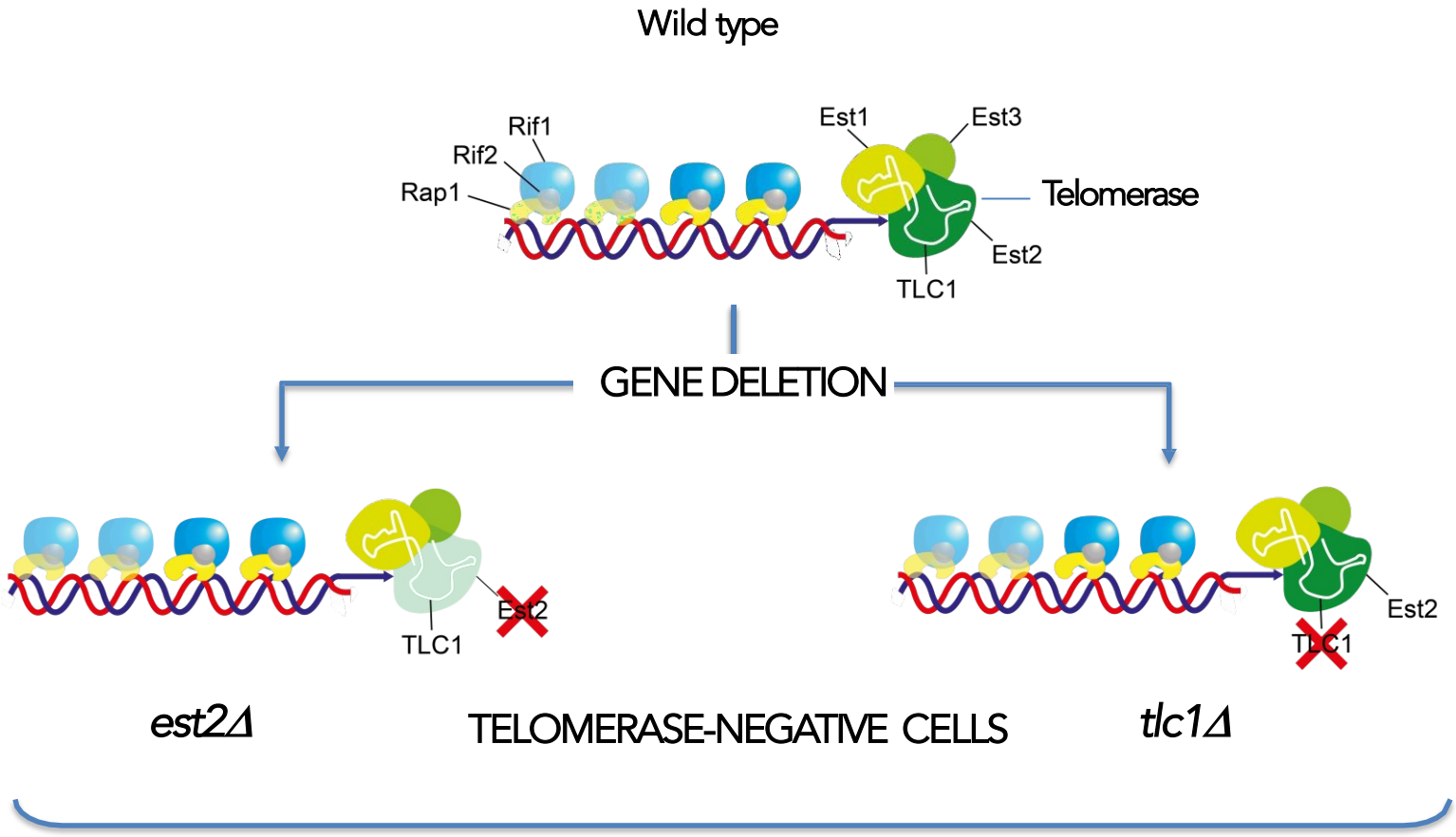


Figure 16. Senescence of yeast EST-1 cells. A) Telomeric yeast DNA fragments from an EST-1 mutant strain are visualized by Southern blotting. Lanes 1 through 8 represent increasing numbers of generations of growth. B) A mutant EST-1 strain streaked out on an agar plate after 25, 46, 67, and 87 generations of prior growth.

Szostak et al., 2010; Armstrong and Tomita, 2017

Yeast as a model to study replicative senescence



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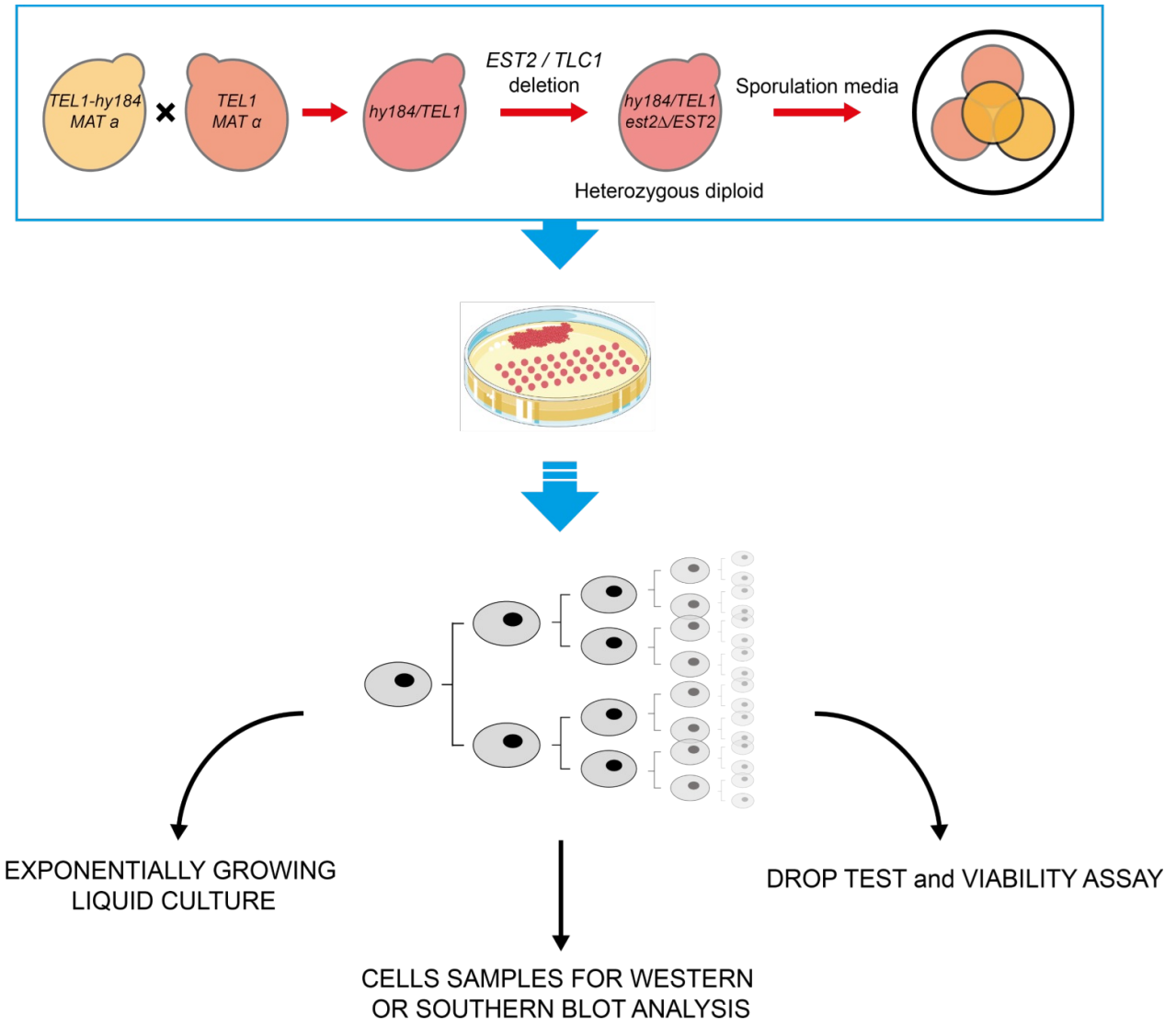

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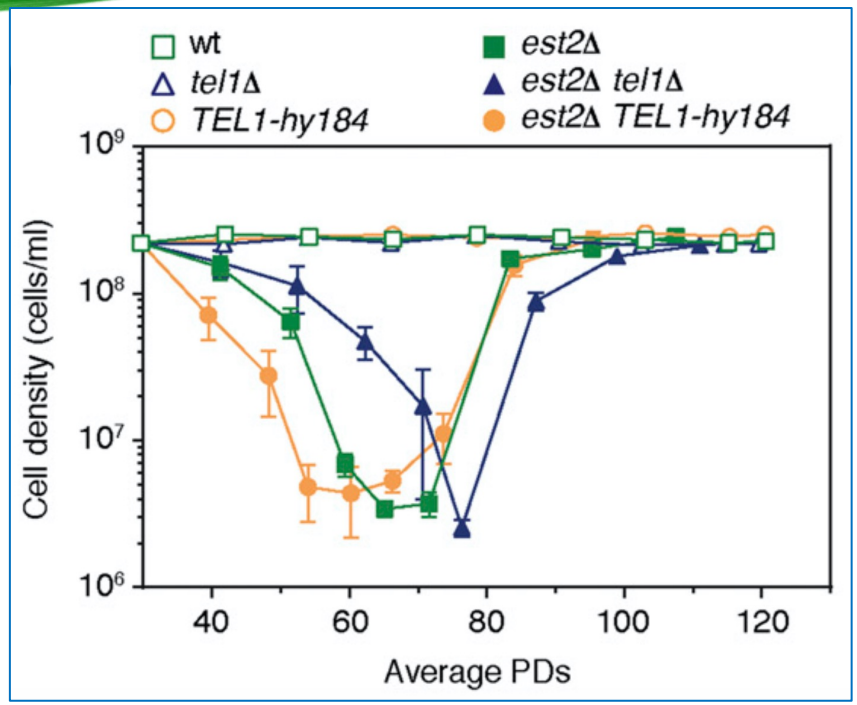

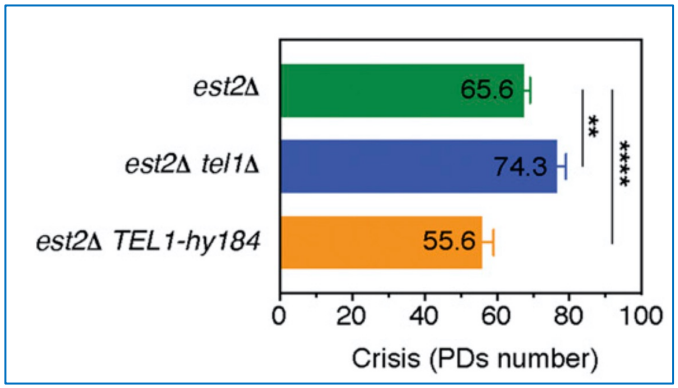

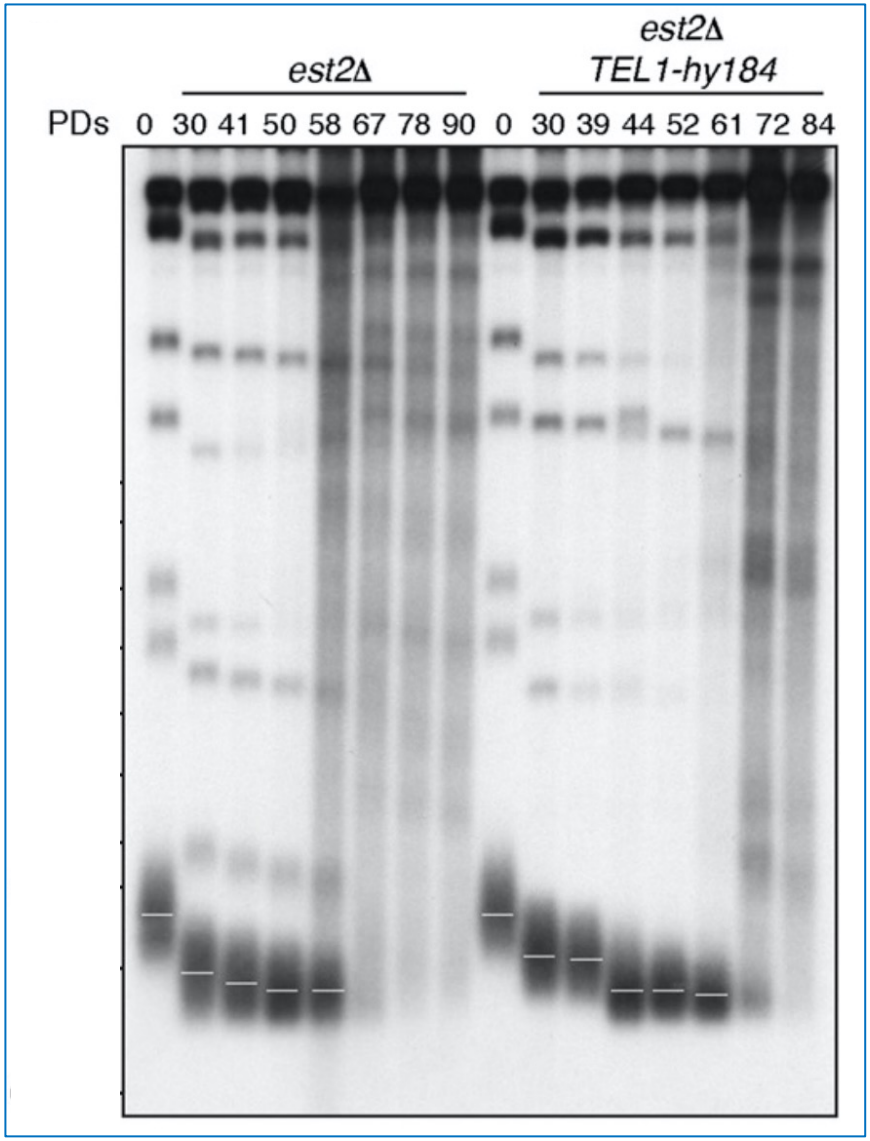

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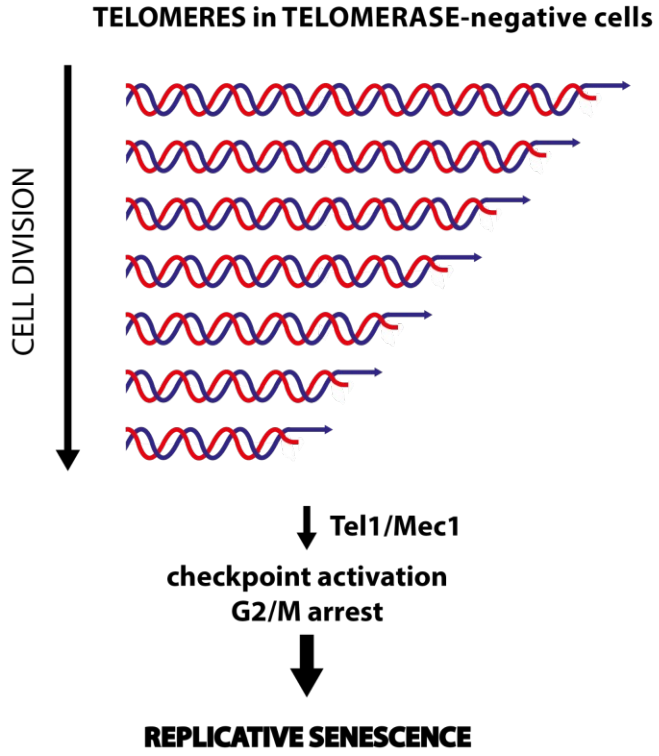
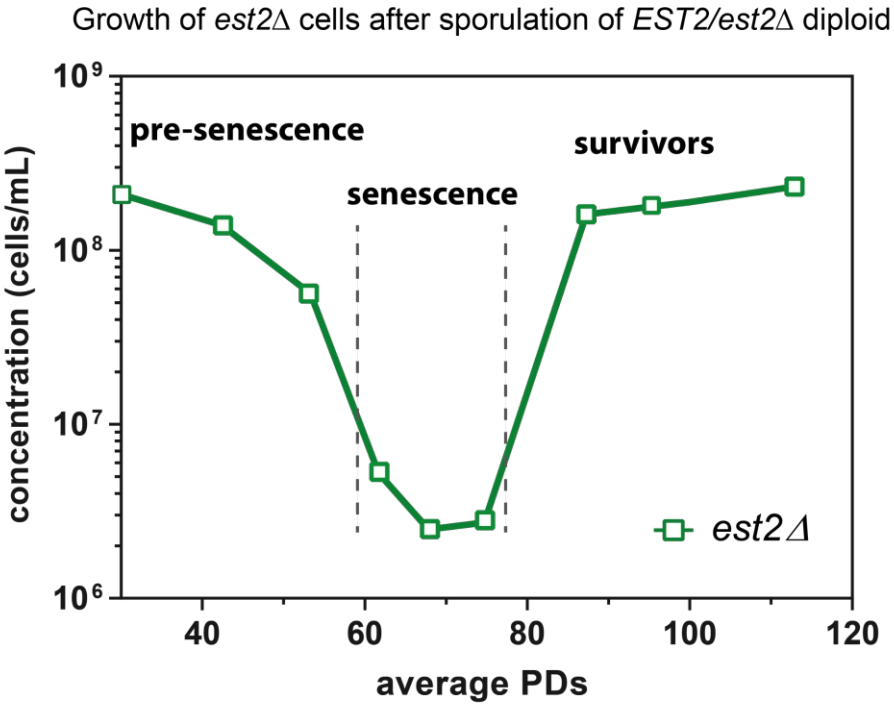
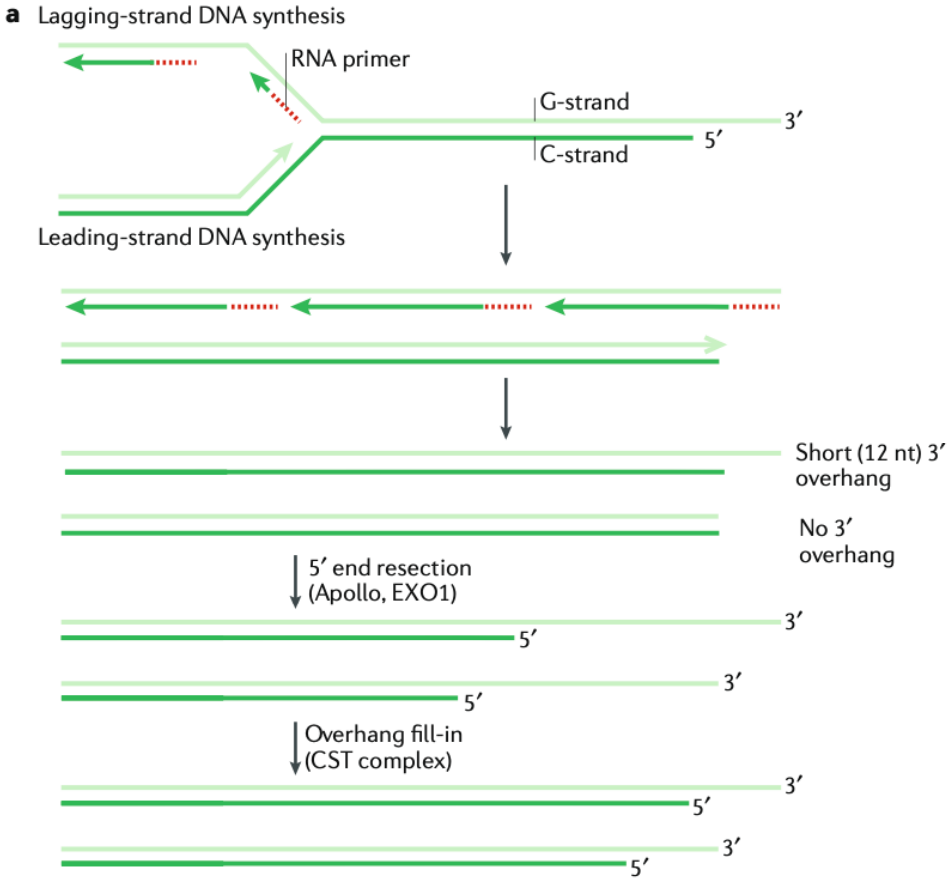
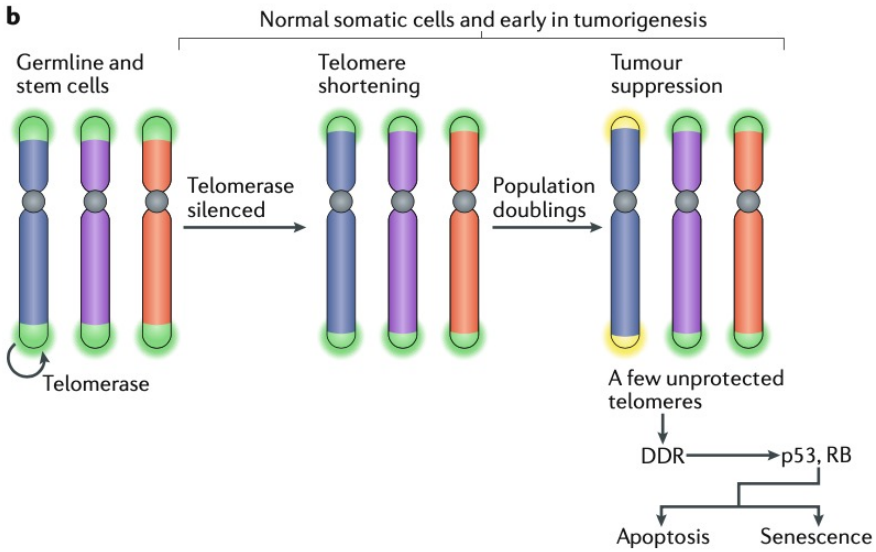




La telomerasi





Maciejowski and de Lange, 2018