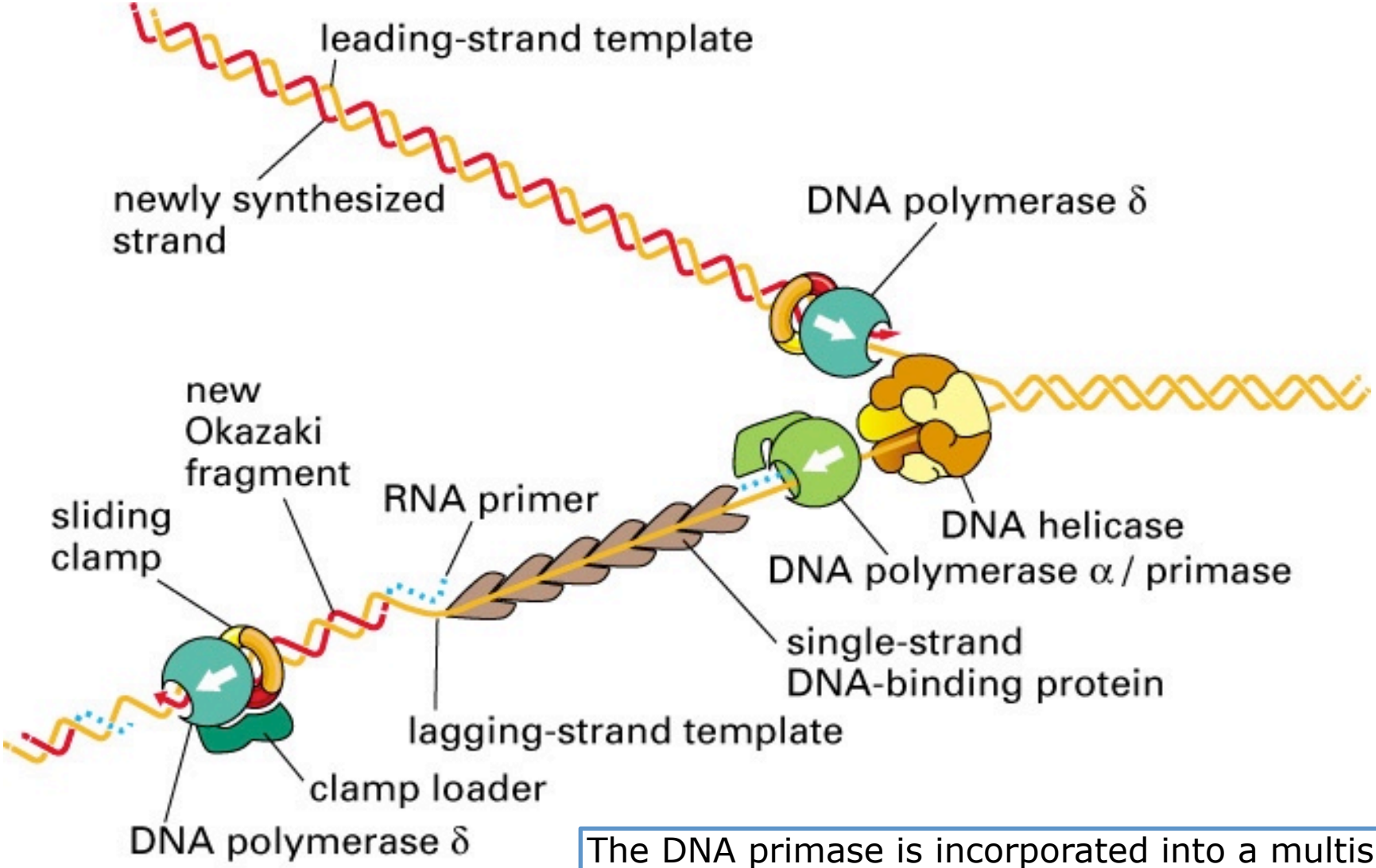
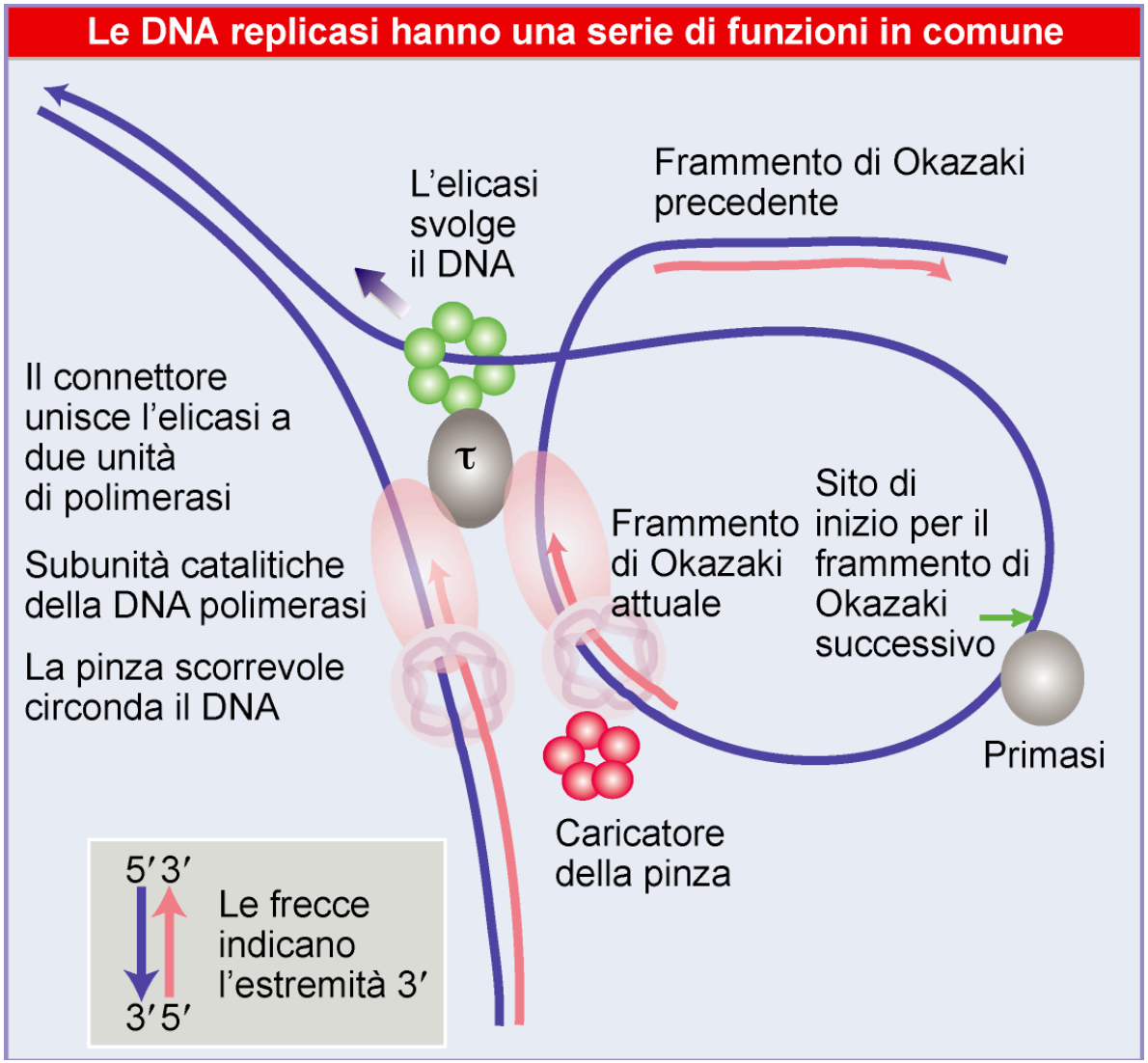
DNA replication is similar in eucaryotes and bacteria



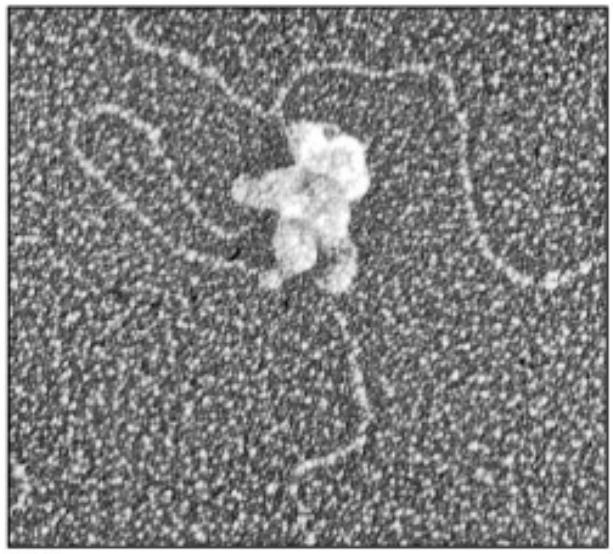
The DNA primase is incorporated into a multisubunit polymerase alfa. DNA polymerase delta then synthesizes the remainder of each Okazaki fragment

Tabella 6.2 **Comparazione tra le proteine più note coinvolte nella replicazione del DNA negli eucarioti e nella replicazione del DNA di *E. coli*.**

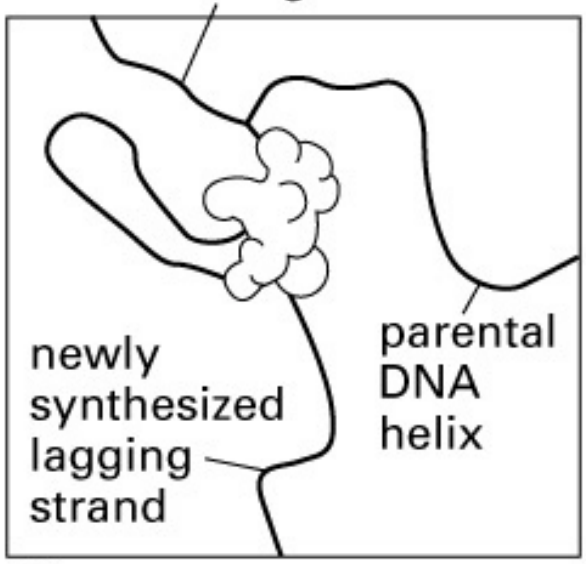
E. coli	Eucarioti	Struttura e funzione della proteina negli eucarioti
DnaA	ORC (T-Ag in SV-40)	Riconoscimento dell'origine; è costituita da 6 subunità
DnaB-DnaC	MCM 2-7 (T-Ag in SV-40)	DNA elicasi; è costituita da 6 subunità
DnaG (primasi)	DNA polimerasi α-primasi	La polimerasi che sintetizza gli RNA primer e corte catene di DNA sul filamento ritardato; è composta da 4 subunità
Subunità α di Pol III	DNA polimerasi δ e DNA polimerasi ε	Le due polimerasi replicative; ciascuna composta da 4 subunità
Subunità β di Pol III	PCNA	La proteina che forma la "clamp" che avvolge e scivola sul DNA; è un omotrimero
Complesso-γ di Pol III	RFC	Il complesso che carica la "clamp" sul DNA; è composto da 5 subunità
SSB	RPA	La proteina che stabilizza il DNA a singolo filamento (ssDNA); è composta da 3 subunità
Pol I	FEN1, RNasi H	Nucleasi che eliminano gli RNA primer dai frammenti di Okazaki
Lig 1	DNA ligasi I	La DNA ligasi che salda i frammenti di Okazaki



L' elicasi (DnaB) che crea la forcella di replicazione e' connessa a due subunita' catalitiche della DNA Pol dalla subunita' τ, ciascuna delle quali e' tenuta al DNA dalla pinza scorrevole. La pol che sintetizza il frammento stampo si muove continuamente, mentre quella che sintetizza il filamento ritardato si dissocia alla fine di un frammento di Okasaki e si riassocia con un primer nell' ansa stampo a singolo filamento per sintetizzare il frammento successivo.

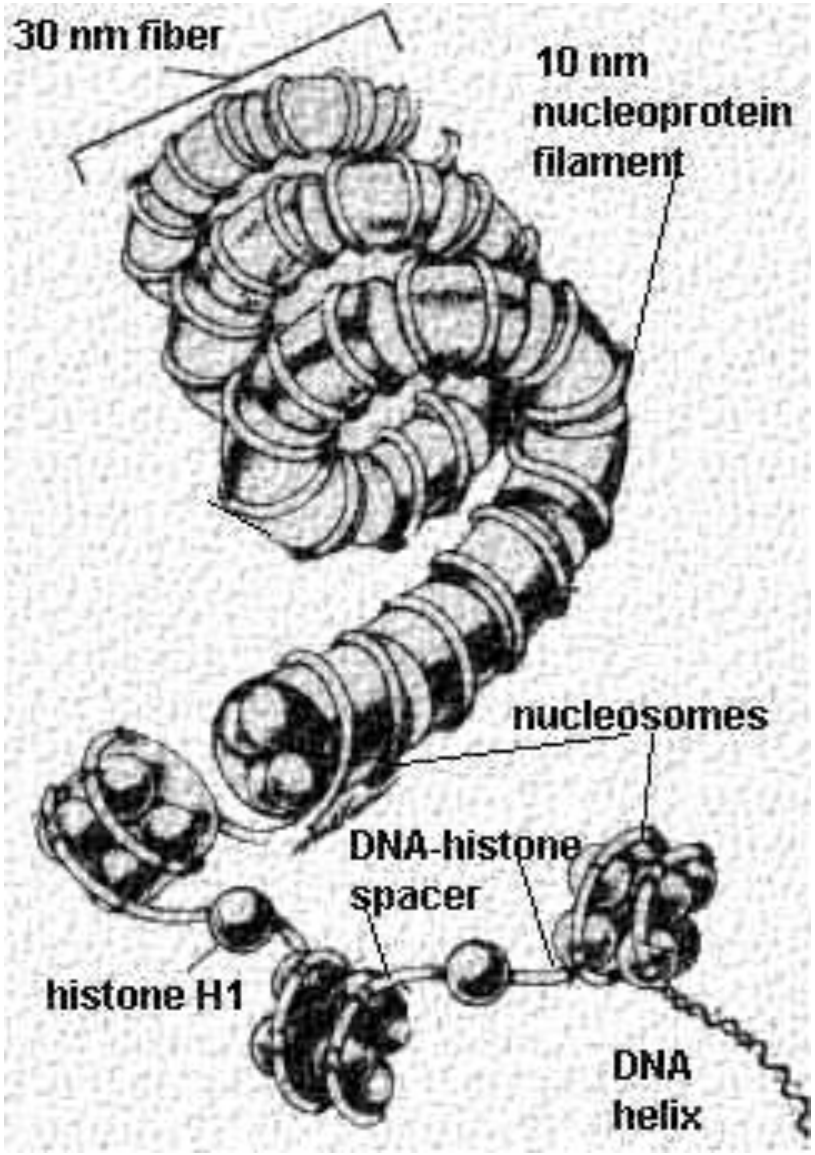


(B)

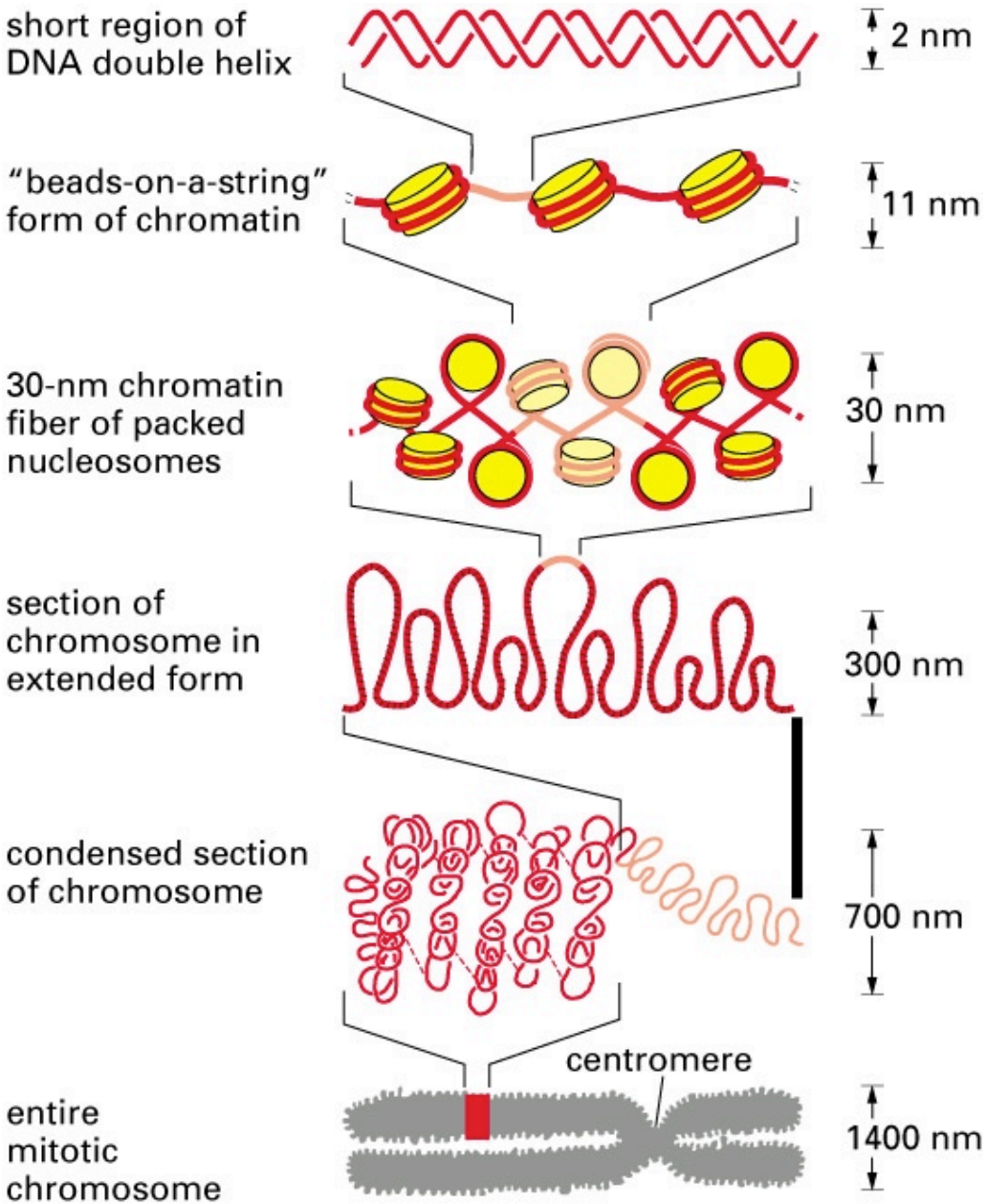

newly synthesized
leading strand
newly
synthesized
lagging
strand
parental
DNA
helix

(C)

The eukaryotic replication machinery has the complication of having to replicate through nucleosomes, spaced at intervals of about 200 nucleotides.



Okazaki fragments are synthesized at intervals of 100-200 nucleotides in eucaryotes, instead of 1000-2000 as in bacteria.



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

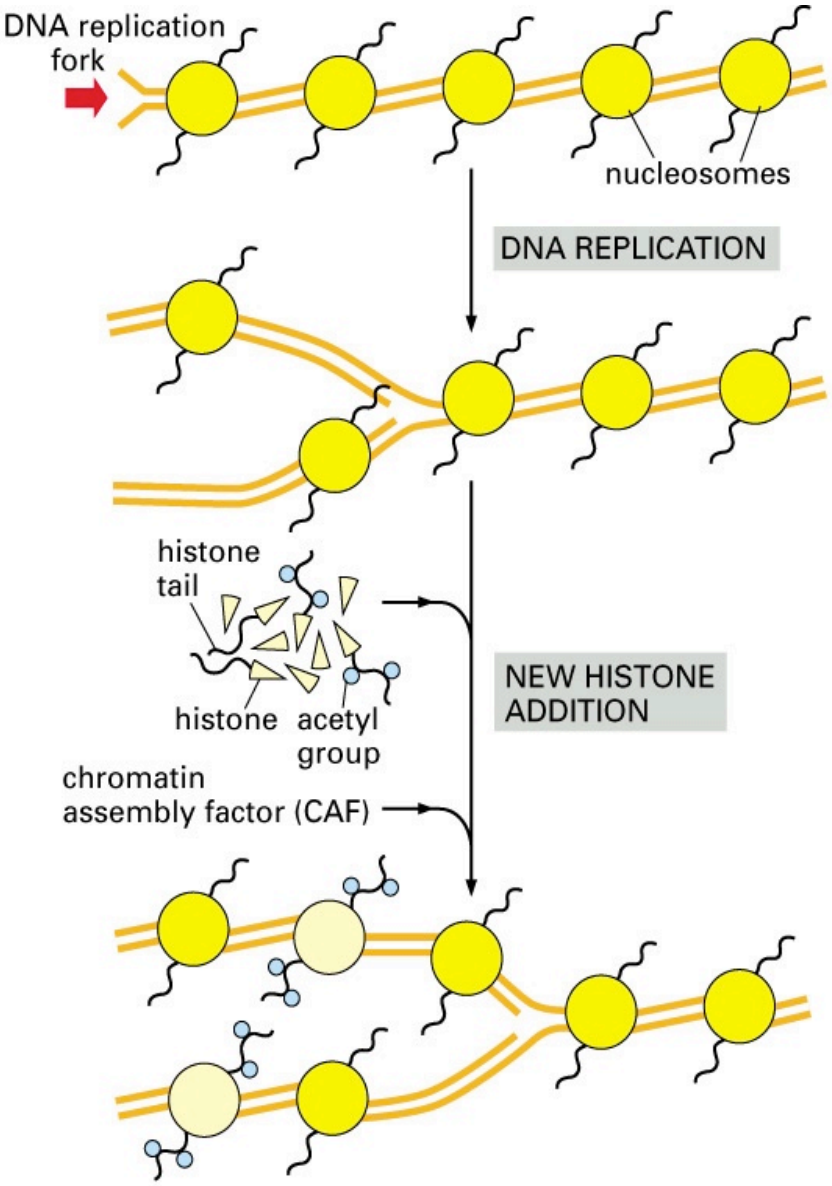
New Nucleosomes Are Assembled Behind the Replication Fork

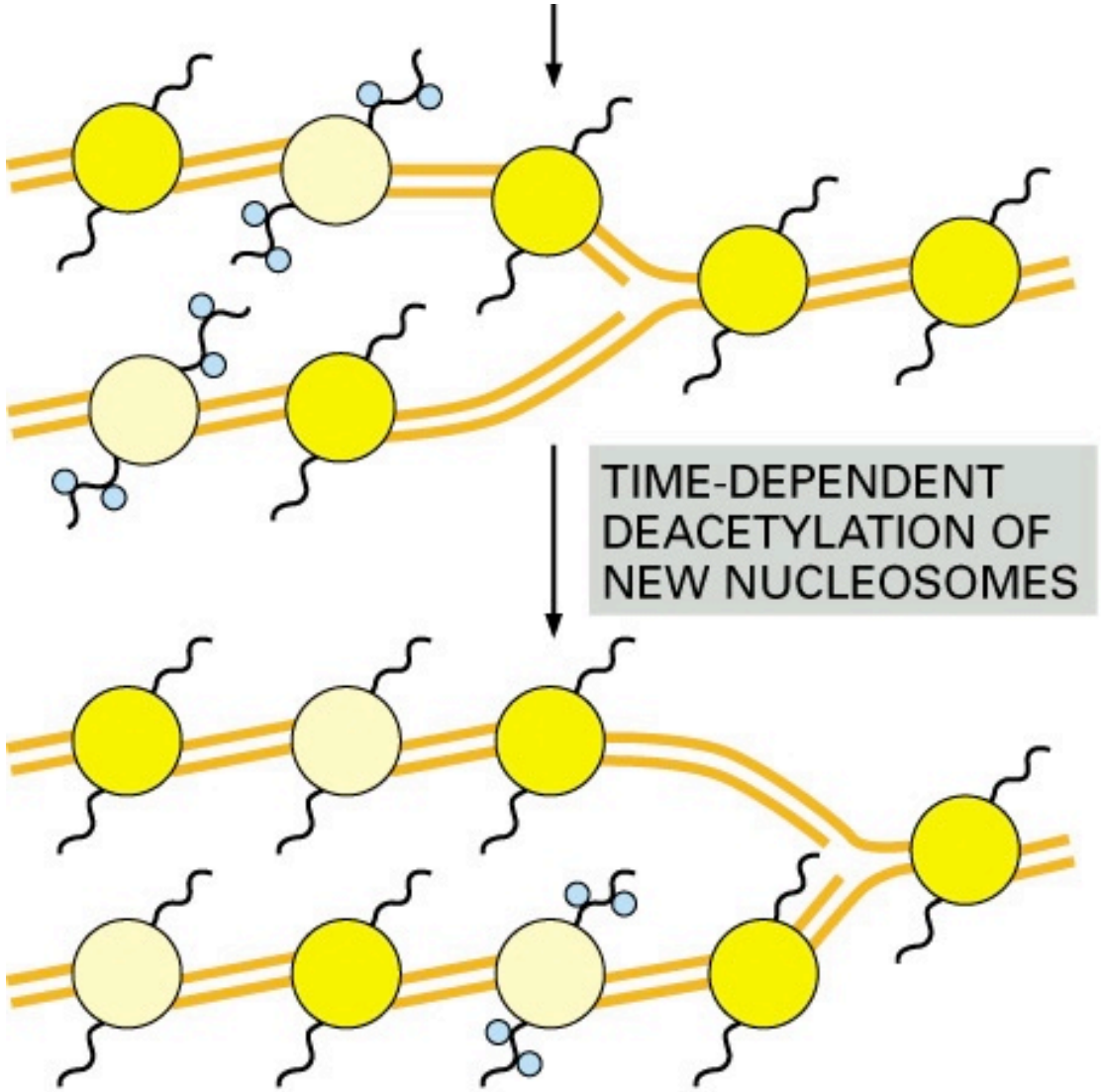
A large amount of new histone protein, approximately equal in mass to the newly synthesized DNA, is required to make the new nucleosomes in each cell cycle.

Unlike most proteins, which are made continuously throughout interphase, histones are synthesized mainly in S phase, when the level of histone mRNA increases about fifty folds.

Both the new helices inherit old histones, but, since the amount of DNA has doubled, an equal amount of new histones is needed.

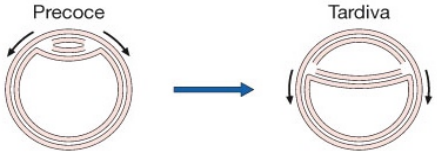
The addition of new histones is aided by chromatin assembly factors (**CAFs**), which are proteins that associate with replication forks and package the newly synthesized DNA as soon as it emerges from the replication machinery.



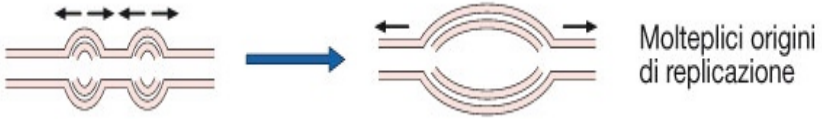


The newly synthesized H3 and H4 histones are rapidly acetylated on their N-terminal tails; after they have been incorporated into chromatin, these acetyl groups are removed

DNA synthesis begins at replication origins

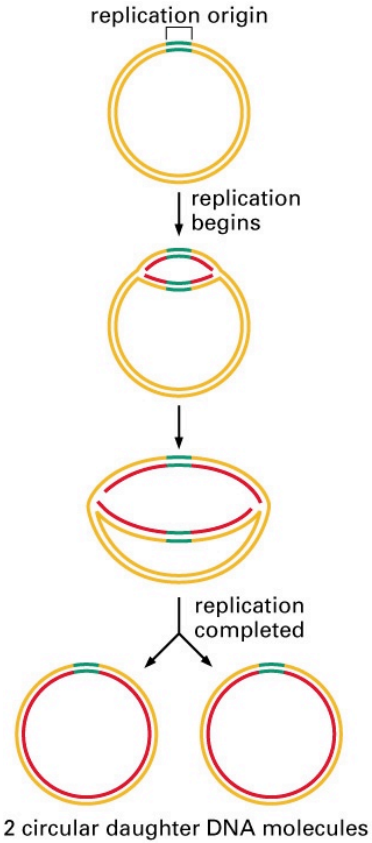


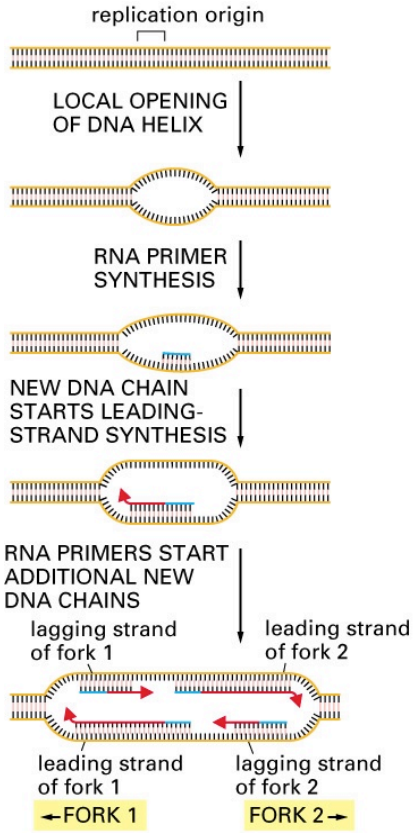
Replicazione bidirezionale (struttura theta)



The genome of E. coli is contained in a single circular DNA molecule (4.6 x 10^6 nucleotide pairs).

DNA replication begins at a single origin and the two forks proceed (500-1000 nn/sec) until they meet up roughly halfway around the chromosome





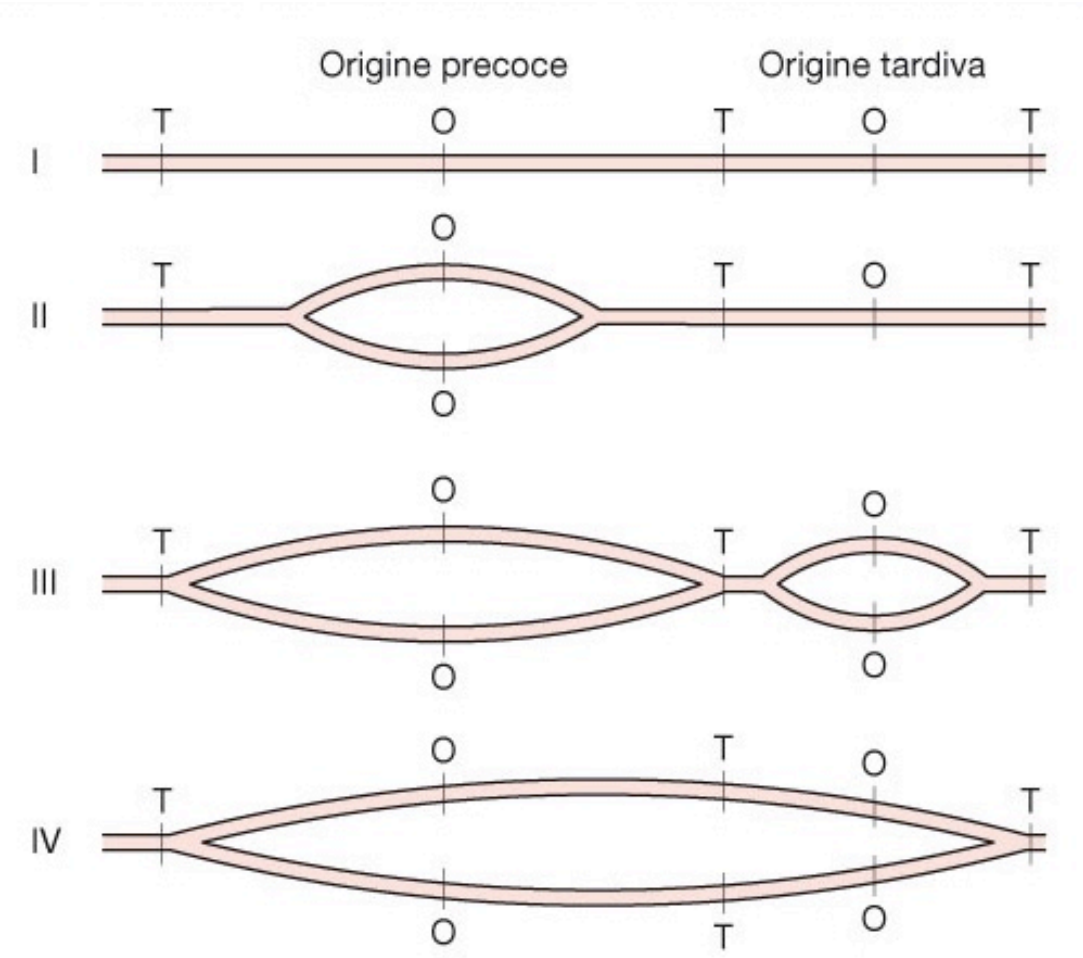
Ciascun cromosoma degli organismi eucarioti contiene molteplici origini di replicazione.

Replication speed: 50 nn/sec

Average chromosome length: 150 million nn

One single replication fork would require about 800 hours

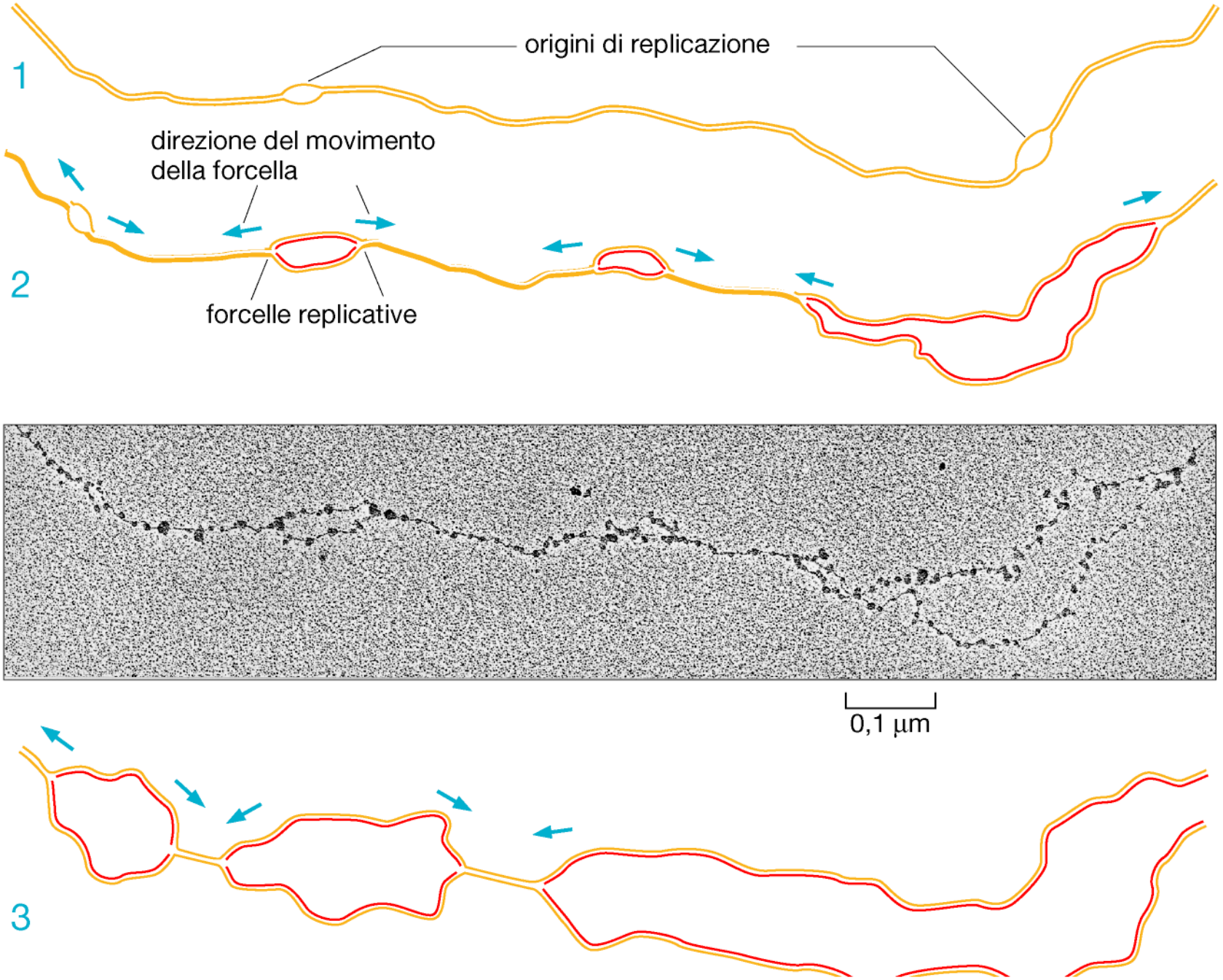
EUCARYOTIC REPLICATION ORIGINS

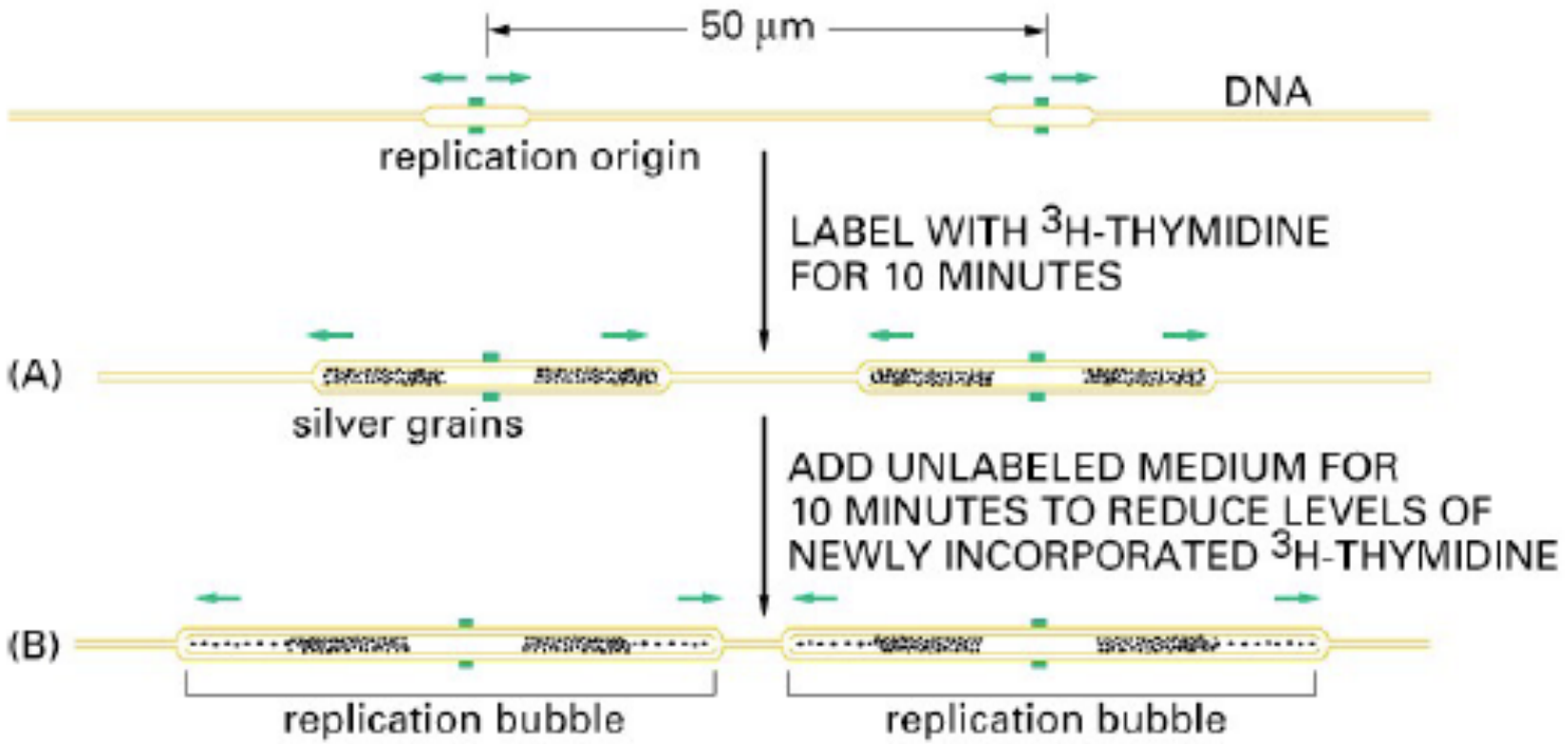


Ciascun cromosoma eucariota e' organizzato in molteplici Unita' di Replicazione, dette **repliconi**, che comprendono 50-80 origini, spaziate da 30.000-300.000 nt.

Ognuna di queste e' organizzata con un punto di origine da cui partono due forcelle di replicazione opposte. I punti di terminazione di due repliconi adiacenti coincidono, cosi' che a tempi tardivi di replicazione, repliconi adiacenti si fondono l'uno con l'altro.

L'accensione delle origini di replicazione non e' simultanea, ma alcune (precoci o early) vengono accese durante la fase S, prima di altre (tardive o late)


origini di replicazione
1
direzione del movimento
della forcella
2
forcelle replicative
0,1 µm
3

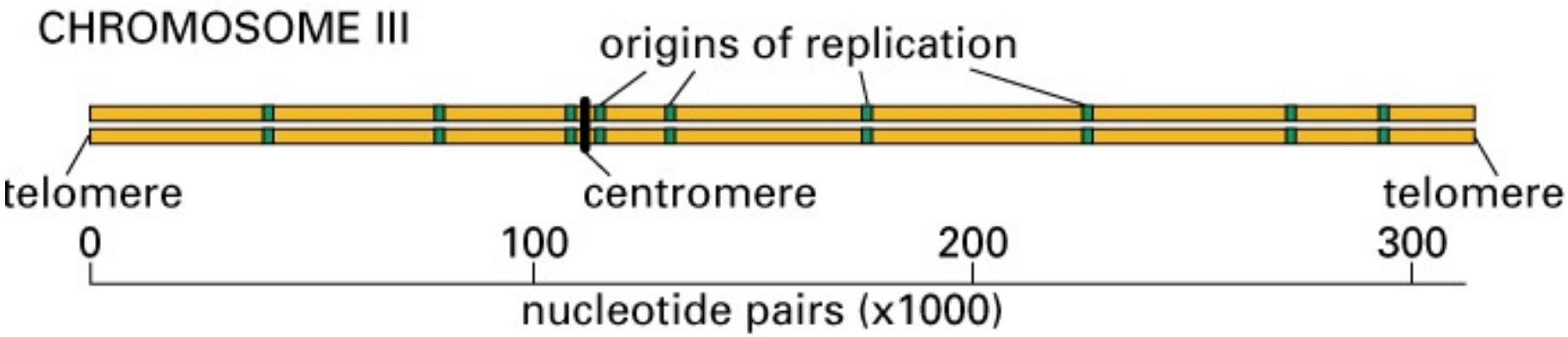

50 µm
DNA
replication origin
LABEL WITH ^{3}H-THYMIDINE
FOR 10 MINUTES
(A)
silver grains
ADD UNLABELED MEDIUM FOR
10 MINUTES TO REDUCE LEVELS OF
NEWLY INCORPORATED ^{3}H-THYMIDINE
(B)
replication bubble
replication bubble

Quali sono le caratteristiche di un’origine di replicazione eucariota?

Well-defined DNA sequences serve as replication origins in the budding yeast: the ARS sequences (autonomously replicating sequences)

Sono state individuate ~350 ARS nel genoma di lievito, indicando che la dimensione media di un replicone e' di **~40 kbps** (13.5 Mpb/350).

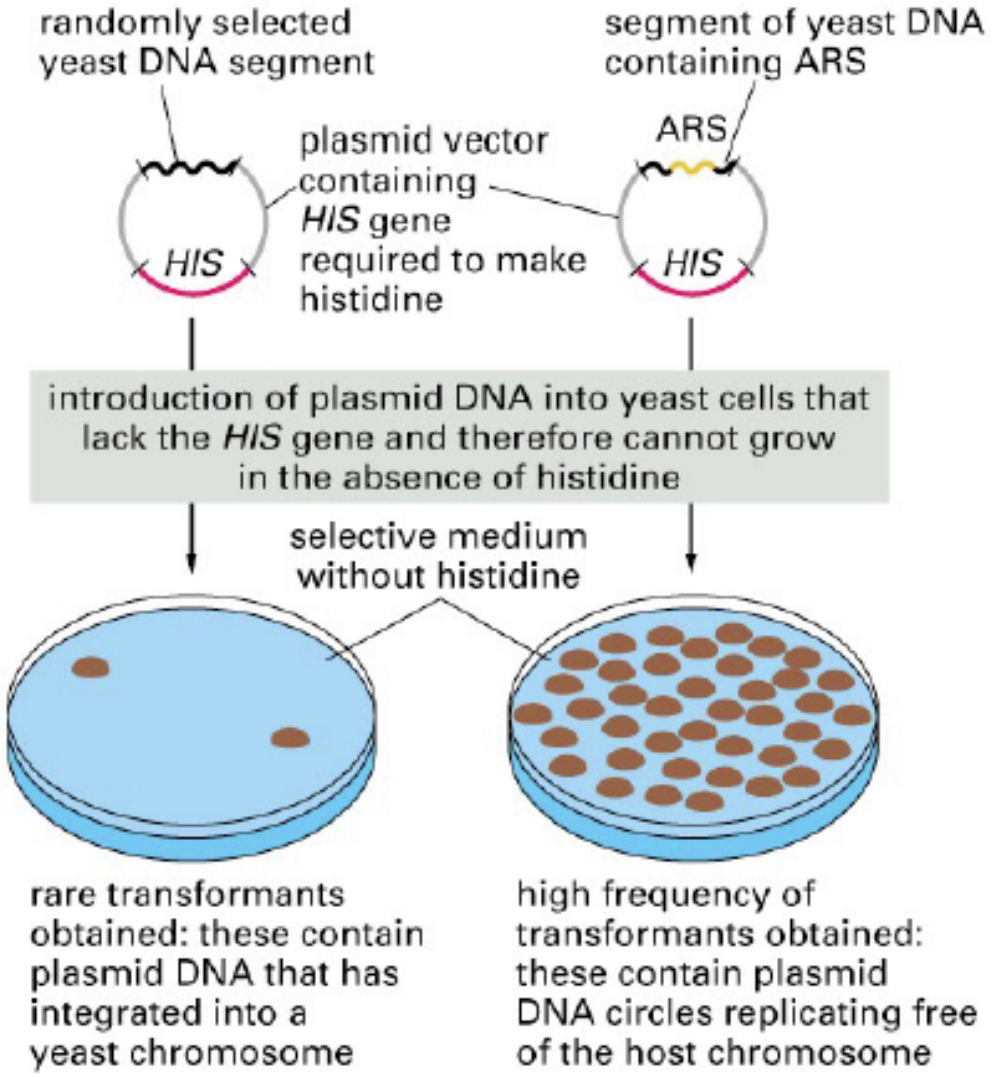
Origins in S. cerevisiae are spaced **~40,000** nn, allowing the whole chromosome to be replicated in about 8 minutes



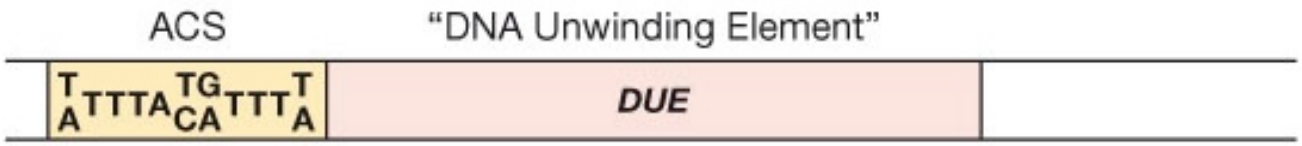
Effect of deleting various replication origins on chromosome III: removing a few origins has little effect, because replication forks that begin at neighboring origins can continue into the regions that lack their own origins: however, as more replication origins are deleted from this chromosome, the chromosome is gradually lost as the cells divide, presumably because it is replicated too slowly.

Well-defined DNA sequences serve as replication origins in the budding yeast: the ARS sequences

Solo le cellule di lievito che, dopo la trasformazione, portano nel vettore plasmidico un frammento di DNA contenente una ARS sono in grado di conferire a quel plasmide la capacita' di replicarsi in lievito e quindi di poter crescere su un terreno selettivo, privo di istidina.



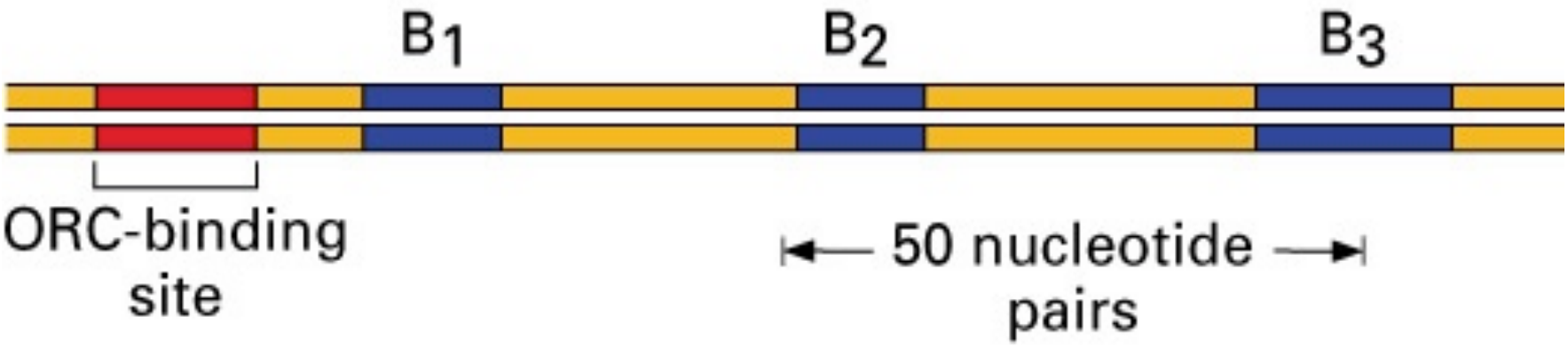
Che caratteristiche ha una Autonomous Replicating Sequence (ARS) in *S. Cerevisiae?*



- Tutte le ARS contengono almeno una *ARS consensus sequence (ACS)* **di 11 pb**, ricca di A e T.
- Mutazioni nelle ACS aboliscono la funzione della ARS
- Fiancheggiano la ACS altre sequenze di lunghezza variabile con sequenza non particolarmente conservata: sono dette *DNA unwinding elements (DUE)*, ricche di A e T, coinvolte nell'apertura della doppia elica.

Origin Recognition Complex (ORC)

Each of the yeast replication origins contains a binding site for a large, multisubunit initiator protein called **ORC (**origin recognition complex), and several auxiliary binding sites for proteins that help attract ORC to the origin DNA



- Binds to ARS Consensus Sequence (**ACS**) in an ATP-dependent manner
- Six subunits (Orc1p - Orc6p); 120, 72, 62, 56, 53, 50 kDa
- Essential for viability
- Conserved in different species
- Absence of any biochemical activity besides origin binding

Origin Recognition Complex (ORC)

- Binds to ARS Consensus Sequence (ACS) in an ATP-dependent manner
- Six subunits (Orc1p - Orc6p); 120, 72, 62, 56, 53, 50 kDa
- Essential for viability
- Mutations that disrupt ORC binding in vitro also disrupt origin function in vivo
- Mutations ORC2 and ORC5 cause defects in S-phase entry and/or origin function
- Conserved in different species
- Orc1p, Orc2p, Orc5p have ATP-binding motifs
- Absence of any biochemical activity besides origin binding
- Functions in silencing
- Binds to the ACS throughout the cell cycle: existance of a post-replicative complex in S, G2, M (ORC alone?) and a pre-replicative complex in G1 (ORC + ??)
- Inactive ARSes also bind to ORC

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Advance Access Publication 9 June 2010

Review

DNA replication origins, ORC/DNA interaction, and assembly of pre-replication complex in eukaryotes

Jingya Sun[1] and Daochun Kong[2*]

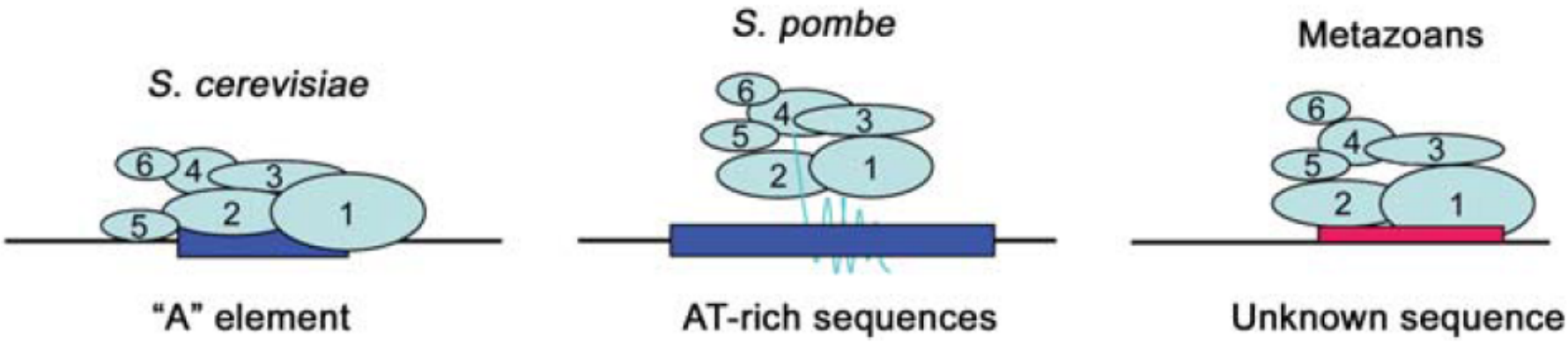
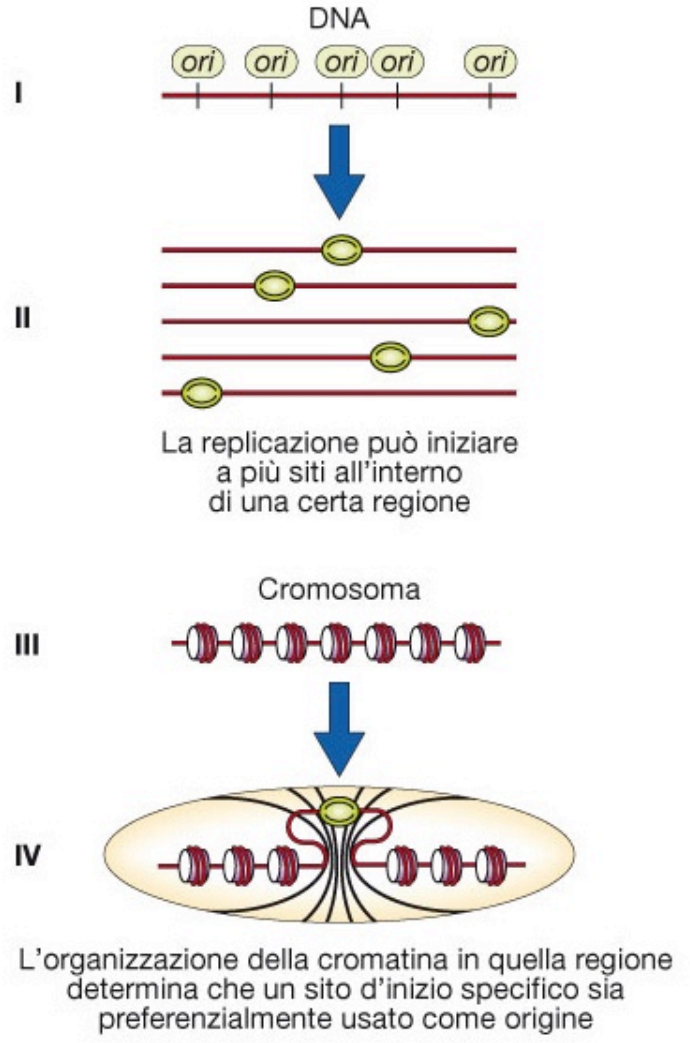


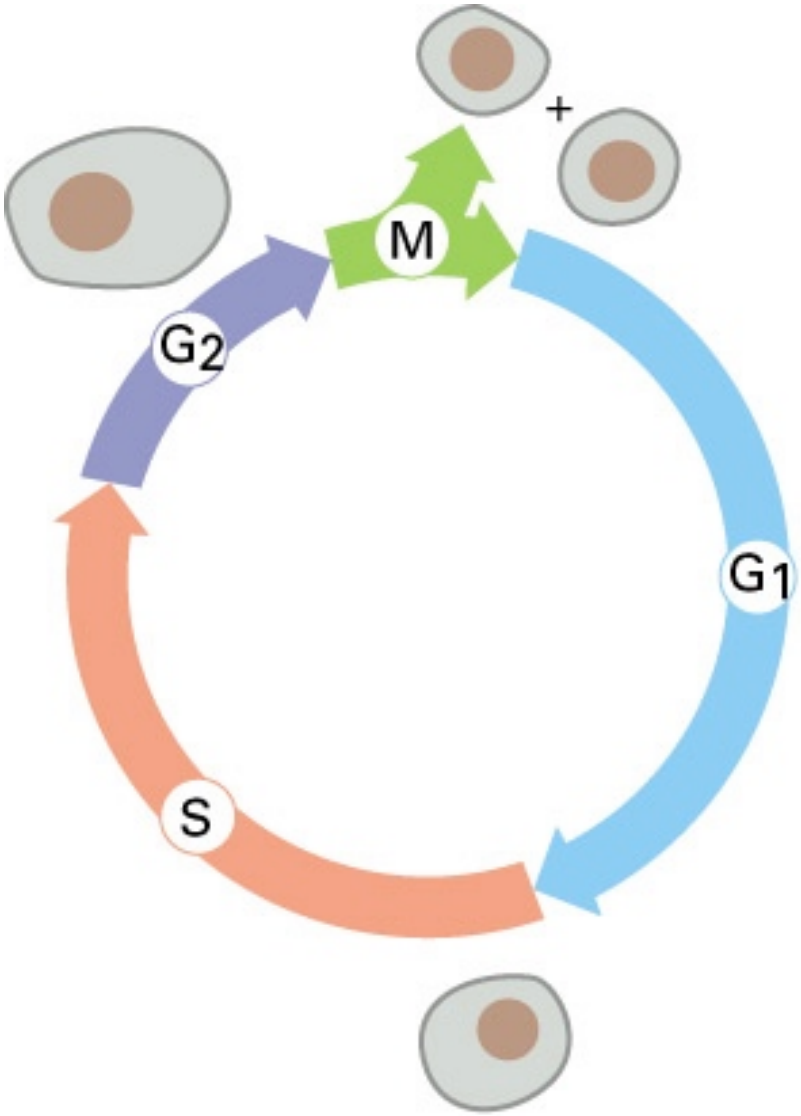
Figure 1 The interaction of ORC and DNA in the budding yeast *S. cerevisiae*, the fission yeast *S. pombe* and metazoans *Sc*ORC uses its five subunits (Orc1−5) to bind specifically to the 'A' element in the presence of ATP. Orc6 does not contact with DNA. *Sp*ORC uses the 9 AT-hook motifs located in the N-terminal half of Orc4 to bind to asymmetric AT-rich sequences. ATP does not affect *Sp*ORC/DNA interaction. The interaction between metazoan ORC and DNA is still not defined. It remains to be determined regarding what sequences metazoan ORC prefers binding to and whether an intrinsic ORC accessory protein guides it to a specific sequence.

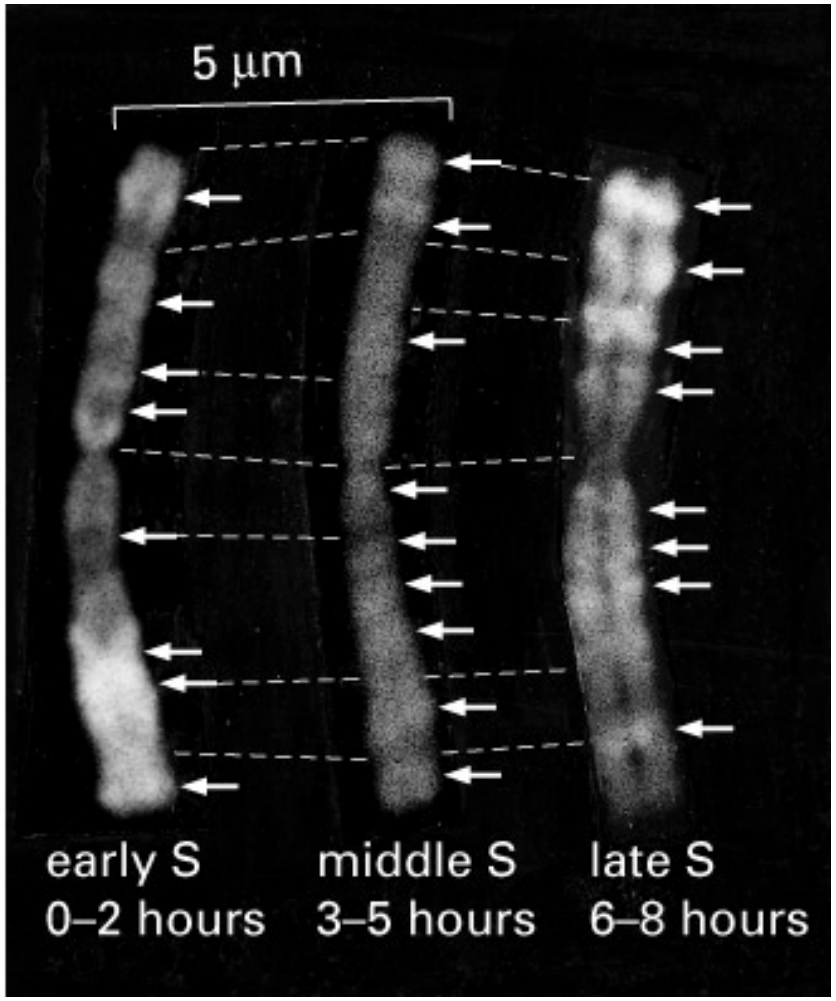
In homo sapiens…

- Le origini di replicazione si stima possano essere 10^4
- In certe regioni del DNA la replicazione puo' iniziare in siti diversi, identificando una "*zona*" preferenziale d'inizio di replicazione.
- Quale sequenza funzioni davvero come origine e' influenzato dalla *struttura della cromatina, dalla trascrizione e dal differenziamento cellulare*.



DNA replication takes place during S phase

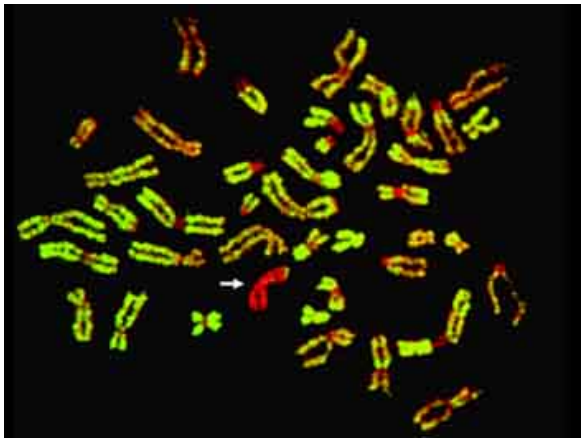




In a mammalian cell, the S phase lasts for about 8 hours. Different regions on the same chromosome replicate at distinct times in S phase.

Transacting factors in G1 rendono le cellule competenti alla replicazione, ma la replicazione puo' attivarsi solo in S

Highly condensed chromatin replicates late, while genes in less condensed chromatin replicate earlier



Two X chromosomes in a female mammalian cell

- the two chromosomes contain essentially the same DNA sequence

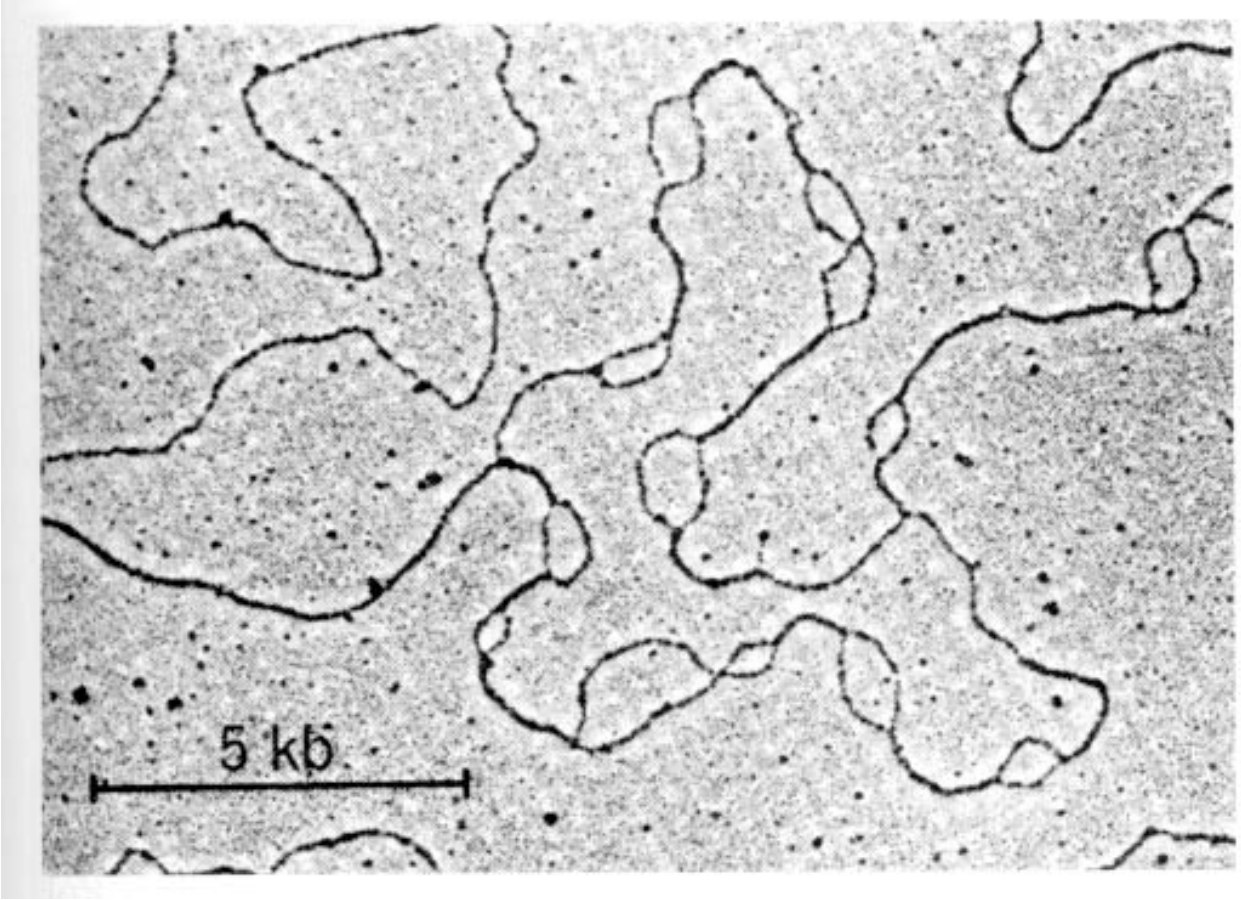
- one is inactive for transcription and is condensed into heterochromatin --> its DNA replicates late in S phase

- one is active for transcription and is less condensed --> it replicates throughout S phase

ETEROCROMATINA (stato più condensato): replicazione tardiva

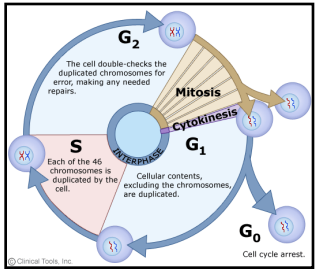
EUCROMATINA (stato meno condensato): replicazione precoce

Multiple Replication Forks During Eukaryotic DNA Synthesis

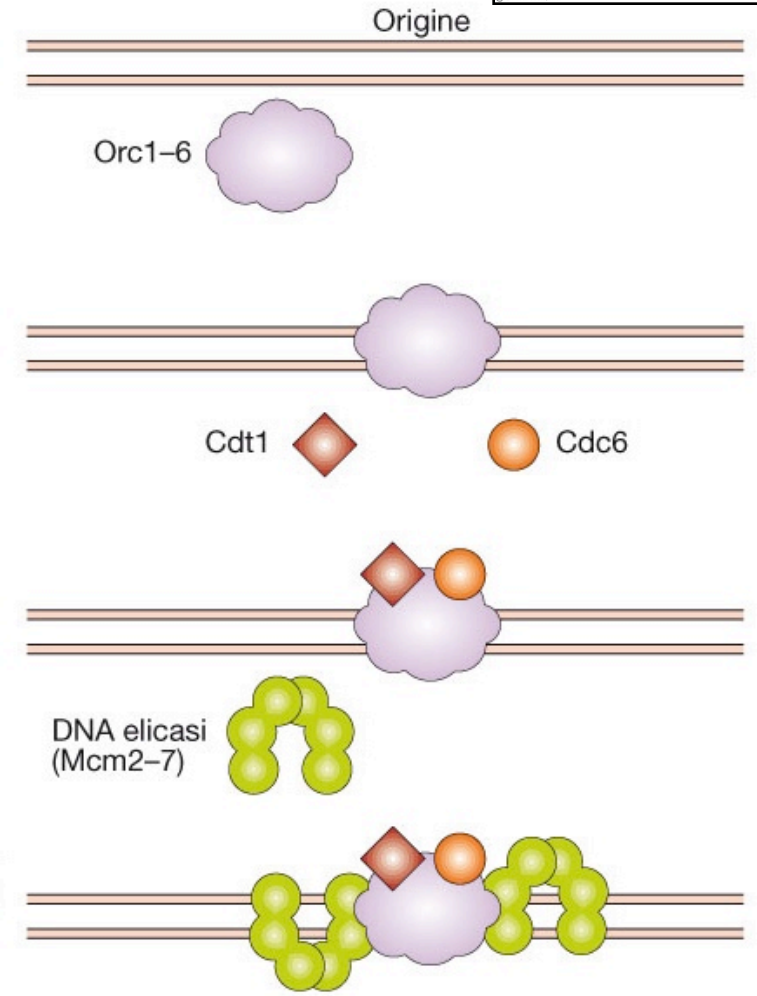


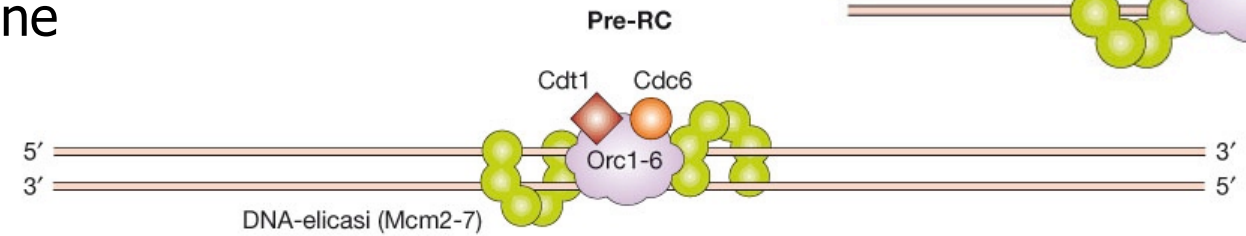
During the cell cycle,
when are new replication origins formed?

I- The Pre-RC **assembly** (G1)



La preparazione delle origini alla replicazione del DNA avviene quando le cellule *escono dalla mitosi* e *proseguono nella fase G1* del ciclo cellulare.
In questo momento si forma su ciascuna origine il **complesso di pre-replicazione** (pre-RC), il cui costituente principale e' il **COMPLESSO ORC**.
Nelle cellule eucariote si assembla sulle origini in **late M** and **early G1**.
Ad ORC si associano due proteine chiave del controllo replicativo, **cdc6** e **cdt1**, richieste per l'attivazione dell'elicasi, nota come **Mcm2-7.**
A questo punto il DNA e' "**licenced**" per la replicazione

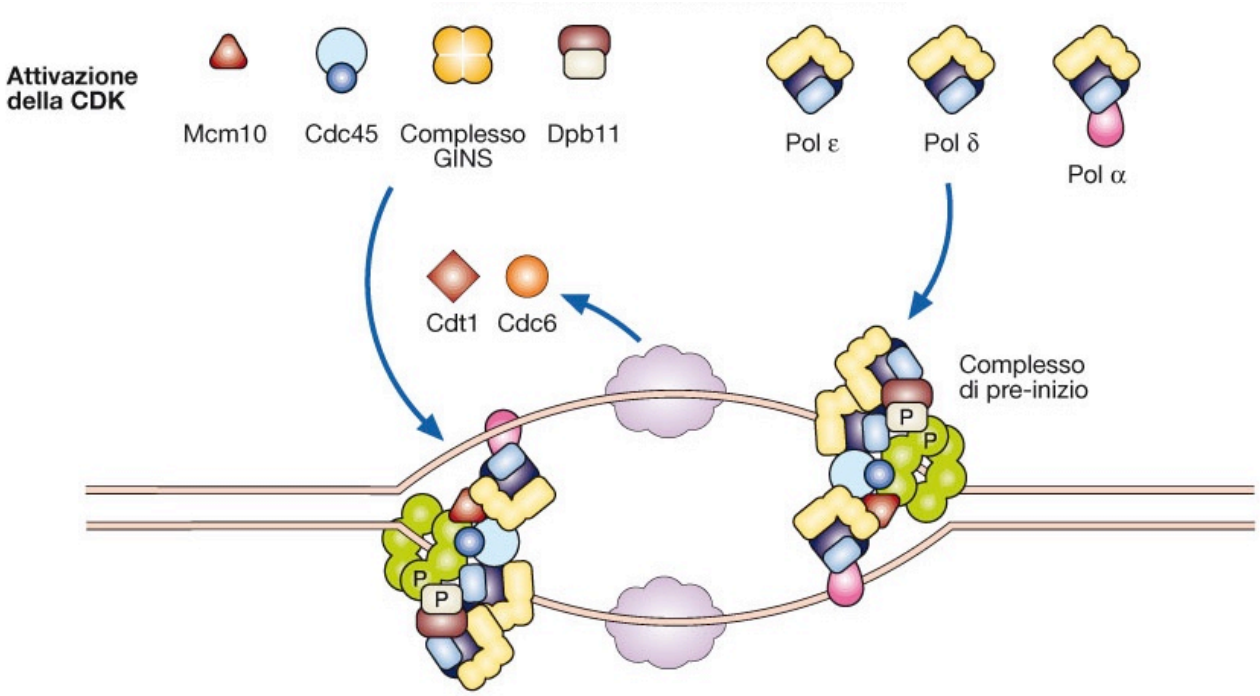




The MCM family

- Mcm2, Mcm3, Mcm5 (CDC46), CDC47, CDC54; at least 7 mcm proteins in Drosophila, that assemble in a ~600 kDa complex
- Essential for viability
- ~10 times more abundant than ORC
- Tightly associated with chromatin in G1, disassociate when replication occurs, reassociate at the end of mitosis
- Component of the licensing factor?

II- The Pre-IC assembly (G1)



L'attivita' delle CDKs coinvolte nella regolazione del ciclo cellulare trasforma il pre-RC in **pre-initiation complex** competente per la replicazione e innesca la sintesi del DNA.

La fosforilazione di ORC da parte delle CDKs provoca il distacco di **Cdt1** e **Cdc6** (che se fosforilati, vengono degradati) e il contemporaneo aggancio di altre proteine, tra cui Mcm10, cdc45, Dpb11 e il complesso GINS (post-RC).
Questi eventi attivano l'attivita' elicasica di Mcm2-7, portando all'apertura dell'origine e al caricamento delle polimerasi e delle altre proteine richieste dal processo replicativo.
I rapporti di formazione tra pre-RC a attivita' delle CDKs assicurano che il DNA venga duplicato una sola volta per ciclo cellulare.

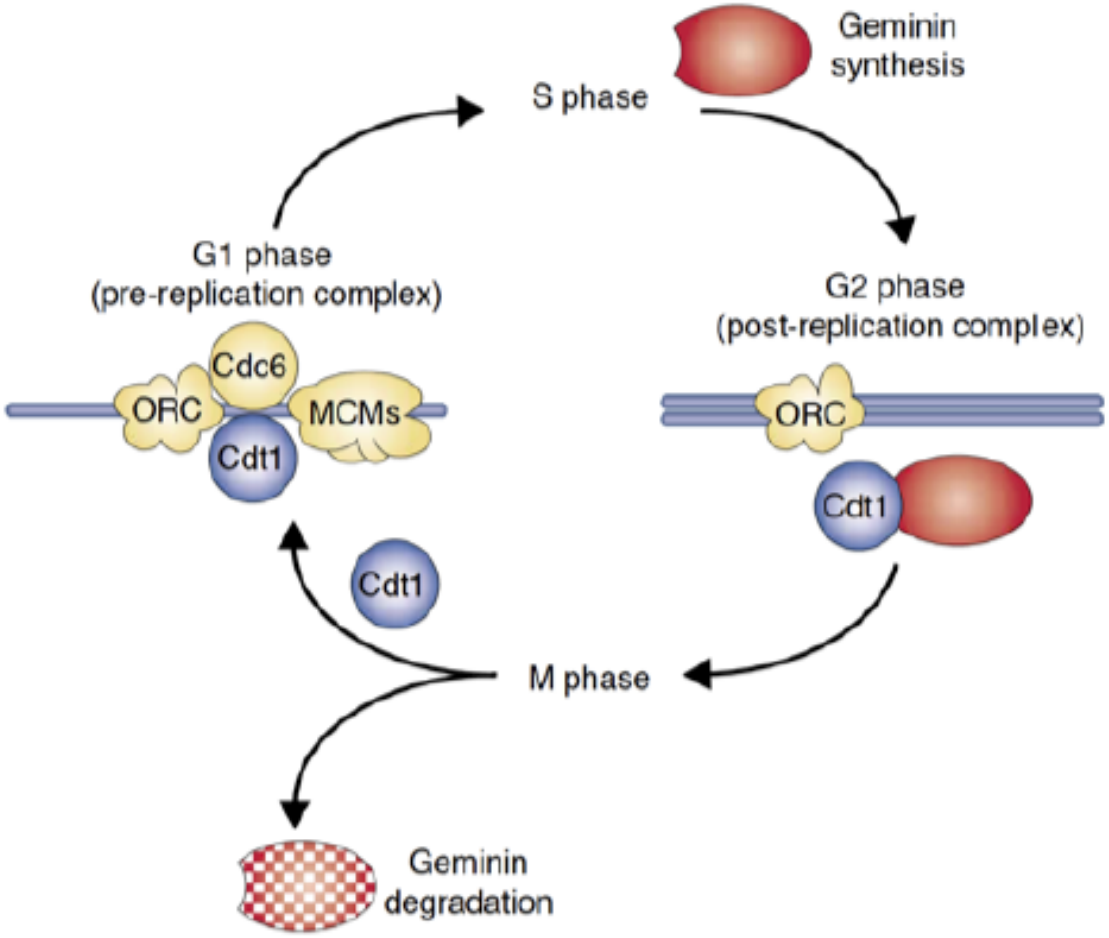
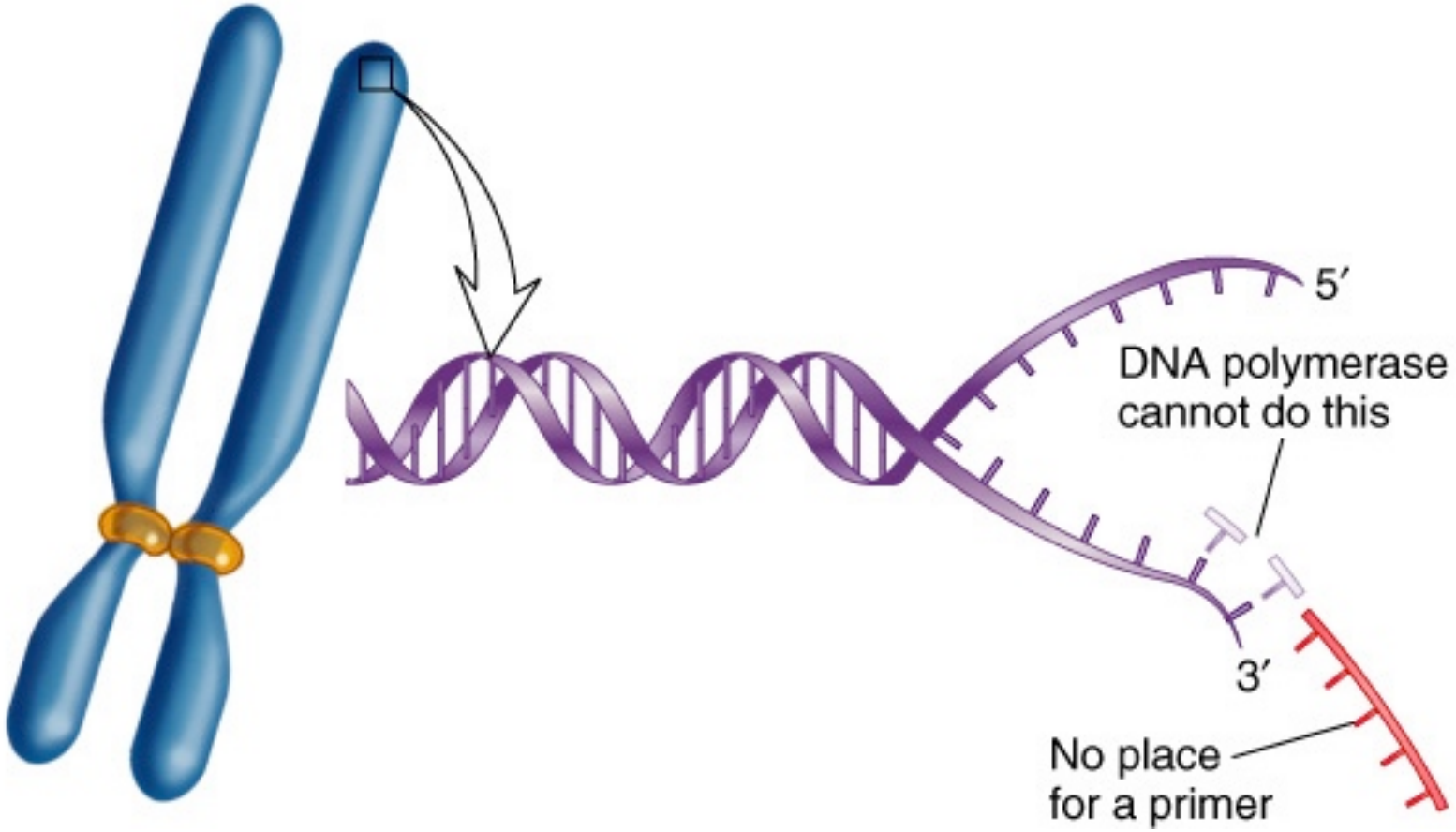


Figure 2 **Model illustrating how Cdt1 and geminin limit DNA replication to exactly one round per cell cycle. The origin-recognition complex (ORC) remains bound throughout the cell cycle. During mitosis Cdt1 is sequestered by geminin; upon exit from metaphase, geminin is degraded, releasing Cdt1. Cdt1 and Cdc6 bind to DNA, allowing the mini-chromosome maintenance (MCM) complex to bind to DNA during G1 phase, thereby 'licencing' DNA for a single round of replication. The MCM complex, Cdt1 and possibly Cdc6 are displaced from DNA during S phase. Newly synthesized geminin binds to displaced Cdt1 during S, G2 and M phases, preventing re-licencing of DNA within the same cell cycle.**

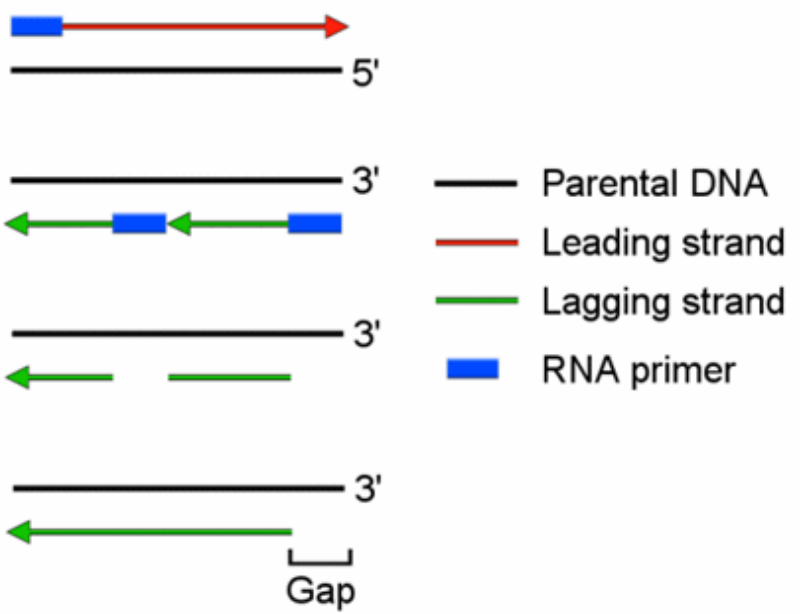
The Problem at the ends of eukaryotic linear Chromosomes



- DNA polymerases can only synthesize DNA only in the 5’ to 3’ direction and cannot initiate DNA synthesis
- These two features pose a problem at the 3’ end of linear chromosomes

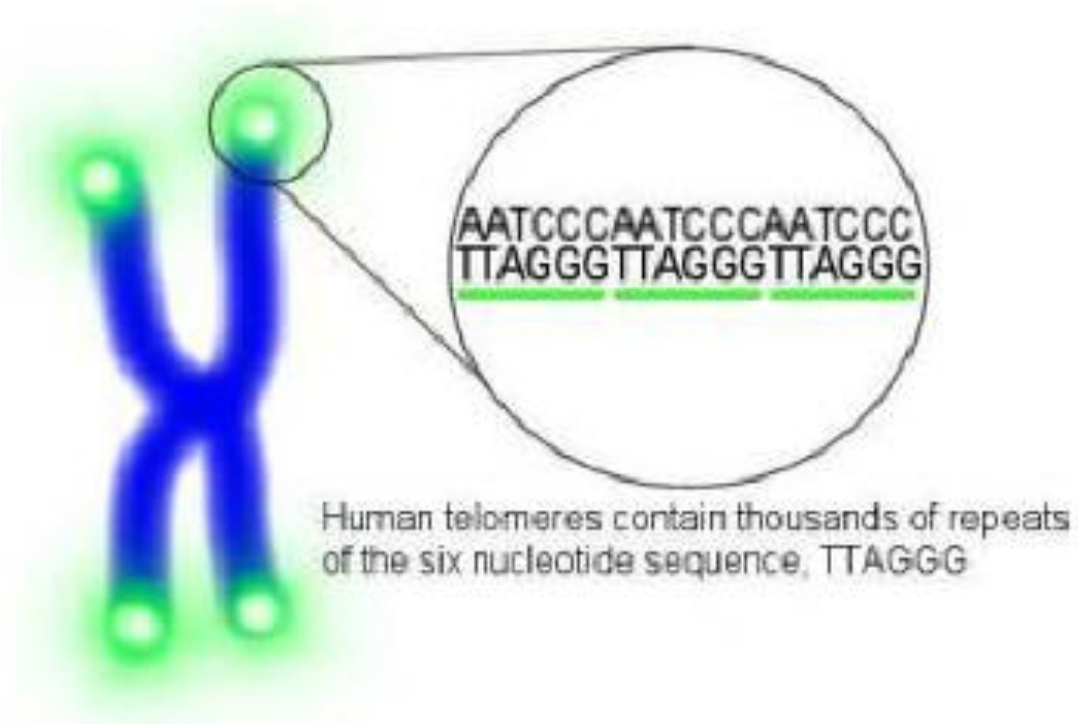
The Problem at the ends of eukaryotic linear Chromosomes

This mechanism encounters a special problem when the replication fork reaches an end of a linear chromosome: there is no place to produce the RNA primer needed to start the last Okazaki fragment at the very tip of a linear DNA molecule (the **end replication problem**)



- Bacteria solve this "end-replication" problem by having circular DNA molecules as chromosomes
- Eucaryotes have special nucleotide sequences at the ends of their chromosomes, which are incorporated into telomeres, and attract an enzyme called telomerase.

Telomeres



Telomere DNA sequences are similar in organisms as diverse as protozoa, fungi, plants, and mammals.

They consist of many **tandem repeats** of a short sequence that contains a block of neighboring G nucleotides.

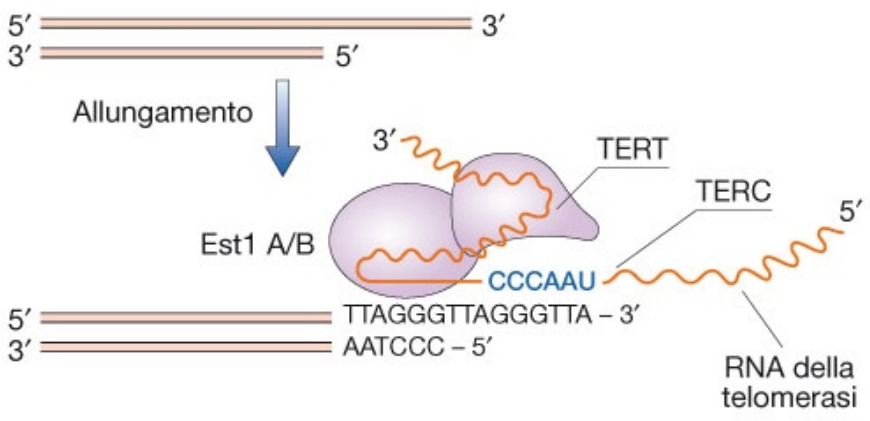
In humans, this sequence is **TTAGGG**, extending for about 10,000 nucleotides.

La Telomerasi

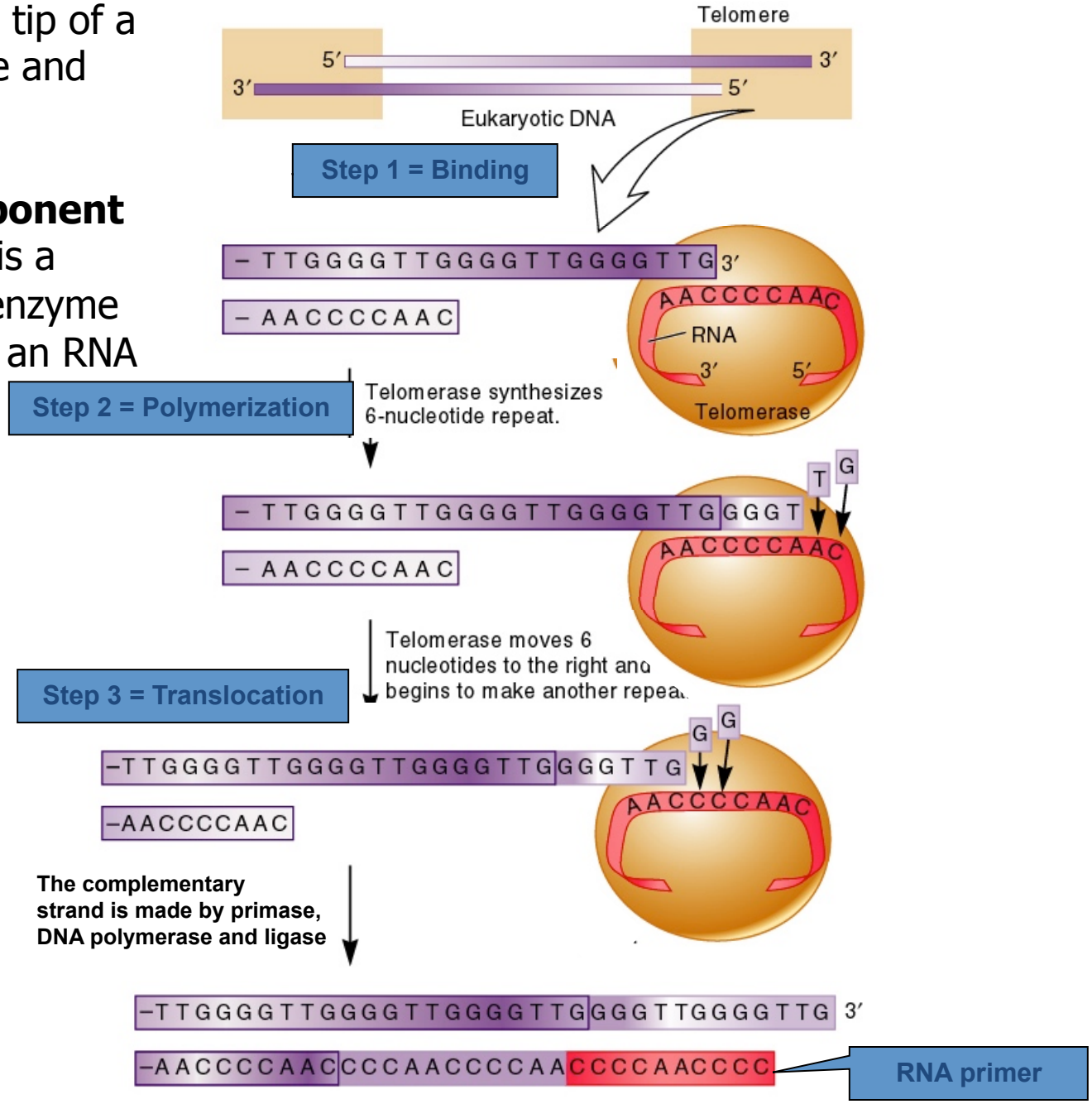
E’ una ribonucleoproteina costituita da due componenti principali:

1. Una proteina (TERT: *Telomerase Reverse Transcriptase*) che agisce come una trascrittasi inversa, essendo capace di sintetizzare DNA copiando uno stampo di RNA;
2. Una molecola di RNA stampo, chiamata TERC (*Telomerase RNA Component*)

La subunita’ catalitica TERT si associa con altre proteine accessorie a formare la macchina proteica coinvolta nel mantenimento dei telomeri.



Telomerase recognizes the tip of a G-rich strand of a telomere and elongates it in the 5′-to-3′ direction, using an **RNA template that is a component of the enzyme itself.** It is a **reverse transcriptase**, enzyme that synthesize DNA using an RNA template



Three Scientists Win Nobel Prize in Medicine

Their work involved the health of cells and the aging process. *Transcript of*

2009: Elisabeth Blackburn, Carol Greider e Jack Szostak

Three scientists based in the United States have won the two thousand nine Nobel Prize for Physiology or Medicine. They are being honored for their work in the nineteen eighties about the health of cells and the aging process.

The winners are Elizabeth Blackburn from the University of California, San Francisco; Jack Szostak from Harvard Medical School in Massachusetts and Carol Greider from Johns Hopkins University in Maryland. They will share the one million four hundred thousand dollar prize.

Elizabeth Blackburn, left, and Carol Greider after receiving a science prize in Frankfurt, Germany, earlier this year

The scientists' work begins with telomeres. These are like protective coverings on the ends of chromosomes. Elizabeth Blackburn compares them to the plastic tips on the ends of shoelaces. She says without telomeres the chromosome and the genes it holds would come apart.

Telomeres are necessary for a cell to divide. They also are involved in directing the number of divisions.

Jack Szostak

Mizz Blackburn and Mister Szostak discovered the special system of genetic information in the telomeres that protects the chromosomes from ruin. Later, Mizz Blackburn and Mizz Greider discovered the substance in the body that builds telomeres. The scientists named the enzyme telomerase.

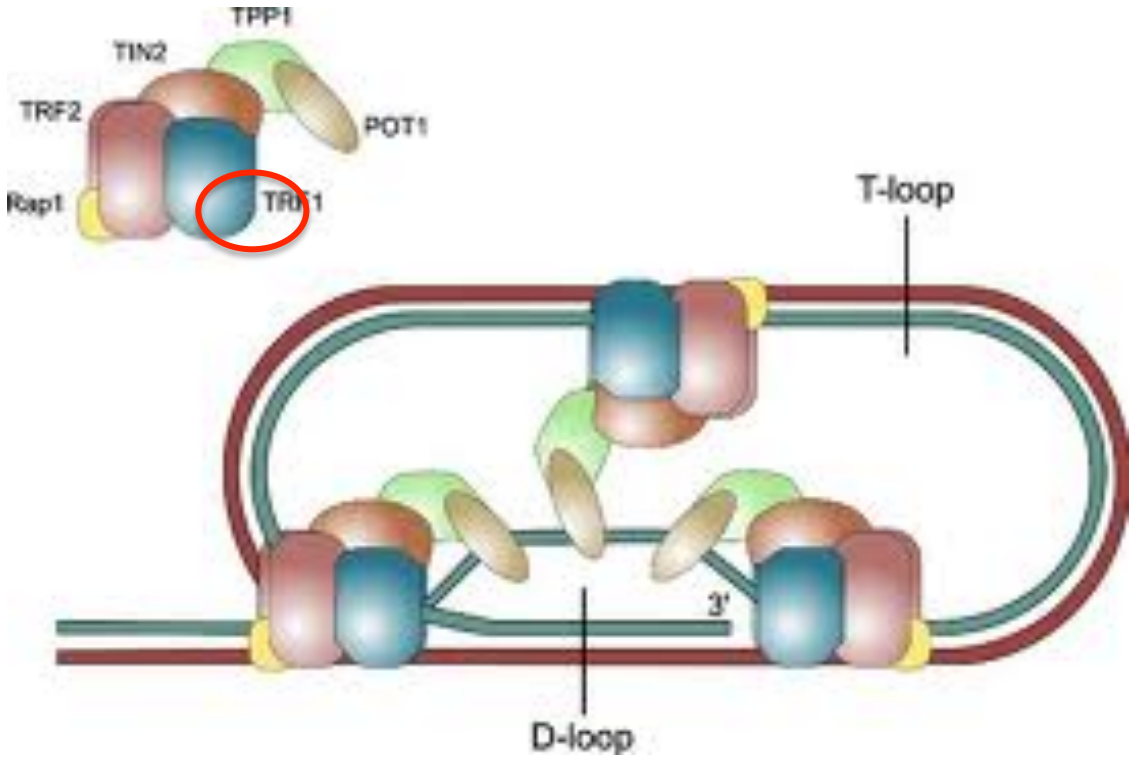
Their research showed that cells age if telomeres are shortened. But, cell death is delayed if a lot of the enzyme telomerase is produced.

Rune Toftgard is a Nobel Committee member from Sweden's Karolinska Institute. He says the work of telomeres is important to the understanding of how genetic material is copied and saved.

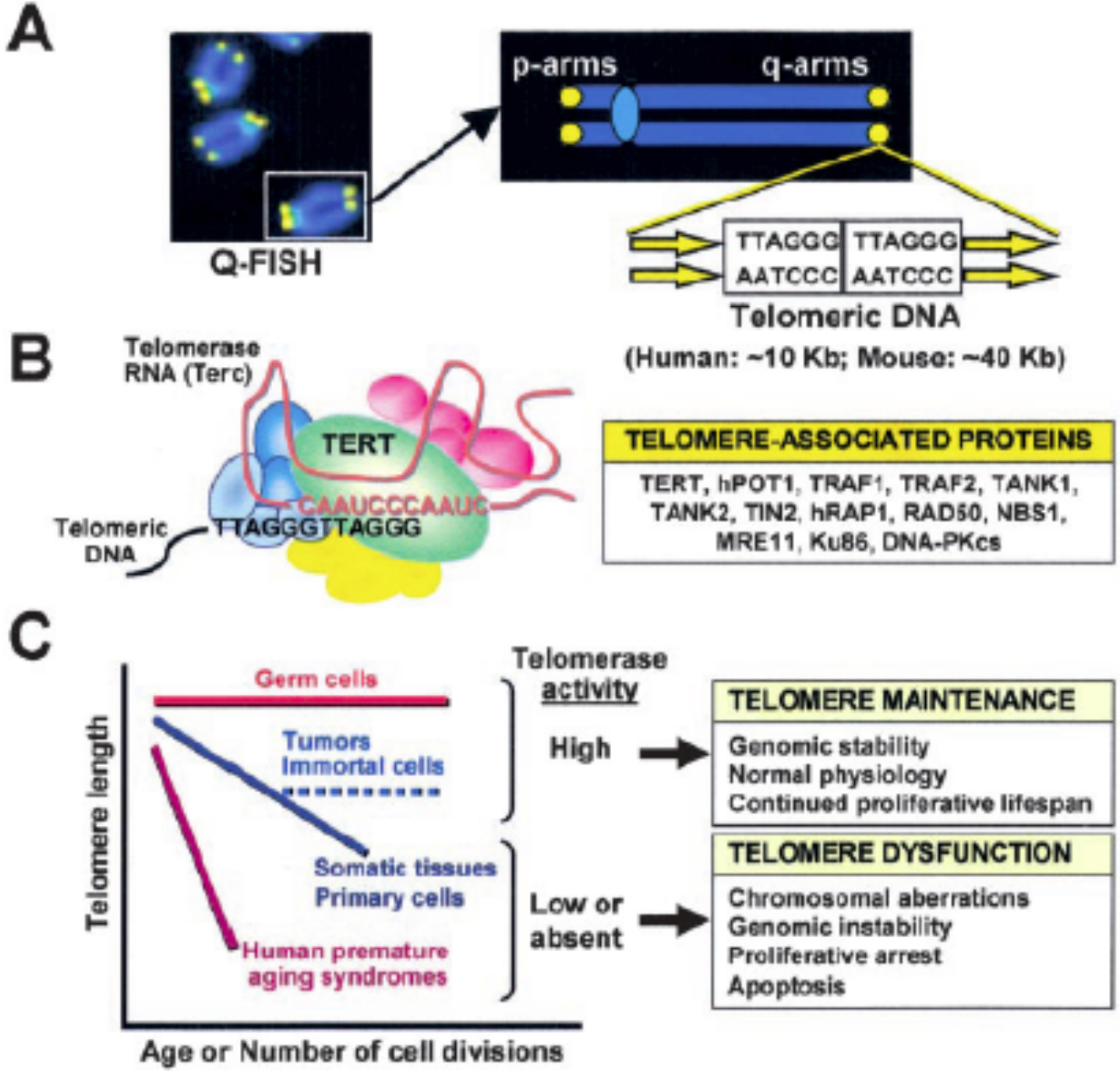
Protezione dei telomeri

La lunghezza dei telomeri condiziona l'accesso della telomerasi ed è quindi CONTROLLATA:

a) Proteina **POT1**: quando il numero delle ripetizioni ricche di G è elevato si lega ad altri fattori proteici sul telomero, fa ripiegare la cromatina ed impedisce l'accesso della telomerasi



The extended telomeric cap helps to maintain the stability of the genome


A
p-arms
q-arms
Q-FISH
TTAGGG
TTAGGG
AATCCC
AATCCC
Telomeric DNA
(Human: ~10 Kb; Mouse: ~40 Kb)
B
Telomerase
RNA (Terc)
TERT
CAAUCCCAAUC
Telomeric
DNA
TTAGGGTTAGGG
TELOMERE-ASSOCIATED PROTEINS
TERT, hPOT1, TRAF1, TRAF2, TANK1,
TANK2, TIN2, hRAP1, RAD50, NBS1,
MRE11, Ku86, DNA-PKcs
C
Telomere length
Germ cells
Tumors
Immortal cells
Somatic tissues
Primary cells
Human premature
aging syndromes
Age or Number of cell divisions
Telomerase
activity
High
Low or
absent
TELOMERE MAINTENANCE
Genomic stability
Normal physiology
Continued proliferative lifespan
TELOMERE DYSFUNCTION
Chromosomal aberrations
Genomic instability
Proliferative arrest
Apoptosis

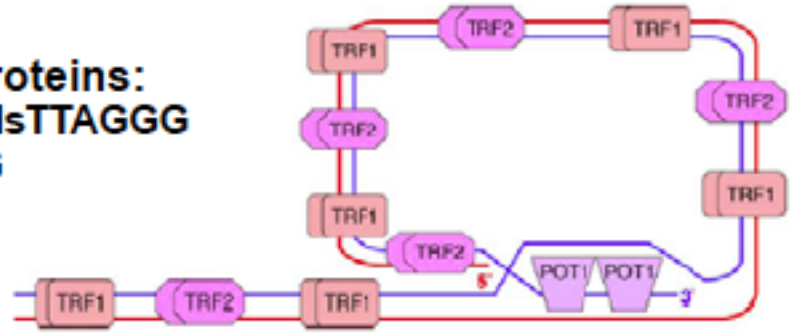
Telomere structure

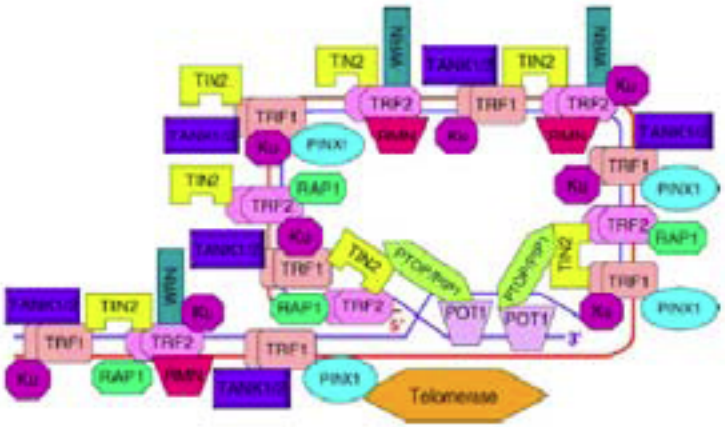
Vertebrate telomeres are long stretches (1-50 kb) of dsDNA containing the repetitive sequence TTAGGG, which terminate in 100-200 bases of ss TTAGGG at the 3' end. This 3' overhang circles back end embed in the duplex DNA

Terminal 3' ssDNA tail (G strand overhang) buried into adjacent ds repetitive telomeric DNA, forming a protective "t loop" structure. The "t loop" is stabilized by a "D loop" (displacement) loop. The G strand overhang is the substrate of telomerase (Terc), which employs an RNA template.

3 telomere-associated proteins:

- **TRF1 and TRF2 bind dsTTAGGG**
- **POT1 bind ssTTAGGG**





The extended telomeric cap helps maintain the stability of the genome

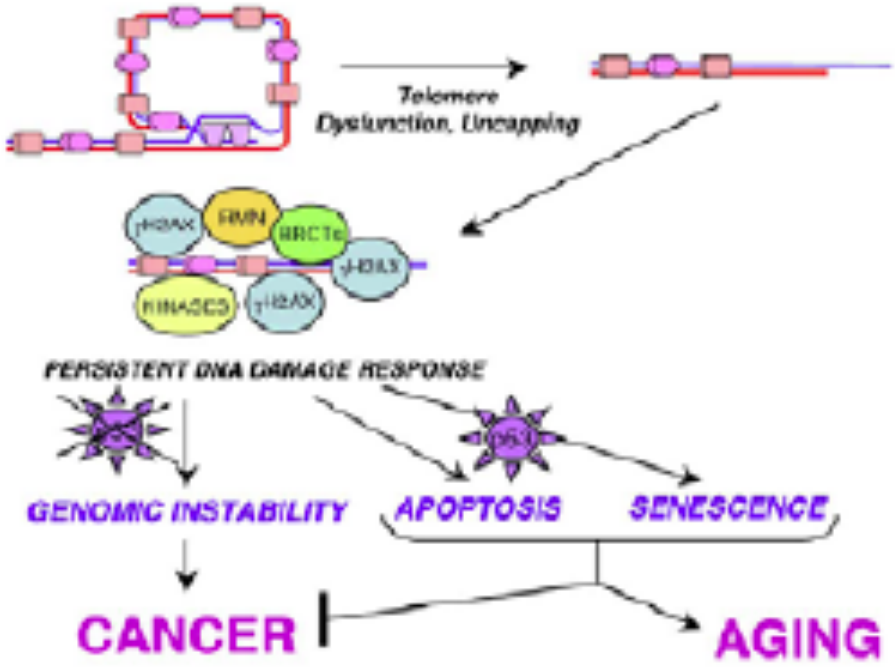
In teoria l'attività della telomerasi allunga i cromosomi ad ogni divisione cellulare.
In pratica in molti tipi cellulari ci sono bassi livelli di telomerasi e ciascuna cellula nasce con un ben definito numero di unità ripetitive a livello dei telomeri.
(**Eccezioni**: cellule in attiva divisione: cellule ovaio, testicolo, cellule epiteliali proliferanti, linfociti, cellule embrione).
Aldilà delle cellule che fanno eccezione, nella maggior parte delle cellule somatiche umane ad ogni divisione cellulare vengono persi 50-100 nucleotidi (accorciamento dei telomeri) e dopo varie generazioni le cellule iniziano ad avere cromosomi difettivi e vanno in **senescenza** (cioè cessano di dividersi).
In tal modo si evita la proliferazione incontrollata e questo sarebbe un meccanismo di protezione dallo sviluppo di tumori, caso in cui infatti si registra spesso una riattivazione della telomerasi.

Telomeres uncapping

During the proliferation of human cells, a gradual shortening of the average length of telomeres is observed because of the "**end-replication problem**" - the inability of the DNA replication to complete DNA synthesis at the very beginning of the replicated lagging strand.

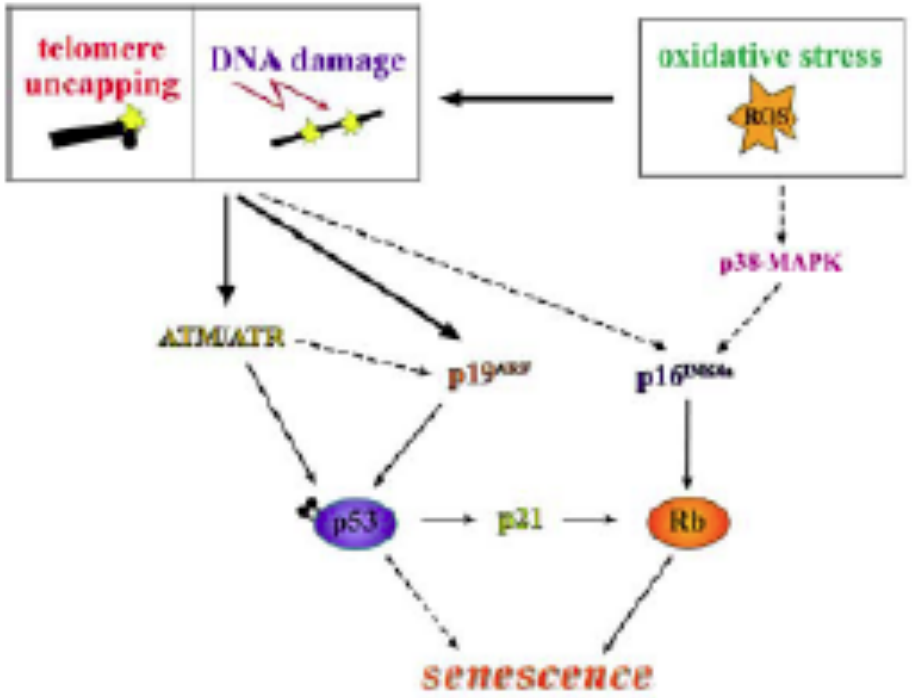
Senescent telomeres lose some of their single-stranded portion - the telomeric overhang - which is crucial for the maintenance of the T-loop and the subsequent formation of the cap

*Telomere uncapping (disruption of the proper structure of the protective cap) seems to be recognized as a **dsDNA break**, activating the DNA damage machinery.*



Telomeres uncapping causes a DNA damage response

DNA damage foci appear at the telomeres of senescent cells, containing many DNA-damage-response proteins, including γ-H2AX, 53BP1, MDC1, NBS1, MRE11 and RAD17



How do cells choose between senescence and apoptosis upon DNA damage-induced p53 activation?

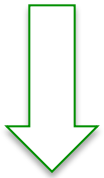
-Different post-translational modifications of p53 in response to different stimuli?

-Binding of different proteins to p53?

-Activation of different sets of transcriptional targets?

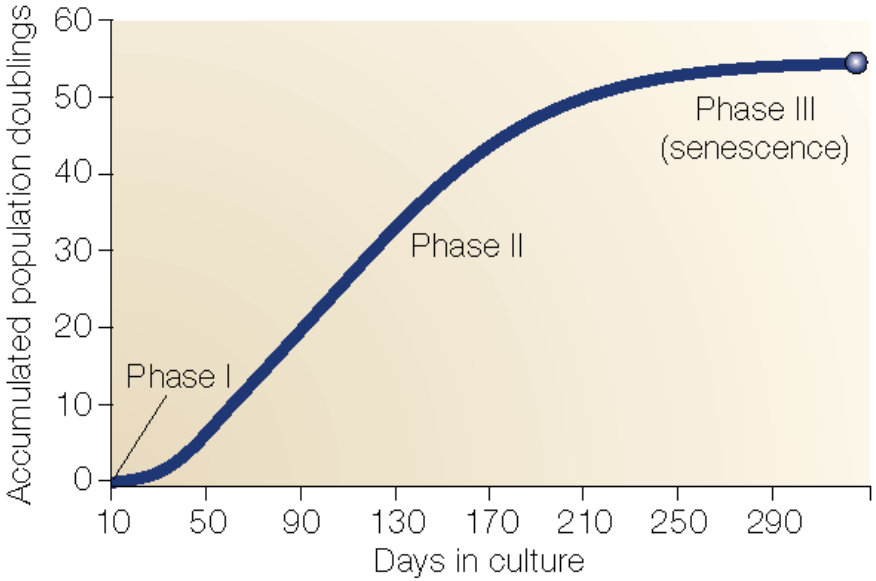
Regolazione lunghezza telomeri

La maggior parte delle cellule somatiche umane non esprime abbastanza telomerasi per mantenere una lunghezza costante dei telomeri:
ACCORCIAMENTO DEI TELOMERI
(50-100 nt/divisione cell.)



Orologio cellulare per l'invecchiamento?

TEORIA DI HAYFLICK (1965)

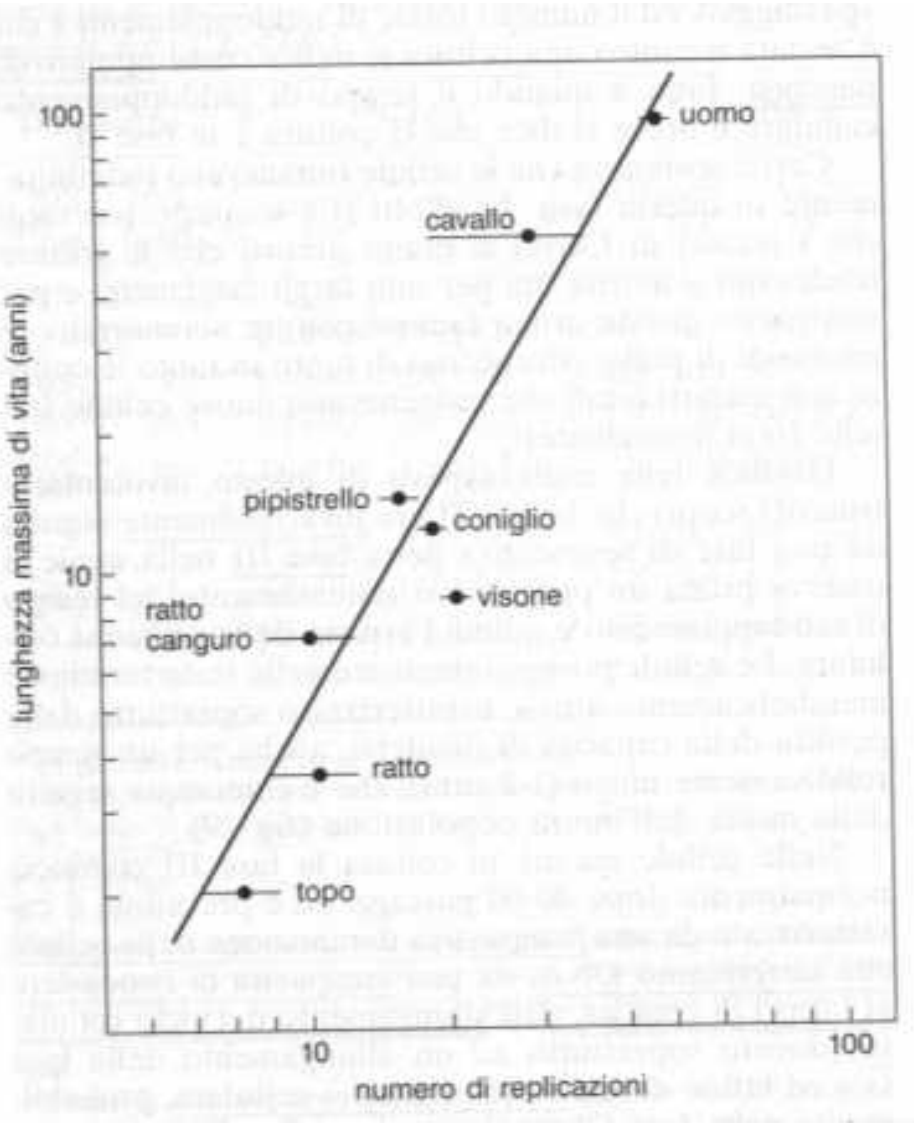


Nature Reviews | Molecular Cell Biology

Hayflick ha basato la sua teoria dell'invecchiamento su esperimenti di coltura "in vitro" di fibroblasti dermici, ma anche in molti altri tessuti.
Hayflick notò che il numero delle replicazioni, cioè del raddoppio delle cellule della coltura, in presenza di adeguate sostanze nutritive, nei fibroblasti umani era circa di 50; subentrava quindi una fase di senescenza, che, dopo circa altre 10 suddivisioni, portava alla estinzione della colonia, vale a dire alla morte di tutte le cellule.
Il numero delle replicazioni dei fibroblasti diminuiva proporzionalmente all'eta' dell'organismo.

TEORIA DI HAYFLICK

Successivamente Hayflick fu in grado di dimostrare che il numero delle replicazioni di fibroblasti appartenenti a varie specie animali era proporzionale alla lunghezza massima della vita dell'animale stesso (figura).
Ne trasse la conclusione che la durata della vita, per cui ciascuna specie, era legata a fattori genetici, e un individuo possiede come un "orologio interno", che è programmato per una durata di vita prefissata.
(Teoria genetica dell' invecchiamento)



Cell, Vol. 91, 25–34, October 3, 1997, Copyright ©1997 by Cell Press

Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA

María A. Blasco,[1,3,7] Han-Woong Lee,[2,7]
M. Prakash Hande,[4] Enrique Samper,[3]
Peter M. Lansdorp,[4,5] Ronald A. DePinho,[2,8]
and Carol W. Greider[1,6,8]

To examine the role of telomerase in normal and neoplastic growth, the telomerase RNA component (*mTR*) was deleted from the mouse germline.

Telomeres were shown to shorten at a rate of 4.8± 2.4 kb per generation.

Cells from the fourth generation onward possessed chromosome ends lacking detectable telomere repeats, aneuploidy, and chromosomal abnormalities, including end-to-end fusions.

These results indicate that telomerase is essential for telomere length maintenance.

Extension of Life-Span by Introduction of Telomerase into Normal Human Cells

Andrea G. Bodnar,* Michel Ouellette,* Maria Frolkis, Shawn E. Holt, Choy-Pik Chiu, Gregg B. Morin, Calvin B. Harley, Jerry W. Shay, Serge Lichtsteiner,† Woodring E. Wright†

Normal human cells undergo a finite number of cell divisions and ultimately enter a nondividing state called replicative senescence. It has been proposed that telomere shortening is the molecular clock that triggers senescence. To test this hypothesis, two telomerase-negative normal human cell types, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding the human telomerase catalytic subunit. In contrast to telomerase-negative control clones, which exhibited telomere shortening and senescence, telomerase-expressing clones had elongated telomeres, divided vigorously, and showed reduced staining for β-galactosidase, a biomarker for senescence. Notably, the telomerase-expressing clones have a normal karyotype and have already exceeded their normal life-span by at least 20 doublings, thus establishing a causal relationship between telomere shortening and in vitro cellular senescence. The ability to maintain normal human cells in a phenotypically youthful state could have important applications in research and medicine.

Constitutive telomerase expression in several independent Tert-transgenic mouse models resulted in increased incidence of spontaneous tumors
(Gonzalez-Suarez et al., 2001; Gonzalez-Suarez et al., 2002; Artandi et al., 2002; Canela et al., 2004).

- Together, these evidences strongly suggests that telomerase activity and telomere length are rate limiting for mammalian life span and supports a model in which short telomeres actively contribute to aging by limiting tissue renewal.

- Together, these evidences strongly suggests that telomerase activity and telomere length are rate limiting for mammalian life span and supports a model in which short telomeres actively contribute to aging by limiting tissue renewal.

- An important prediction of this model is that slowing the rate of telomere shortening should delay aging.

- However, to address experimentally this prediction, it is necessary to take into account the role of telomere biology in cancer.

LETTER

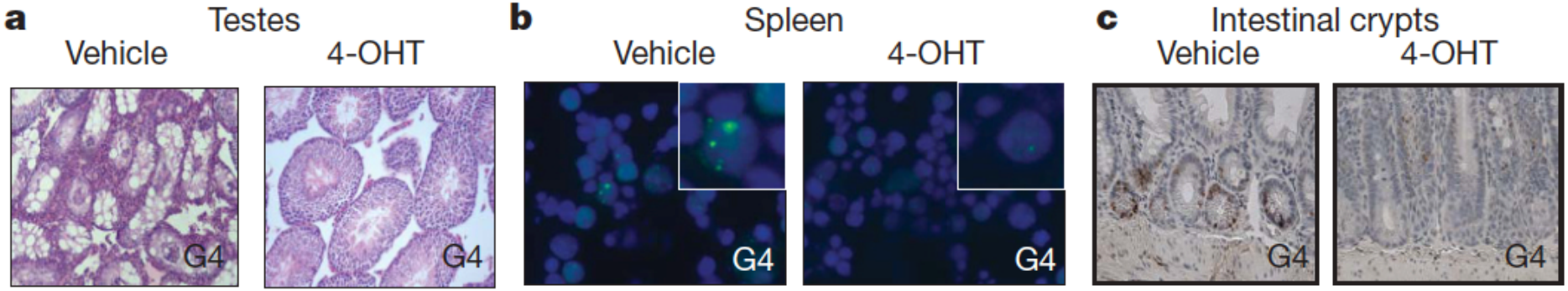
102 | NATURE | VOL 469 | 6 JANUARY 2011

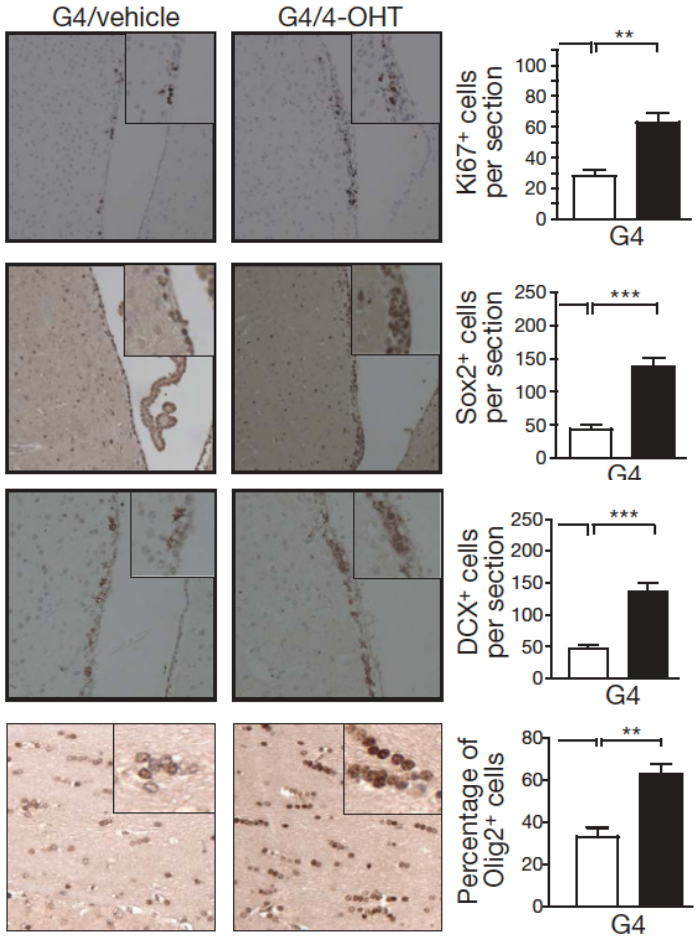
doi:10.1038/nature09603

Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice

Mariela Jaskelioff[1], Florian L. Muller[1], Ji-Hye Paik[1], Emily Thomas[1], Shan Jiang[1], Andrew C. Adams[2], Ergun Sahin[1], Maria Kost-Alimova[1], Alexei Protopopov[1], Juan Cadiñanos[1], James W. Horner[1], Eleftheria Maratos-Flier[2] & Ronald A. DePinho[1]

- A novel mouse model to explore the impact of physiological telomerase reactivation across diverse adult cell types and organ systems.
- Notably, the mice enlisted into this study are adults exhibiting significant **progeroid phenotypes** (mice null for mTerc or mTert).
- In TERT-ER mice with advanced degenerative phenotypes, short-term telomerase reactivation restored telomere reserves, quelled DNA damage signalling, and alleviated cellular checkpoint responses in several high-turnover organ systems with significant functional impact including increased fecundity….





Telomerase reactivation extends telomeres, reduces DNA damage signalling and associated cellular checkpoint responses, allows resumption of proliferation in quiescent cultures, and eliminates degenerative phenotypes across multiple organs including testes, spleens and intestines.
Notably, somatic telomerase reactivation reversed neurodegeneration with restoration of proliferating neural progenitors.

The brief course of telomerase reactivation was not sufficient to promote carcinogenesis.
However, it remains possible that more prolonged telomerase reactivation schedules or applications in later life may provoke carcinogenesis.

Cell *135*, 609–622, November 14, 2008 ©2008 Elsevier Inc.

Telomerase Reverse Transcriptase Delays Aging in Cancer-Resistant Mice

Antonia Tomás-Loba,[1,5] Ignacio Flores,[1,5] Pablo J. Fernández-Marcos,[2] María L. Cayuela,[1,6] Antonio Maraver,[2] Agueda Tejera,[1] Consuelo Borrás,[3] Ander Matheu,[2] Peter Klatt,[1,2] Juana M. Flores,[4] José Viña,[3] Manuel Serrano,[2] and Maria A. Blasco[1,*]

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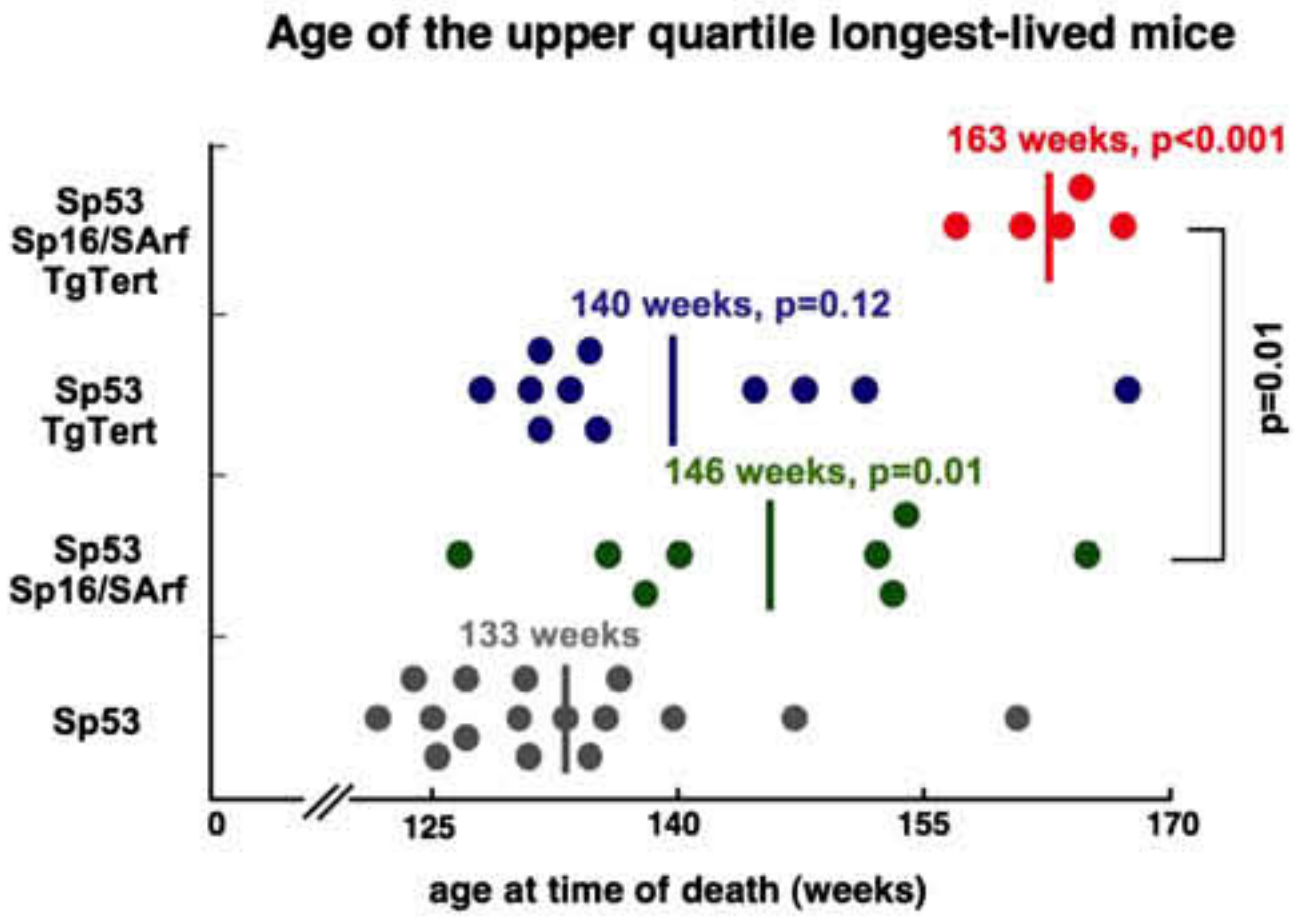
DOI 10.1016/j.cell.2008.09.034

Telomerase confers limitless proliferative potential to most human cells through its ability to elongate telomeres, the natural ends of chromosomes, which otherwise would undergo progressive attrition and eventually compromise cell viability. However, the role of telomerase in organismal aging has remained unaddressed, in part because of the cancer-promoting activity of telomerase. To circumvent this problem, we have constitutively expressed telomerase reverse transcriptase (TERT), one of the components of telomerase, in mice engineered to be cancer resistant by means of enhanced expression of the tumor suppressorsp53, p16,andp19ARF (these three tumor suppressors are involved in protection against a large variety of cancers-Collado et al., 2007). In this context,TERToverexpression improves the fitness of epithelial barriers, particularly the skin and the intestine, and produces a systemic delay in aging accompanied by extension of the median life span. These results demonstrate that constitutive expression of Tert provides anti-aging activity in the context of a mammalian organism.


Age of the upper quartile longest-lived mice
163 weeks, p<0.001
140 weeks, p=0.12
146 weeks, p=0.01
133 weeks
p=0.01
Sp53 Sp16/SArf TgTert
Sp53 TgTert
Sp53 Sp16/SArf
Sp53
0
125
140
155
170
age at time of death (weeks)

NATURE|Vol 464|25 March 2010|doi:10.1038/nature08982

Linking functional decline of telomeres, mitochondria and stem cells during ageing

Ergün Sahin[1] & Ronald A. DePinho[1]

The study of human genetic disorders and mutant mouse models has provided evidence that genome maintenance mechanisms, DNA damage signalling and metabolic regulation cooperate to drive the ageing process. In particular, age-associated telomere damage, diminution of telomere 'capping' function and associated p53 activation have emerged as prime instigators of a functional decline of tissue stem cells and of mitochondrial dysfunction that adversely affect renewal and bioenergetic support in diverse tissues. Constructing a model of how telomeres, stem cells and mitochondria interact with key molecules governing genome integrity, 'stemness' and metabolism provides a framework for how diverse factors contribute to ageing and age-related disorders.

Medical advances over the past century have produced a near doubling of human life expectancy in industrialized countries. This longevity boom — due primarily to improved neonatal care, to prevention and treatment of infections and to interventions for cardiovascular and endocrine diseases — is expected to lead to a population of 1.2 billion individuals aged 60 years or older by the year 2025. For medical technology to have a meaningful impact on the continued health and well-being of this ageing population, there is an increasingly urgent need for a more complete understanding of the molecular pathways and biological processes underlying ageing and age-related disorders.

Our progress in understanding the details of the ageing process has been hampered by issues such as the gradual and heterogeneous onset of tissue and organismal decline, the complexity and diversity of phenotypic presentations, and the lack of adequate biomarkers capable of quantifying the 'degree' of ageing at the molecular and cellular levels. Further complicating matters, the pace of mammalian ageing is influenced by external factors such as dietary intake, intrinsic stresses such as reactive oxygen species (ROS) and telomere erosion, and germline variation in genetic elements governing DNA repair, among other processes[1,2]. Studies in organisms ranging from yeast to primates have highlighted the importance of metabolic flux through the phosphatidylinositol-3-OH kinase (PI(3)K) pathway, as well as the activity of the master metabolic regulators such as sirtuins, the preservation of genome integrity by means of an efficient DNA repair and oxidative defence apparatus, the levels of genotoxic stress resulting from eroded telomeres or excessive ROS, the activation of the p53 and p16 tumour suppressor pathways, the robustness of mitochondrial reserve or function, and the integrity of a normal extracellular milieu and cytokine profile[2–4]. However, how these pathways and processes act together either to promote or to retard the ageing process is not entirely clear.

Increasing evidence points to telomeres and p53-mediated DNA damage signalling being core components that drive the senescent or apoptotic depletion of tissue stem-cell reserves and age-related tissue degeneration. Although such cellular checkpoint mechanisms contribute to the functional decline of highly proliferative tissues, how they would adversely affect the more quiescent tissues that are equally ravaged by the ageing process (such as heart, brain and liver) has been more difficult to rationalize. Here, we put forth a speculative model that posits a connection linking telomere damage and p53 activation with stem-cell and mitochondrial dysfunction. This model offers a unifying explanation of how telomeres influence the health of the ageing organism across diverse tissues with wide-ranging proliferative profiles.

The ageing phenotype

Regardless of the precise underlying molecular mechanisms, the fundamental defining manifestation of ageing is an overall decline in the functional capacity of various organs to maintain baseline tissue homeostasis and to respond adequately to physiological needs under stress[3,5]. In many aged tissues, these declines are gradual and modest in middle years but accelerate rapidly late in life and become particularly apparent under conditions that challenge the organism to overcome various stressors with a robust physiological or regenerative response. At the anatomical and physiological levels, diminished tissue cellularity and inadequate regenerative responses seem to be intimately linked to many of the classic age-associated medical maladies, such as muscle atrophy, anaemia, feeble immune responses and impaired wound healing (Box 1).

In accordance with this functional decline, age is the major risk factor for the development of chronic medical conditions. In the United States, nearly 50% of individuals over the age of 65 develop cardiovascular disease; 35% develop arthropathies; 15% develop type 2 diabetes; and 10% develop pulmonary disease. Some of these conditions are particularly debilitating and extract a significant social and economic toll. Stroke and dementia, the most common causes of long-term institutionalization of individuals over 65, cost the US health-care system alone $21 billion per year — a cost projected to rise by 14%, to $24 billion, in 2010 (ref. 6). Advancing age is also the major risk factor for the development of cancer. Specifically, between ages 40 and 80, there is a rapid increase in cancer incidence that produces an overall lifetime cancer risk of nearly one in two individuals in industrialized nations[7]. Thus, diseases of the aged collectively constitute the major causes of human suffering and consume the bulk of our health-care resources. From this perspective, the need to understand the nature of ageing and how its consequences can be managed, and possibly reversed, has never been greater.

[1]Belfer Institute for Applied Cancer Science, Departments of Medical Oncology, Medicine and Genetics, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA.