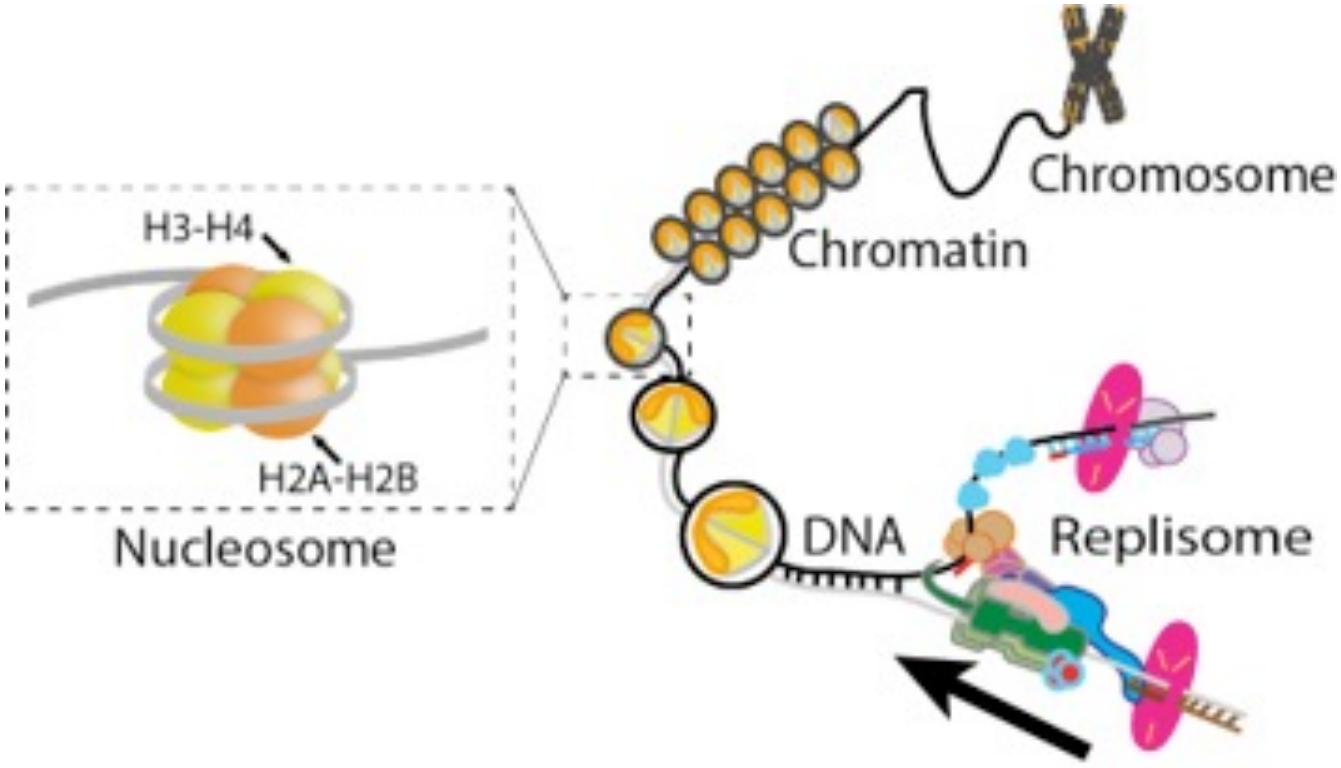
DNA REPLICATION

Objectives

- Essential Question: is DNA replication really similar in procaryotes and eucaryotes?
- Compare the process of DNA replication in prokaryotes and in eukaryotes
- Origins
- Cell cycle dependence
- Telomeres
- Applications of our knowledge

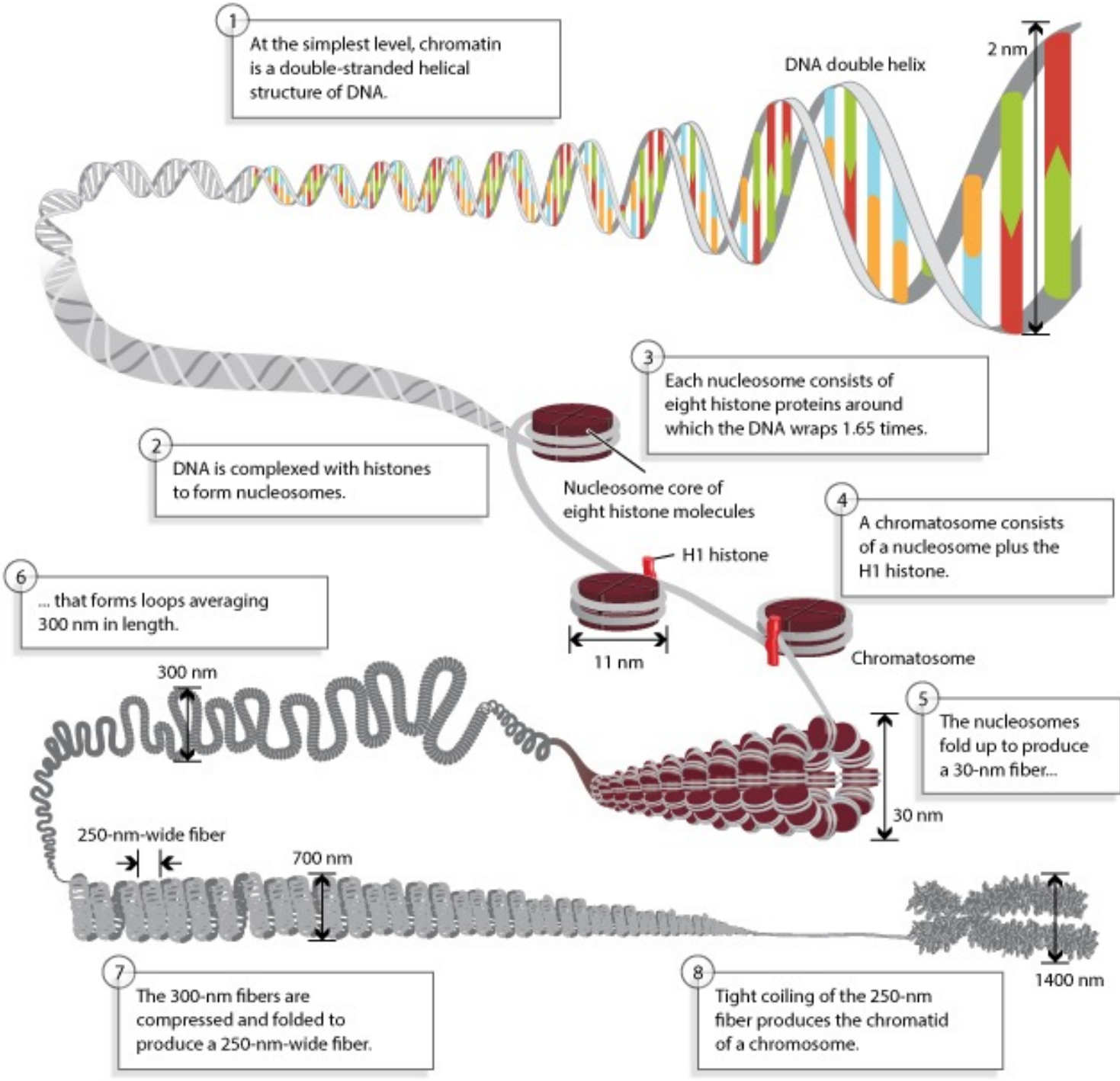
In Eucaryotes, the replication machinery has to deal with the 3D chromatin structure



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

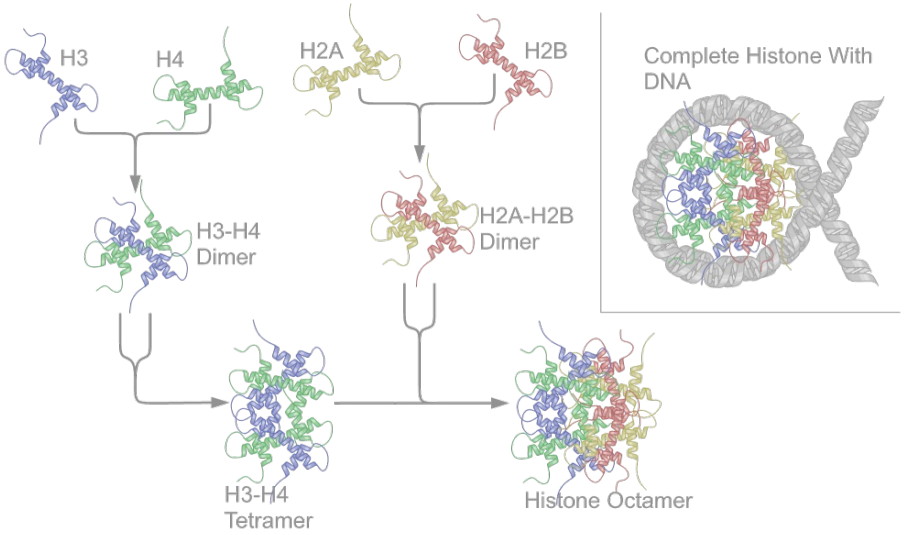
Okazaki fragments are synthesized at intervals of 100-200 nucleotides in eucaryotes, instead of 1000-2000 as in bacteria.

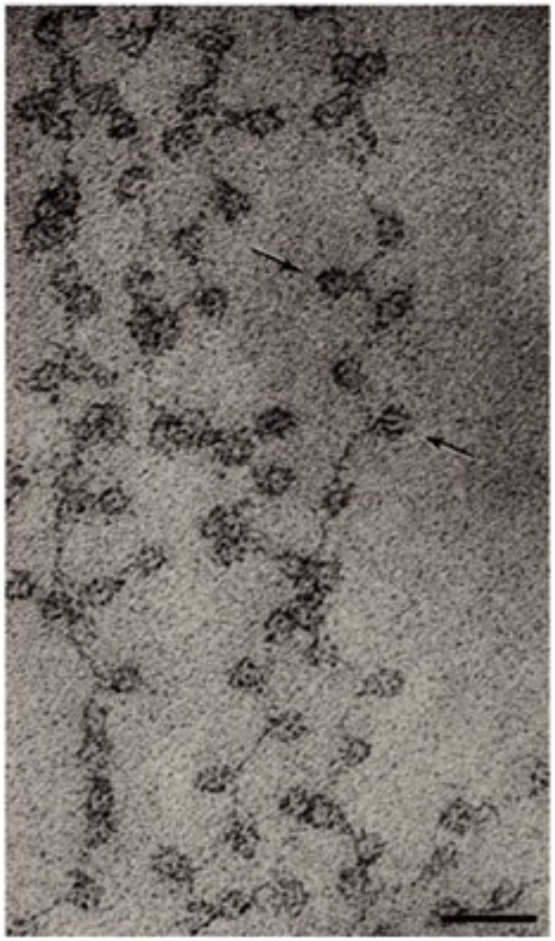
Chromatin structure



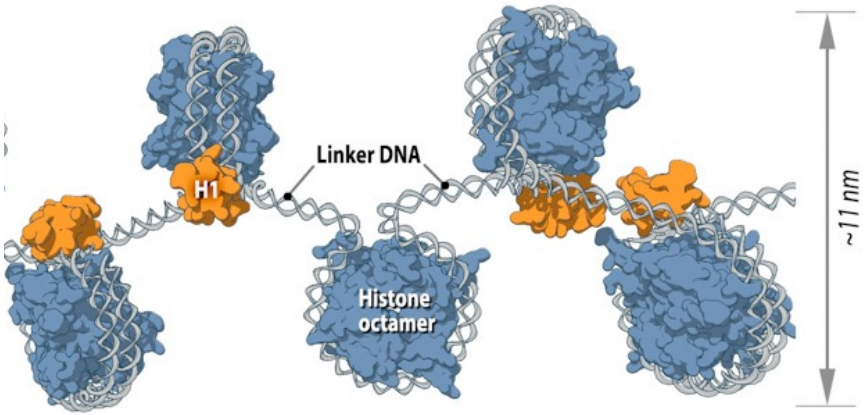
Nucleosomes

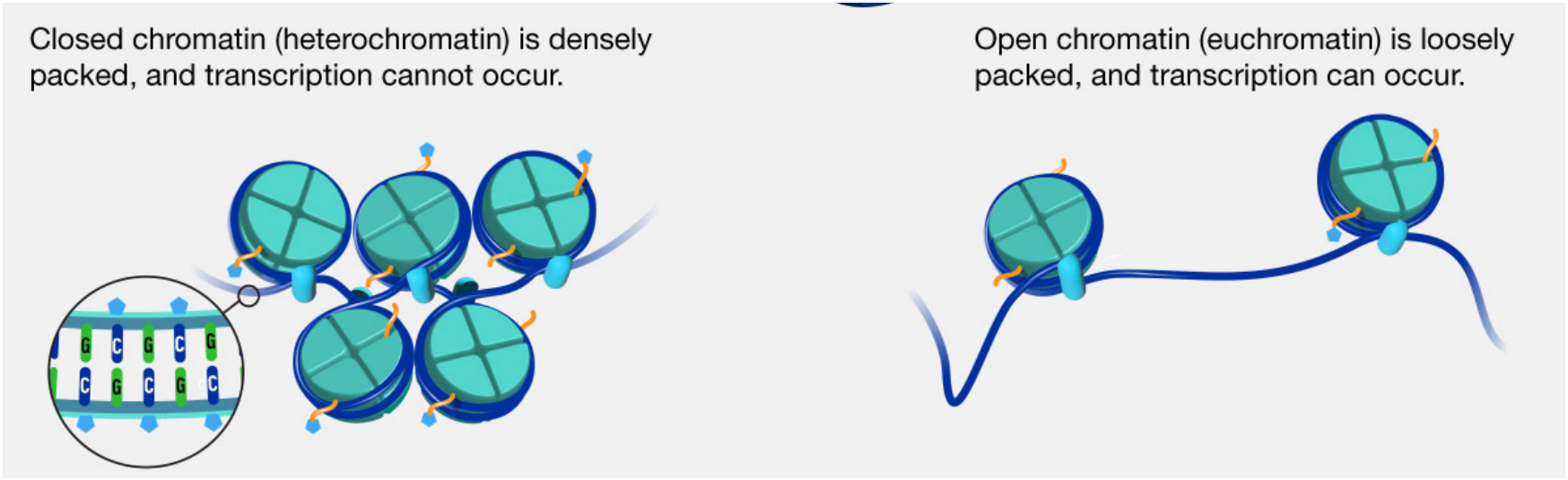
The basic repeating structural (and functional) unit of chromatin is the nucleosome, which contains eight histone proteins and about 146 base pairs of DNA





The addition of one H1 protein wraps another 20 base pairs, resulting in two full turns around the octamer, and forming a structure called a chromatosome





•Histones can be enzymatically modified by the addition of acetyl, methyl, or phosphate groups.

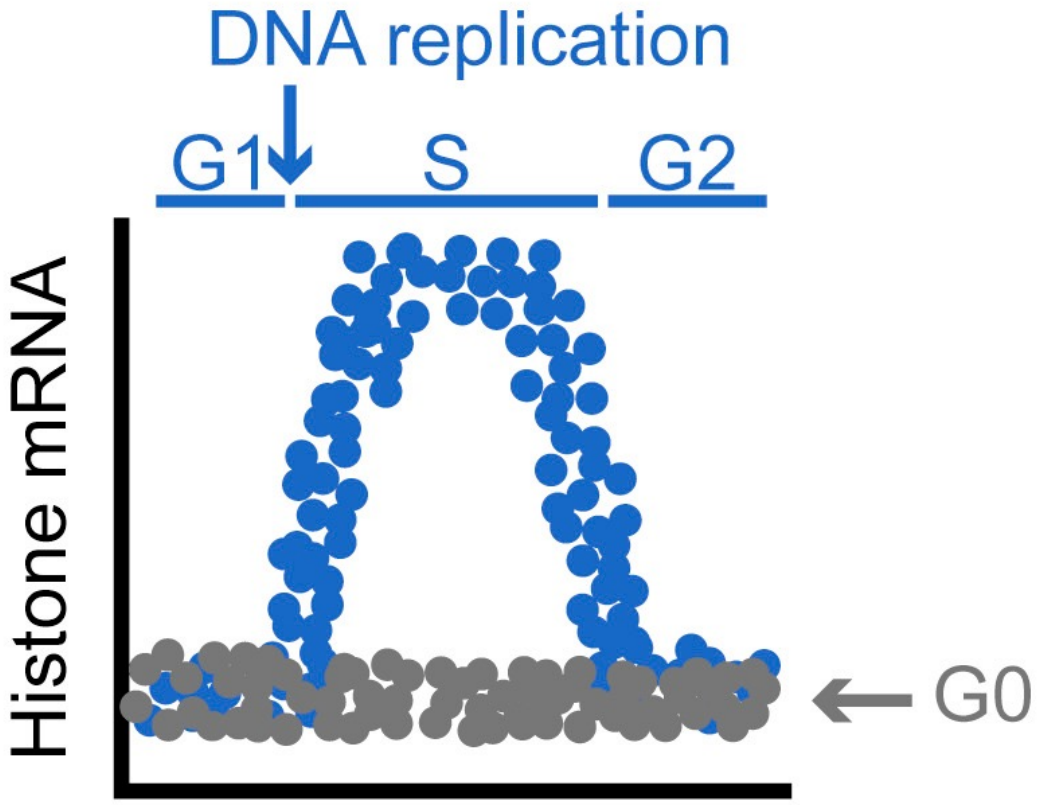
•Histones can be displaced by chromatin remodeling complexes, thereby exposing underlying DNA sequences to polymerases and other enzymes (Smith & Peterson, 2005).

• It is important to remember that these processes are reversible, so modified or remodeled chromatin can be returned to its compact state after transcription and/or replication are complete.

Histones

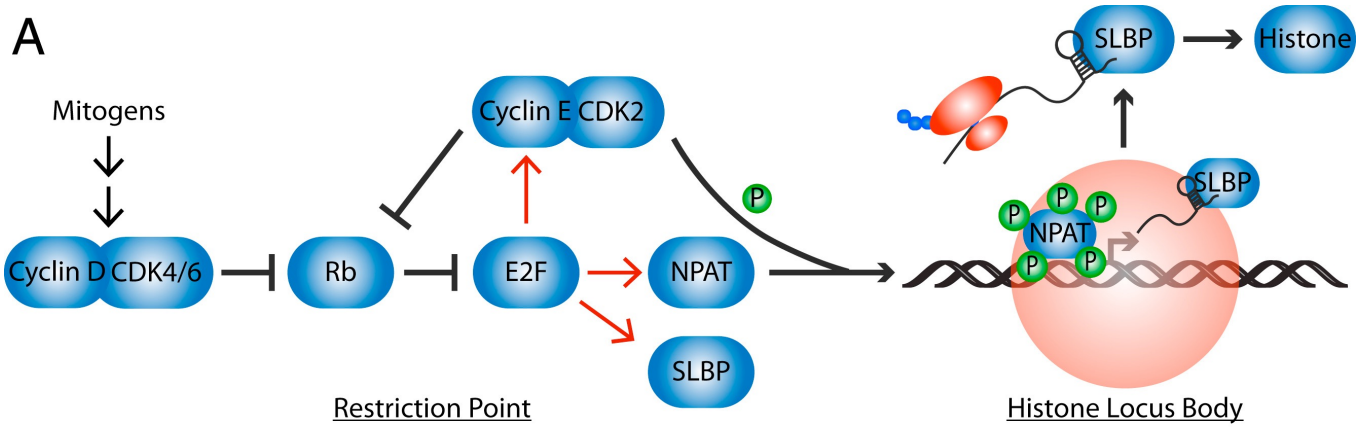
A large amount of new histone protein, approximately equal in mass to the newly synthesized DNA, is required to make the new nucleosomes in each cell cycle.

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.



In metazoans the increase in the rate of histone synthesis is due to the increase in histone mRNA as well as decrease in mRNA degradation; this results in an increase of active mRNA for translation of histone proteins.

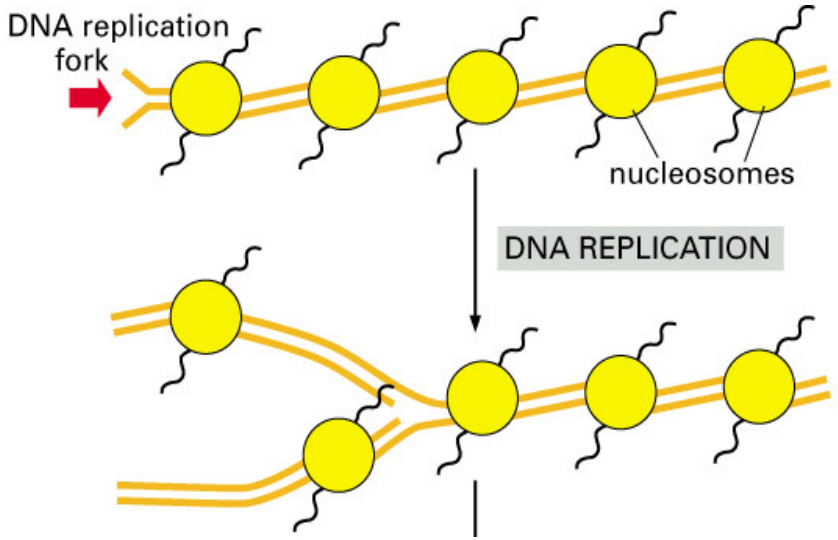
Metazoans also have multiple copies of histone genes clustered on chromosomes 1 and 6 (in structures called Cajal bodies)→ important regulatory link between cell-cycle control and histone synthesis.



Nuclear protein Ataxia-Telangiectasia (NPAT- nuclear protein coactivator of histone transcription), is a transcription factor which activates histone gene transcription. NPAT activates histone gene expression only after it has been phosphorylated by the G1/S-Cdk cyclin E-Cdk2 in early S phase.

New Nucleosomes Are Assembled Behind the Replication Fork

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



Cell *146*, August 19, 2011 ©2011 Elsevier Inc.

Mechanisms for the Inheritance of Chromatin States

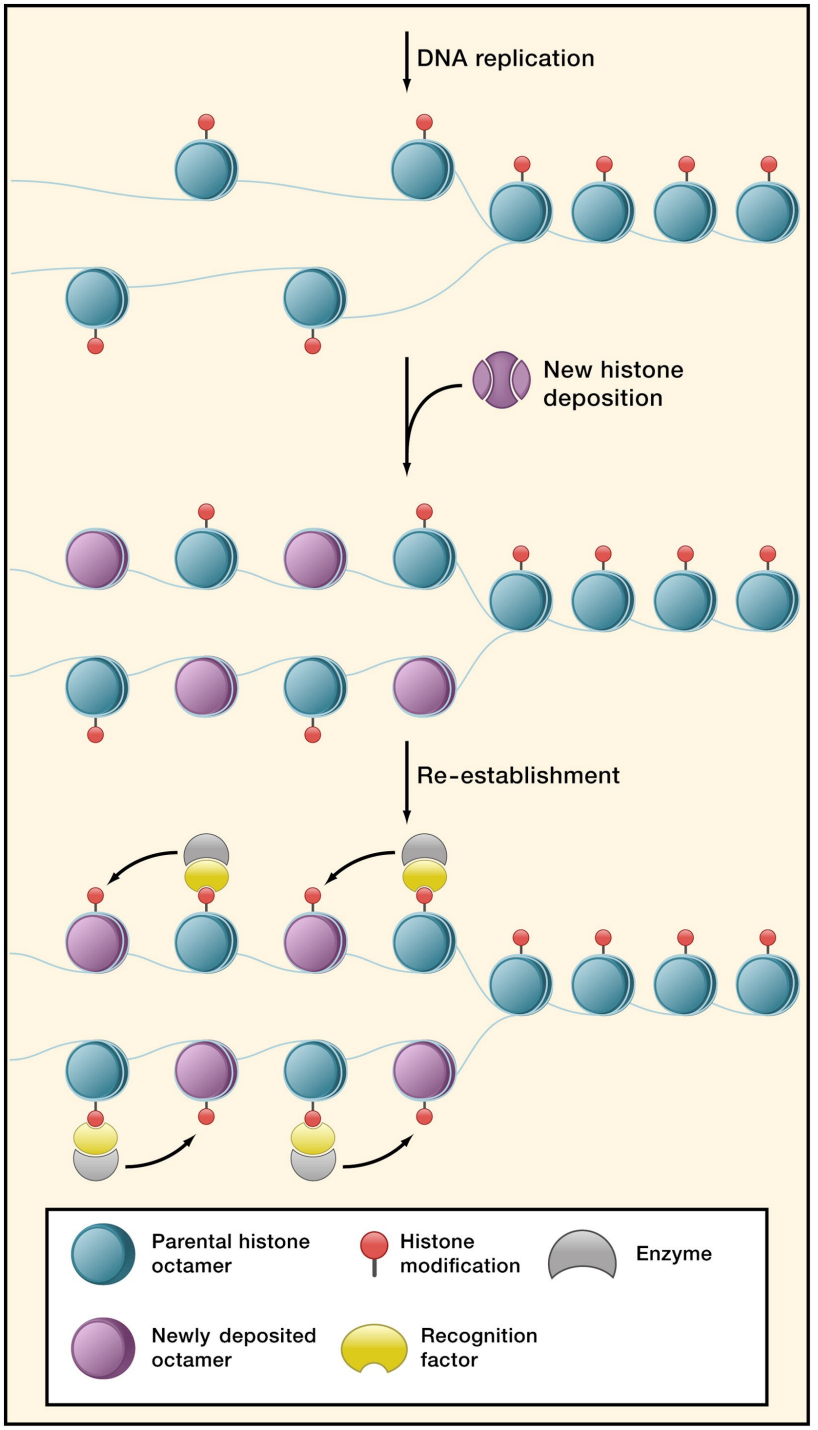
Danesh Moazed[1,*]
[1]Howard Hughes Medical Institute, Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: danesh@hms.harvard.edu
DOI 10.1016/j.cell.2011.07.013

Parental histones and their posttranslational modifications are retained and randomly associate with the newly synthesized daughter DNA strands.

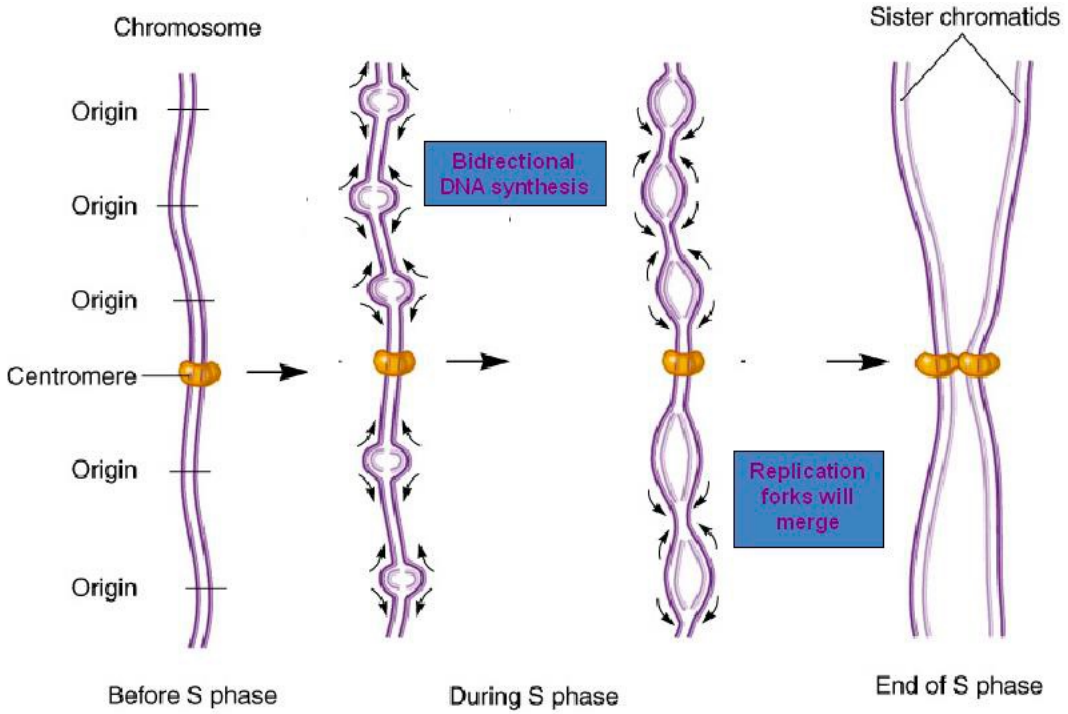
The modifications of parental histones are copied onto newly deposited histones by *chromatin modification complexes:*

- a subunit recognizes the modification on the parental histone
- another subunit catalyzes the same modification on an adjacent nucleosome.

Note that distribution of histones to daughter DNA strands is **random**.



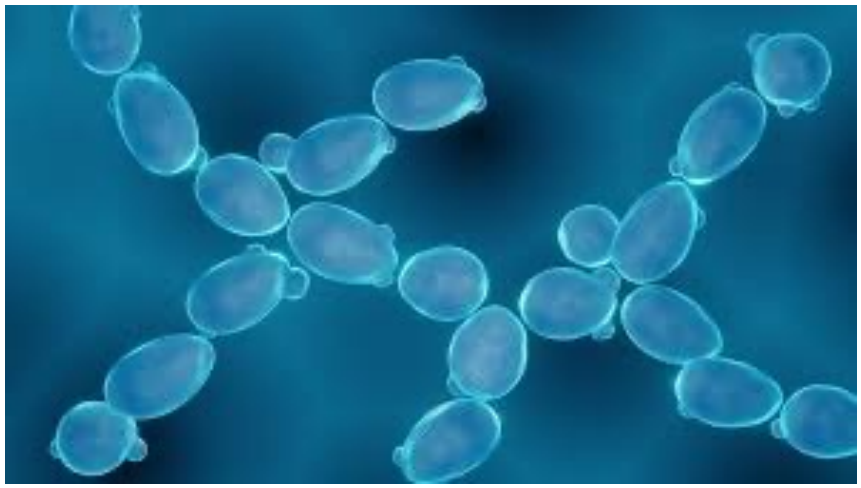
DNA synthesis begins at replication origins



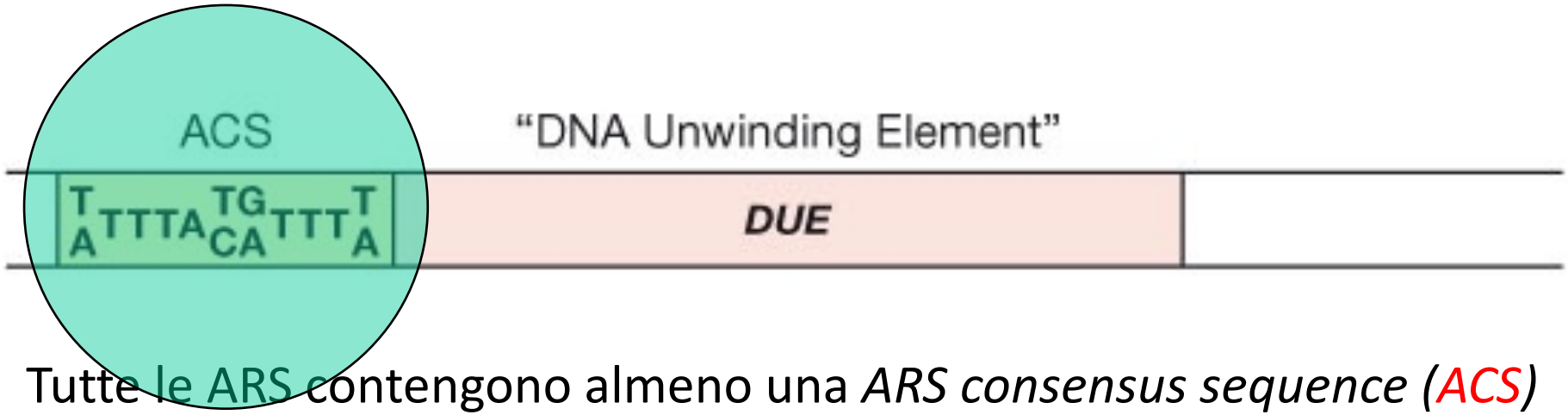
Ciascun cromosoma eucariota e' organizzato in molteplici Unita' di Replicazione, dette **repliconi**, che comprendono 50-80 origini, spaziate da 30.000-300.000 nt.

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.

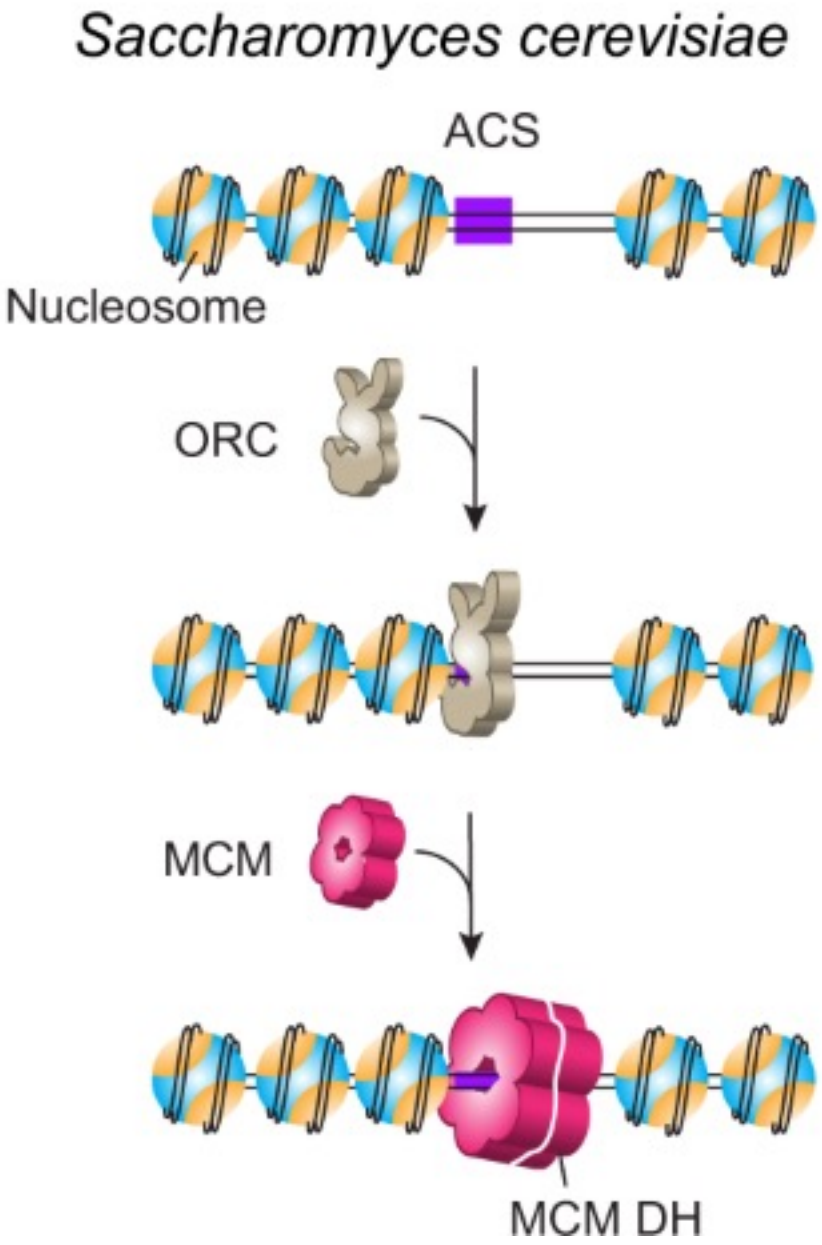
Quali sono le caratteristiche di un’origine di replicazione eucariota?



ARS sequences - autonomously replicating sequences are replication origins in yeast.



- Tutte le ARS contengono almeno una *ARS consensus sequence (ACS)* **di 11 pb**, ricca di A e T, seguita da altre regioni di lunghezza variabile, *DNA unwinding elements (DUE)*, coinvolte nell'apertura della doppia elica.
- Mutazioni nelle ACS aboliscono la funzione della ARS



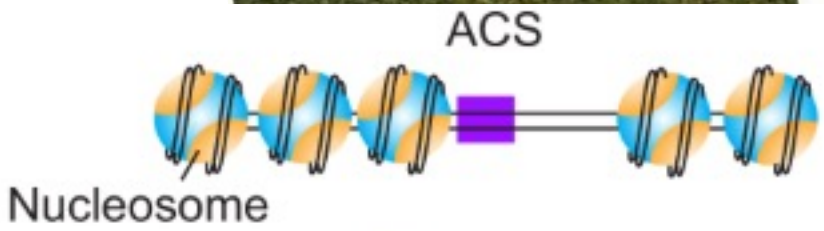
The ACS region is the main binding site for a large, multisubunit initiator protein called **ORC** (origin recognition complex).

ORC binds to ACS in an ATP-dependent manner

ORC behaves as a scaffold for for the assembly of other key initiation factors for replication.

In yeast, also inactive ARSes bind ORC

ORC

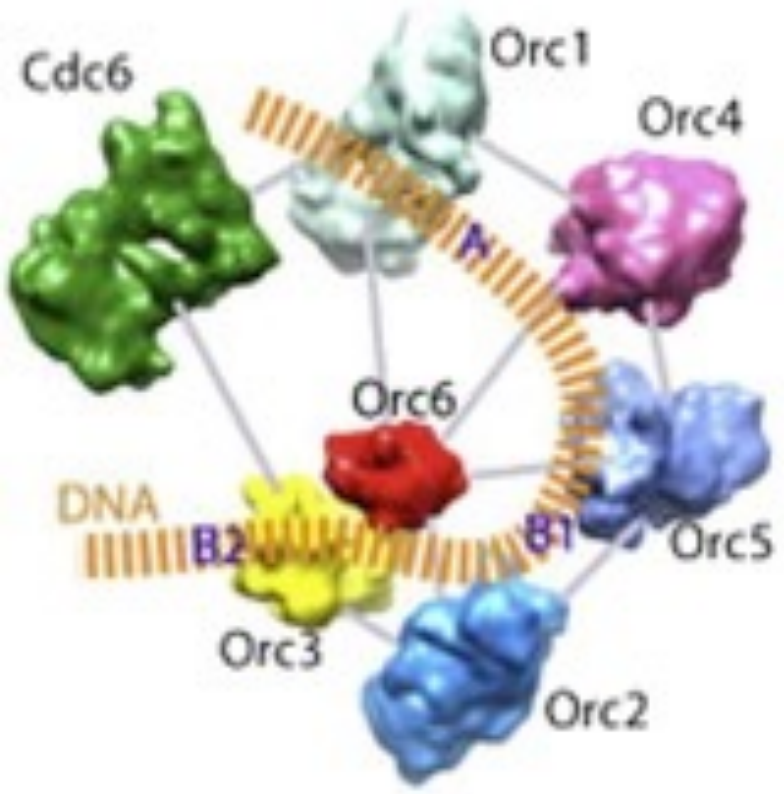


Origin Recognition Complex (ORC)

- Six subunits (Orc1p-6p); 120, 72, 62, 56, 53, 50 kDa)
- Essential for viability
- Conserved in different species

- Absence of any biochemical activity besides origin binding

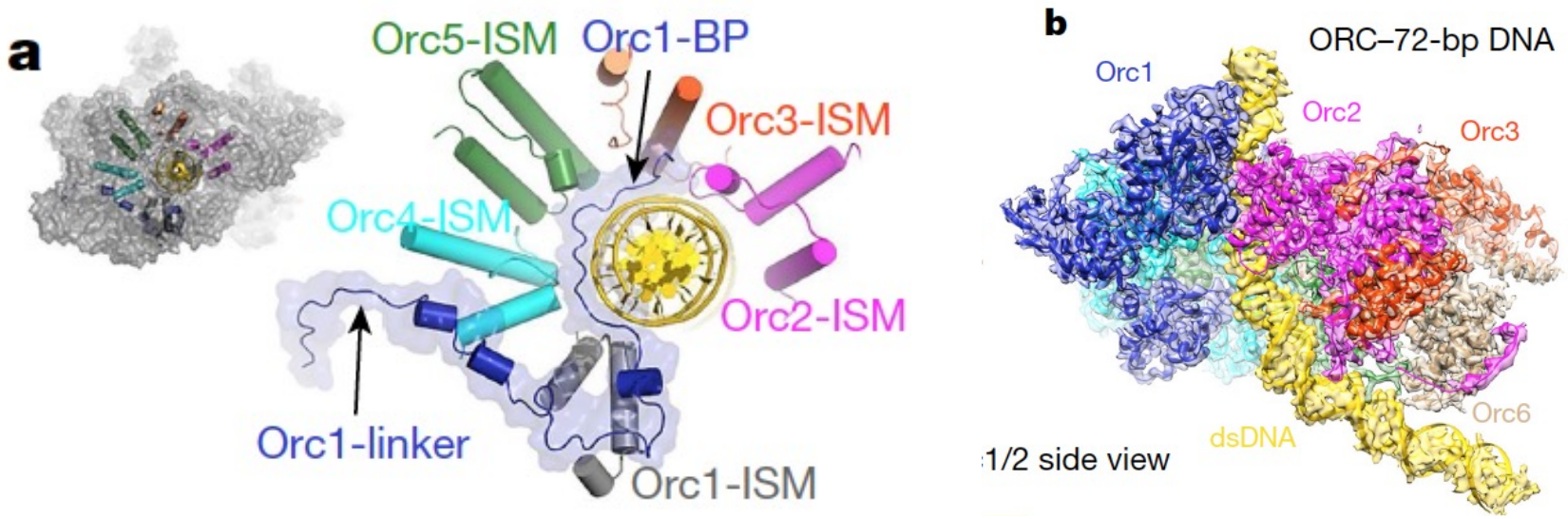
- ORC binds ARSes during the whole cell cycle



ARTICLE 12 JULY 2018 | VOL 559 | NATURE | 217
https://doi.org/10.1038/s41586-018-0293-x

Structure of the origin recognition complex bound to DNA replication origin

Ningning Li[1,7], Wai Hei Lam[2,7], Yuanliang Zhai[2,3,6,7*], Jiaxuan Cheng[4,7], Erchao Cheng[4], Yongqian Zhao[2,3], Ning Gao[1*] & Bik-Kwoon Tye[2,5*]



The six-subunit origin recognition complex (ORC) binds to DNA to mark the site for the initiation of replication in eukaryotes. Here we report a 3 Å cryo-electron microscopy structure of the *Saccharomyces cerevisiae* ORC bound to a 72-base-pair origin DNA sequence that contains the ARS consensus sequence (ACS) and the B1 element. The ORC encircles DNA through extensive interactions with both phosphate backbone and bases, and bends DNA at the ACS and B1 sites. Specific recognition of thymine residues in the ACS is carried out by a conserved basic amino acid motif of Orc1 in the minor groove, and by a species-specific helical insertion motif of Orc4 in the major groove. Moreover, similar insertions into major and minor grooves are also embedded in the B1 site by basic patch motifs from Orc2 and Orc5, respectively, to contact bases and to bend DNA. This work pinpoints a conserved role of ORC in modulating DNA structure to facilitate origin selection and helicase loading in eukaryotes.

Negli eucarioti multicellulari

- esistono da 20.000 a 50.000 origini di replicazione (ne sono state mappate una ventina).

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 7119–7123, July 1994
Biochemistry

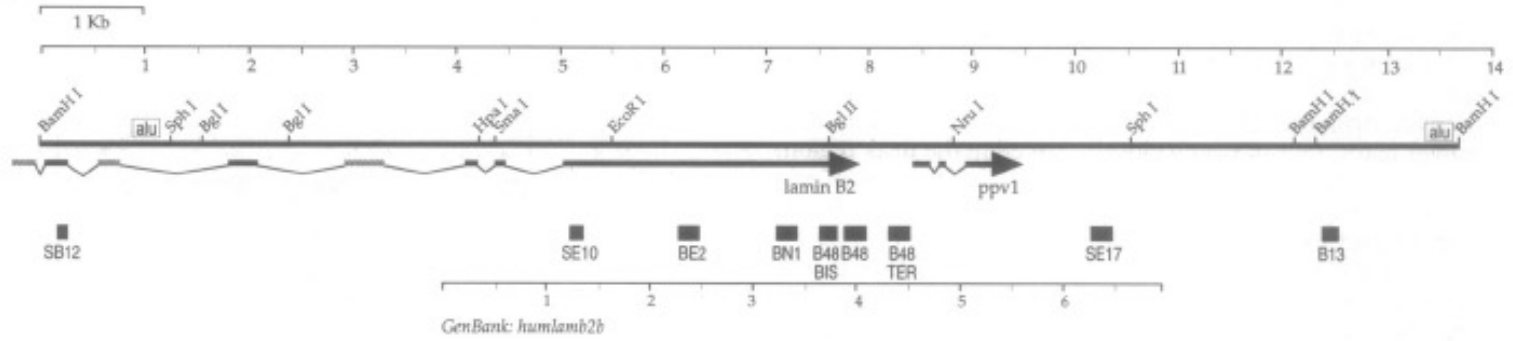
Fine mapping of a replication origin of human DNA

(competitive polymerase chain reaction/DNA replication/lamin B2)

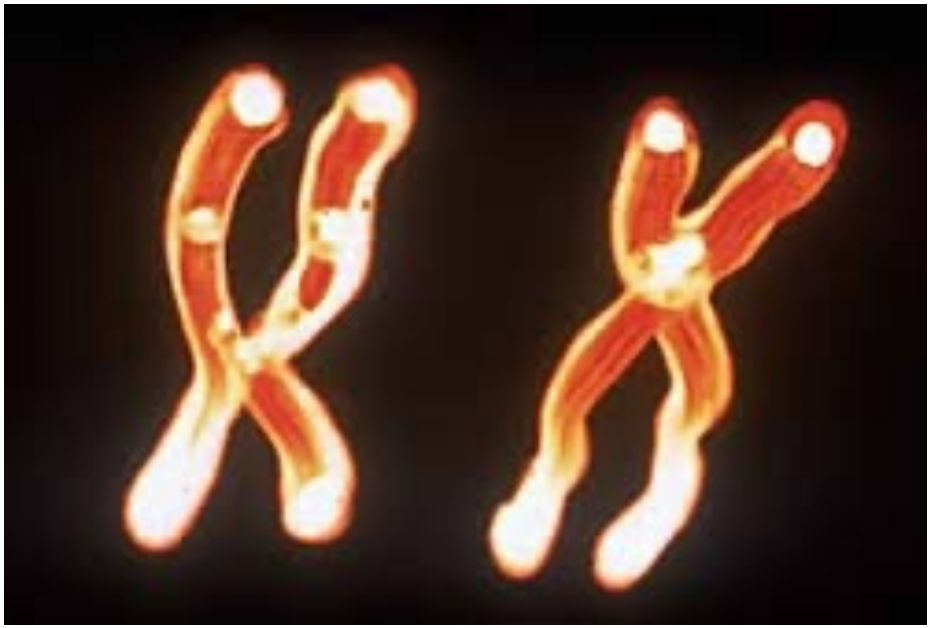
MAURO GIACCA*, LORENA ZENTILIN*, PAOLO NORIO*, SILVIA DIVIACCO*, DANIELA DIMITROVA*, GIOVANNA CONTREAS*, GIUSEPPE BIAMONTI†, GIOVANNI PERINI†, FLORIAN WEIGHARDT†, SILVANO RIVA†, AND ARTURO FALASCHI*‡

*International Centre for Genetic Engineering and Biotechnology, AREA Science Park, Padriciano 99-34012, Trieste, Italy; and †Istituto di Genetica Biochimica ed Evoluzionistica, Consiglio Nazionale delle Ricerche, Via Abbiategrasso 207, 27100 Pavia, Italy

Communicated by Arthur Kornberg, February 1, 1994



L'eterocromatina viene duplicata successivamente all'eucromatina

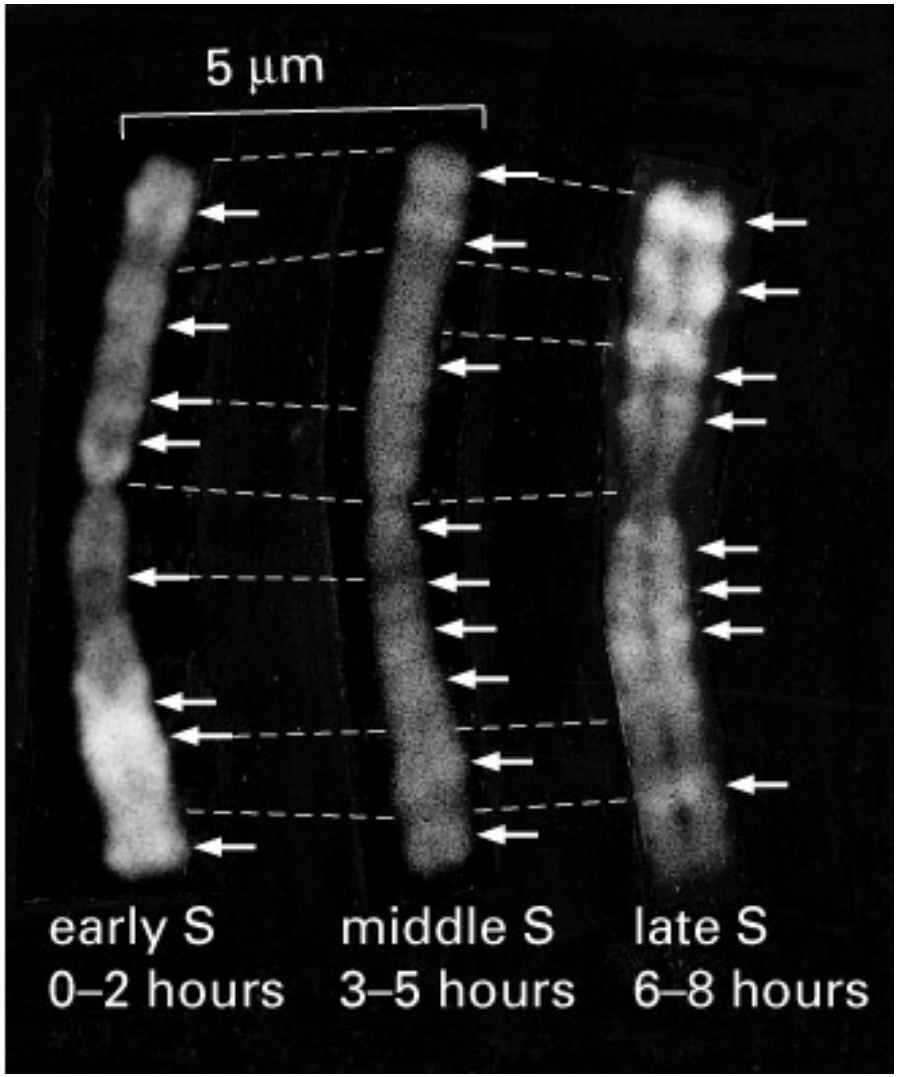


- the two X chromosomes contain the same DNA sequence

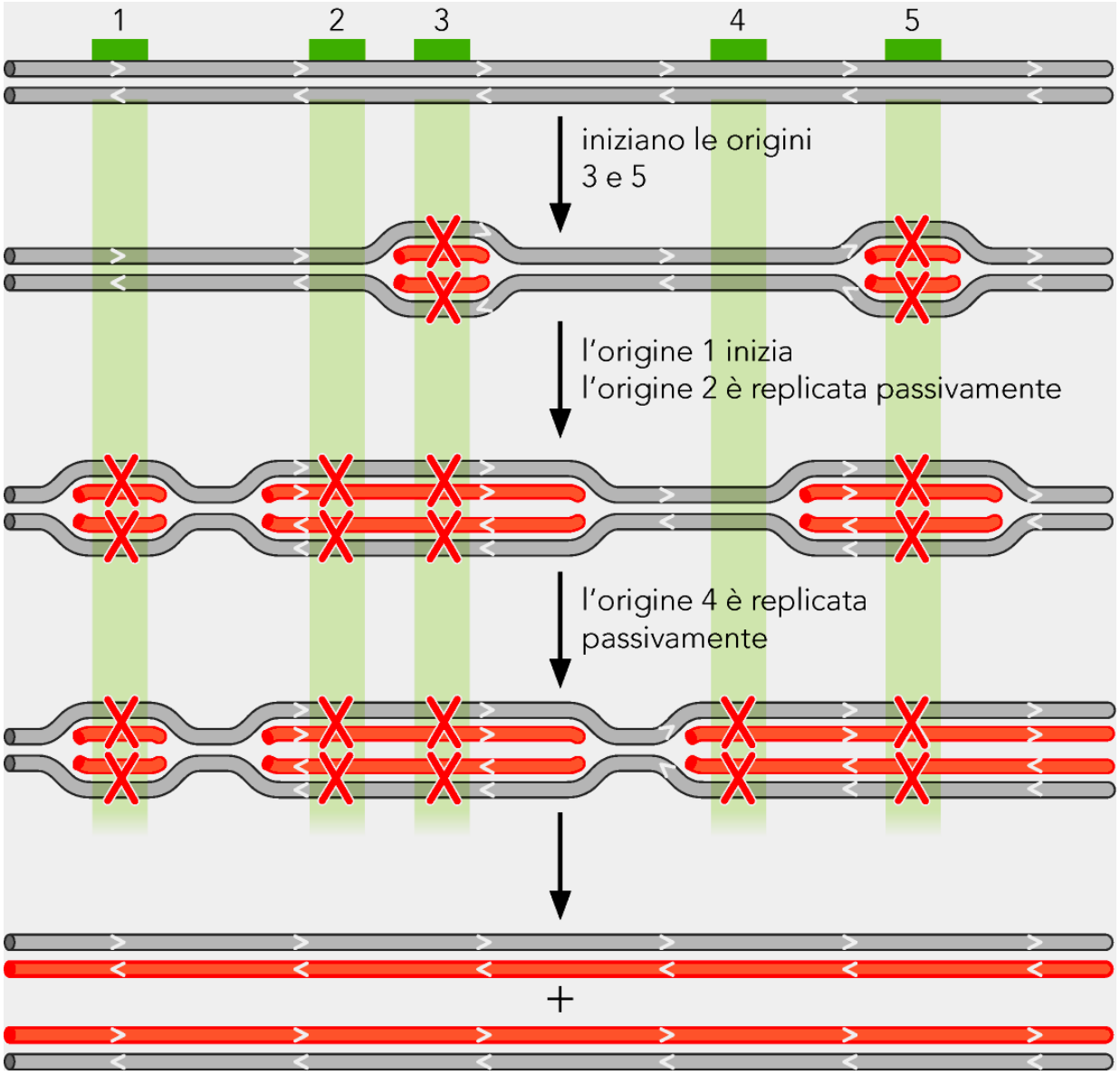
- one is inactive for transcription and is condensed into heterochromatin --> its DNA replicates late in S phase

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

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



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.



Una volta attivate le origini non possono ulteriormente essere attivate (contengono DNA neosintetizzato)

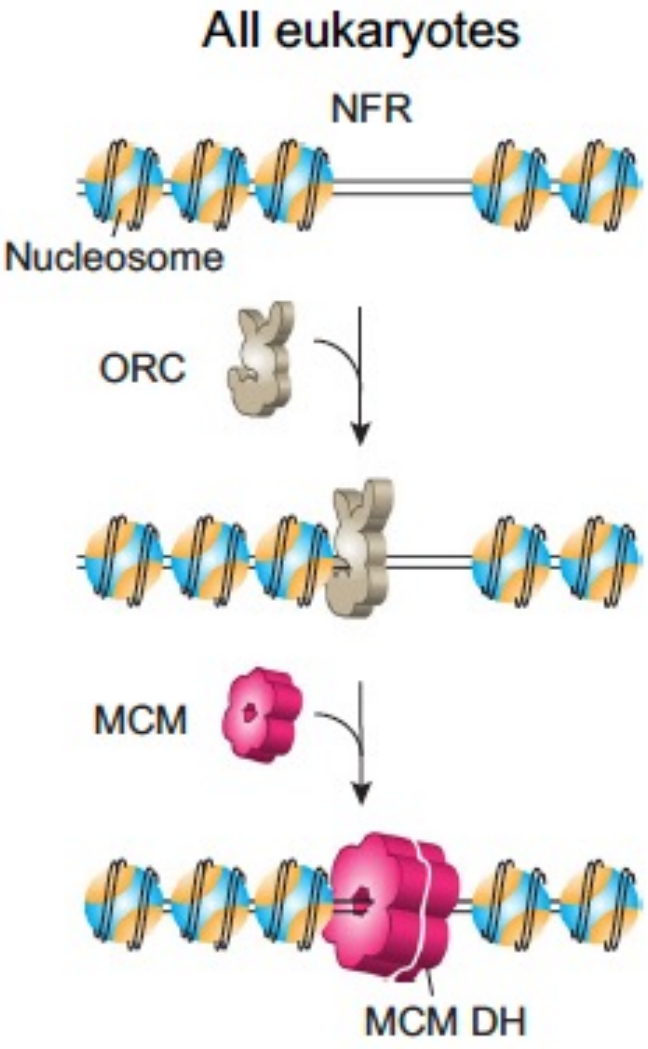
Alcune origini sono replicate passivamente (per estensione della/e bolla/e adiacenti); e non verranno quindi attivate poiché "contengono" DNA neosintetizzato

Article https://doi.org/10.1038/s41467-022-32657-7

Received: 12 April 2022

Accepted: 9 August 2022

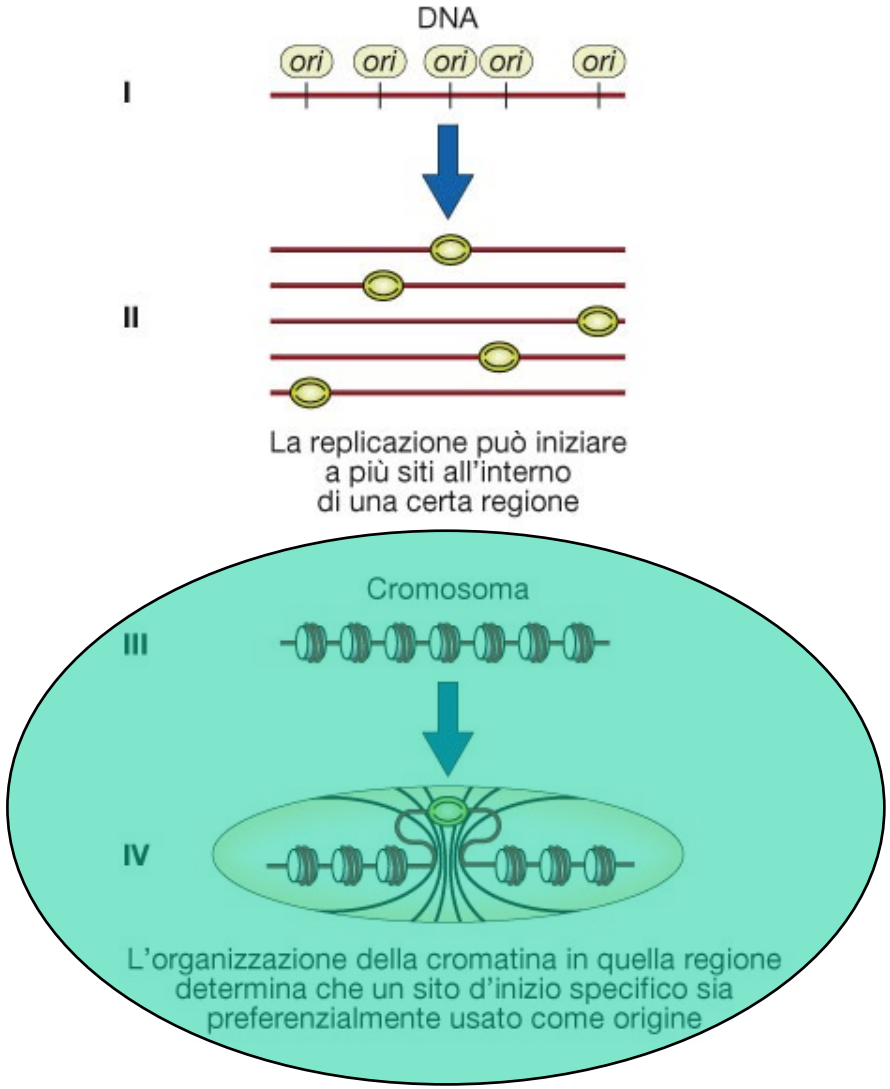
Nucleosome-directed replication origin licensing independent of a consensus DNA sequence



Origins in most eukaryotes lack a consensus ACS motif, but still utilize ORC which is known to bind nucleosomes.

ORC binds nucleosomes and direct MCM loading within nucleosome-free regions (NFRs) that lack ARS consensus sequences.

What about the the *chromatin-based* regulatory mechanisms of origin firing?



Article

576 | Nature | Vol 577 | 23 January 2020

H2A.Z facilitates licensing and activation of early replication origins

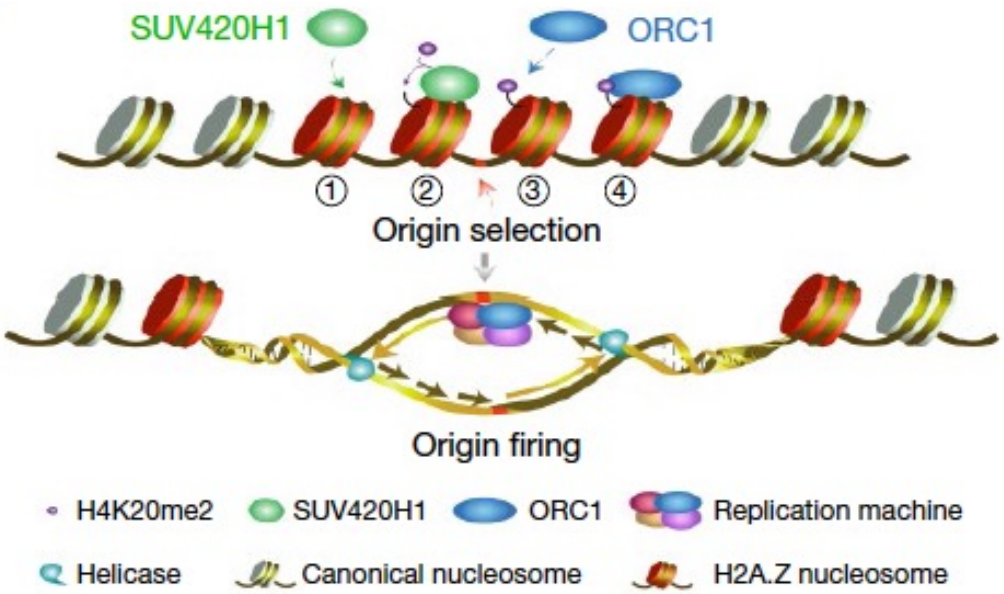
https://doi.org/10.1038/s41586-019-1877-9

Received: 5 December 2018

Accepted: 31 October 2019

Published online: 25 December 2019

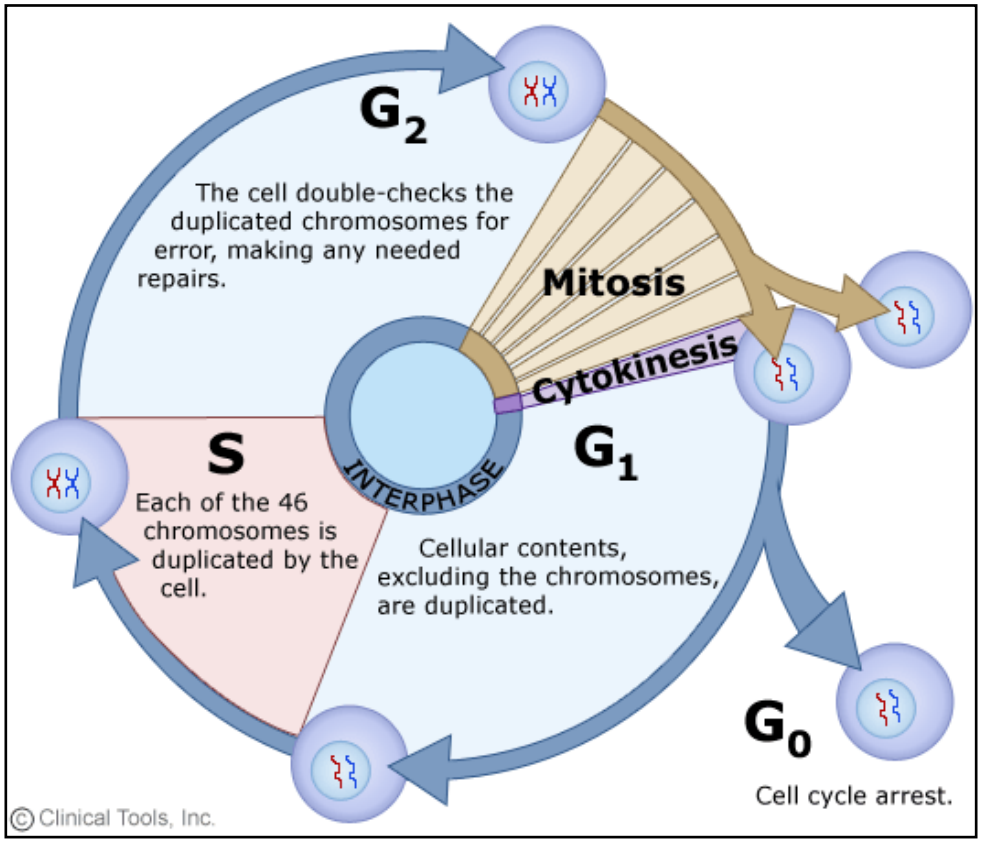
Haizhen Long[1,2,10], Liwei Zhang[1,10], Mengjie Lv[3,10], Zengqi Wen[1,2,10], Wenhao Zhang[4], Xiulan Chen[2,5], Peitao Zhang[6], Tongqing Li[7], Luyuan Chang[1,2], Caiwei Jin[2,3], Guozhao Wu[1,2], Xi Wang[8], Fuquan Yang[2,5], Jianfeng Pei[7], Ping Chen[1], Raphael Margueron[9], Halteng Deng[4], Mingzhao Zhu[2,3]* & Guohong Li[1,2]*



- In HeLa cells, nucleosomes containing the histone variant H2A.Z are enriched with histone H4 that is dimethylated on its lysine 20 residue (H4K20me2): this methylation is required for ORC1 binding.
- Genome-wide studies show that ORC1 and nascent DNA strands co-localize with H2A.Z throughout the genome.
- H2A.Z-regulated replication origins have a higher firing efficiency and early replication timing compared with other origins.
- The histone variant H2A.Z epigenetically regulates the licensing and activation of early replication origins and maintains replication timing through the H4K20me2–ORC1 axis.

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

La preparazione delle origini alla replicazione avviene quando le cellule *escono dalla mitosi* e *proseguono nella fase G1* del ciclo cellulare (late M-early G1).

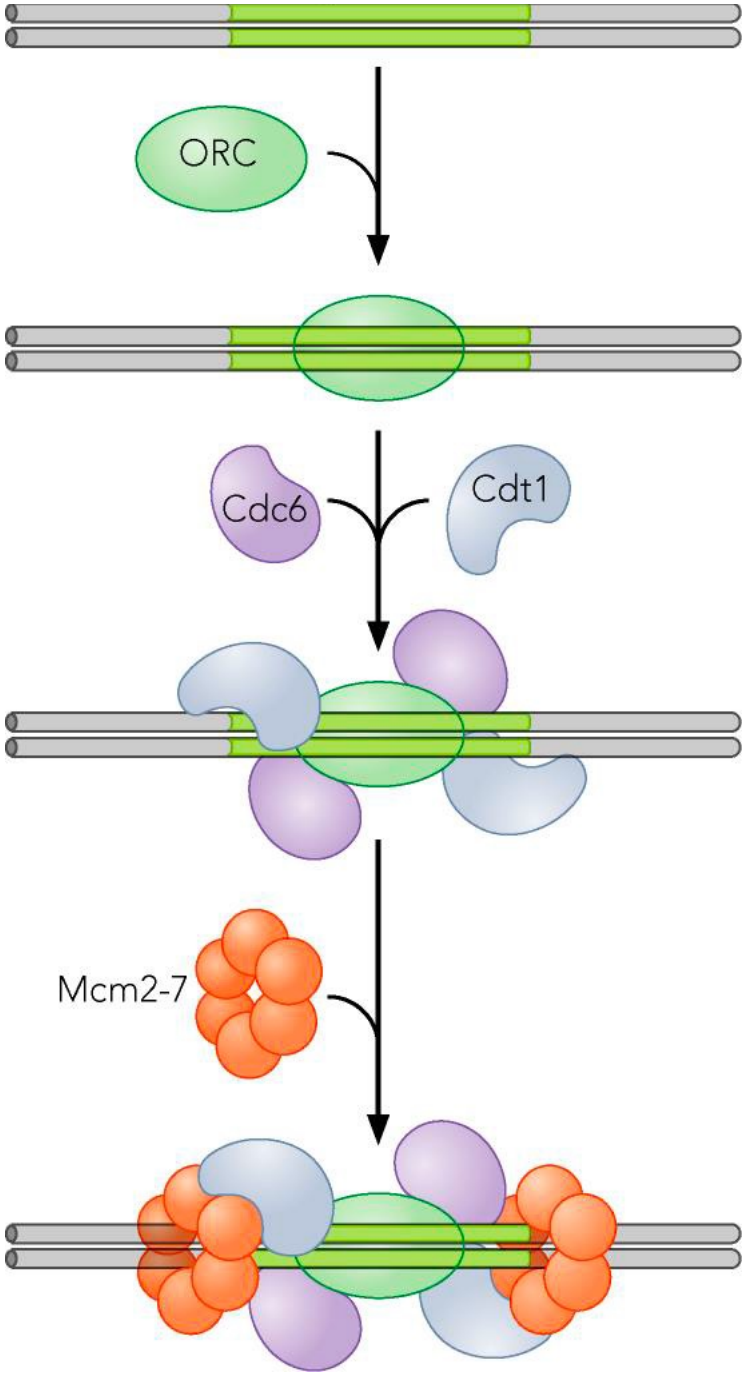


I- The Pre-RC **assembly** (G1)

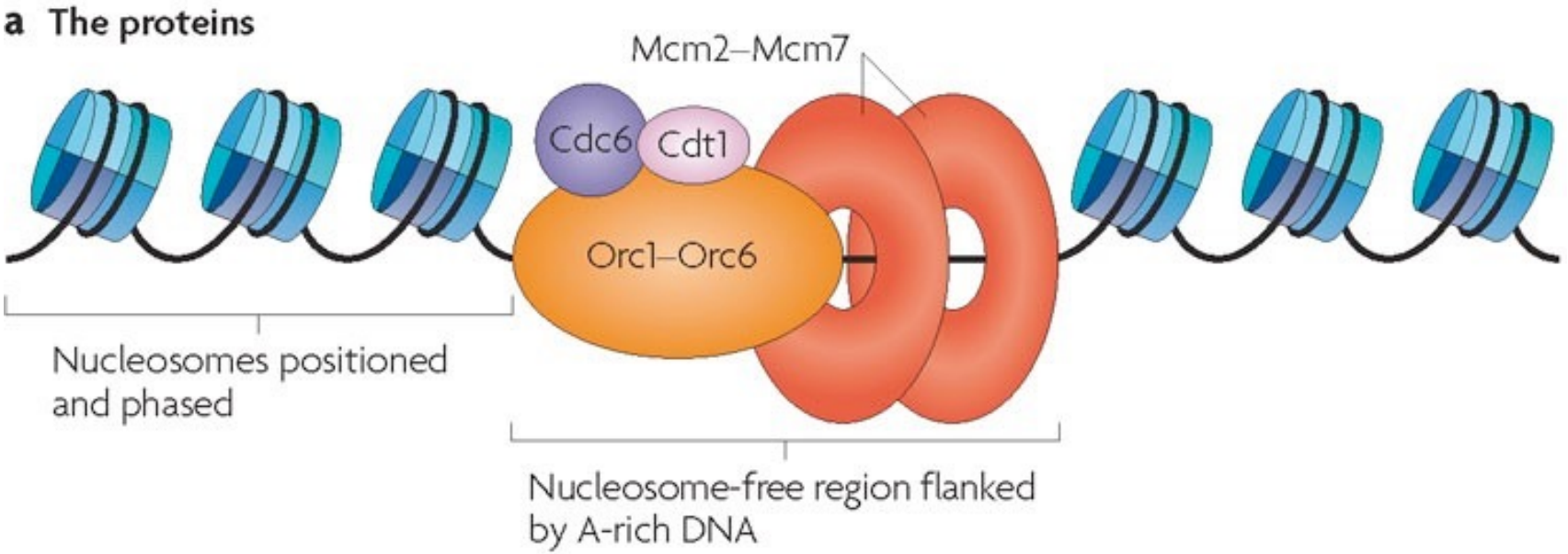
Su ogni origine si assembla il **complesso di pre-replicazione** (pre-RC), il cui cardine e' il **COMPLESSO ORC, gia' legato al DNA**.

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 l'origine e' "**licenced**" per la replicazione

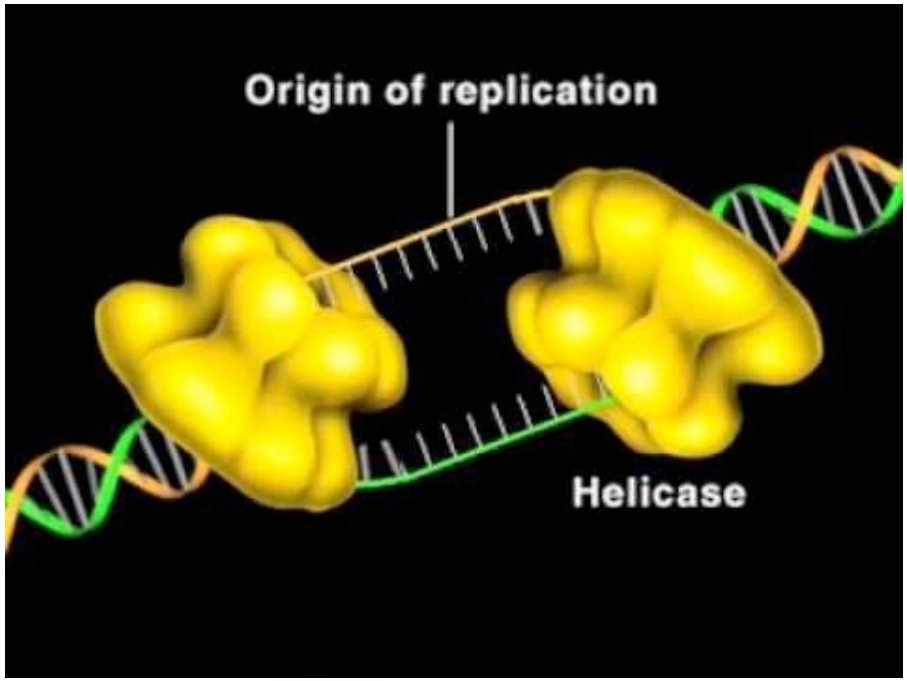


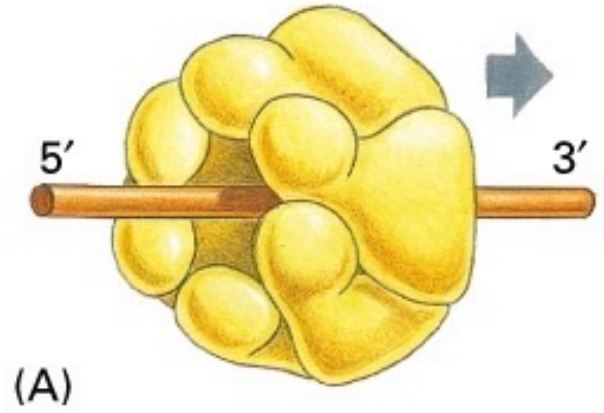
I- The Pre-Replication Complex



Mcm2-7

MCM2-7 helicase function arises from its architecture.



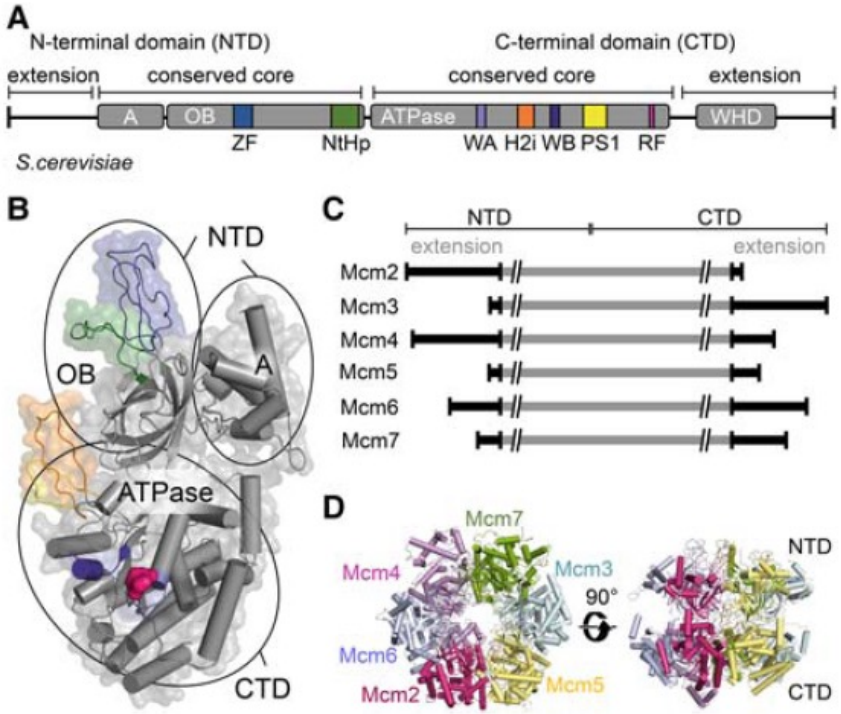


REVIEW

From structure to mechanism—understanding initiation of DNA replication

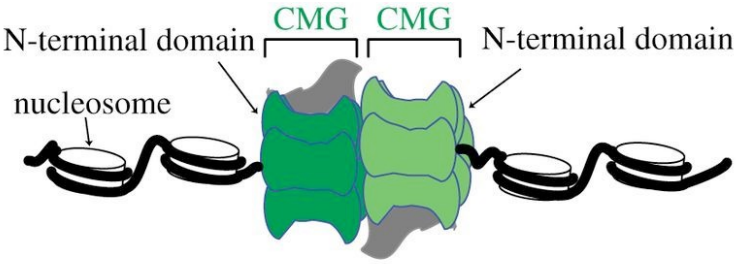
Alberto Riera,[1] Marta Barbon,[1,2,3] Yasunori Noguchi,[1,3] L. Maximilian Reuter,[1,3] Sarah Schneider,[1,3] and Christian Speck[1,2]

GENES & DEVELOPMENT 31:1073–1088 Published by Cold Spring Harbor Laboratory Press; ISSN 0890-9369/17; www.genesdev.org

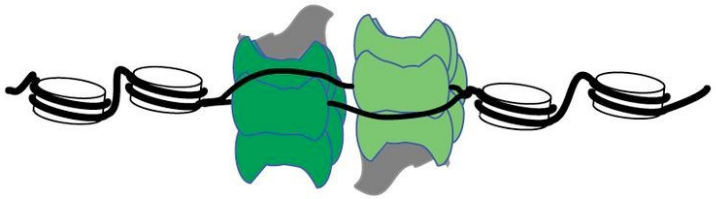


Mechanism of action of Mcm2-7

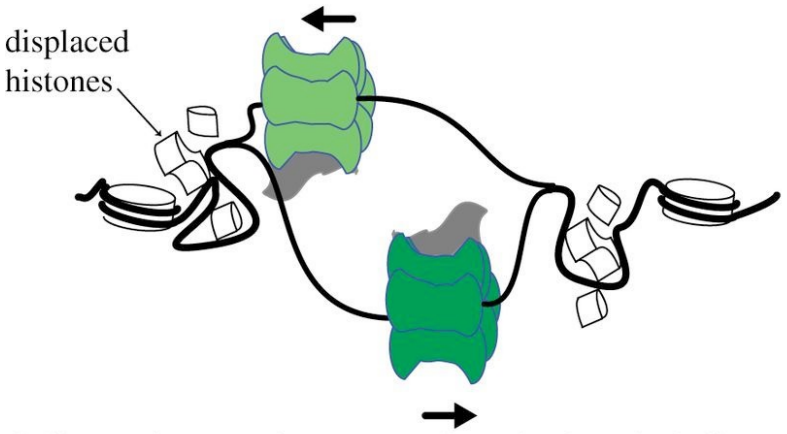
Il complesso elicasico “abbraccia” il DNA a doppia elica



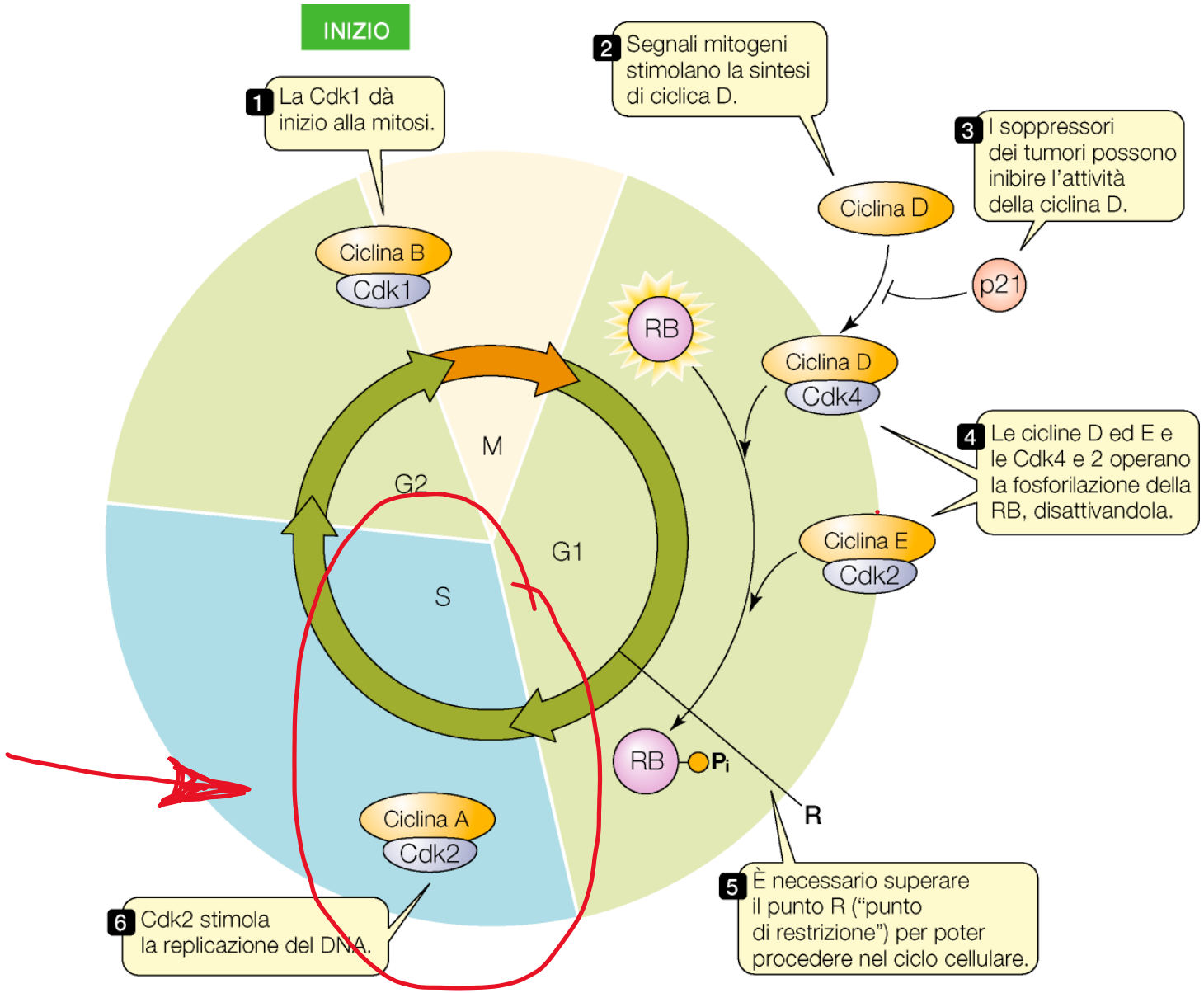
I due filamenti sono separati e uno dei due è incanalato nello spacio centrale del complesso proteico



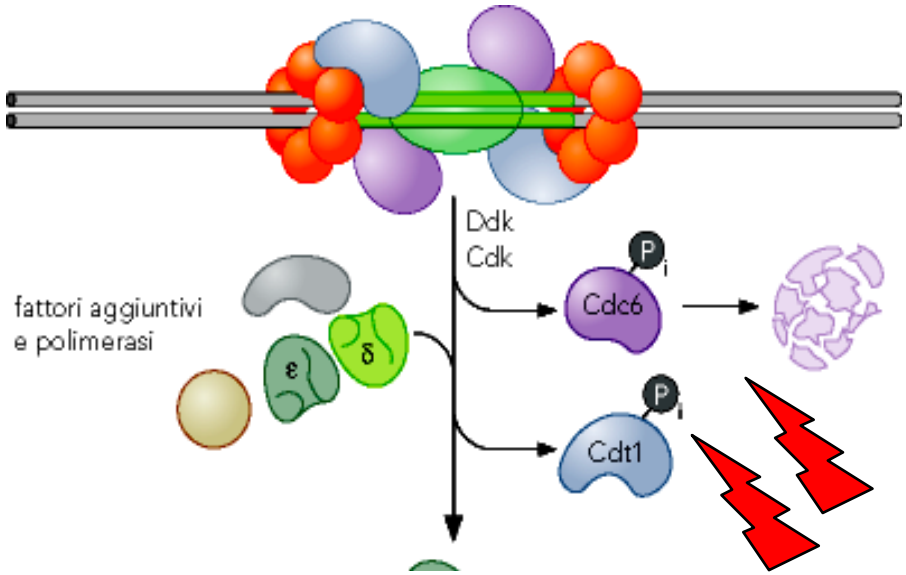
Successivamente le due elicasi si allontanano in direzione opposta, creando la bolla di replicazione



Il ciclo cellulare è regolato da Cicline e Chinasi ciclino-dipendenti (CDKs)



II- Attivazione del Pre-RC: il Pre-IC (G1-S)

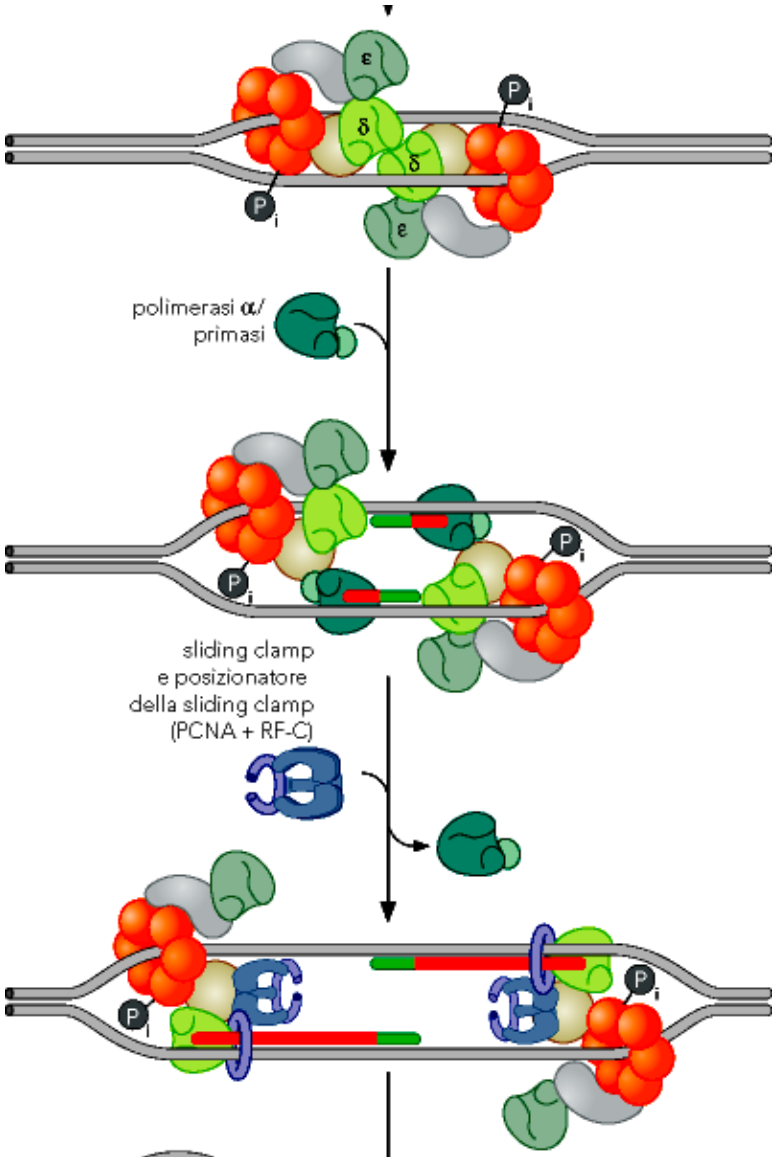


- Il passaggio da Pre-RC a Pre-IC richiede l'attivita' chinasica delle CDKs (chinasi ciclino-dipendenti).

- Le chinasi ciclinaA-dipendenti (espresse SOLO nel passaggio G1-S) fosforilano **Cdc6** e **Cdt1**.

- La forma fosforilata di **Cdc6** e **Cdt1** sono riconosciute da una Ubiquitina-ligasi, che procede alla loro poli-ubiquitinazione, con successiva degradazione da parte del proteasoma.

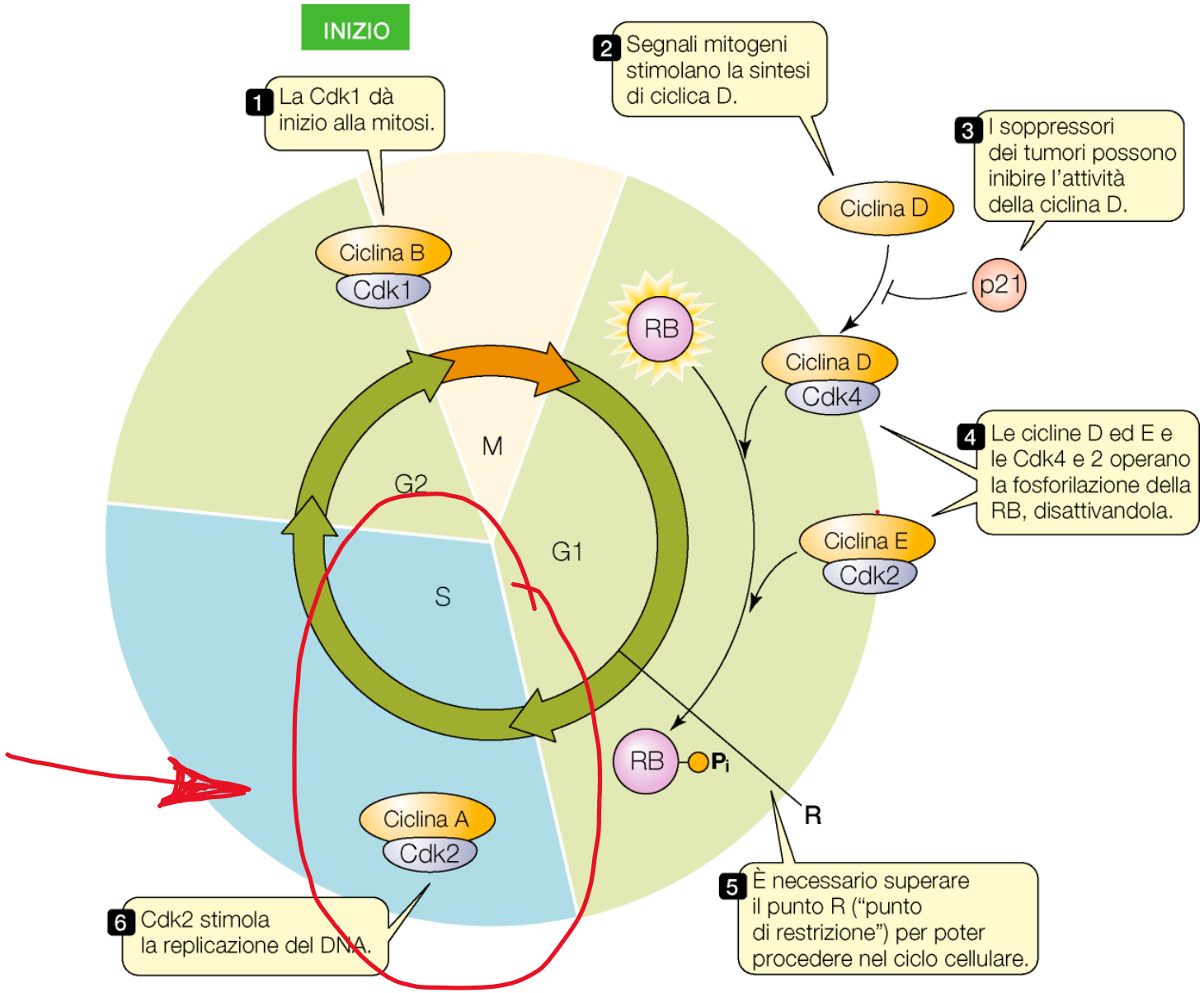
Il Pre-IC (G1-S)

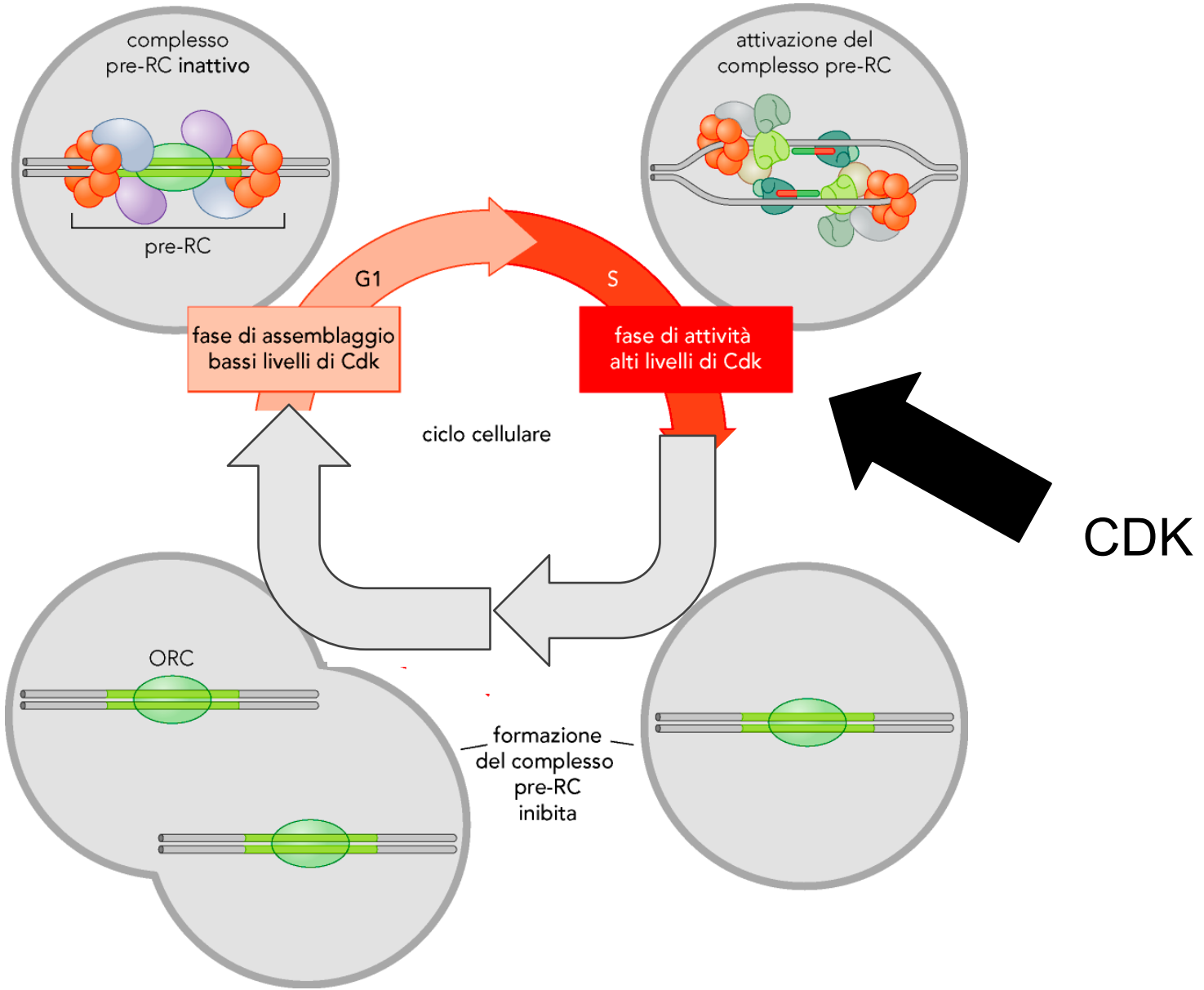


- L'elicasi Mcm2-7 denatura il DNA sull'origine
- Vengono sequenzialmente caricate le proteine implicate nella replicazione: la polimerasi alfa con attività primasica; le polimerasi delta ed epsilon che interagendo con la PCNA=pinza procedono alla replicazione dello stampo.

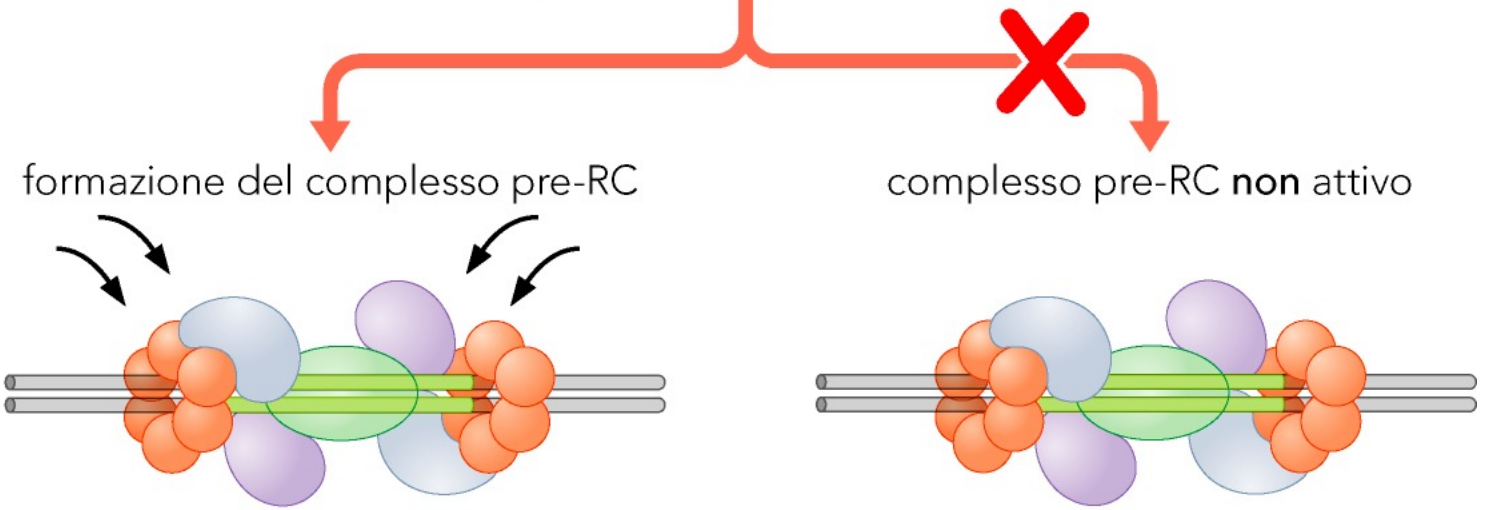
Come viene assicurato che la replicazione avvenga una sola volta/ciclo cellulare?

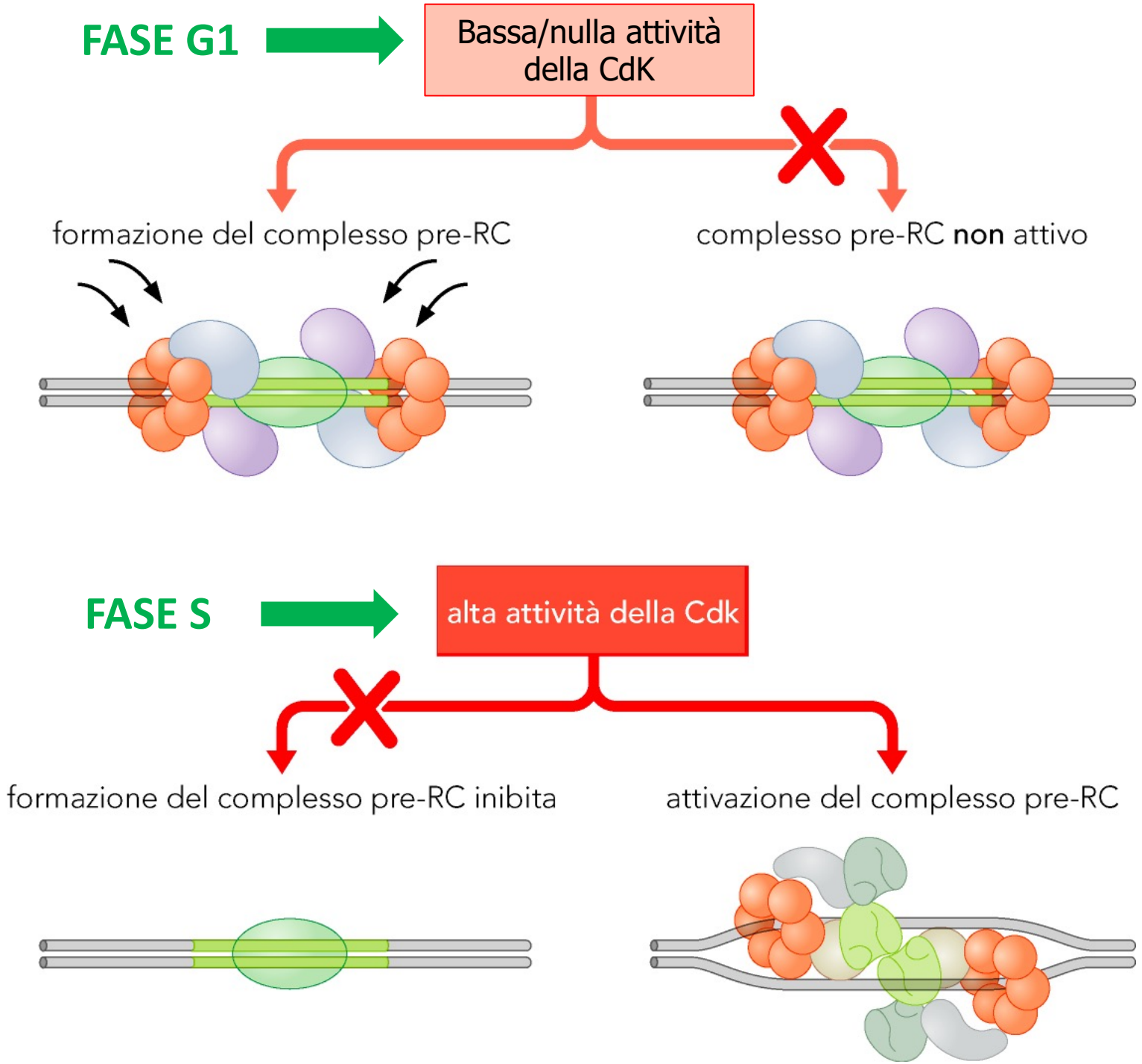
Il ciclo cellulare è regolato da Cicline e Chinasi ciclino-dipendenti (CDKs)



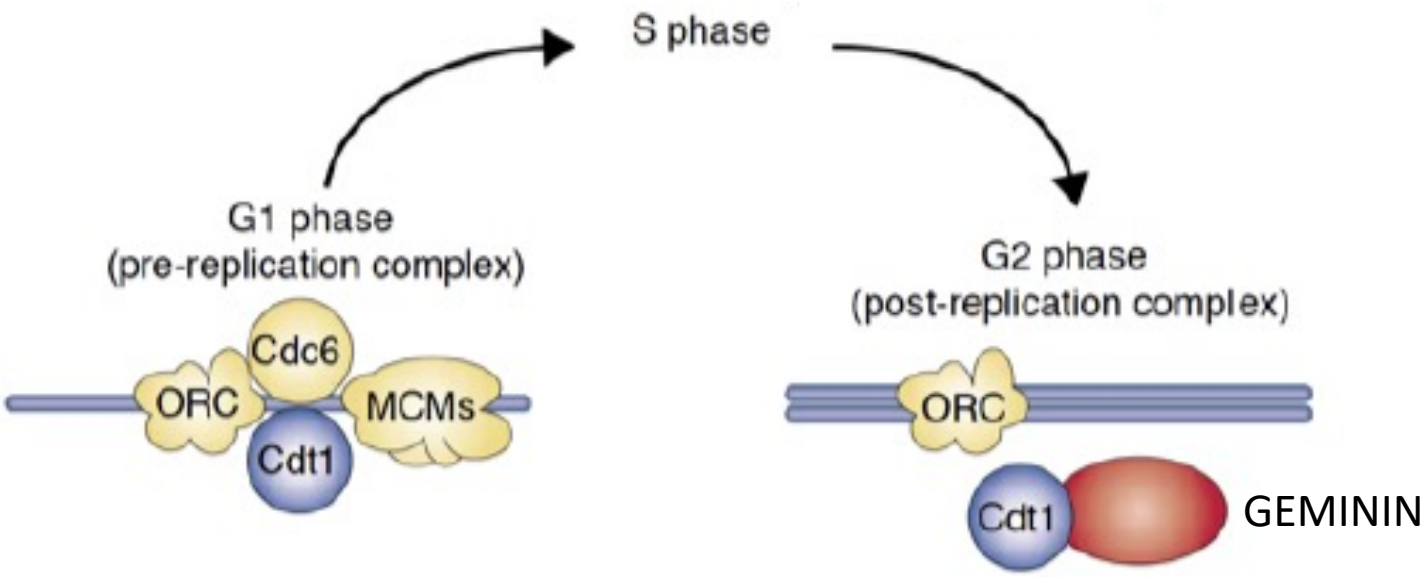

complesso
pre-RC inattivo
pre-RC
attivazione del
complesso pre-RC
G1
S
fase di assemblaggio
bassi livelli di Cdk
fase di attività
alti livelli di Cdk
ciclo cellulare
CDK
ORC
formazione
del complesso
pre-RC
inibita

FASE G1 → Bassa/nulla attività della CdK

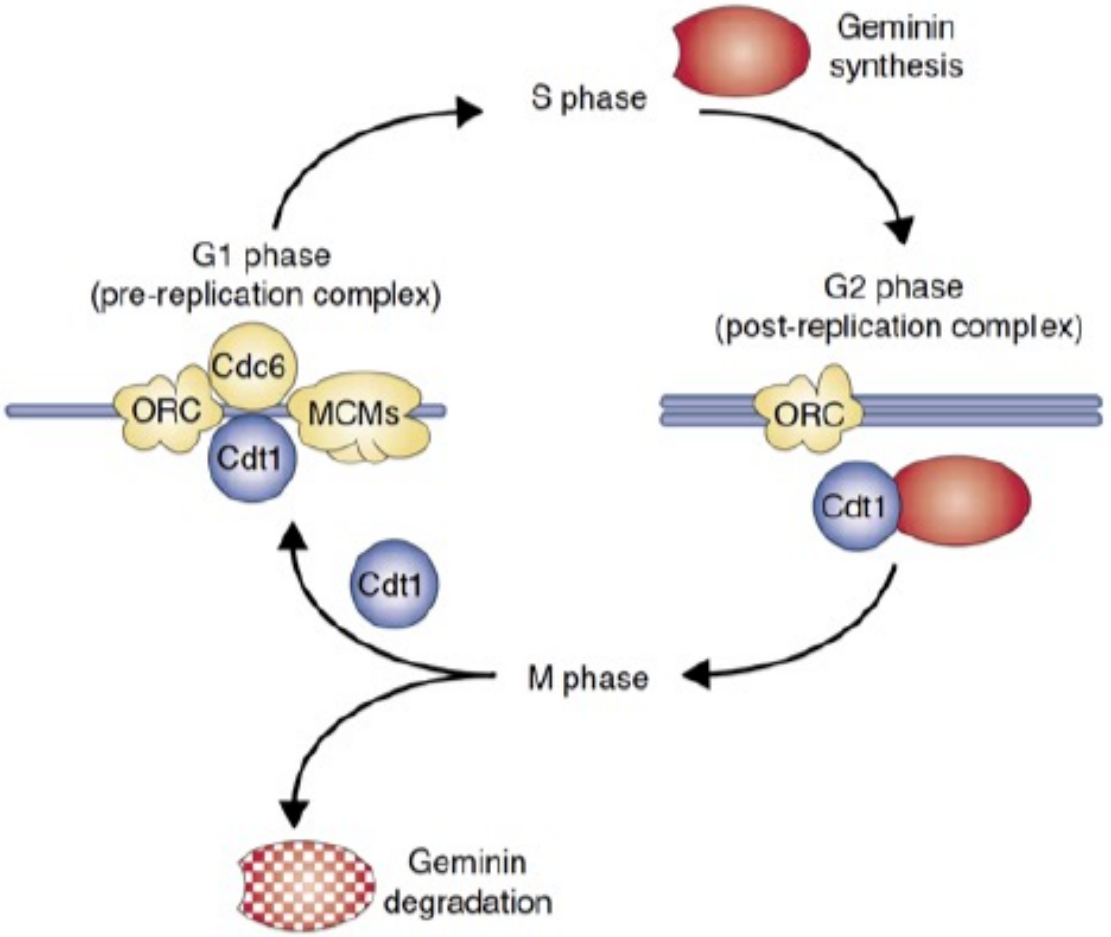



FASE G1
Bassa/nulla attività della CdK
formazione del complesso pre-RC
complesso pre-RC non attivo
FASE S
alta attività della Cdk
formazione del complesso pre-RC inibita
attivazione del complesso pre-RC

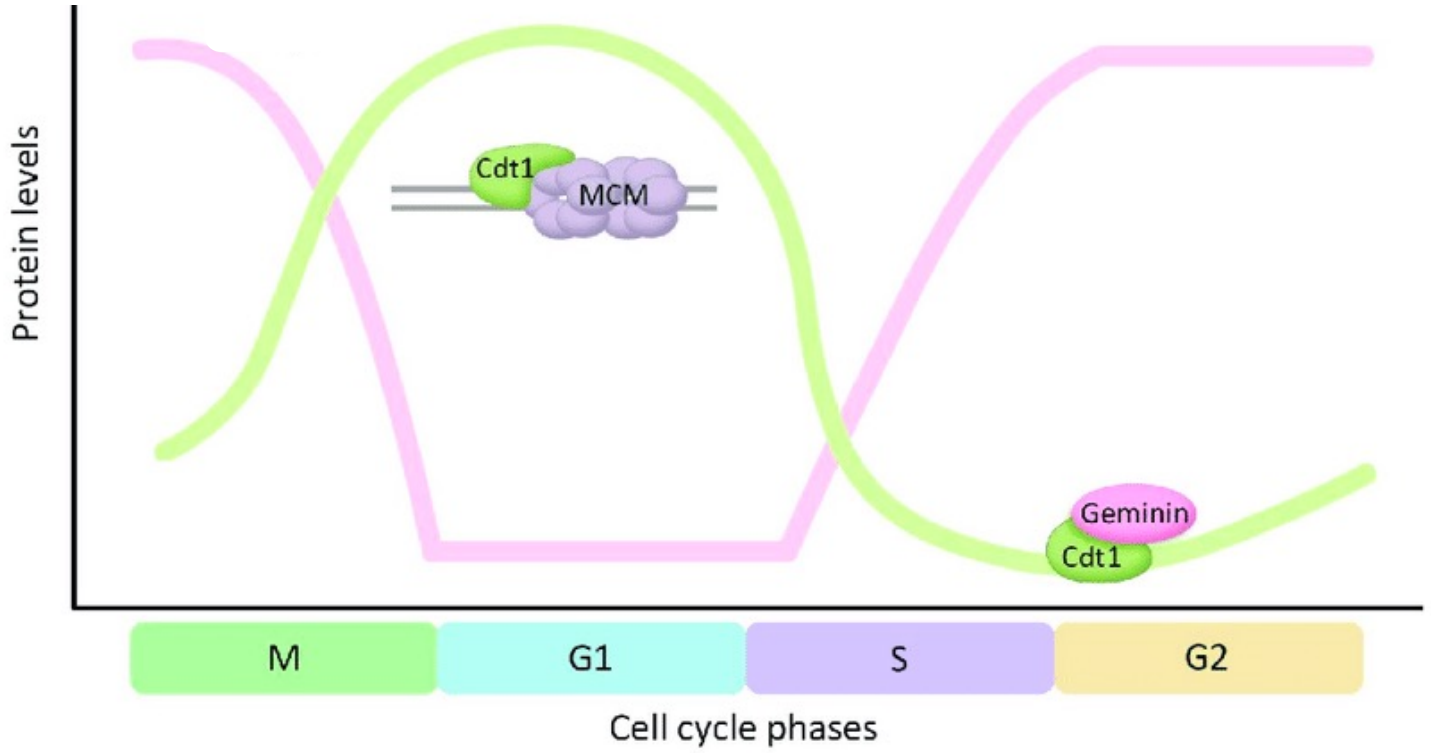
Come viene assicurato che la replicazione avvenga una sola volta/ciclo cellulare?



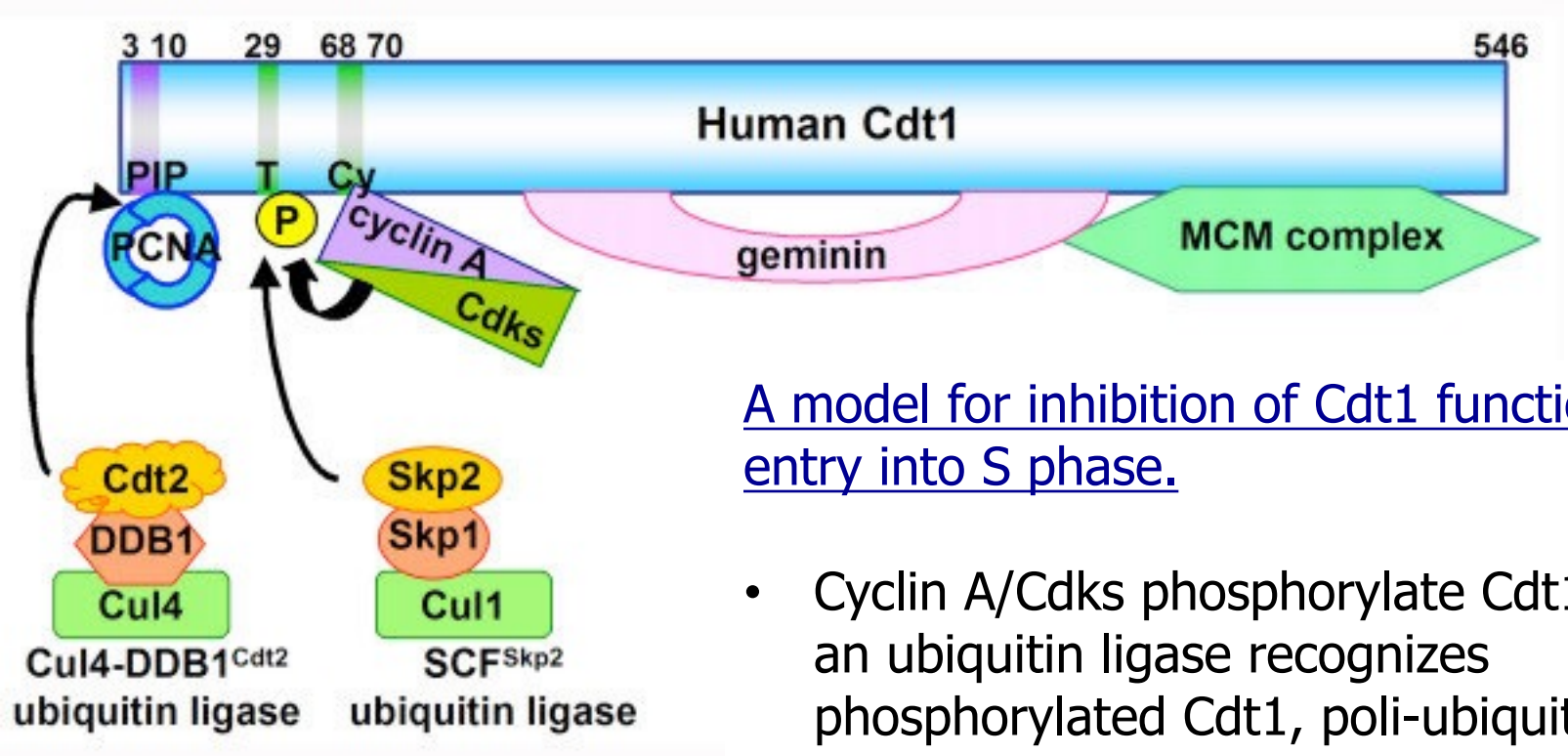
✓ Durante la fase G2, Cdt1 e' sequestrato dalla proteina GEMININ.



- ✓ All'entrata in G1, Geminin e' degradata, rilasciando Cdt1 che quindi puo' legare ORC insieme a cdc6, promuovendo la formazione del Pre-Replication Complex.
- ✓ In S, Geminin è nuovamente sintetizzata, e lega Cdt1 durante le fasi S e G2, impedendo cosi' che il DNA subisca piu' rounds di replicazione all'interno dello stesso ciclo cellulare.


Protein levels
Cdt1
MCM
Geminin
Cdt1
M
G1
S
G2
Cell cycle phases

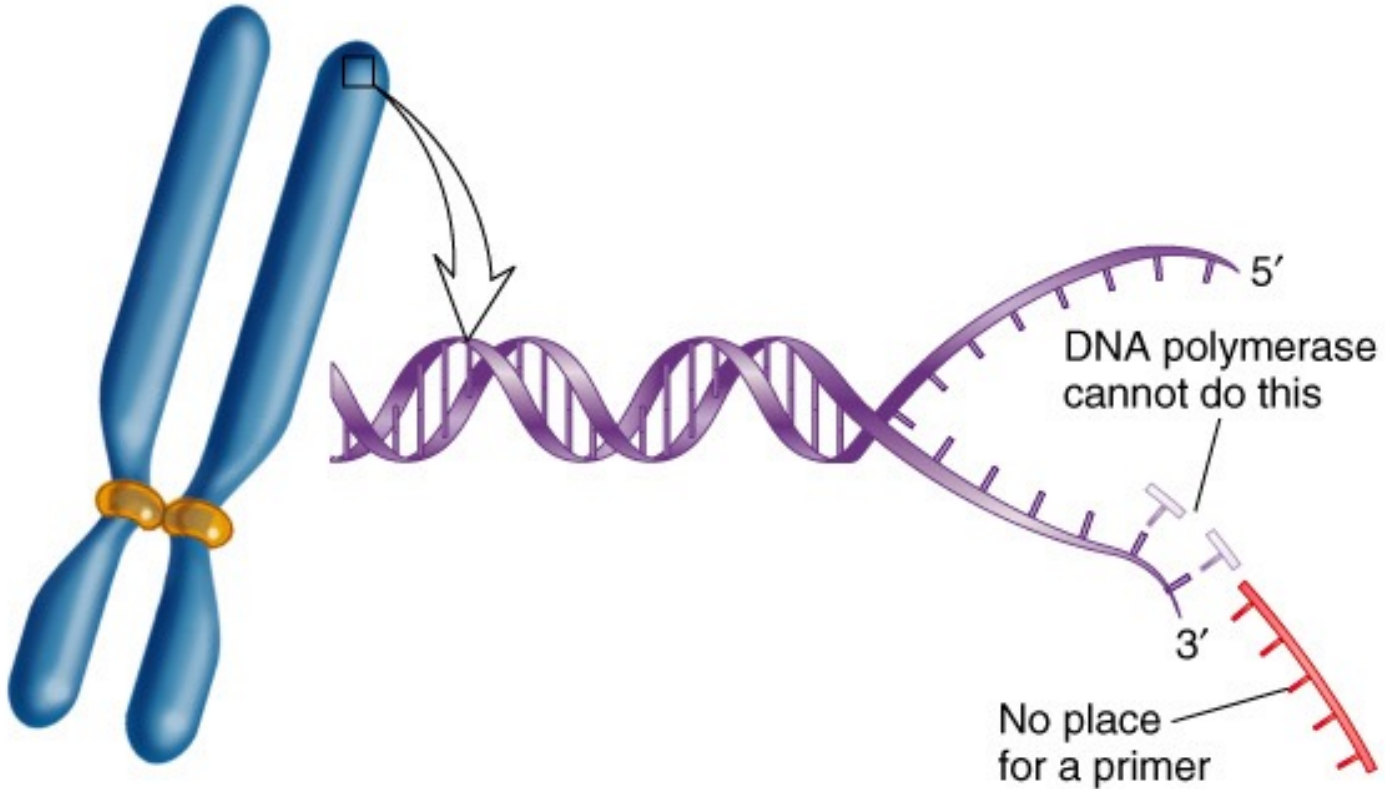
CDT1 (Chromatin licensing and DNA replication factor 1)



A model for inhibition of Cdt1 function after entry into S phase.

- Cyclin A/Cdks phosphorylate Cdt1, then an ubiquitin ligase recognizes phosphorylated Cdt1, poli-ubiquitines it to makes it degraded by proteasome.
- Cdk phosphorylation inhibits Cdt1 DNA binding activity.
- After S phase, geminin protein also accumulates, sequestering Cdt1 by direct binding.

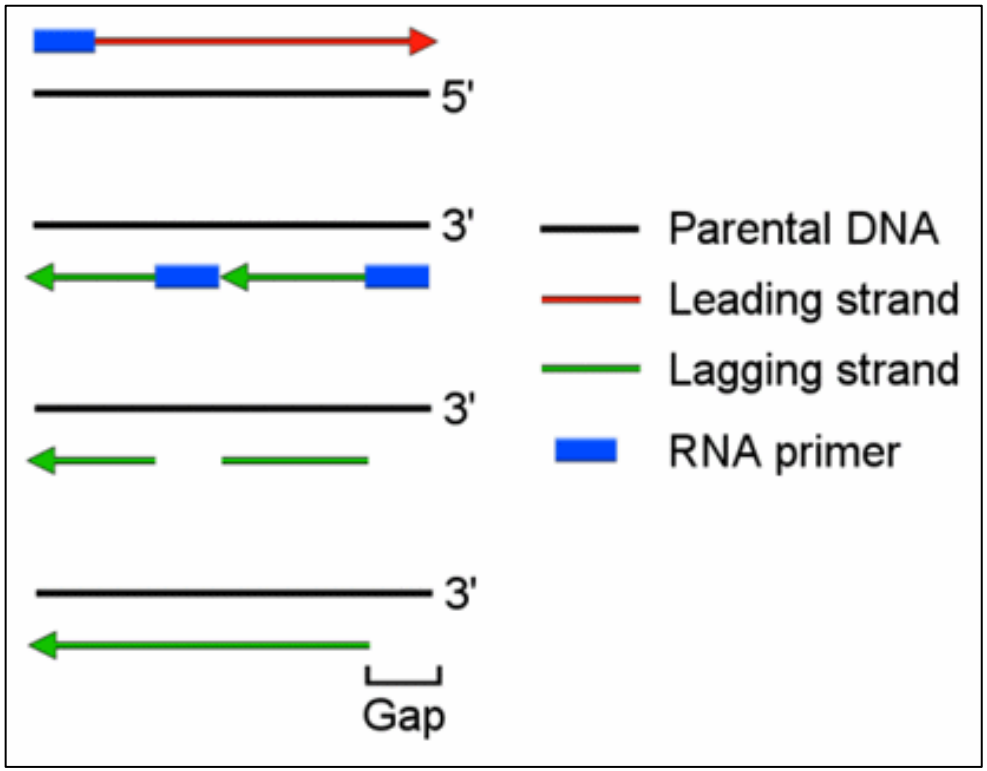
The Problem at the ends of eukaryotic linear Chromosomes



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

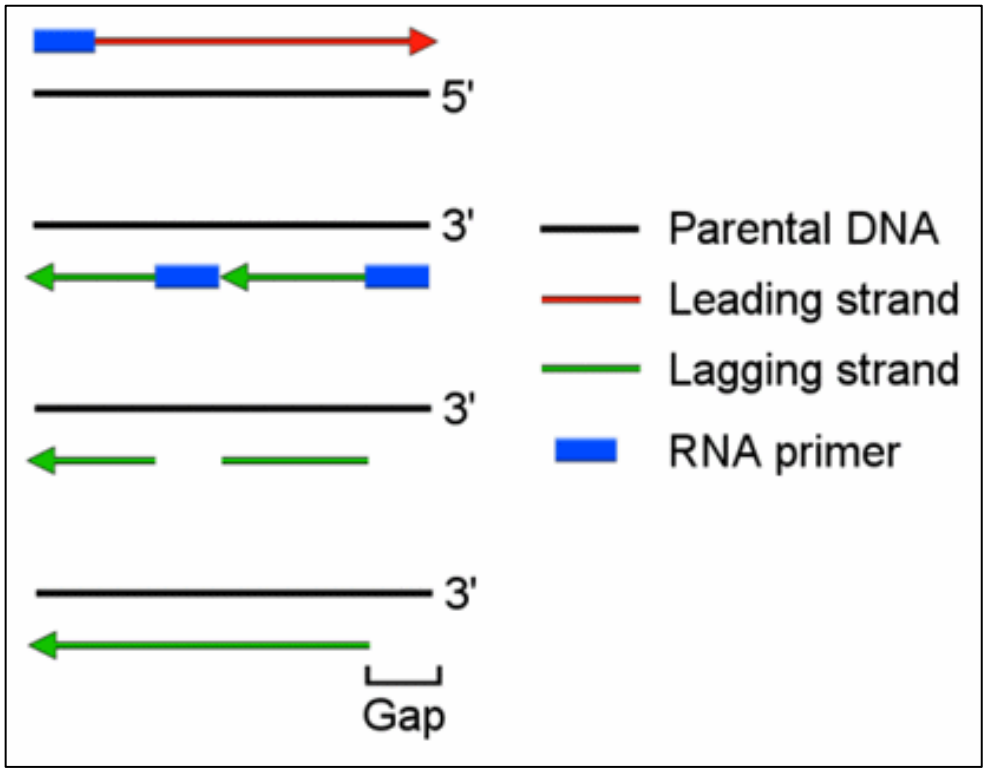
The end replication problem

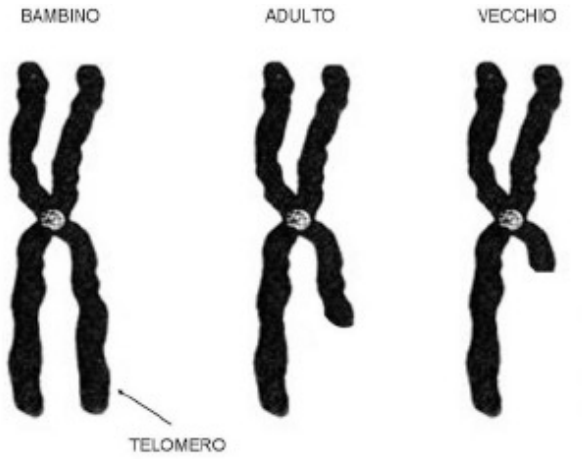
When the replication fork reaches an end of a linear chromosome, there is no place to produce the RNA primer needed to start the last Okazaki fragment at the very tip of a linear DNA molecule



The end replication problem

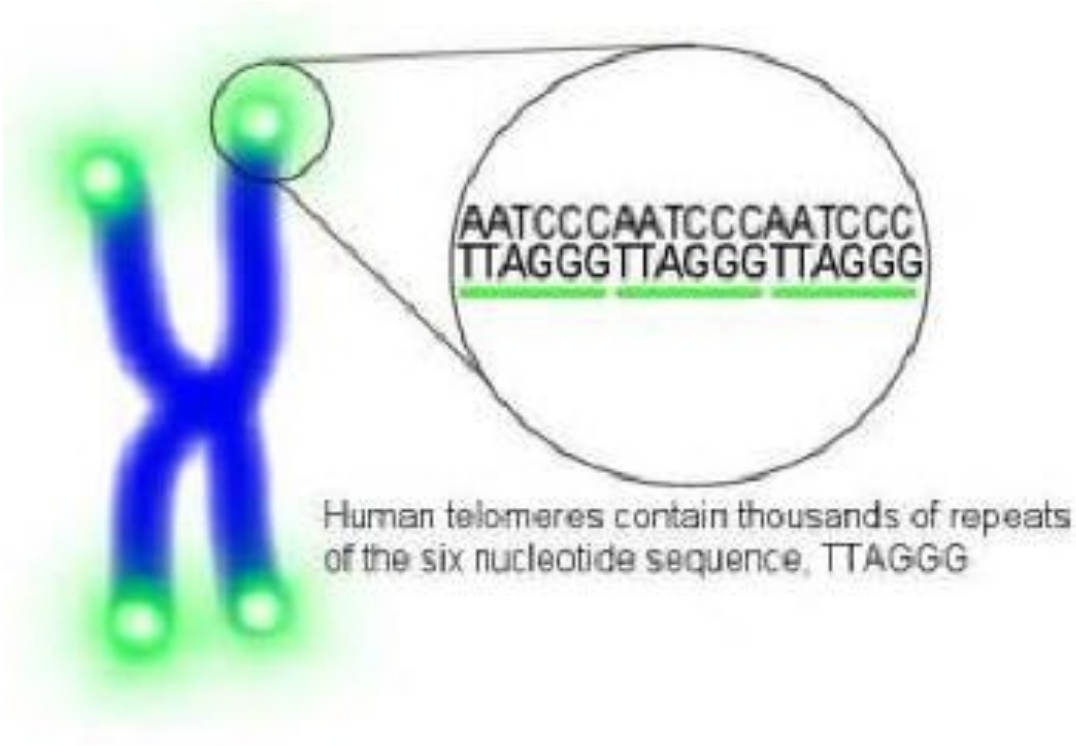
When the replication fork reaches an end of a linear chromosome, there is no place to produce the RNA primer needed to start the last Okazaki fragment at the very tip of a linear DNA molecule





Ad ogni ciclo replicativo si ha perdita ineluttabile di materiale genetico alle estremità dei cromosomi

Telomeres



Human telomeres contain thousands of repeats of the six nucleotide sequence, TTAGGG

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.

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.

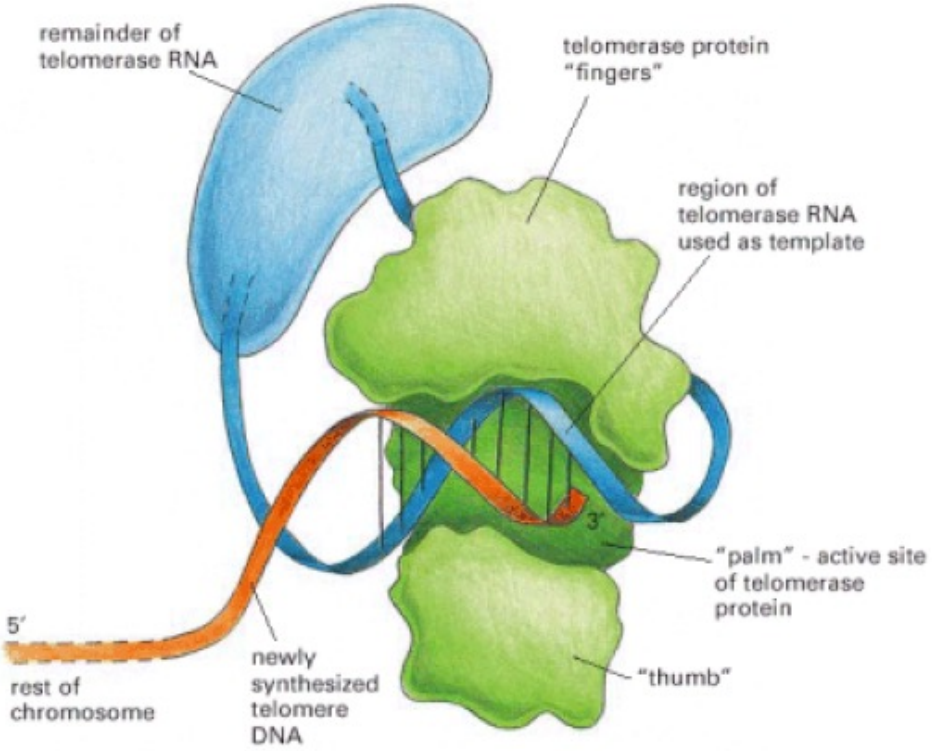
Telomeres are necessary for a cell to divide. They also are involved in directing the number of divisions.

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.

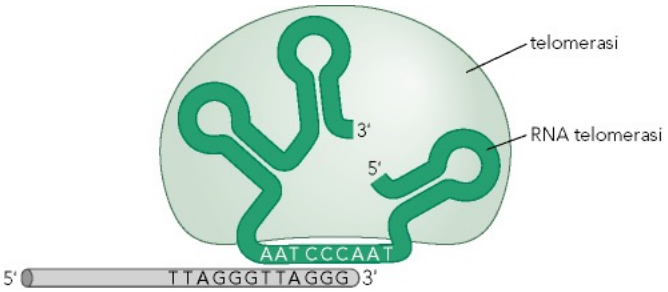


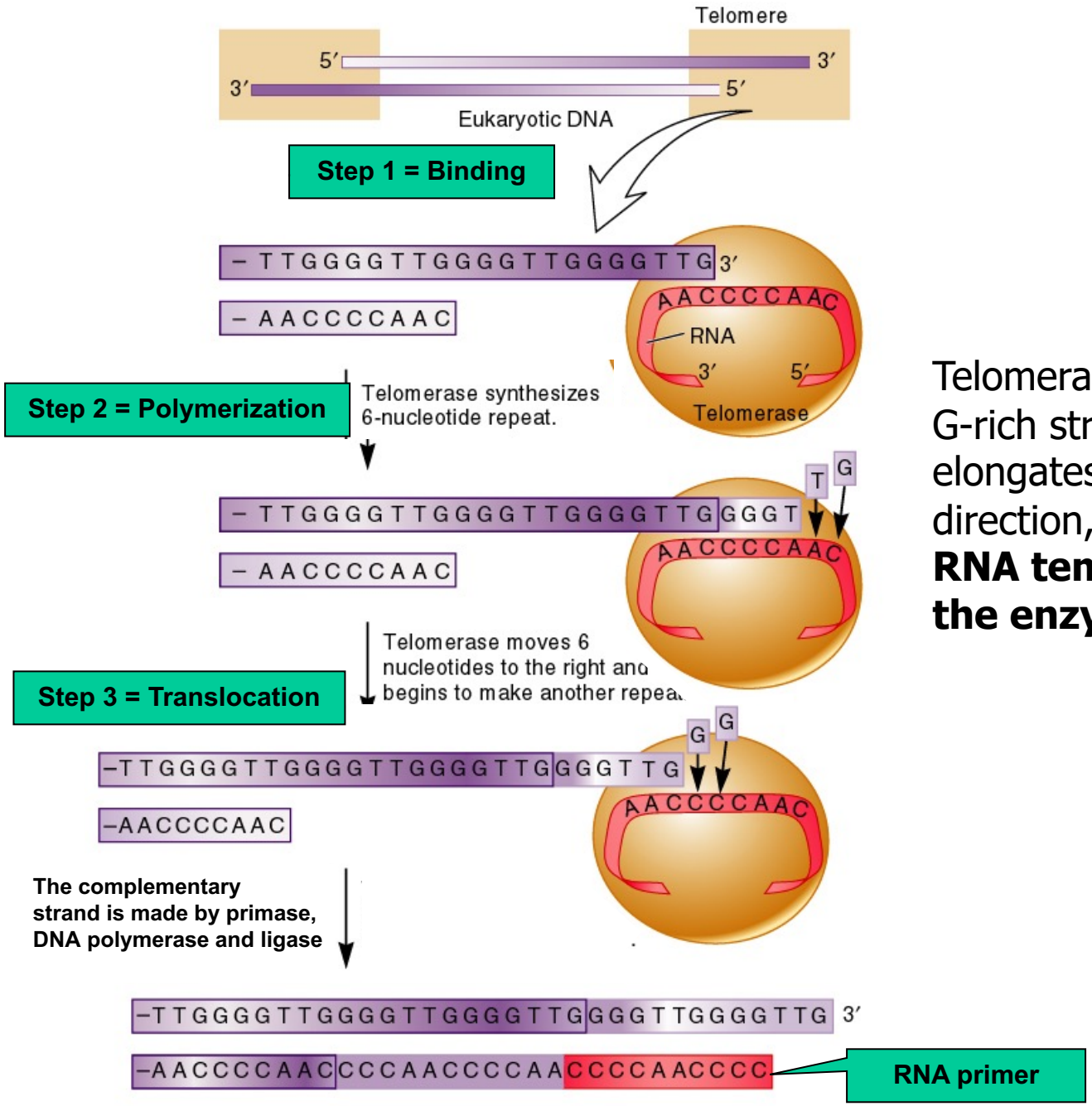
The structure of telomerase.

The telomerase is a protein RNA complex that carries an RNA template for synthesizing a repeating, G-rich telomere DNA sequence. Only the part of the telomerase protein homologous to reverse transcriptase is shown here *(green)*. A reverse transcriptase is a special form of polymerase enzyme that uses an RNA template to make a DNA strand; telomerase is unique in carrying its own RNA template with it at all times. (Modified from J. Lingner and T.R. Cech, *Curr. Opin. Genet. Dev.* 8:226 232, 1998.)

E' una ribonucleoproteina costituita da due componenti principali:

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





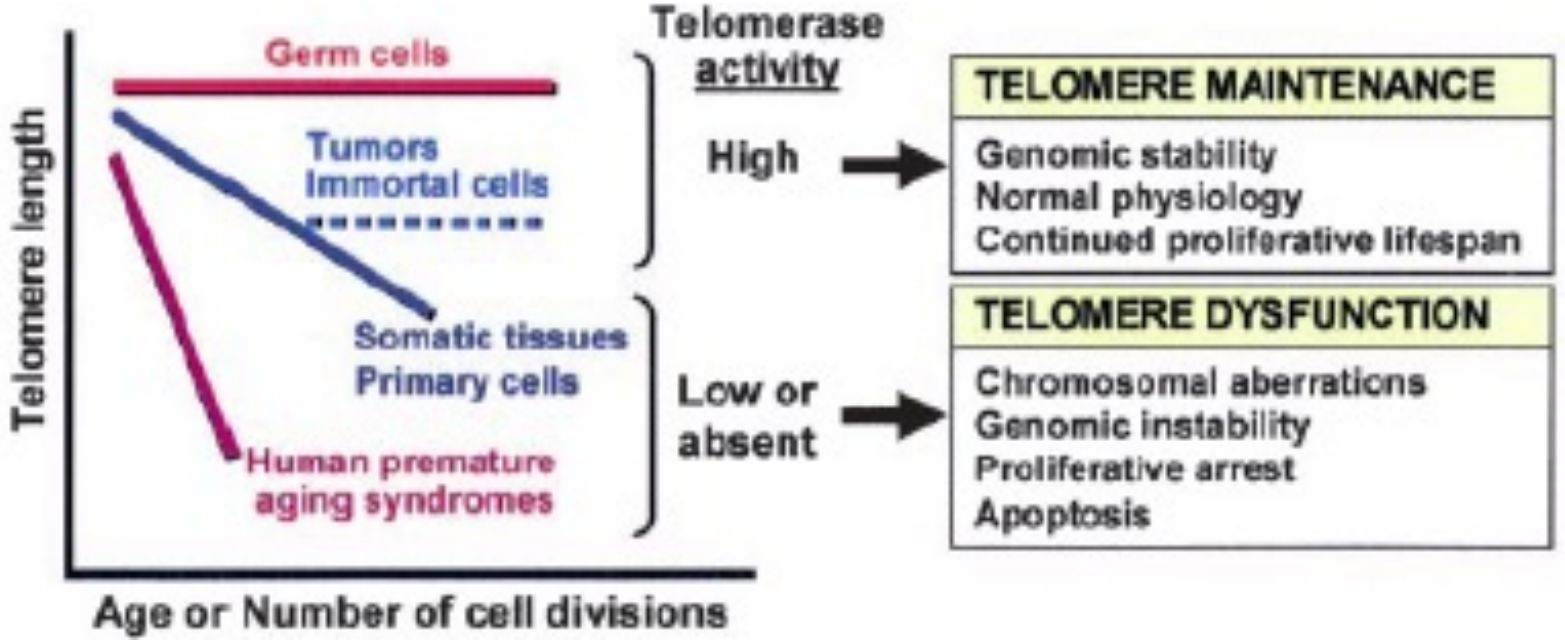
Telomerase recognizes the tip of a G-rich strand of a telomere and elongates it in the 5′-to-3′ direction, using the UAACCC **RNA template in the core of the enzyme.**

La Telomerasi svolge la propria attività durante la vita embrionale

Nella maggior parte delle cellule somatiche umane la telomerasi è inattiva.

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).

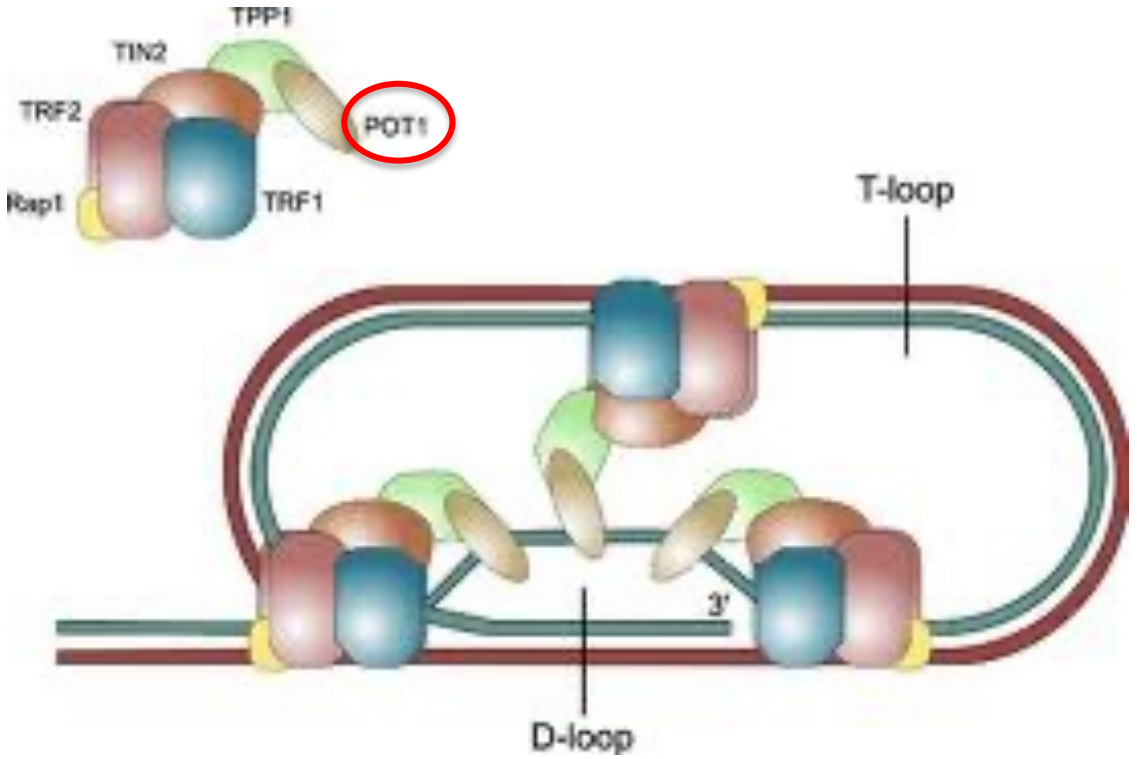
Eccezioni: cellule ovaio, testicolo, linfociti, cellule dell'embrione



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

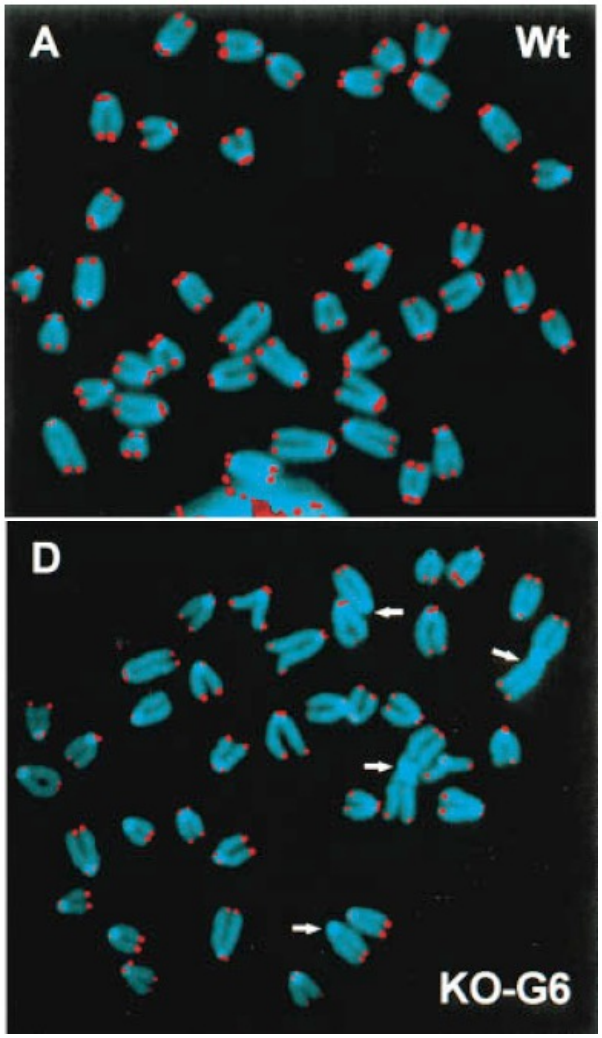
Cell, Vol. 91, 25–34, October 3, 1997, Copyright ©1997 by Cell Press

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/ 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.

- 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.

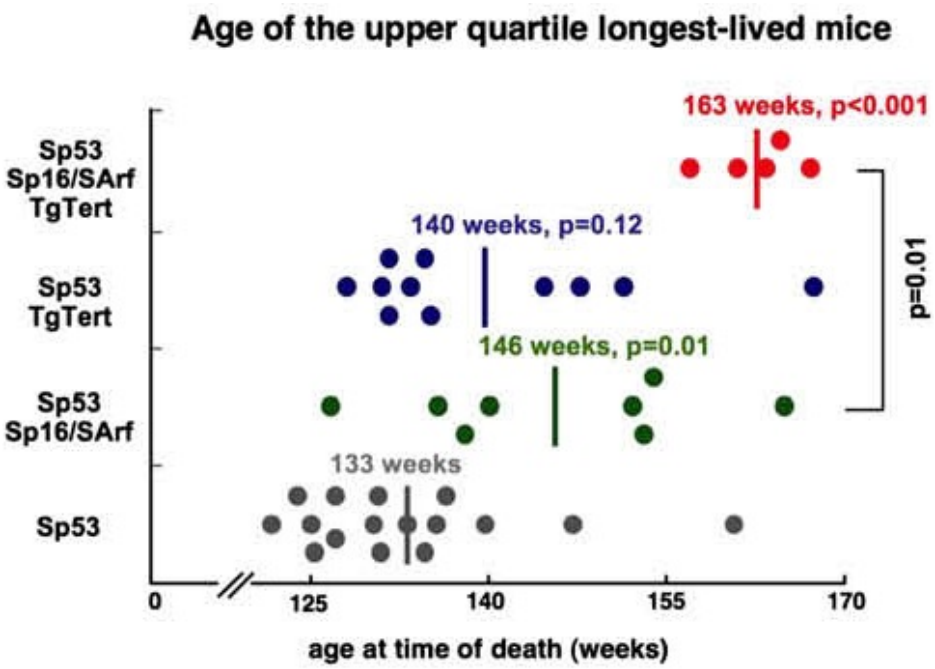
Constitutive telomerase expression in several independent Tert-transgenic mouse models resulted in increased incidence of spontaneous tumors (Gonzalez-Suarez et al., 2001; Gonzalez-Suarez et al., 2002; Artandi et al., 2002; Canela et al., 2004).

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,*]

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) in mice engineered to be cancer resistant by means of enhanced expression of the tumor suppressors p53, p16, and p19ARF (these three tumor suppressors are involved in protection against a large variety of cancers). In this context TERT overexpression 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.

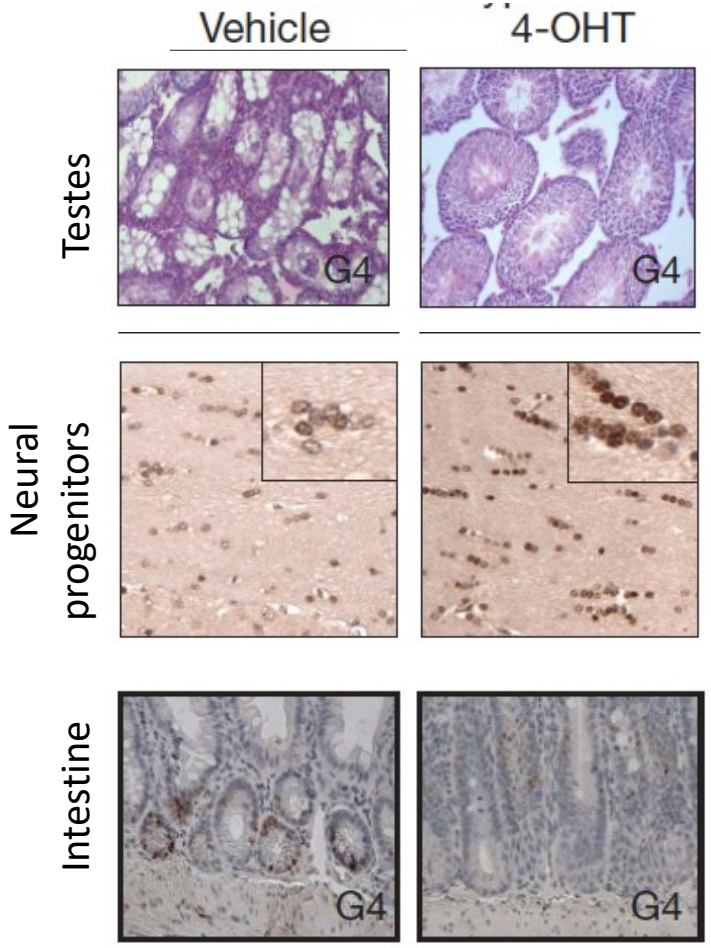
102 | NATURE | VOL 469 | 6 JANUARY 2011

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]

In a mouse model with advanced degenerative phenotypes (mice null for mTerc or mTert), short-term somatic Telomerase reactivation:

- extends telomeres;
- reduces DNA damage signalling;
- resumes proliferation in quiescent cells;
- eliminates degenerative phenotypes in multiple organs (testes, spleen, intestine)
- reversed neurodegeneration with restoration of proliferating neural progenitors.



The brief course of telomerase reactivation did not promote carcinogenesis.