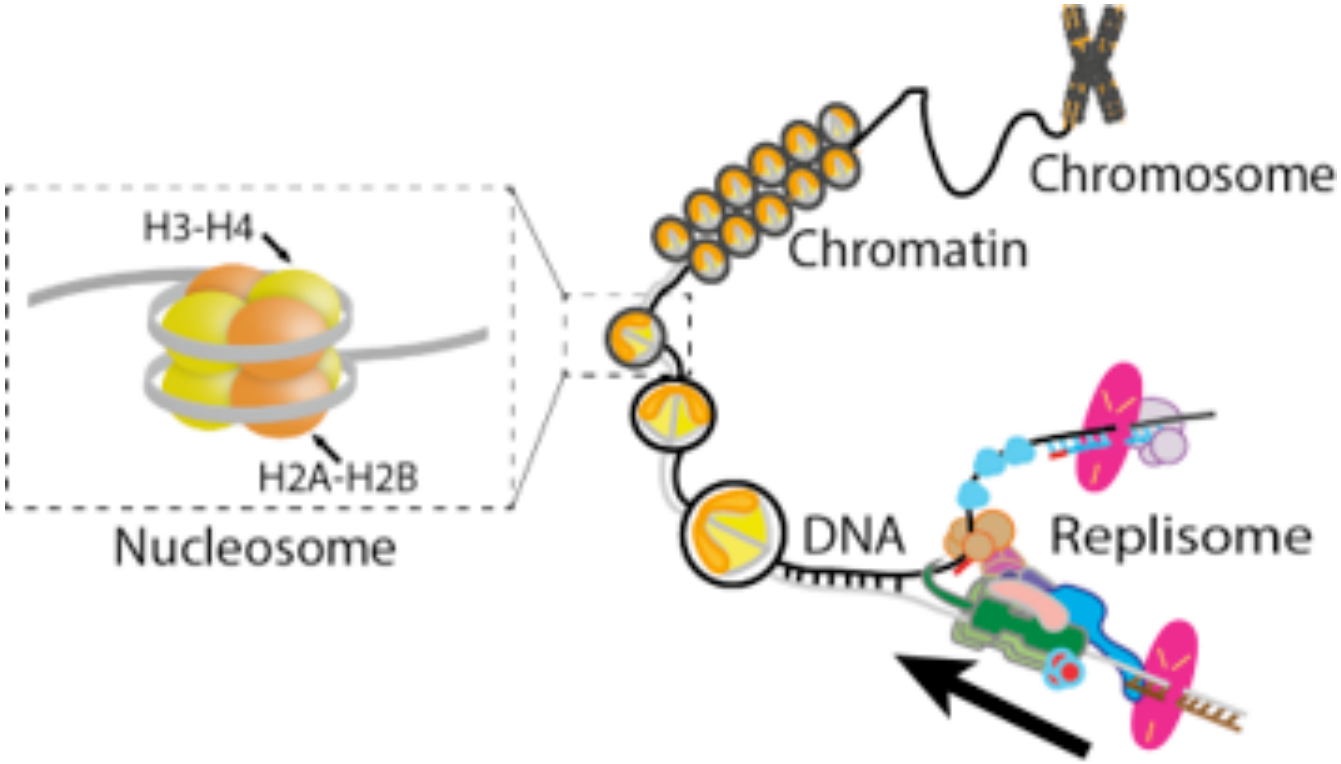
DNA REPLICATION

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

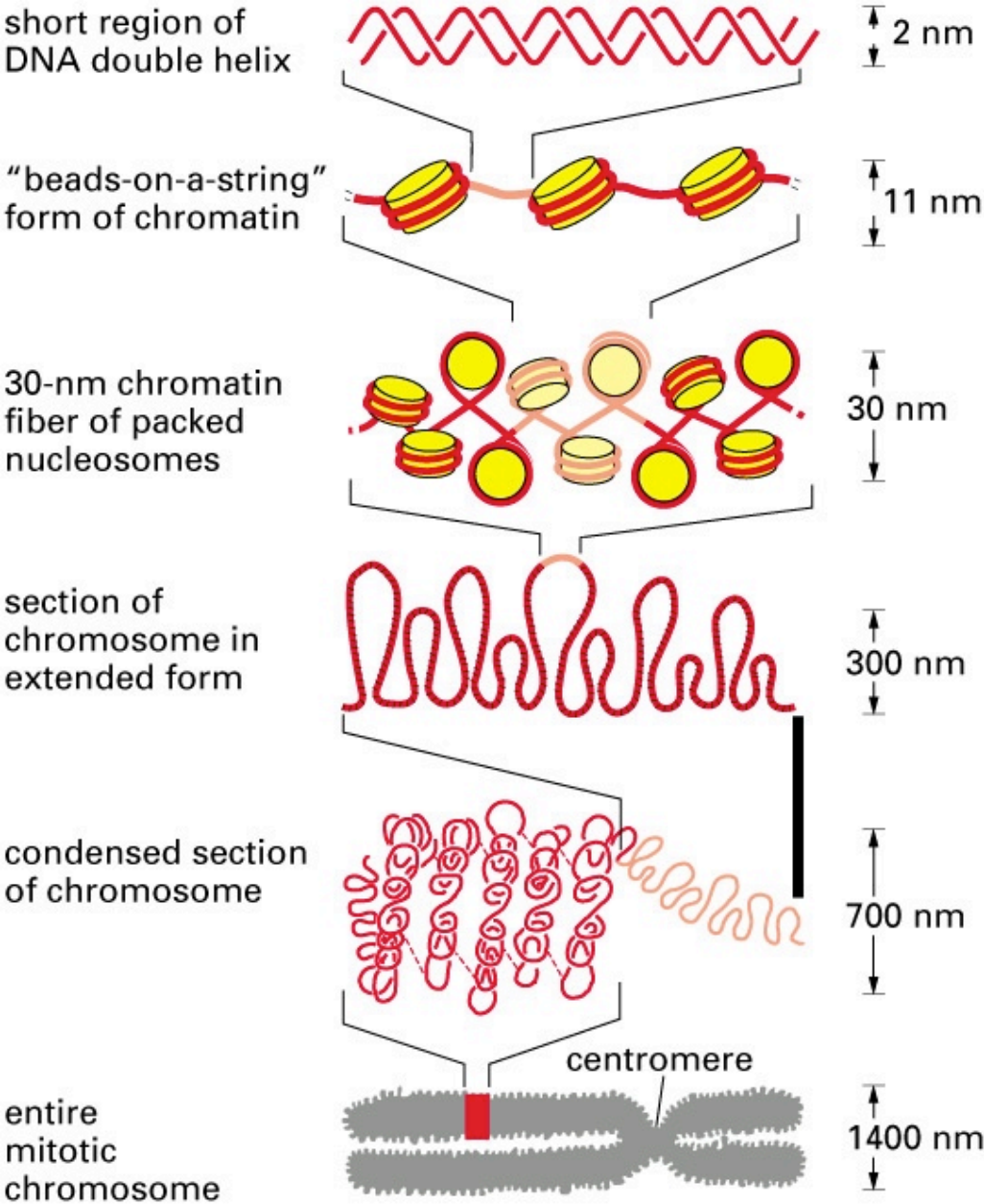
In Eucaryotes, the replication machinery has to deal with the 3D chromatin structure



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

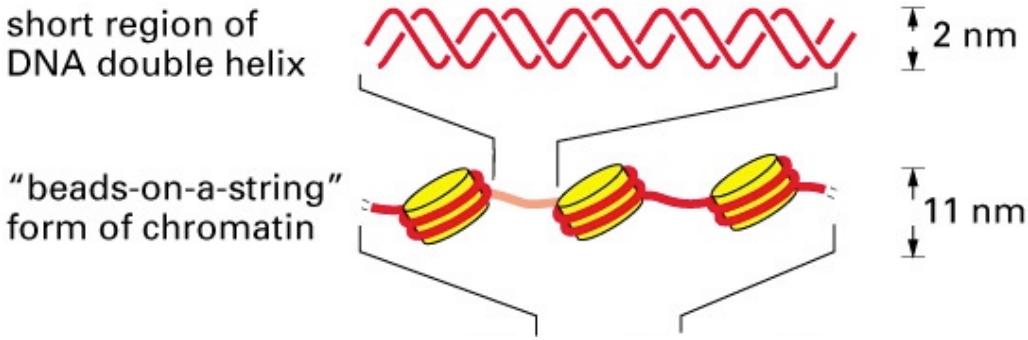
Okazaki fragments are synthesized at intervals of 100-200 nucleotides in eucaryotes, instead of 1000-2000 as in bacteria.

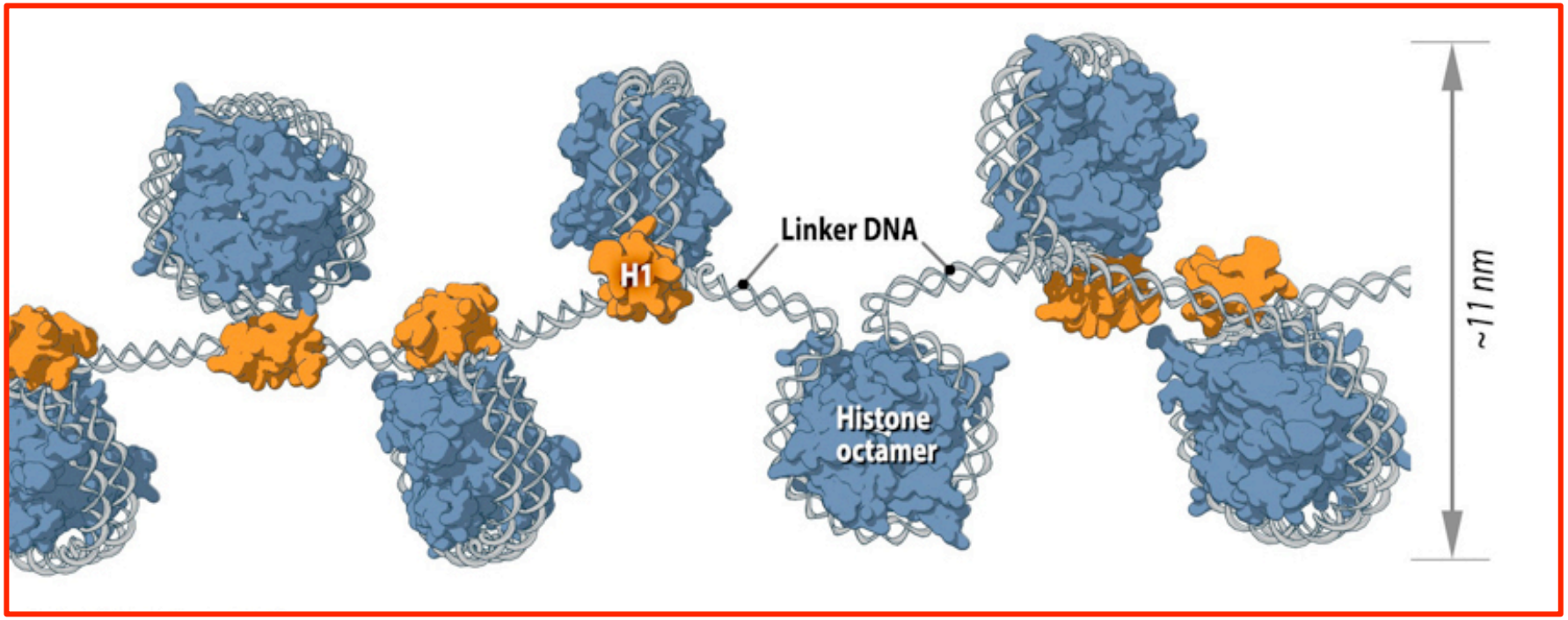
Chromatin structure



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

Chromatin structure

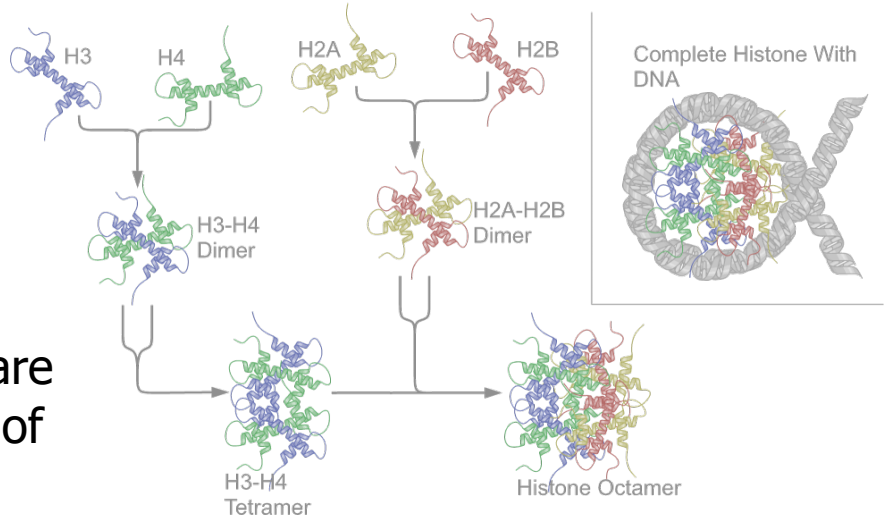




Histones

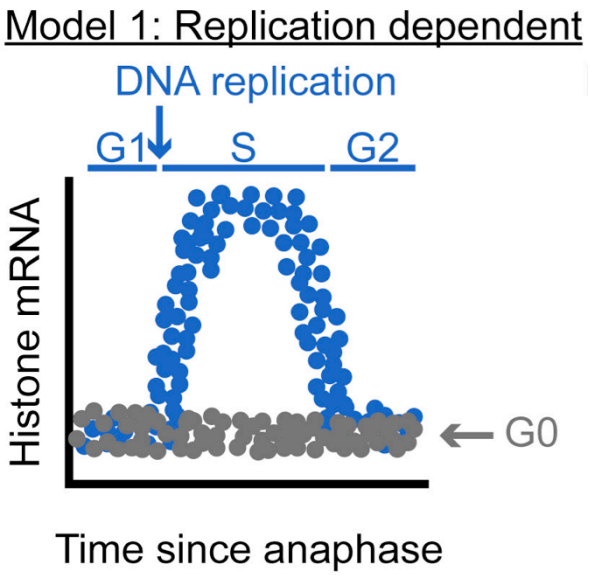
A large amount of new histone protein, approximately equal in mass to the newly synthesized DNA, is required to make the new nucleosomes in each cell cycle.

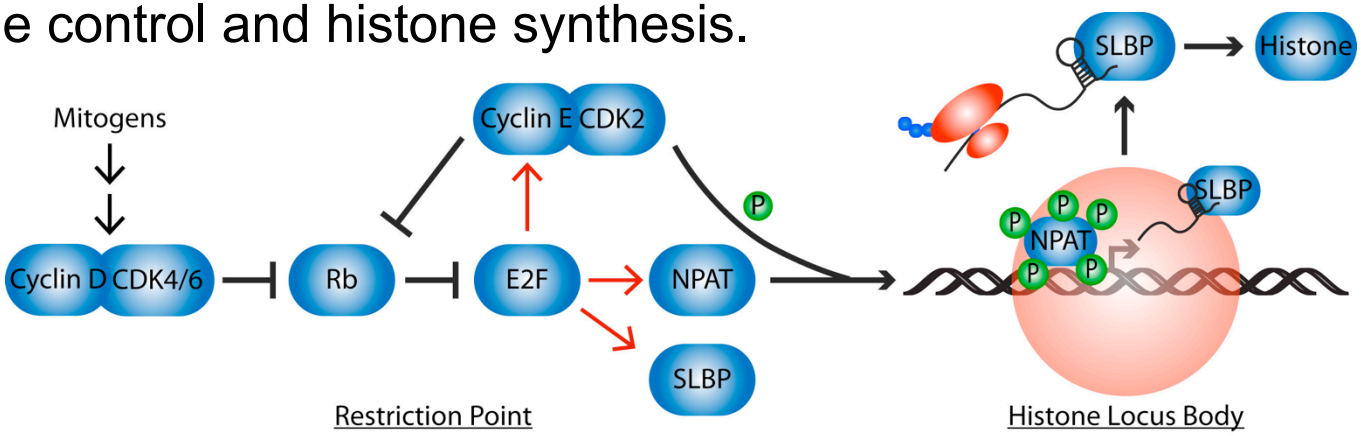
Unlike most proteins, which are made continuously throughout interphase, histones are synthesized mainly in S phase, when the level of histone mRNA increases about fifty folds.



Super family	Family	Subfamily	Members
Linker	H1	H1F	H1F0, H1FNT, H1FOO, H1FX
		H1H1	HIST1H1A, HIST1H1B, HIST1H1C, HIST1H1D, HIST1H1E, HIST1H1T
Core	H2A	H2AF	H2AFB1, H2AFB2, H2AFB3, H2AFJ, H2AFV, H2AFX, H2AFY, H2AFY2, H2AFZ
		H2A1	HIST1H2AA, HIST1H2AB, HIST1H2AC, HIST1H2AD, HIST1H2AE, HIST1H2AG, HIST1H2AI, HIST1H2AJ, HIST1H2AK, HIST1H2AL, HIST1H2AM
		H2A2	HIST2H2AA3, HIST2H2AC
	H2B	H2BF	H2BFM, H2BFS, H2BFWT
		H2B1	HIST1H2BA, HIST1H2BB, HIST1H2BC, HIST1H2BD, HIST1H2BE, HIST1H2BF, HIST1H2BG, HIST1H2BH, HIST1H2BI, HIST1H2BJ, HIST1H2BK, HIST1H2BL, HIST1H2BM, HIST1H2BN, HIST1H2BO
		H2B2	HIST2H2BE
	H3	H3A1	HIST1H3A, HIST1H3B, HIST1H3C, HIST1H3D, HIST1H3E, HIST1H3F, HIST1H3G, HIST1H3H, HIST1H3I, HIST1H3J
		H3A2	HIST2H3C
		H3A3	HIST3H3
	H4	H41	HIST1H4A, HIST1H4B, HIST1H4C, HIST1H4D, HIST1H4E, HIST1H4F, HIST1H4G, HIST1H4H, HIST1H4I, HIST1H4J, HIST1H4K, HIST1H4L
		H44	HIST4H4

In metazoans the increase in the rate of histone synthesis is due to the increase in processing of pre-mRNA to its mature form 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).
This shows an important regulatory link between cell-cycle control and histone synthesis.

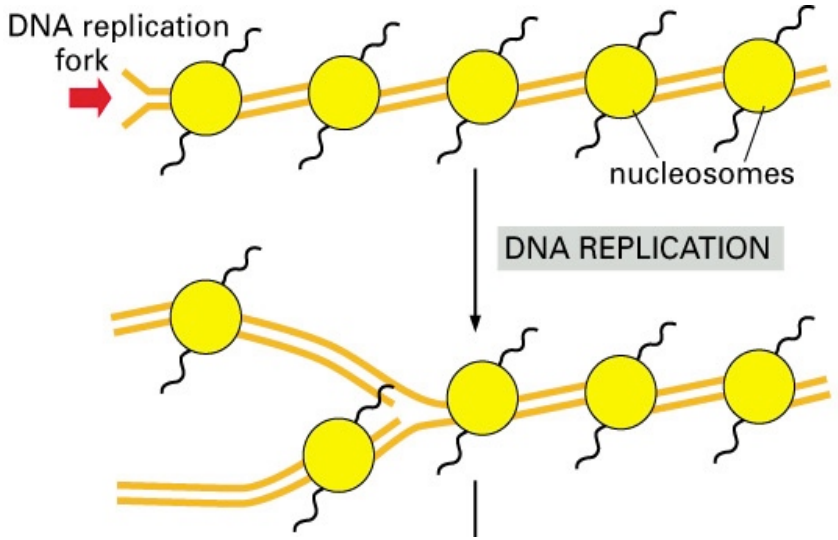


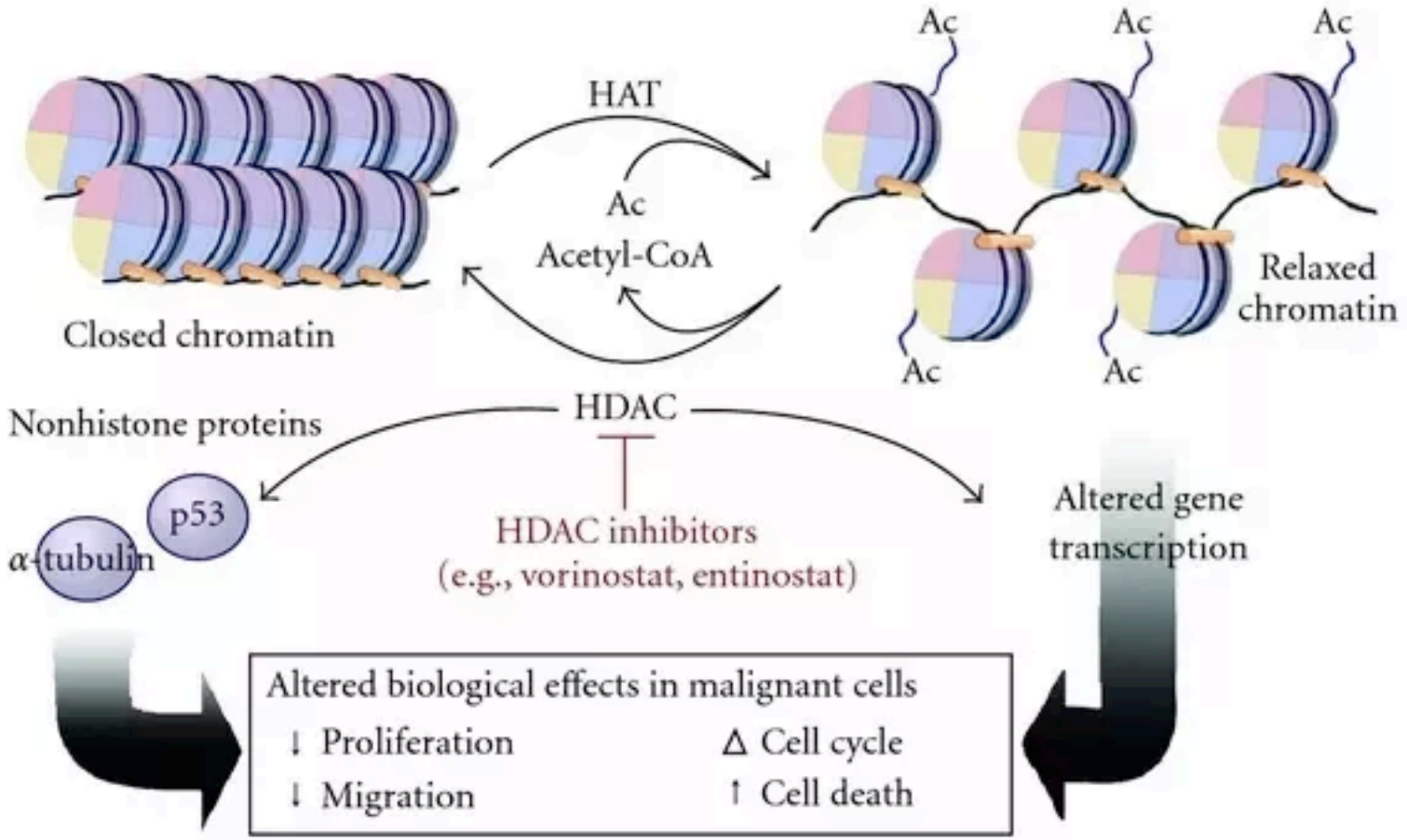


Nuclear protein Ataxia-Telangiectasia (NPAT- nuclear protein coactivator of histone transcription), is a transcription factor which activates histone gene transcription.
NPAT activates histone gene expression only after it has been phosphorylated by the G1/S-Cdk cyclin E-Cdk2 in early S phase.

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.




HAT
Ac
Acetyl-CoA
Closed chromatin
Ac
Ac
Ac
Relaxed chromatin
Ac
Ac
HDAC
Nonhistone proteins
p53
α-tubulin
HDAC inhibitors
(e.g., vorinostat, entinostat)
Altered gene transcription
Altered biological effects in malignant cells
↓ Proliferation
↓ Migration
Δ Cell cycle
↑ Cell death

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Mechanisms for the Inheritance of Chromatin States

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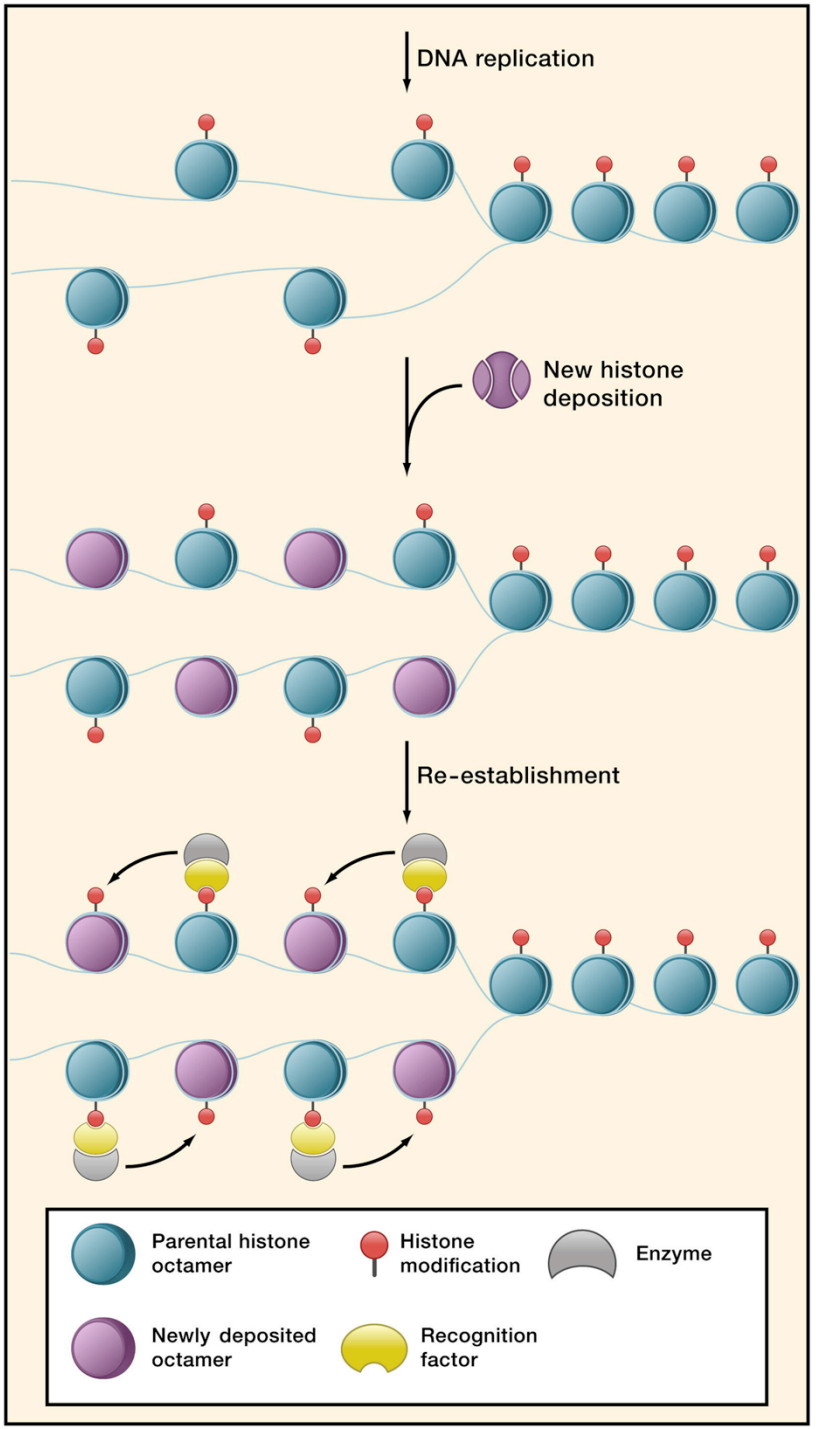
Parental histones and their posttranslational modifications are retained and randomly associate with the newly synthesized daughter DNA strands.

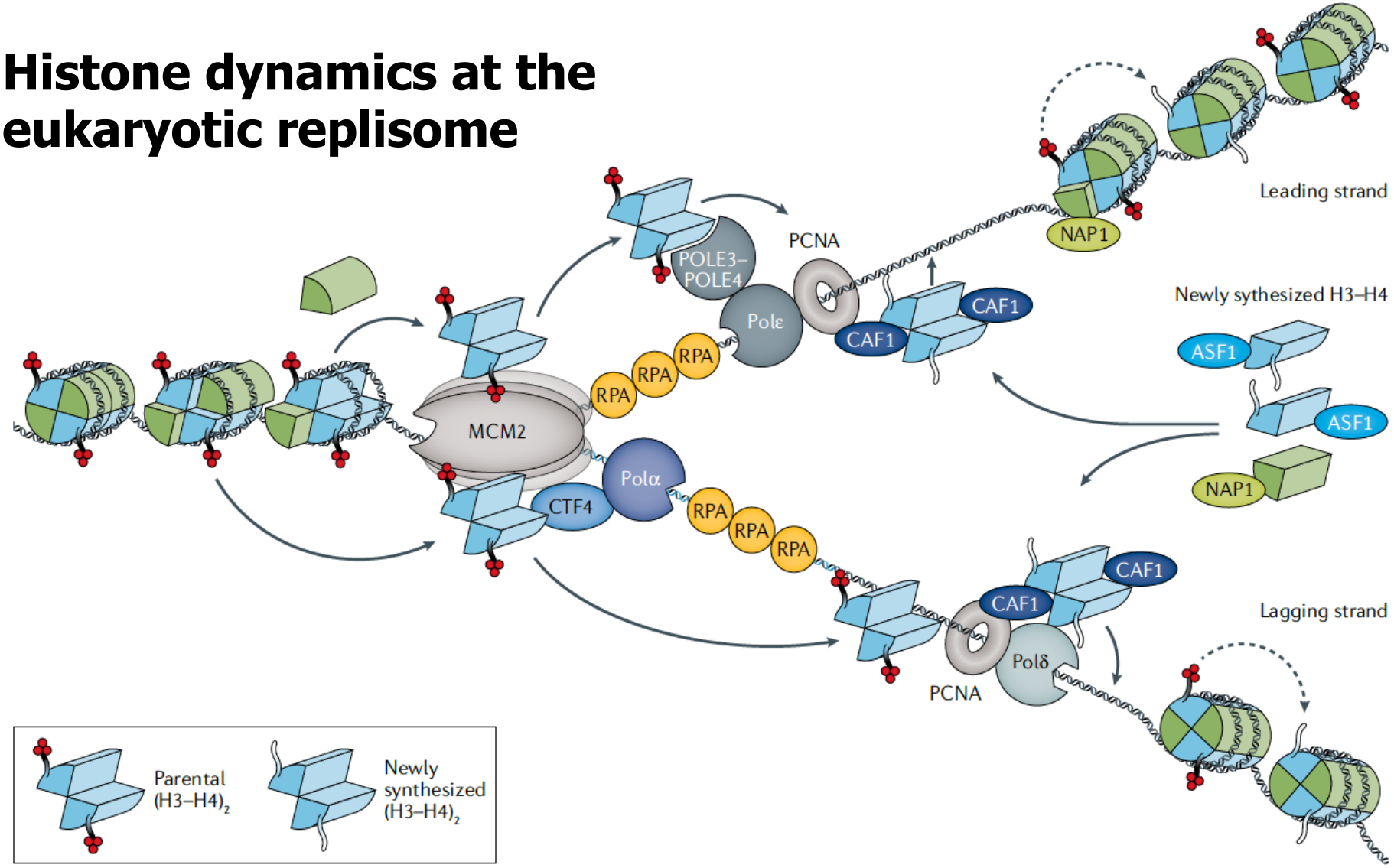
The modifications of parental histones are copied onto newly deposited histones by *chromatin modification complexes:*

- 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**.



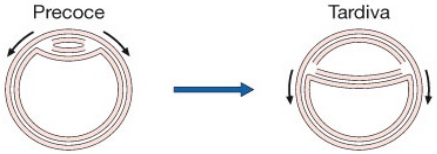
Histone dynamics at the eukaryotic replisome



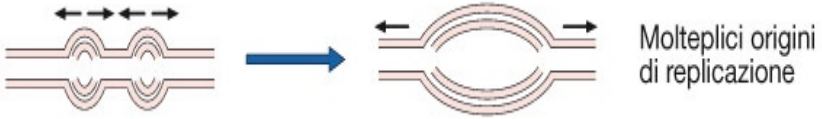
Parental nucleosome segregation and the inheritance of cellular identity

Thelma M. Escobar[1,2,5], *Alejandra Loyola*[3,4,5] *and Danny Reinberg*[1,2] ✉

DNA synthesis begins at replication origins

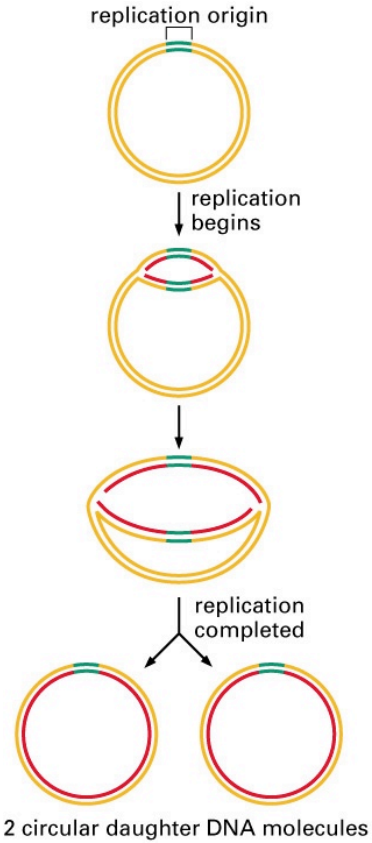


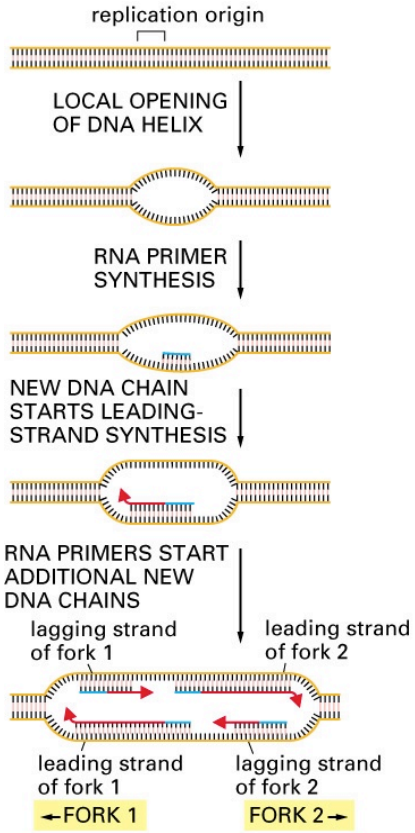
Replicazione bidirezionale (struttura theta)



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





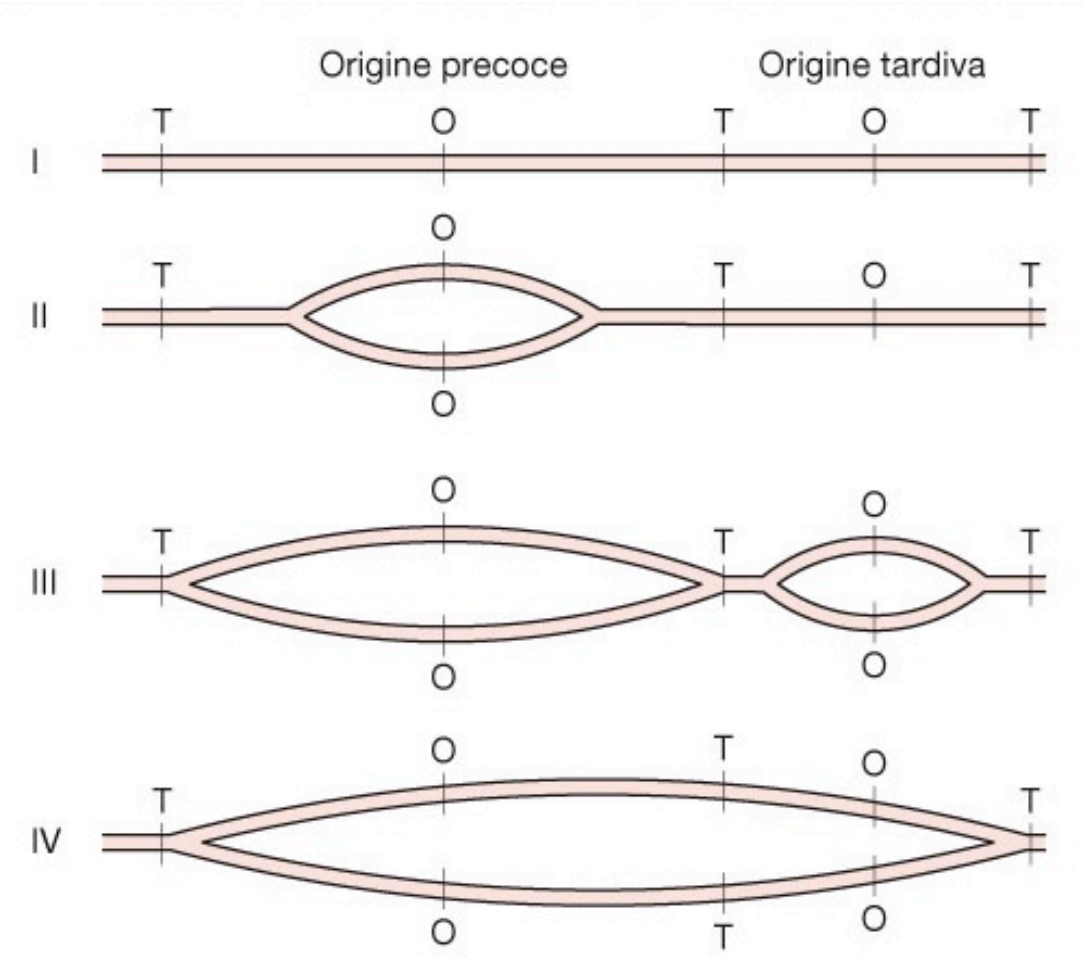
Ciascun cromosoma degli organismi eucarioti contiene molteplici origini di replicazione.

Replication speed: 50 nn/sec

Average chromosome length: 150 million nn

One single replication fork would require about 800 hours

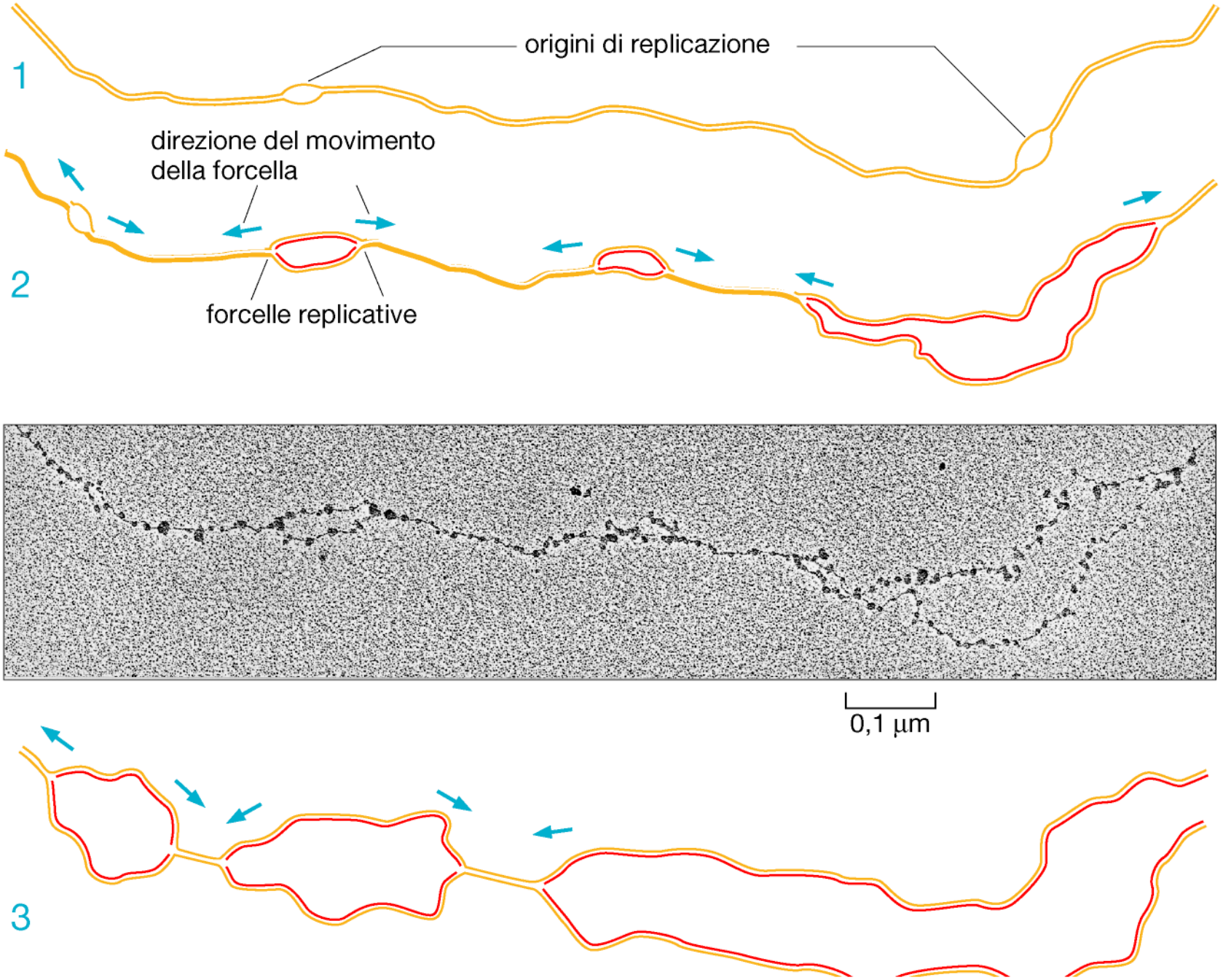
EUCARYOTIC REPLICATION ORIGINS



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

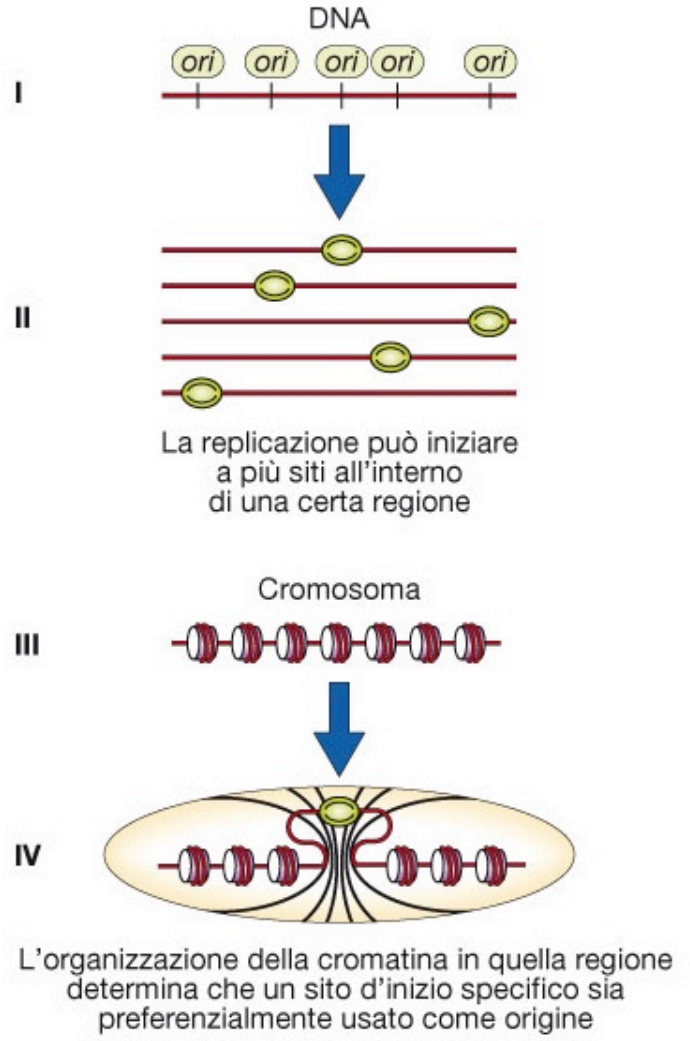
Ognuna di queste e' organizzata con un punto di origine da cui partono due forcelle di replicazione opposte. I punti di terminazione di due repliconi adiacenti coincidono, cosi' che a tempi tardivi di replicazione, repliconi adiacenti si fondono l'uno con l'altro.

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)

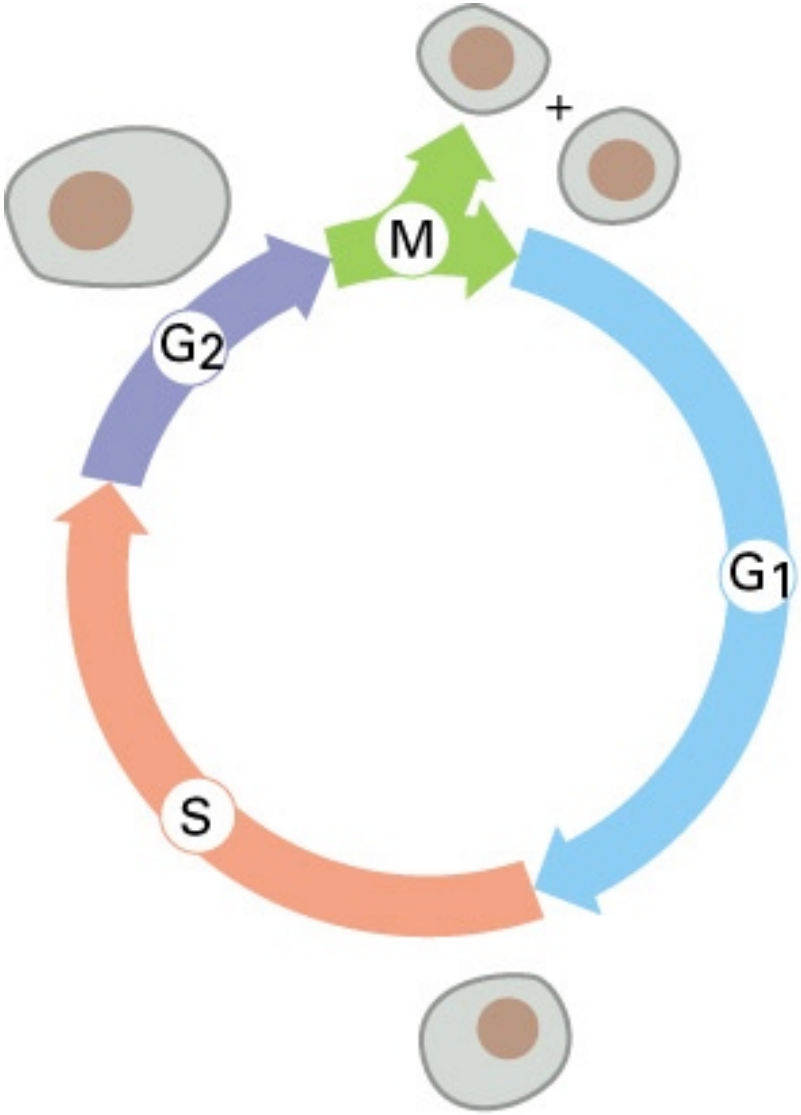

origini di replicazione
1
direzione del movimento
della forcella
2
forcelle replicative
0,1 µm
3

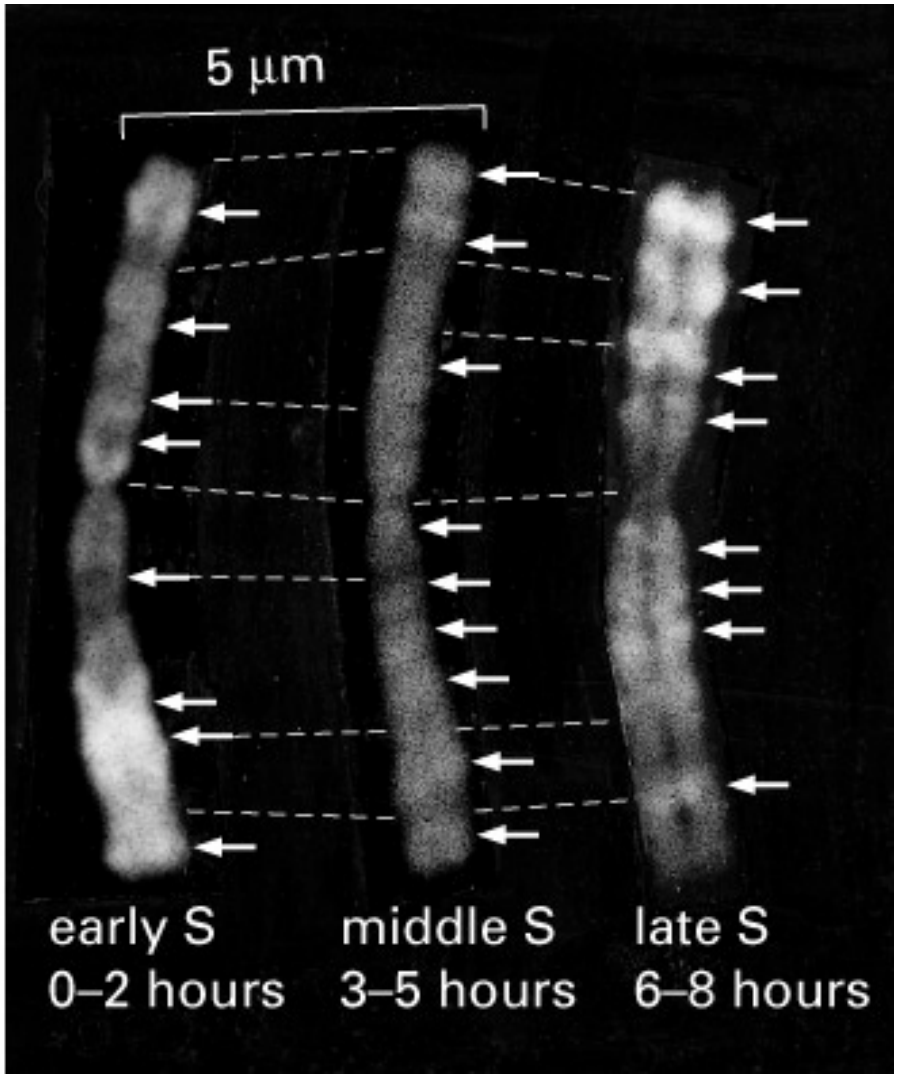
In homo sapiens…

- Le origini di replicazione si stima possano essere 10^4
- In alcune 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

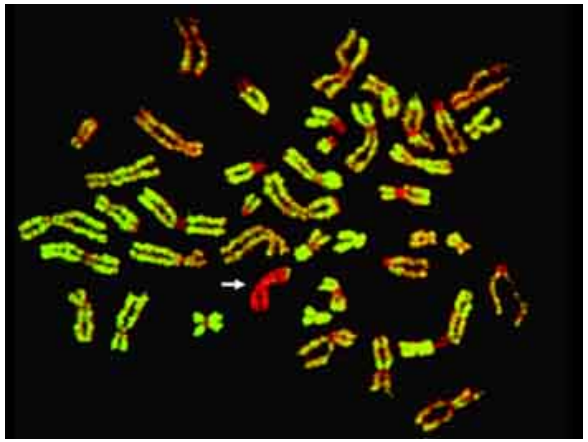




In a mammalian cell, the S phase lasts for about 8 hours. Different regions on the same chromosome replicate at distinct times in S phase.

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

Two X chromosomes in a female mammalian cell



- 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

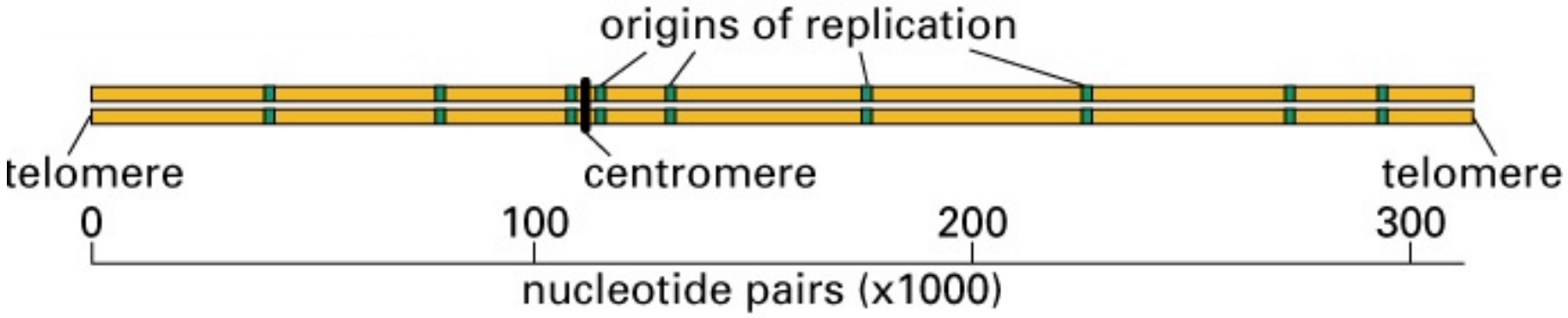
- one is active for transcription and is less condensed --> it replicates throughout S phase

ETEROCROMATINA: replicazione tardiva
EUCROMATINA: replicazione precoce

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

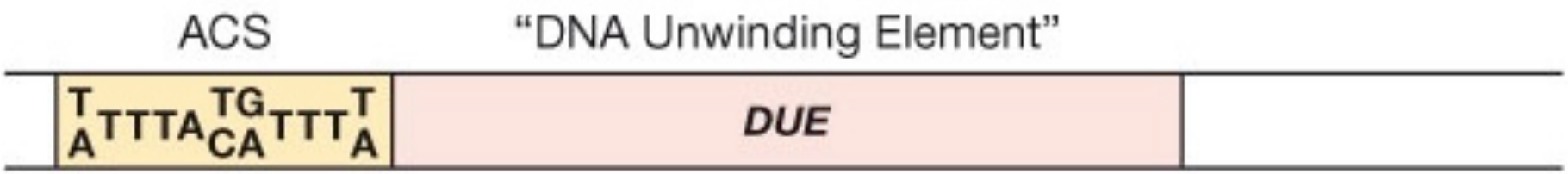
Well-defined DNA sequences serve as replication origins in yeast: the ARS sequences (autonomously replicating sequences)

Origins in S. cerevisiae are spaced **~40,000** nn



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, the chromosome is gradually lost as the cells divide, presumably because it is replicated too slowly.

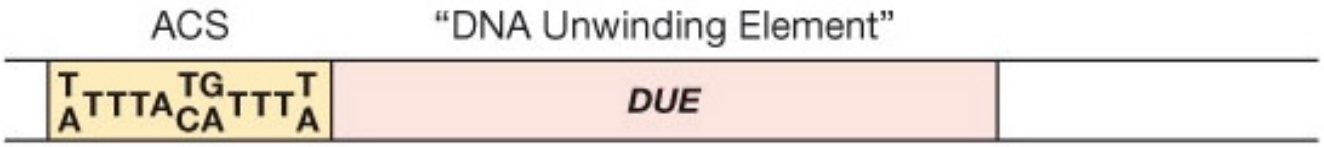
Quali sono le caratteristiche di una Autonomous Replicating Sequence (ARS) in *S. Cerevisiae?*



- Tutte le ARS contengono almeno una *ARS consensus sequence (ACS)* **di 11 pb**, ricca di A e T, 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

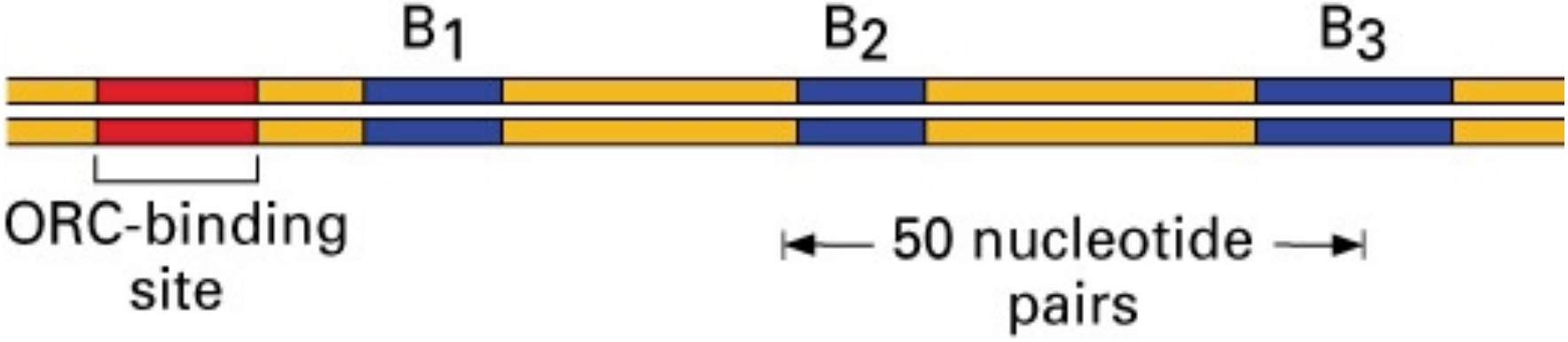
Origin Recognition Complex (ORC)



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

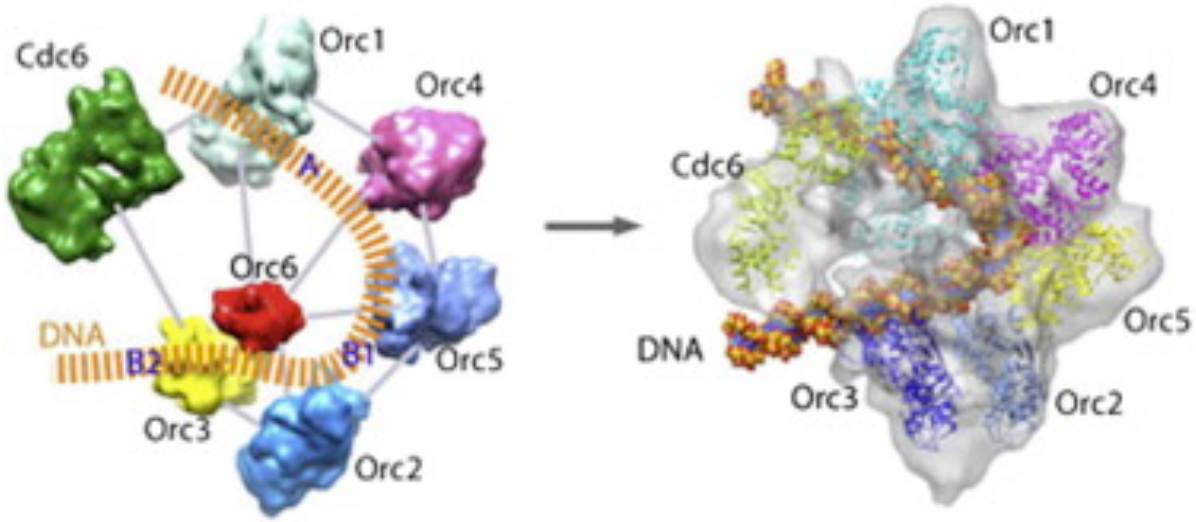
Several auxiliary binding sites (B1, B2 etc) exist for ORC subunits on the ARS in yeast.

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



Origin Recognition Complex (ORC)

- Six subunits (Orc1p-6p); 120, 72, 62, 56, 53, 50 kDa)
- Essential for viability
- Binds to ACS in an ATP-dependent manner
- Orc 1-2-and 5 have ATP binding motifs
- Mutantions that disrupt ORC binding to DNA also disrupt origin function in vivo
- Conserved in different species
- Absence of any biochemical activity besides origin binding
- ORC binds ARSes during the whole cell cycle
- In yeast, also inactive ARSes bind ORC



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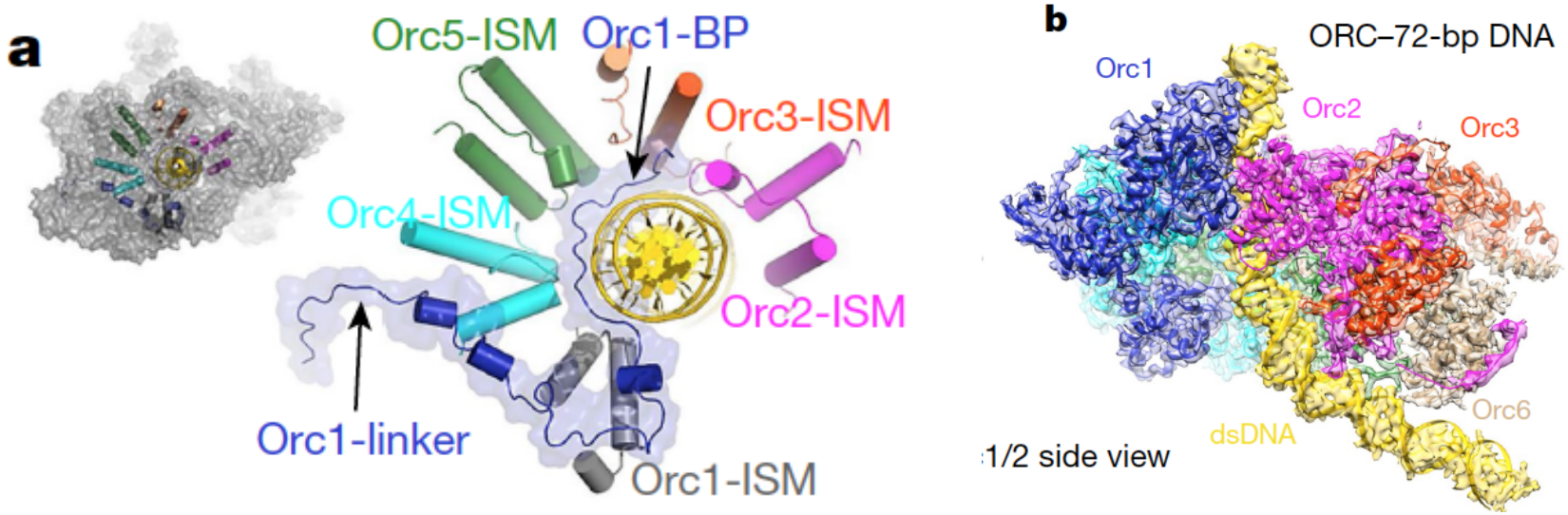
https://doi.org/10.1038/s41586-018-0293-x

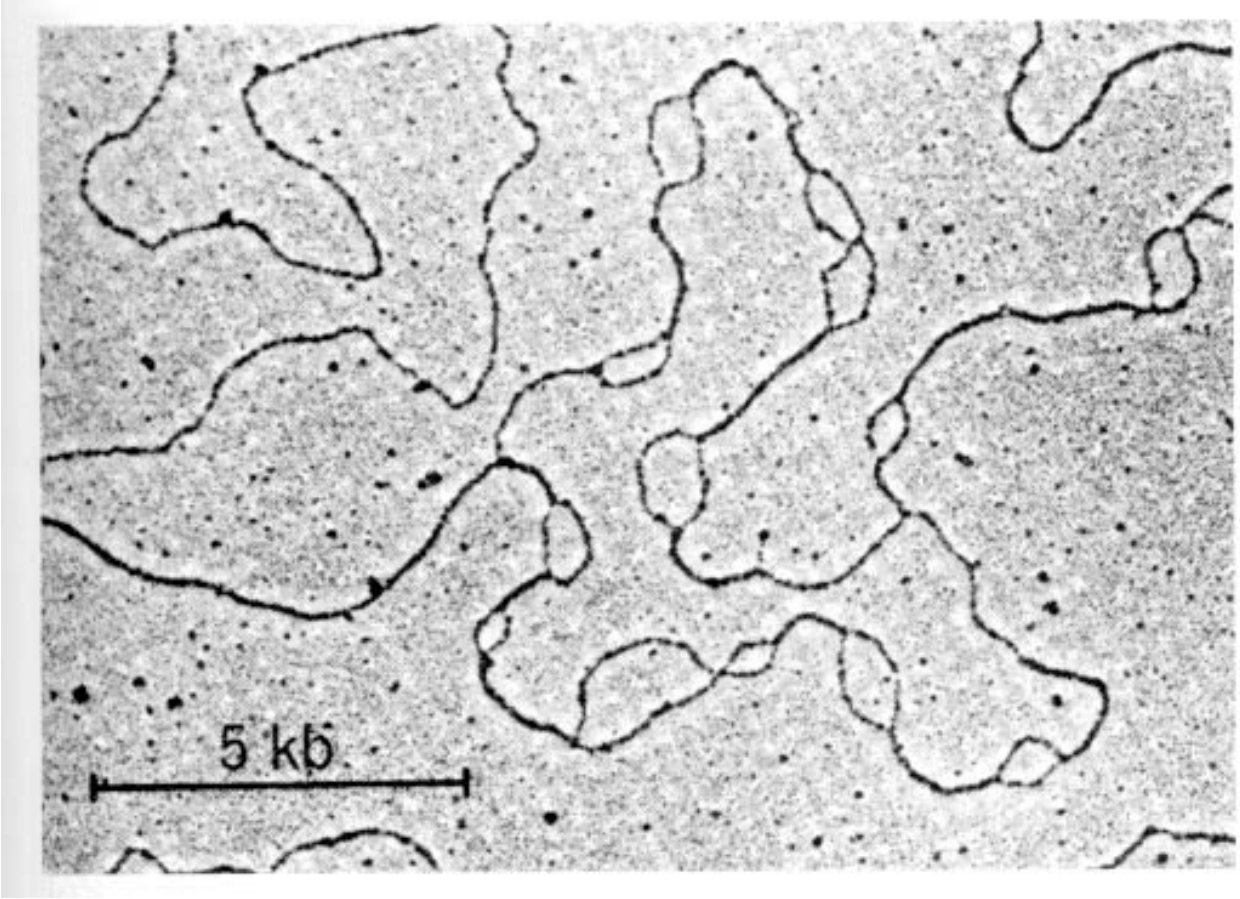
Structure of the origin recognition complex bound to DNA replication origin

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*]

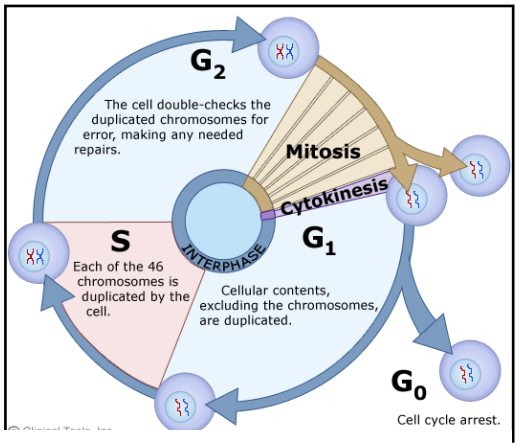


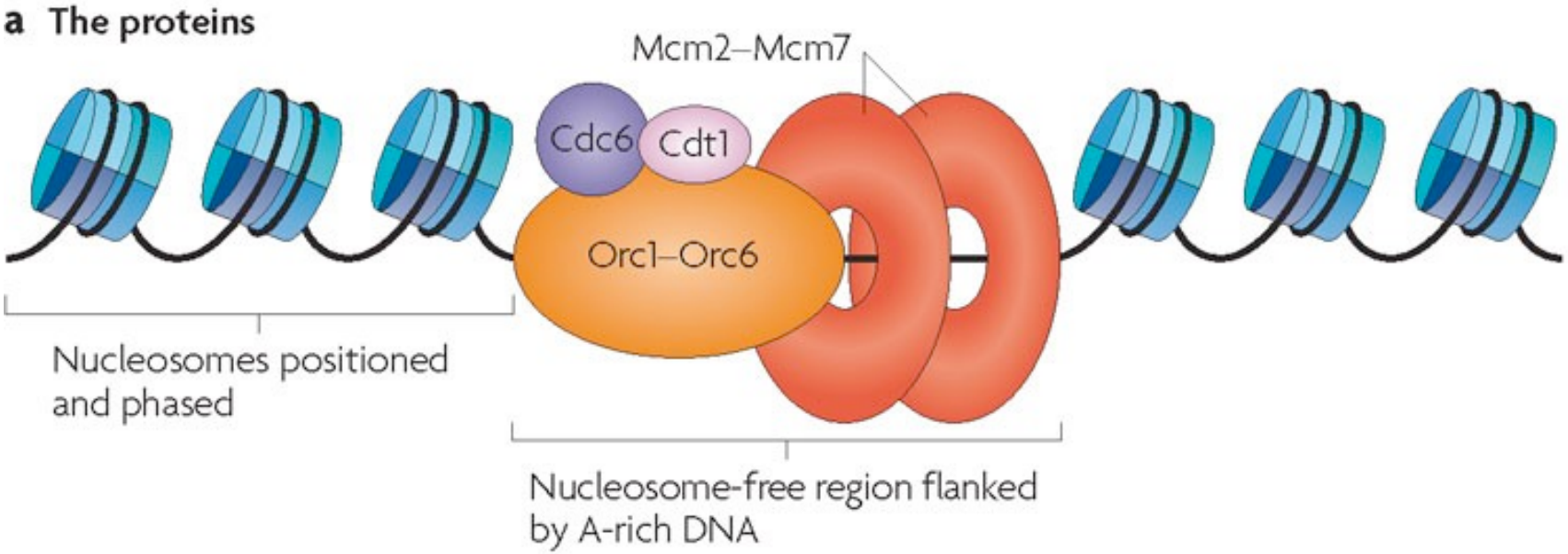
The six-subunit origin recognition complex (ORC) binds to DNA to mark the site for the initiation of replication in eukaryotes. Here we report a 3 Å cryo-electron microscopy structure of the *Saccharomyces cerevisiae* ORC bound to a 72-base-pair origin DNA sequence that contains the ARS consensus sequence (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.

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

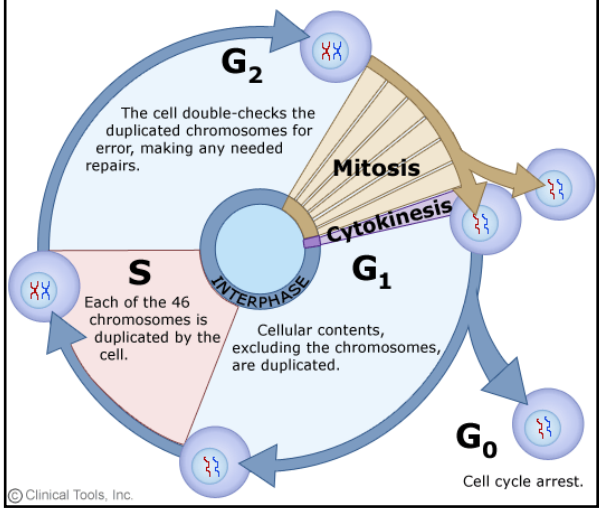


I- The Pre-Replication Complex





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

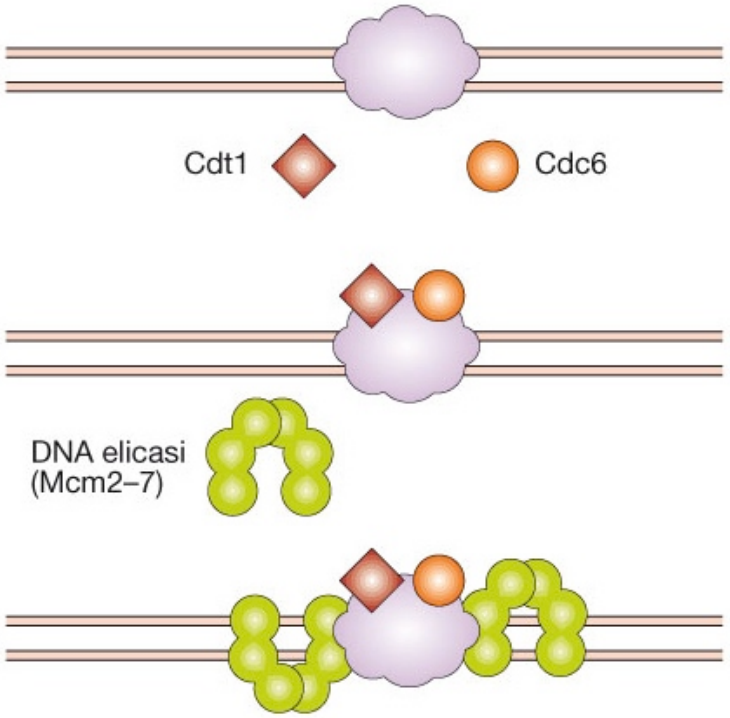


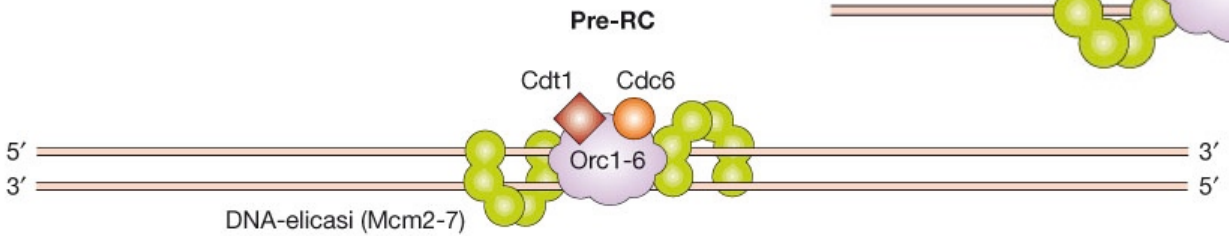
La preparazione delle origini alla replicazione avviene quando le cellule *escono dalla mitosi* e *proseguono nella fase G1* del ciclo cellulare (late M-early G1).

In questo momento si forma su ciascuna origine il **complesso di pre-replicazione** (pre-RC), il cui costituente principale e' il **COMPLESSO ORC**.

Ad ORC si associano due proteine chiave del controllo replicativo, **cdc6** e **cdt1**, richieste per l'attivazione dell'elicasi, nota come **Mcm2-7.**

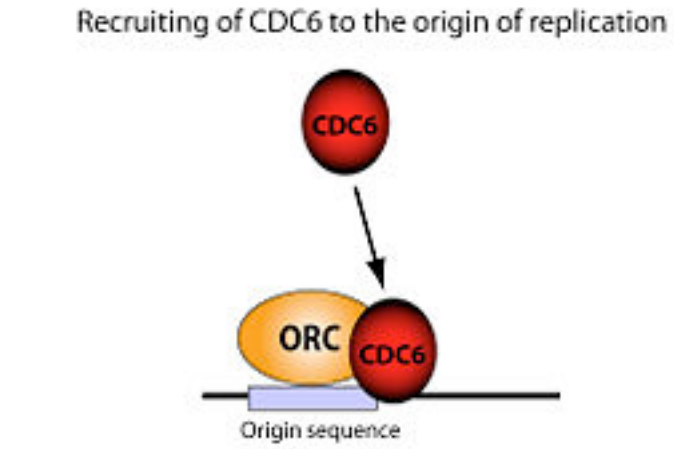
A questo punto l'origine e' "**licenced**" per la replicazione

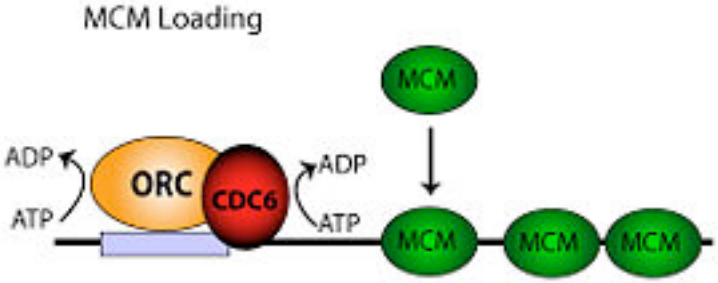


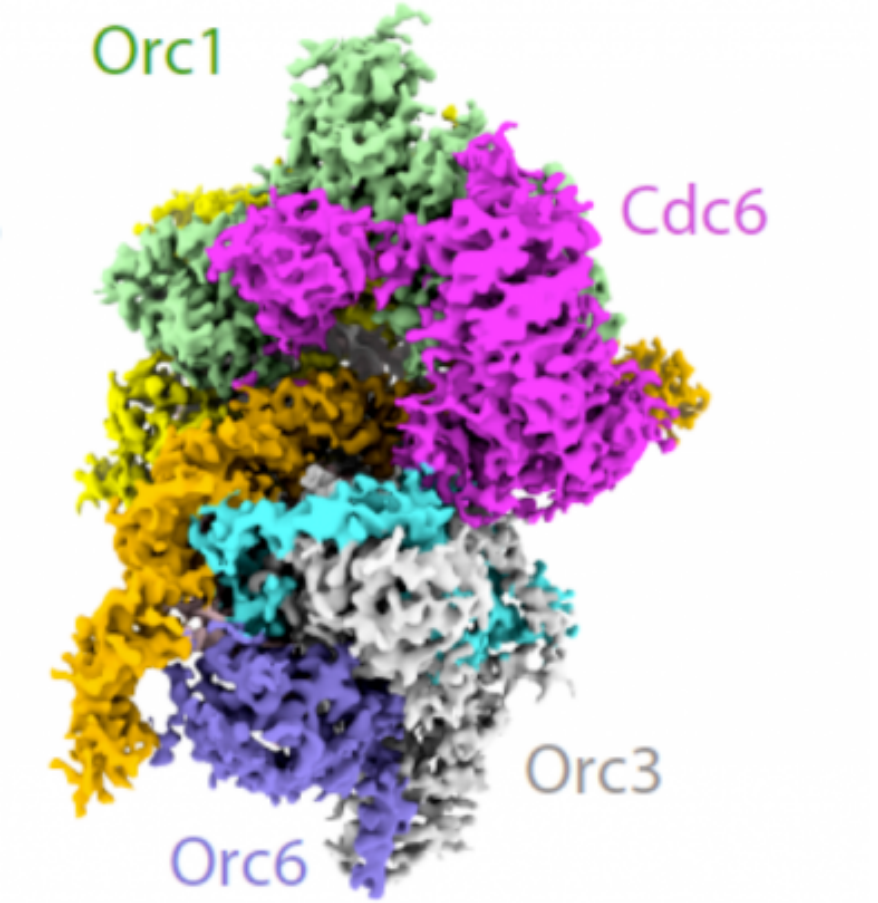


cdc-6

CDC6 is an ATP binding protein; CDC6 assembles after ORC in an ATP dependent manner and is required for loading MCM proteins onto the DNA.

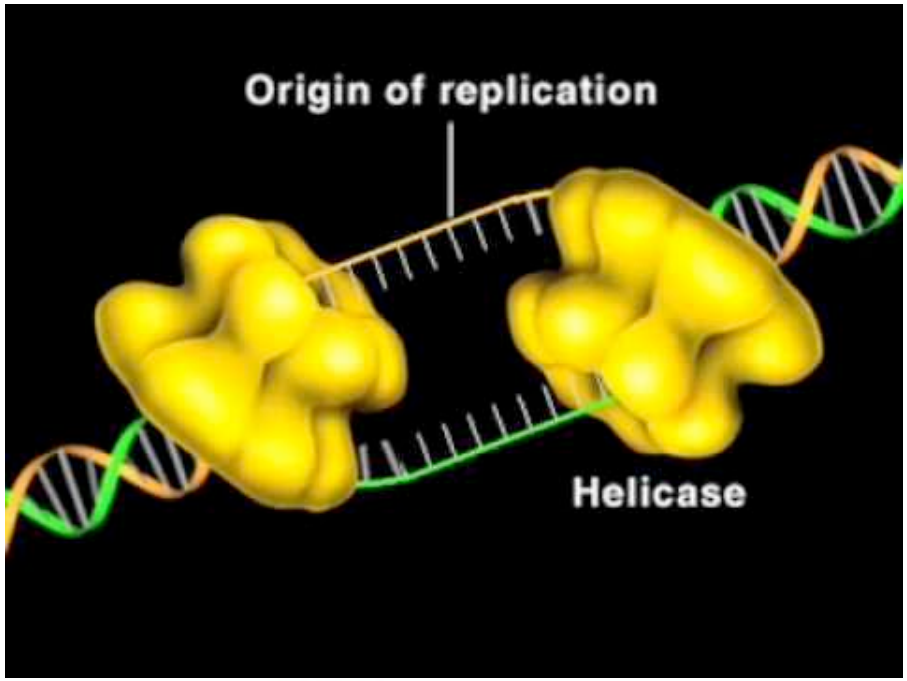


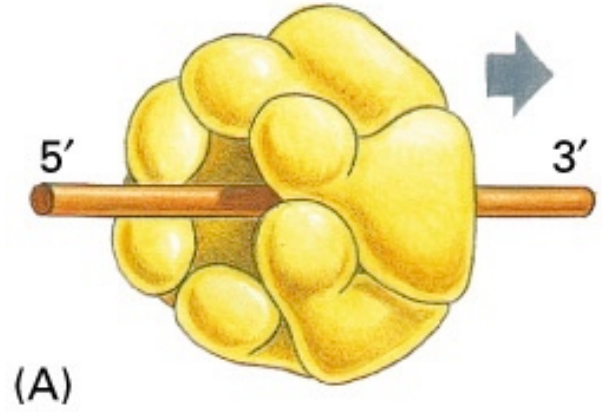




Mcm2-7

MCM2-7 helicase function arises from its architecture.



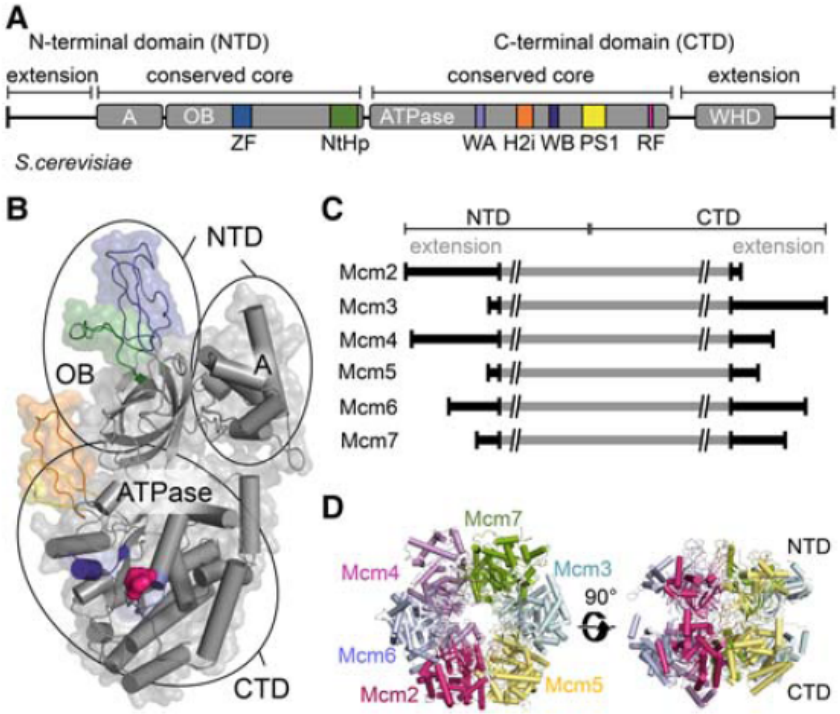


REVIEW

From structure to mechanism—understanding initiation of DNA replication

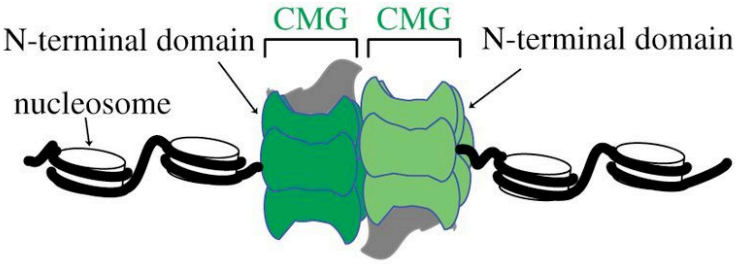
Alberto Riera,[1] Marta Barbon,[1,2,3] Yasunori Noguchi,[1,3] L. Maximilian Reuter,[1,3] Sarah Schneider,[1,3] and Christian Speck[1,2]

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

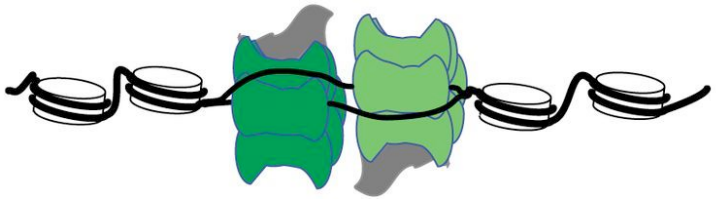


Mechanism of action of Mcm2-7

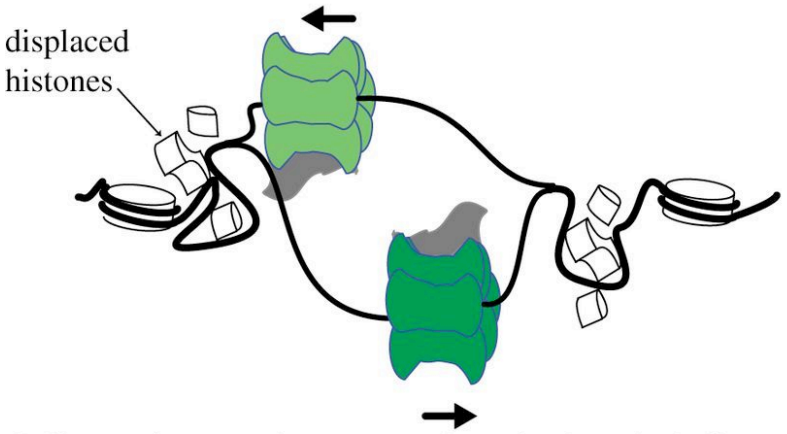
Il complesso elicasico “abbraccia” il DNA a doppia elica



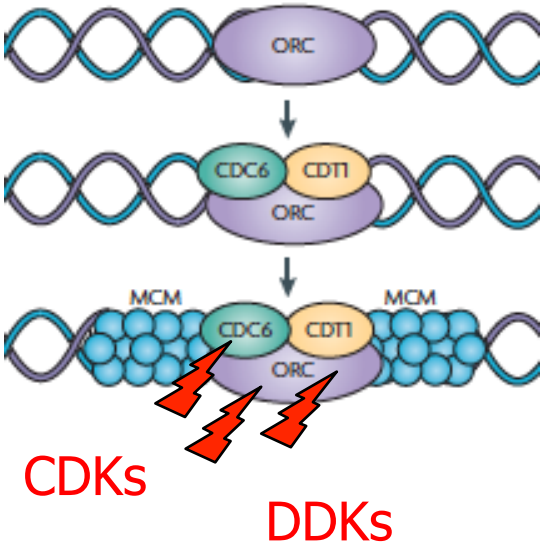
I due filamenti sono separati e uno dei due è incanalato nello spacio centrale del complesso proteico



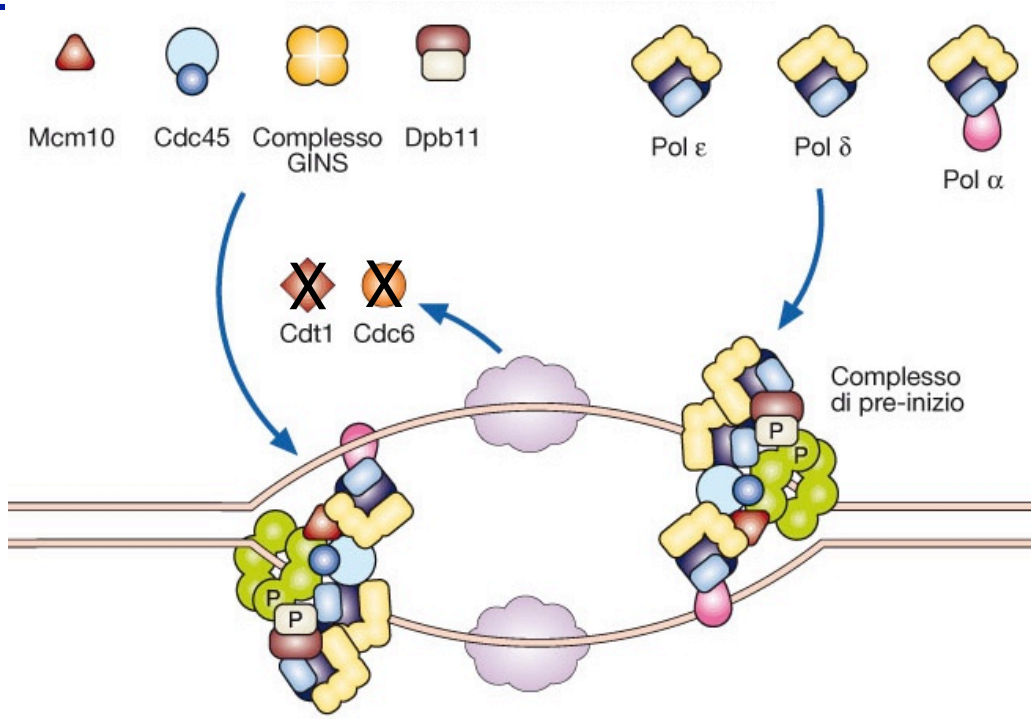
Successivamente le due elicasi si allontanano in direzione opposta, creando la bolla di replicazione



I- The Pre-RC (G1)



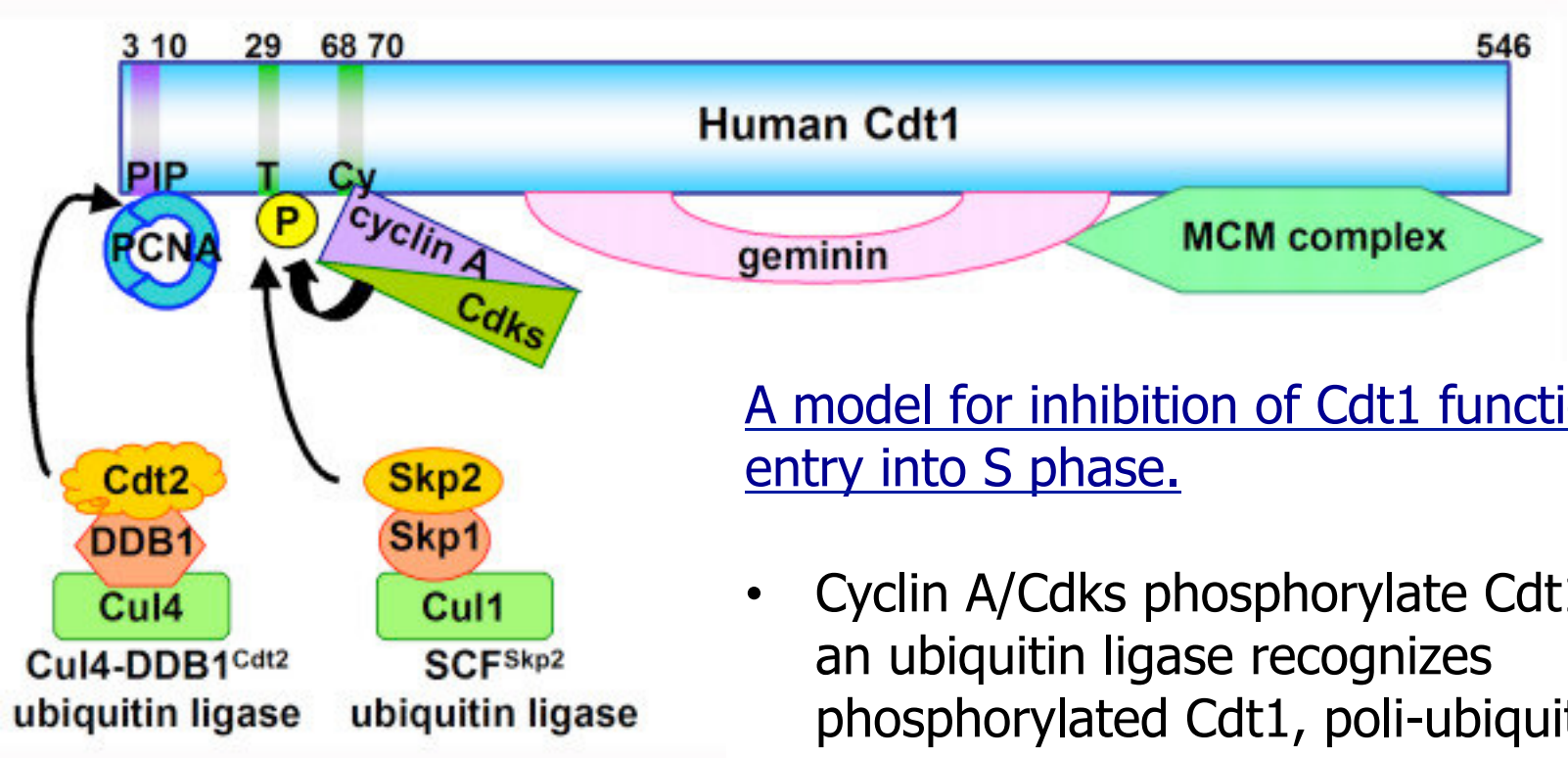
II- The Pre-IC (G1-S)



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

- La fosforilazione di ORC da parte delle chinasi ciclina-dipendenti (CDKs) provoca il distacco di **Cdc6** e **Cdt1** (che se fosforilato, viene degradato) e il contemporaneo aggancio di altre proteine (tra cui Mcm10, cdc45, Dpb11 e il complesso GINS).
- 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.

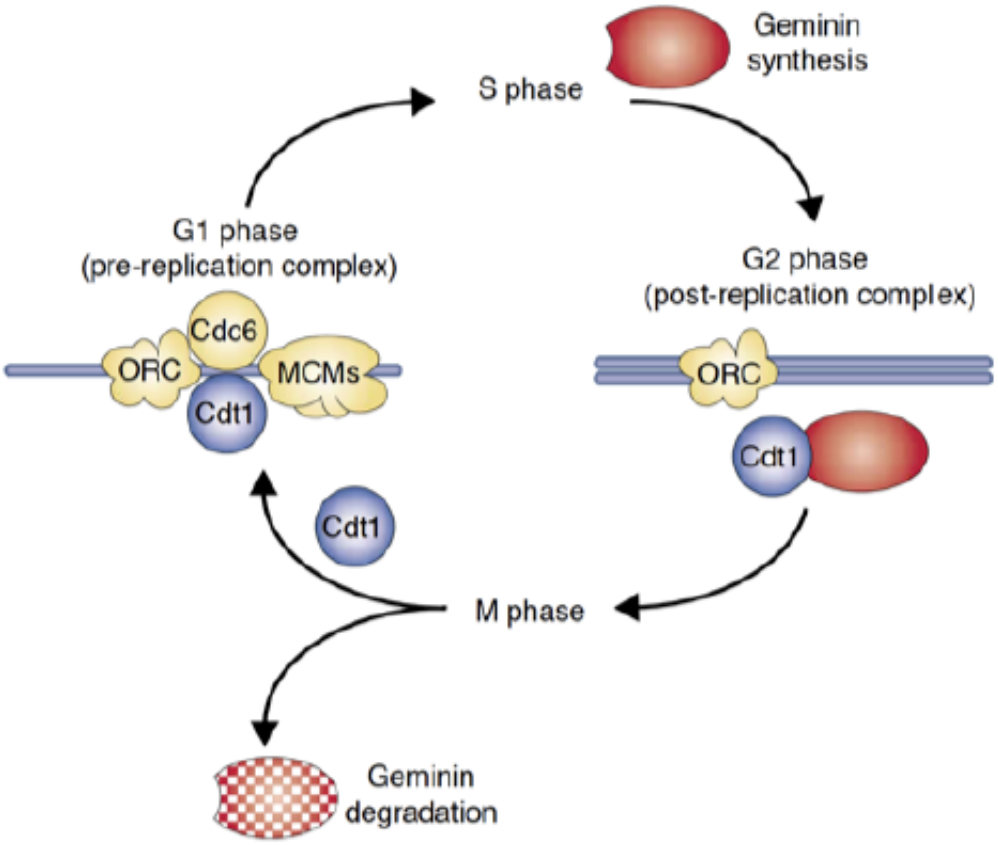
CDT1 (Chromatin licensing and DNA replication factor 1)

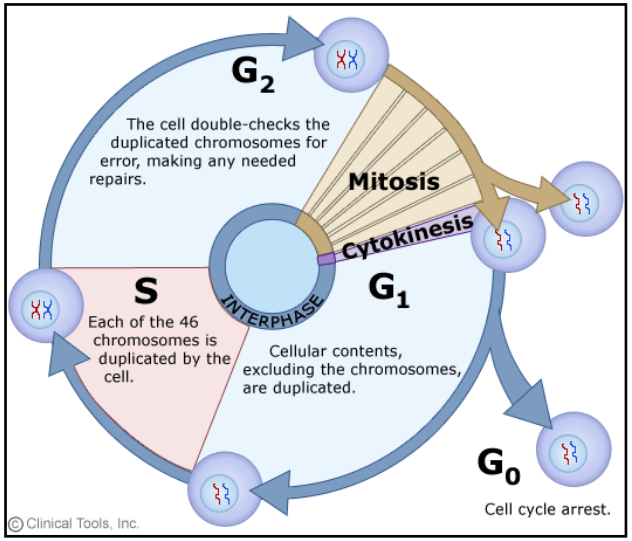


A model for inhibition of Cdt1 function after entry into S phase.

- 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.

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



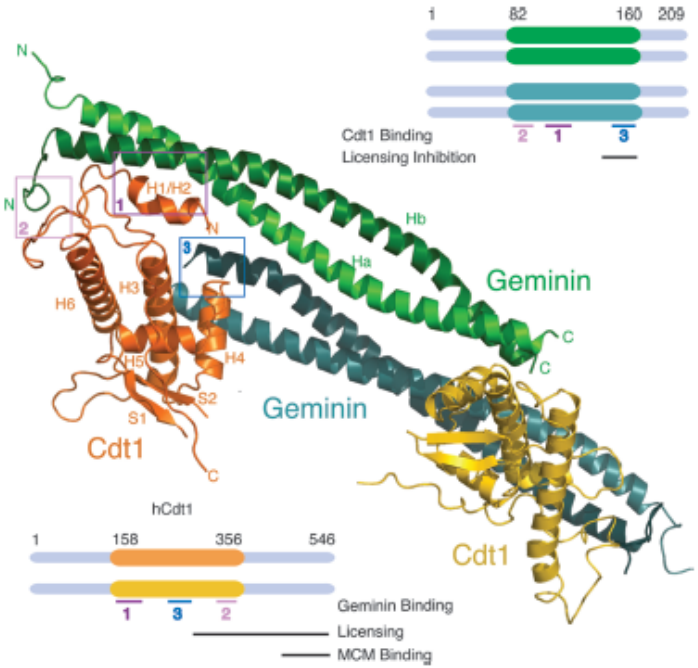


- ✓ Durante la fase G2, Cdt1 e' sequestrato da GEMININ
- ✓ All'entrata in G1, geminin e' degradata, rilasciando Cdt1 che quindi puo' legare ORC insieme a cdc6, promuovendo il legame di mcm2-7 e la formazione del Pre-Replication Complex.
- ✓ In S, Geminin, nuovamente sintetizzata, lega Cdt1 durante le fasi S e G2, impedendo cosi' che il DNA subisca piu' rounds di replicazione all'interno dello stesso ciclo cellulare.

PNAS | November 24, 2009 | vol. 106 | no. 47 | 19807–19812

Quaternary structure of the human Cdt1-Geminin complex regulates DNA replication licensing

V. De Marco[a,1], P. J. Gillespie[b,2], A. Li[b,2,3], N. Karantzelis[c], E. Christodoulou[a,1], R. Klompmaker[d], S. van Gerwen[a], A. Fish[a], M. V. Petoukhov[e], M. S. Iliou[f,4], Z. Lygerou[f], R. H. Medema[d], J. J. Blow[b,5], D. I. Svergun[e], S. Taraviras[c], and A. Perrakis[a,5]



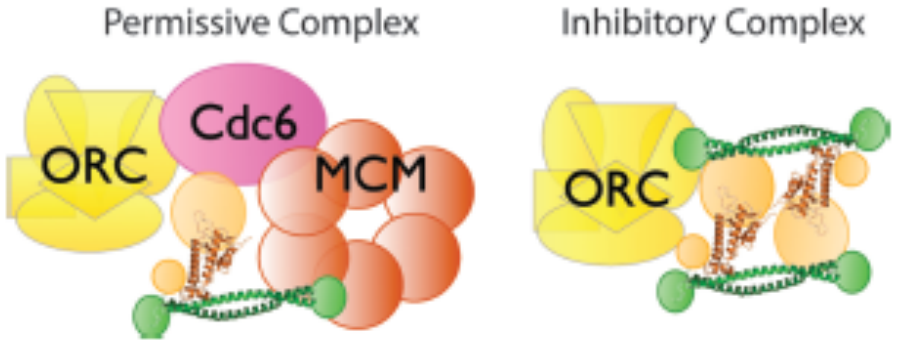
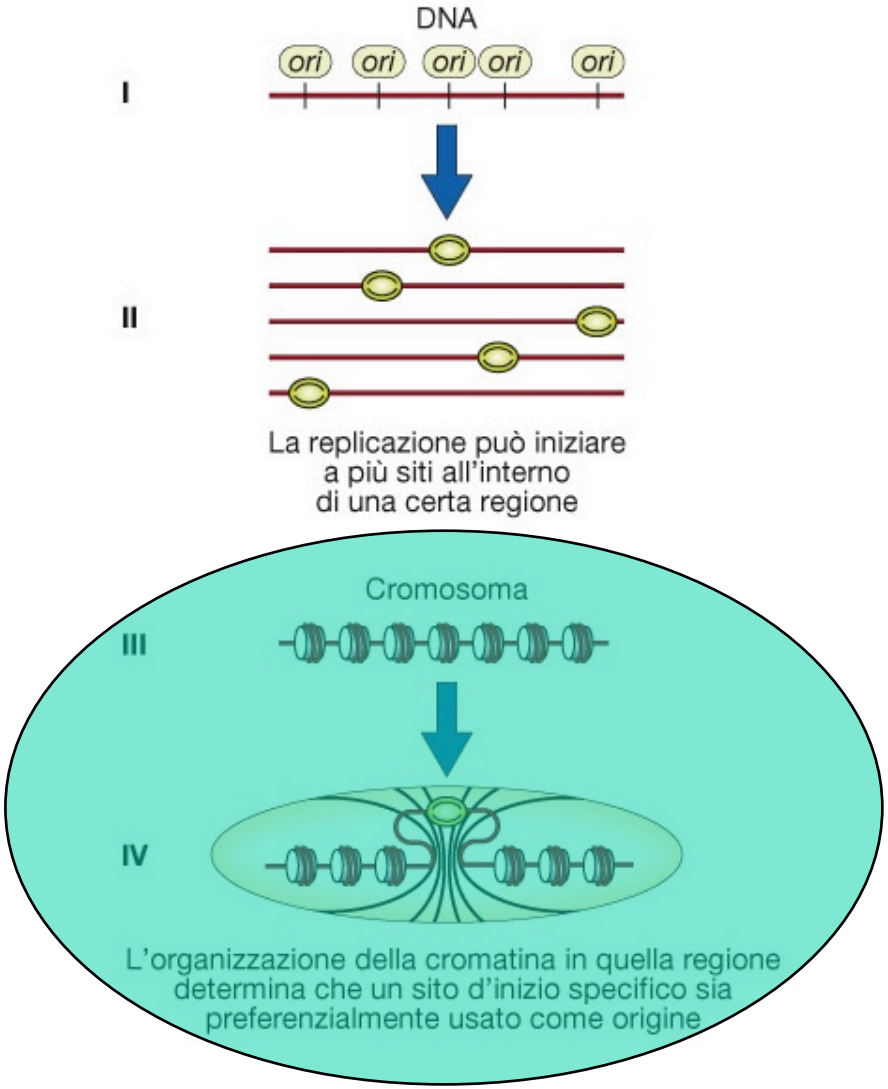


Fig. 6. Proposed model for the mechanism of DNA licensing inhibition by Geminin.

The molecular model for Geminin activity involves an equilibrium between a heterotrimer and a heterohexamer, whose relative abundance is regulated during the cell cycle. The heterohexamer represents an inhibitory complex, which prohibits DNA licensing.

The heterotrimer, where critical Cdt1 residues are exposed to engage in replication licensing (by promoting MCM chromatin association) represent a permissive complex that allows licensing.

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

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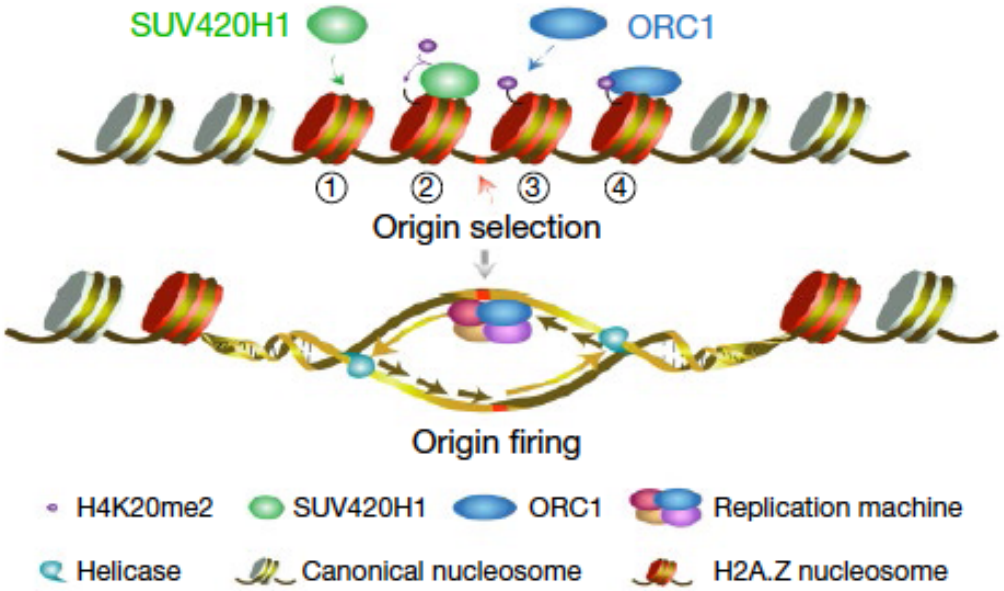
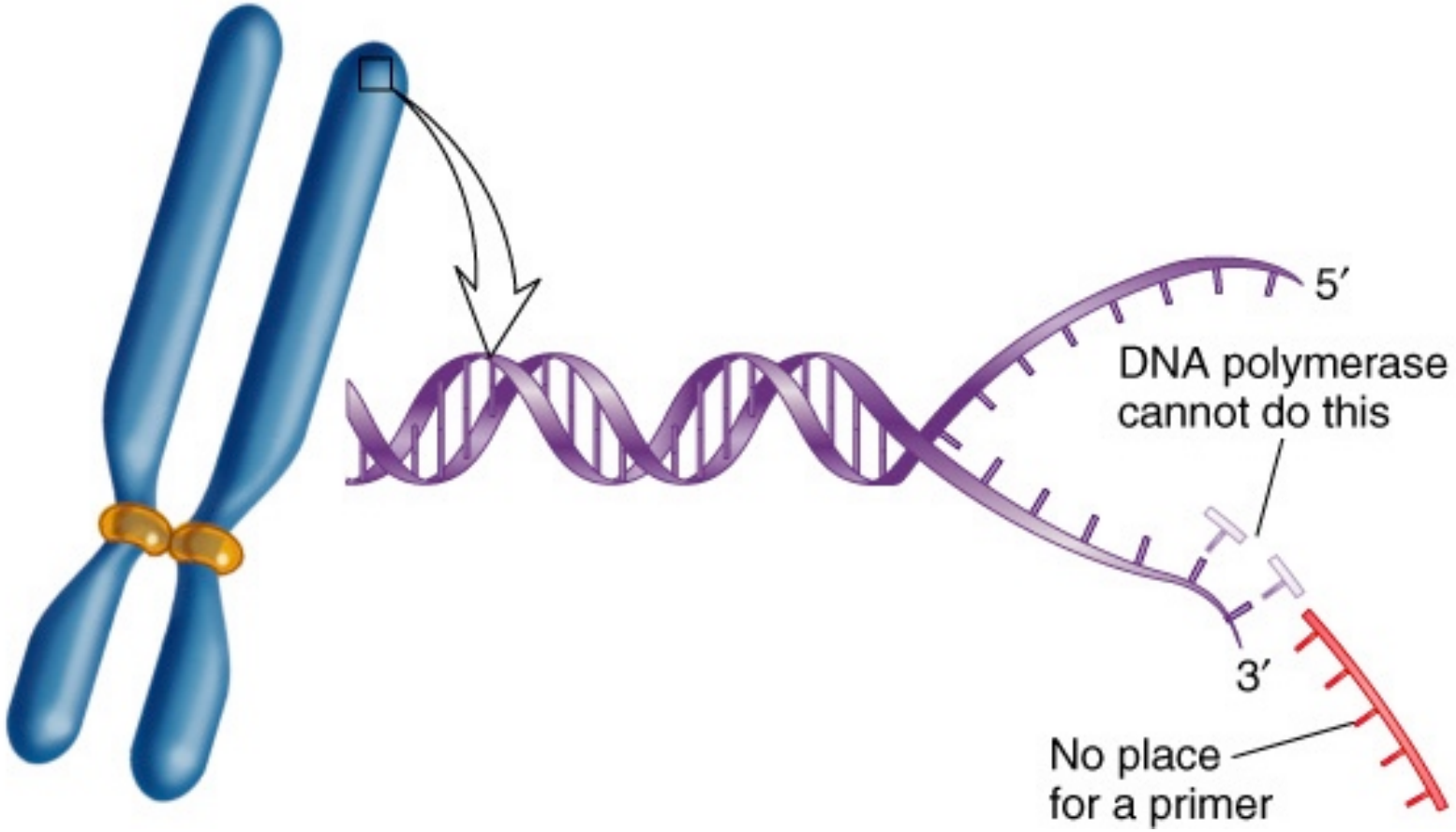
Haizhen Long[1,2,10], Liwei Zhang[1,10], Mengjie Lv[3,10], Zengqi Wen[1,2,10], Wenhao Zhang[4], Xiulan Chen[2,5], Peitao Zhang[6], Tongqing Li[7], Luyuan Chang[1,2], Caiwei Jin[2,3], Guozhao Wu[1,2], Xi Wang[8], Fuquan Yang[2,5], Jianfeng Pei[7], Ping Chen[1], Raphael Margueron[9], Halteng Deng[4], Mingzhao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

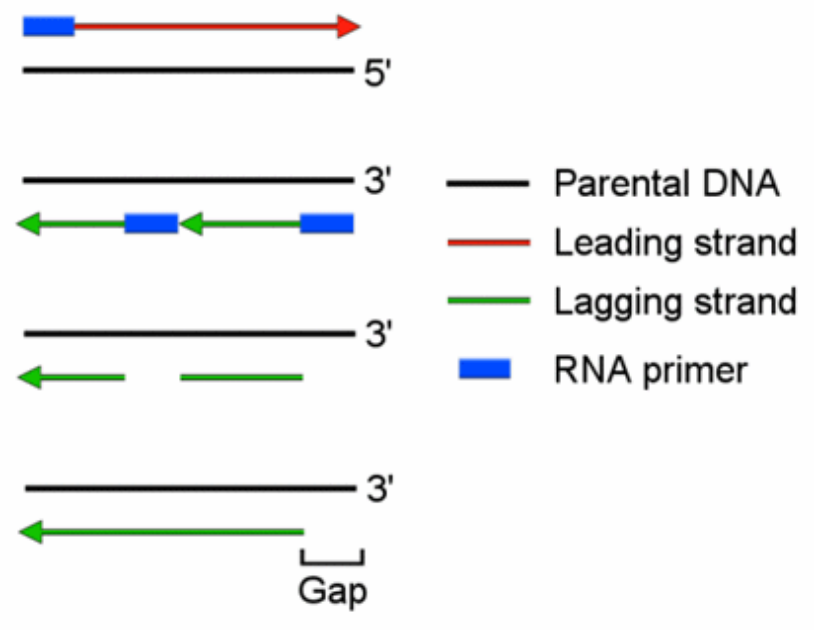
The Problem at the ends of eukaryotic linear Chromosomes



- 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

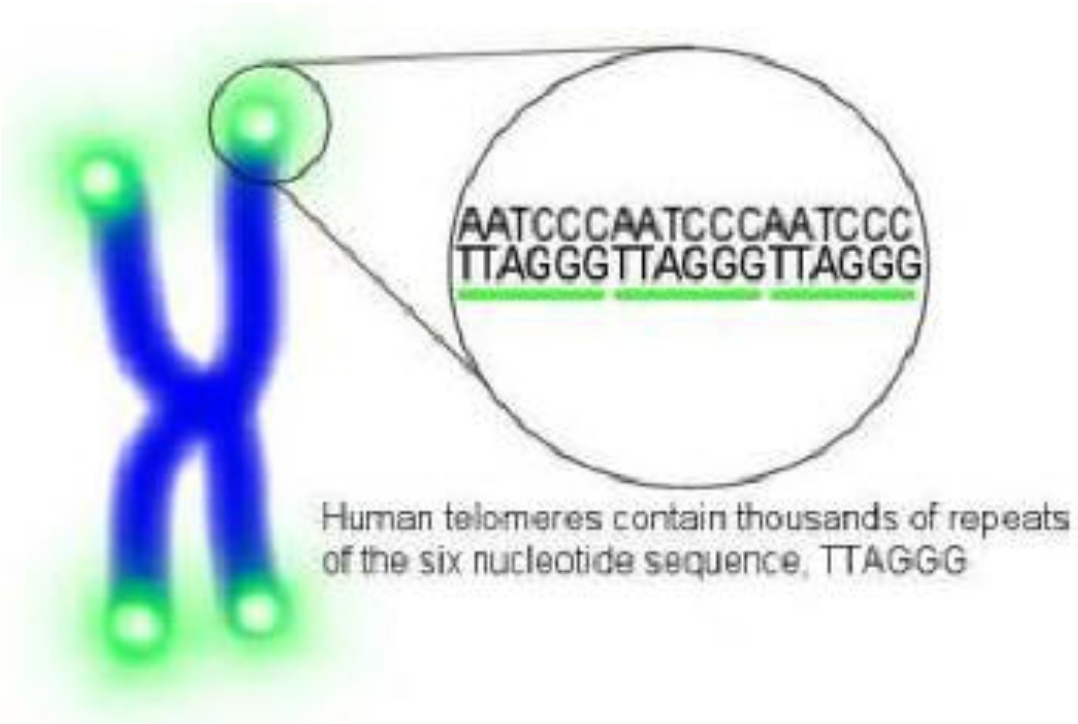
The Problem at the ends of eukaryotic linear Chromosomes

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.

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.

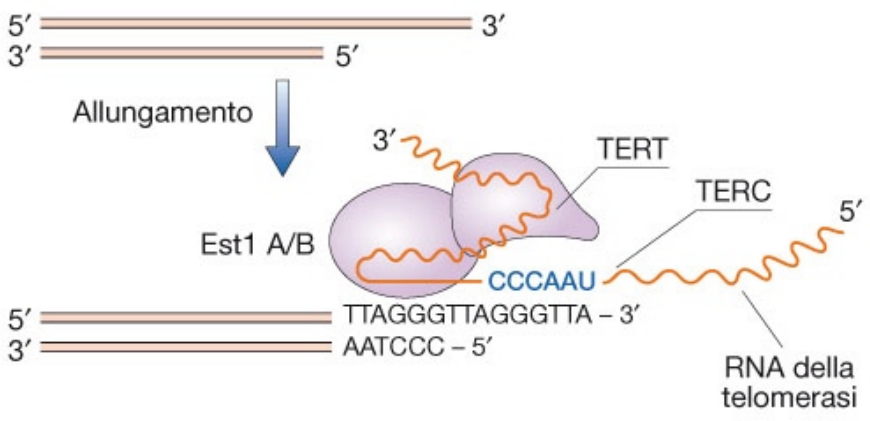
Rune Toftgard is a Nobel Committee member from Sweden's Karolinska Institute. He says the work of telomeres is important to the understanding of how genetic material is copied and saved.

La Telomerasi

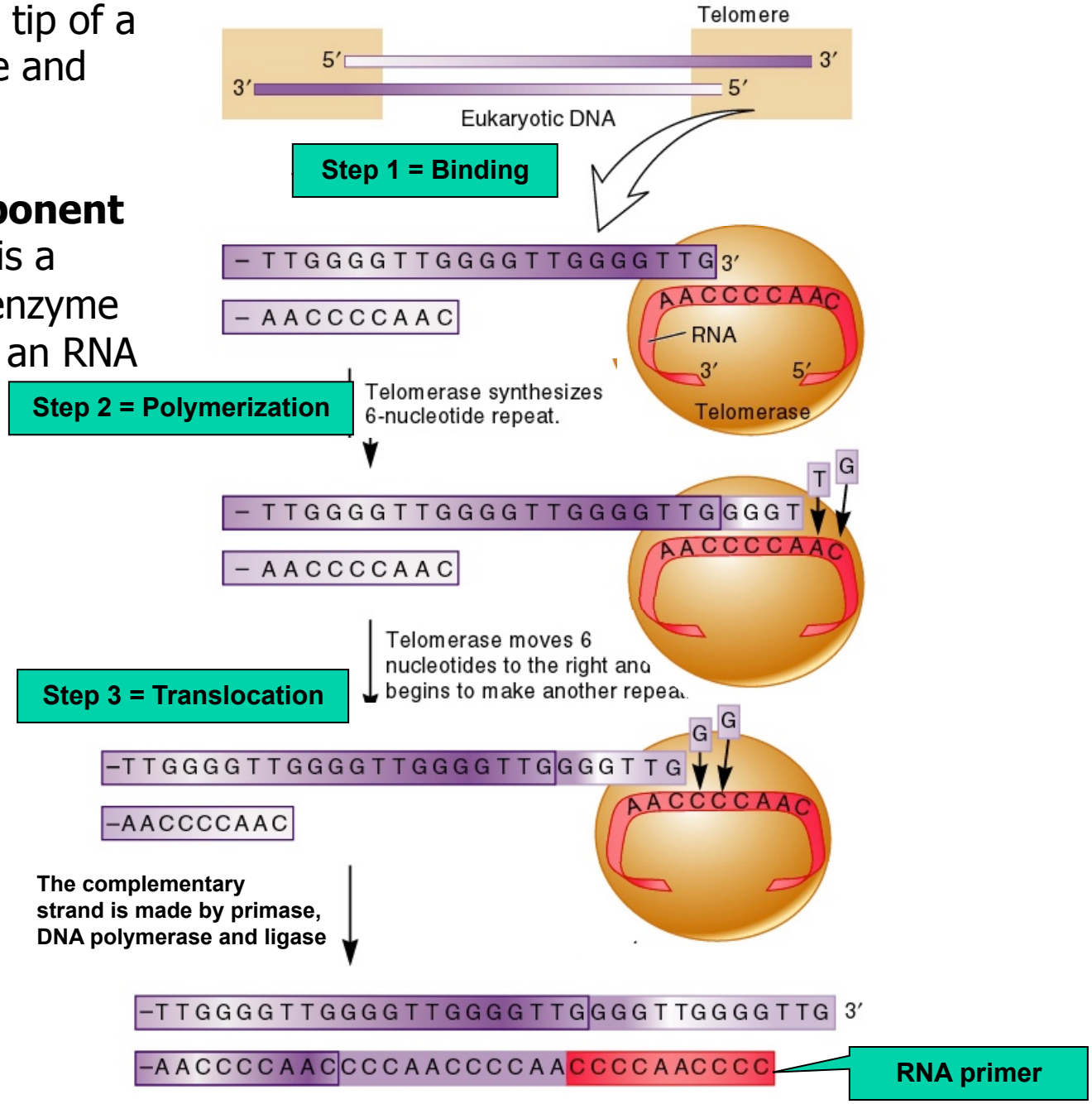
E’ una ribonucleoproteina costituita da due componenti principali:

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



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.

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).

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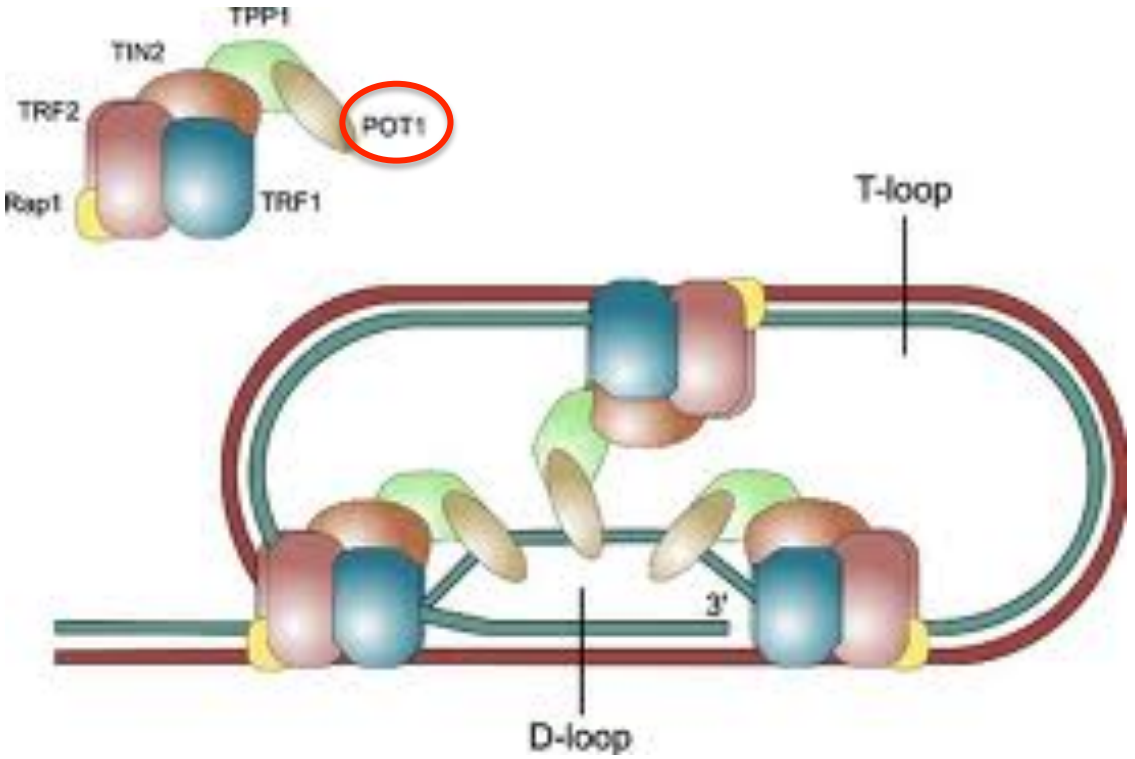
Eccezioni: cellule in attiva divisione: cellule ovaio, testicolo, cellule epiteliali proliferanti, linfociti, cellule embrione

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



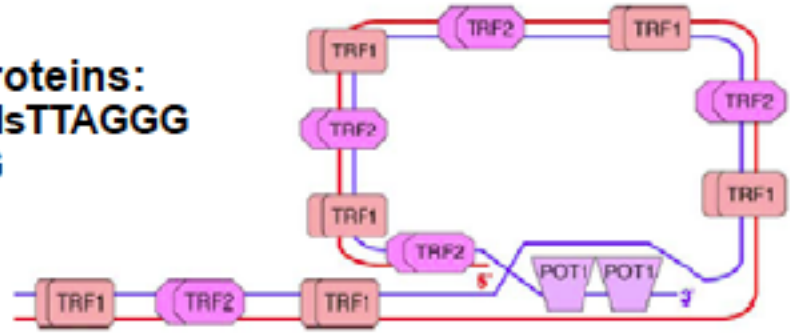
Telomere structure

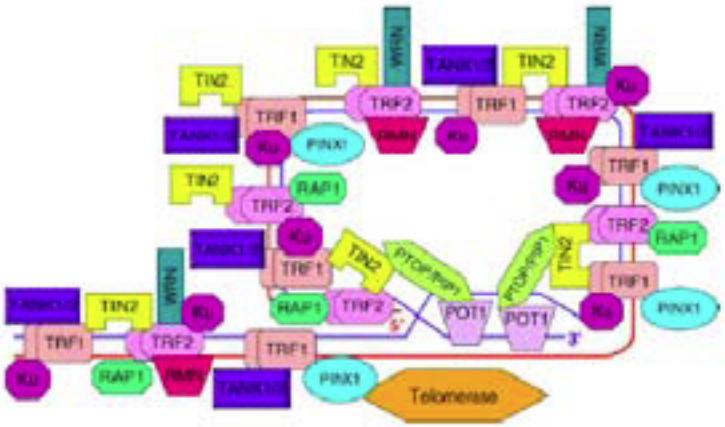
Vertebrate telomeres are long stretches (1-50 kb) of dsDNA containing the repetitive sequence TTAGGG, which terminate in 100-200 bases of ss TTAGGG at the 3' end. This 3' overhang circles back end embed in the duplex DNA

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**



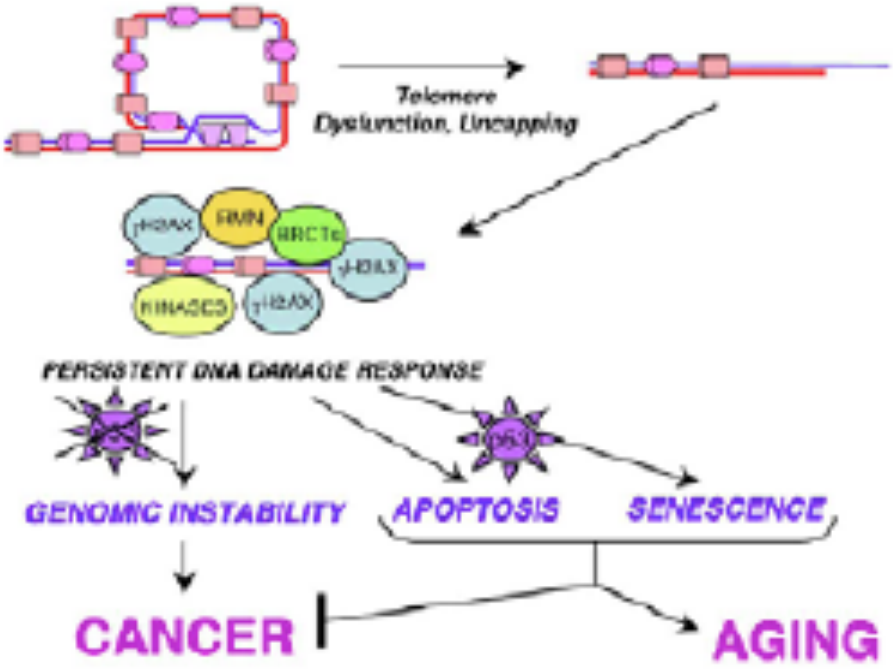


The extended telomeric cap helps maintain the stability of the genome

Telomere uncapping

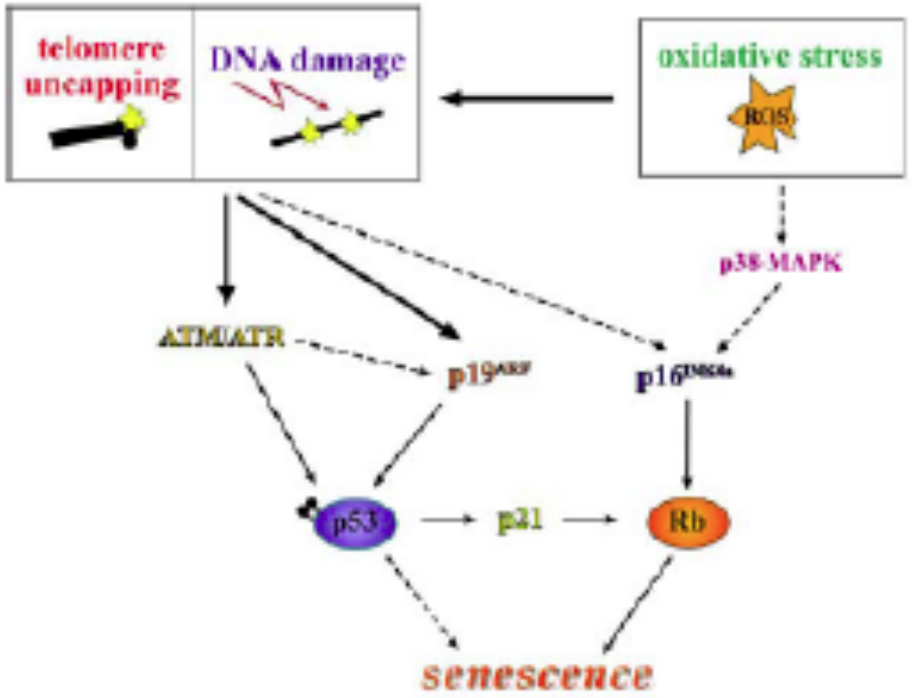
Senescent telomeres lose some of their single-stranded portion - the telomeric overhang - which is crucial for the maintenance of the T-loop and the subsequent formation of the cap

*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?

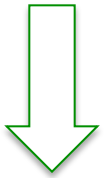
-Different post-translational modifications of p53 in response to different stimuli?

-Binding of different proteins to p53?

-Activation of different sets of transcriptional targets?

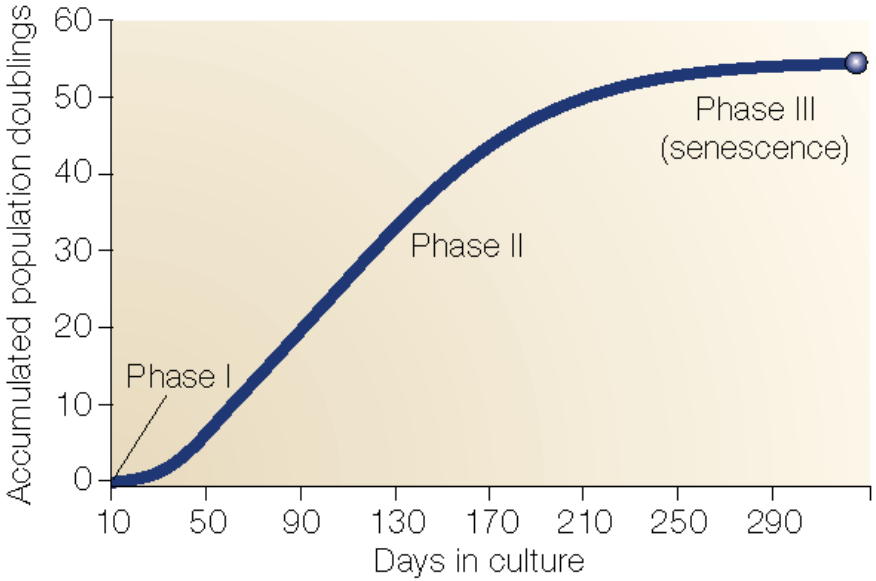
Regolazione lunghezza telomeri

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)

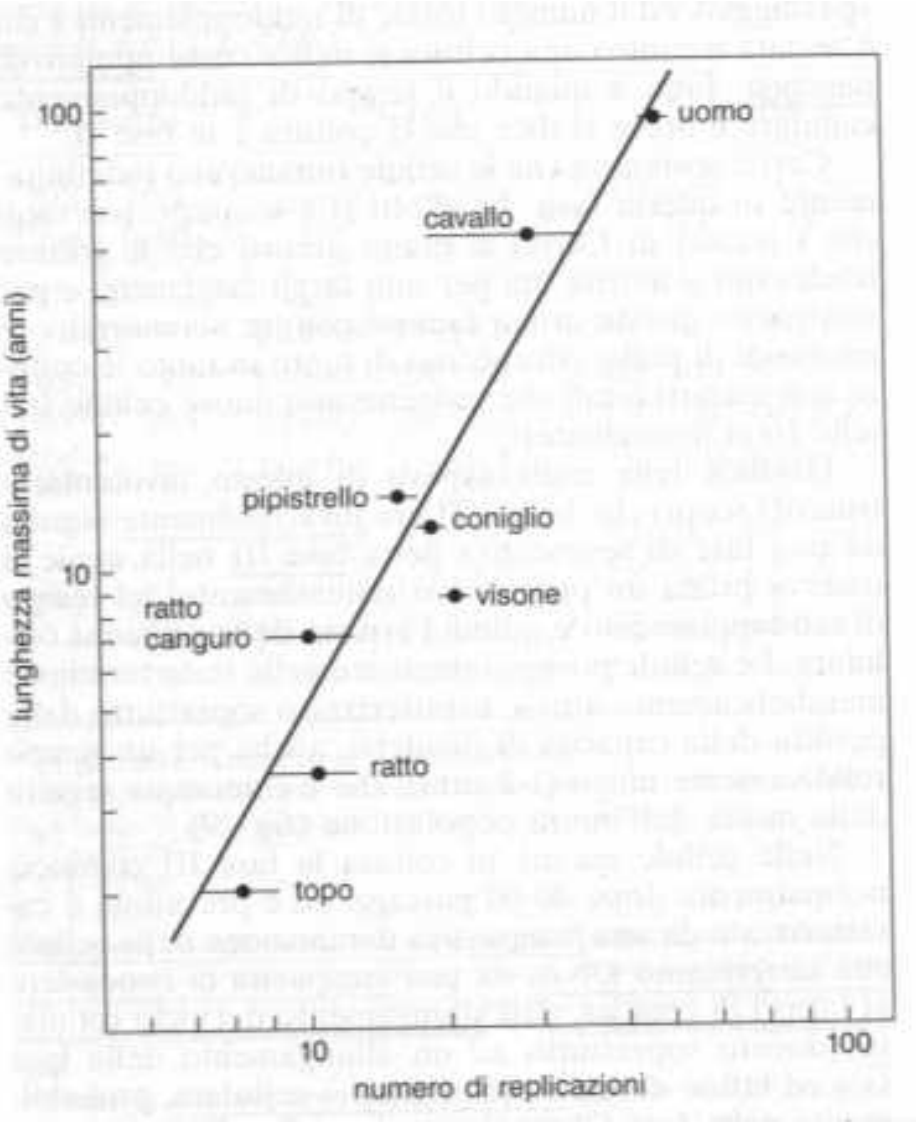


Nature Reviews | Molecular Cell Biology

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.
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)



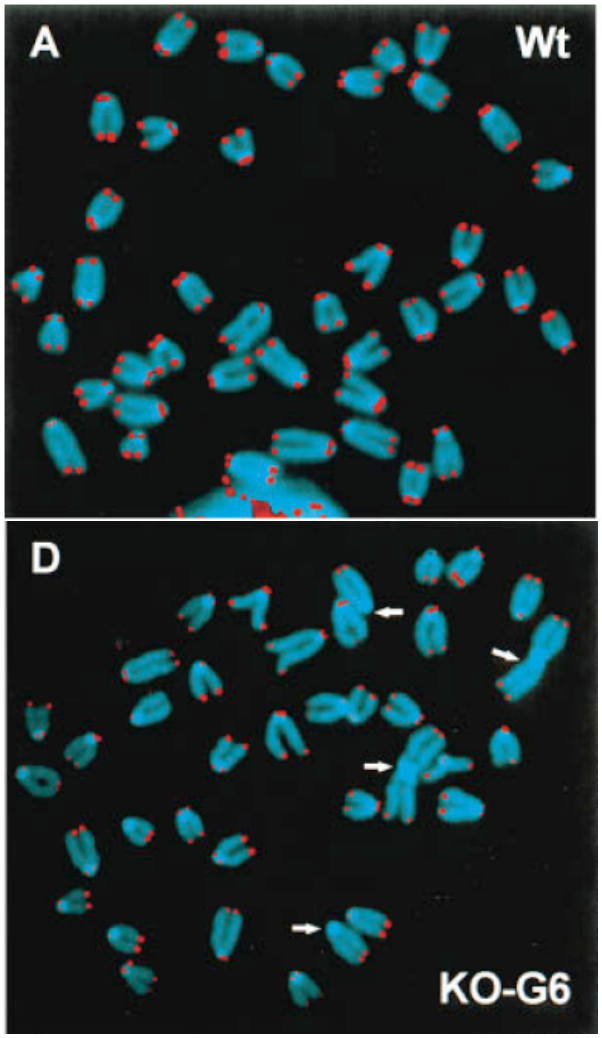
Cell, Vol. 91, 25–34, October 3, 1997, Copyright ©1997 by Cell Press

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/ 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).

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Telomerase Reverse Transcriptase Delays Aging in Cancer-Resistant Mice

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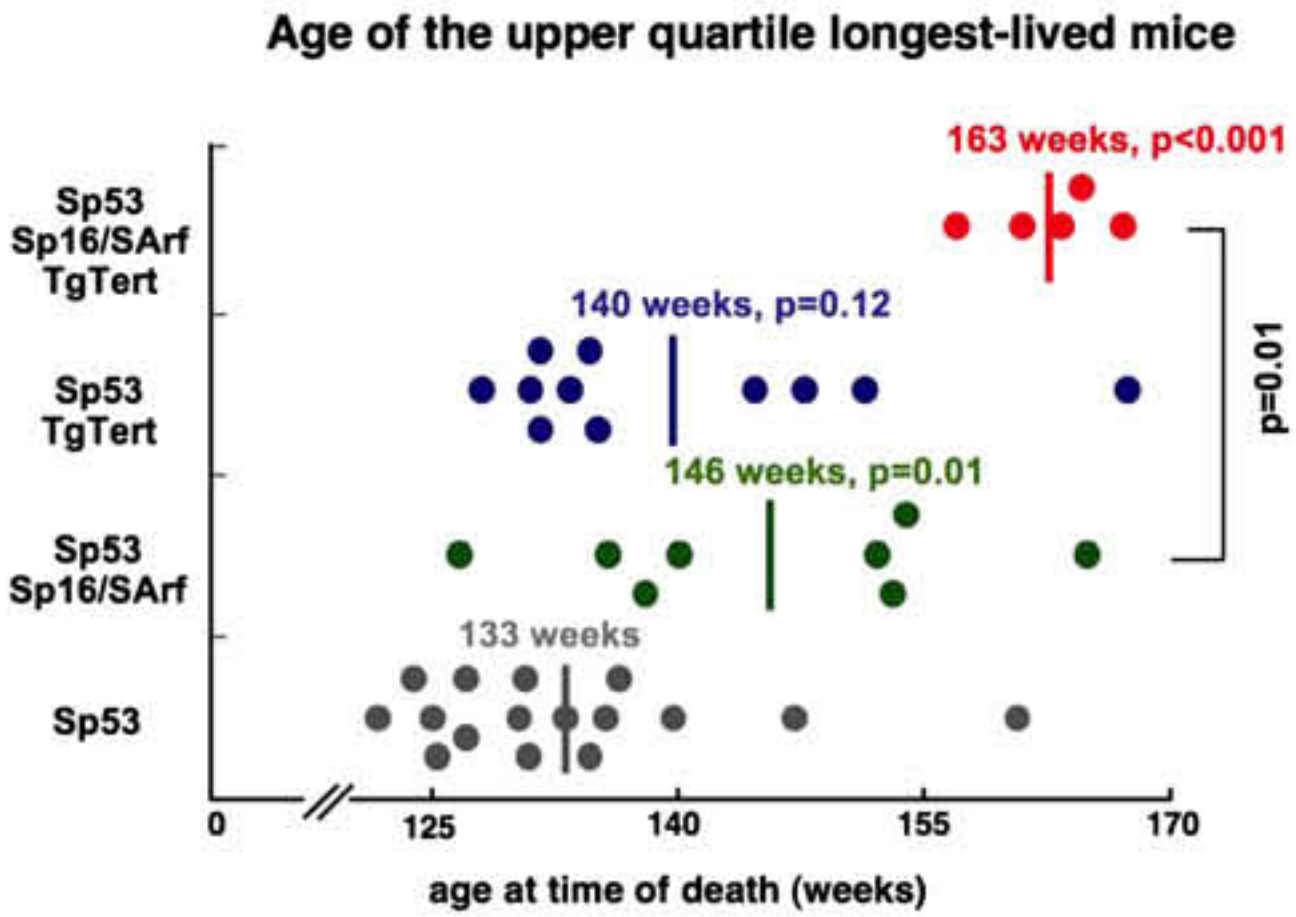
Telomerase confers limitless proliferative potential to most human cells through its ability to elongate telomeres, the natural ends of chromosomes, which otherwise would undergo progressive attrition and eventually compromise cell viability. However, the role of telomerase in organismal aging has remained 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 suppressors p53, p16, and p19ARF (these three tumor suppressors are involved in protection against a large variety of cancers-Collado et al., 2007). In this context TERT overexpression 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)

LETTER

102 | NATURE | VOL 469 | 6 JANUARY 2011

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Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice

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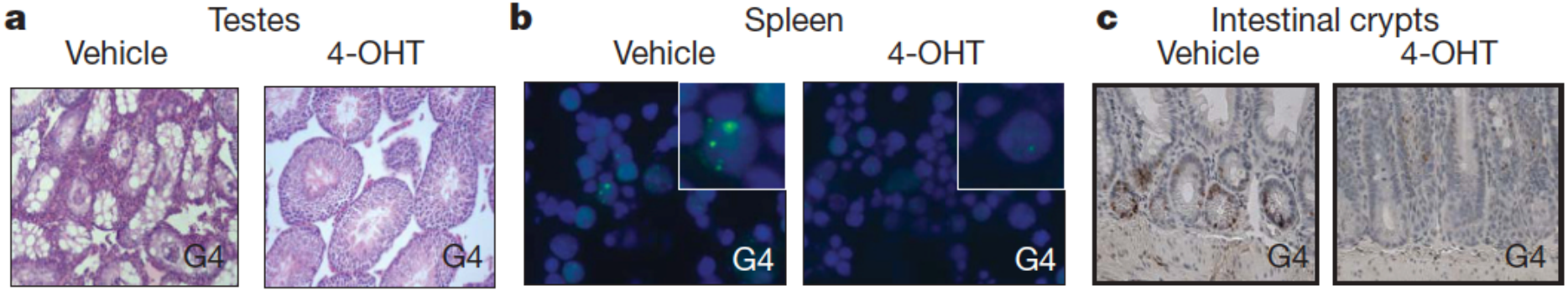
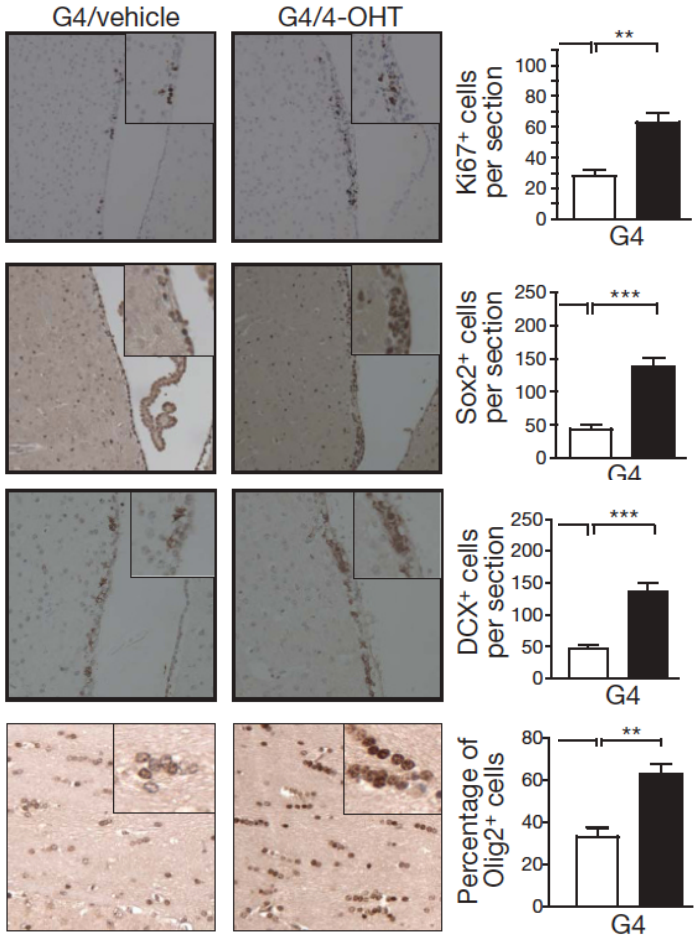
- A novel mouse model to explore the impact of physiological telomerase reactivation across diverse adult cell types and organ systems.
- Notably, the mice enlisted into this study are adults exhibiting significant **progeroid phenotypes** (mice null for mTerc or mTert).
- In TERT-ER mice with advanced 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.