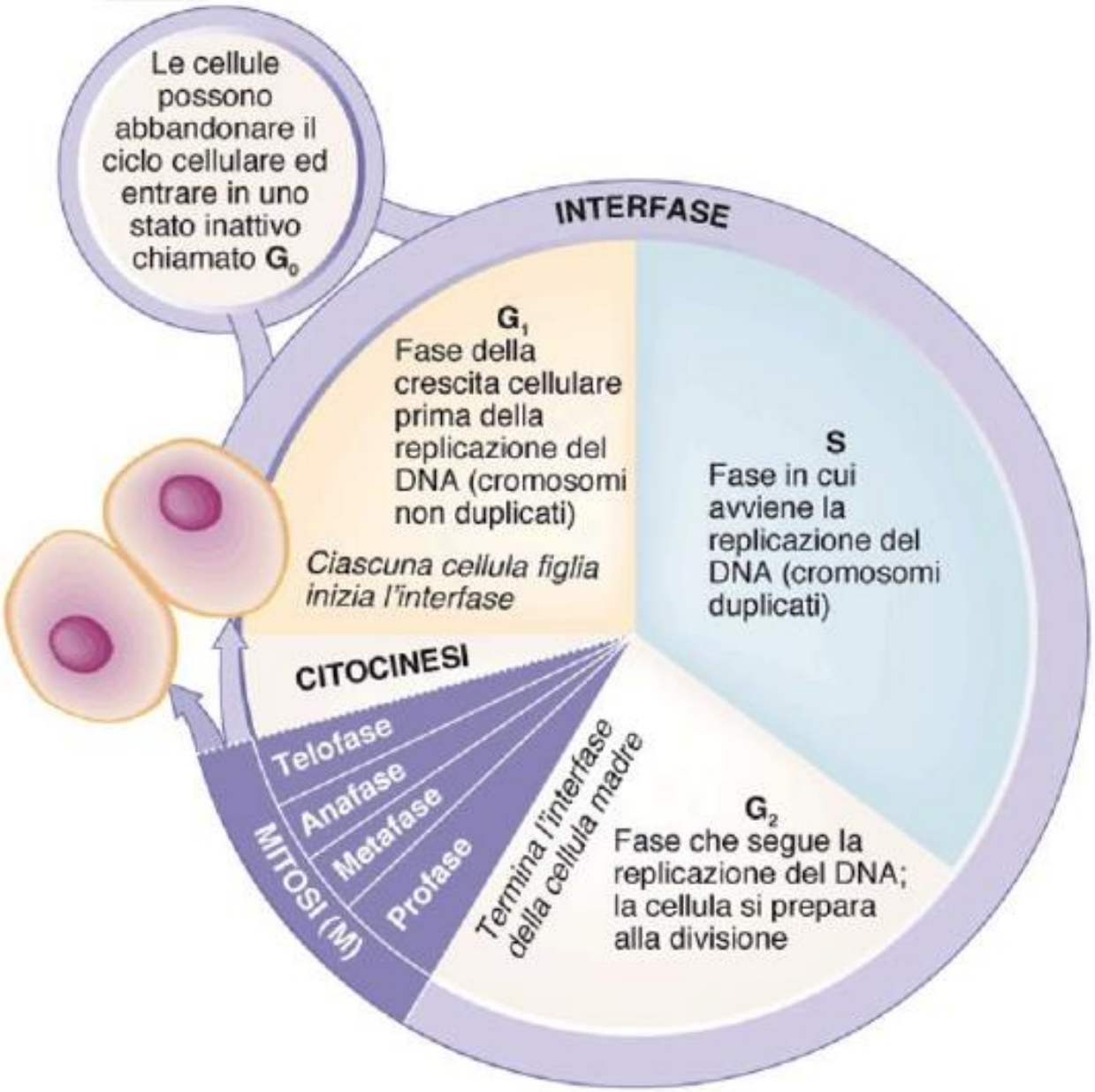
CICLO CELLULARE

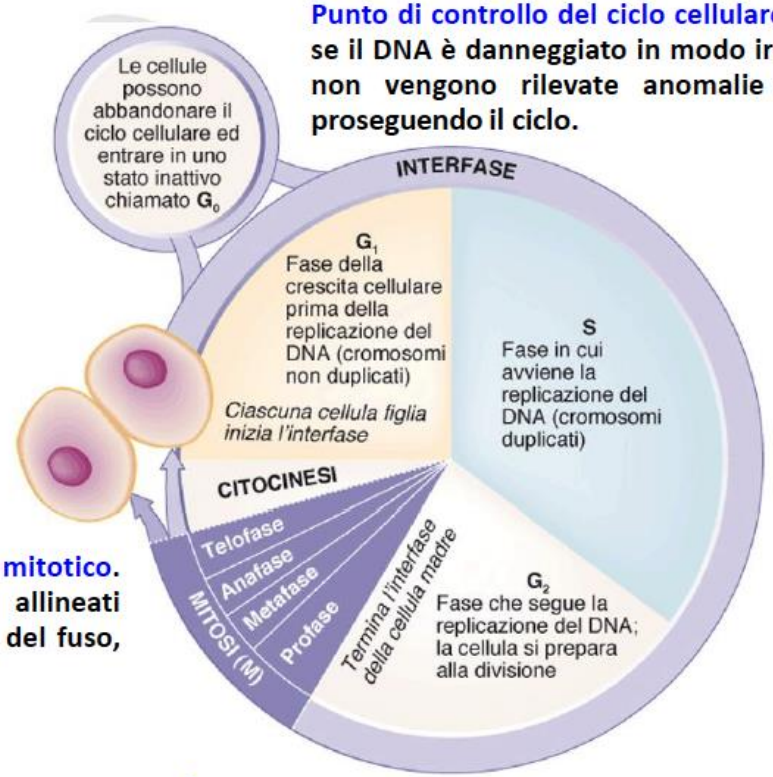


CICLO CELLULARE: checkpoints

Il ciclo cellulare presenta precisi punti di controllo che consentono la continuazione del ciclo solo se non vengono riscontrate anomalie. In caso contrario, la cellula va incontro al processo di apoptosi (morte cellulare programmata).

1. Punto di controllo G_1

Punto di controllo del ciclo cellulare. La cellula entra nella fase G_0 o, se il DNA è danneggiato in modo irreparabile, avviene l'apoptosi. Se non vengono rilevate anomalie la cellula entra in divisione, proseguendo il ciclo.



3. Punto di controllo M

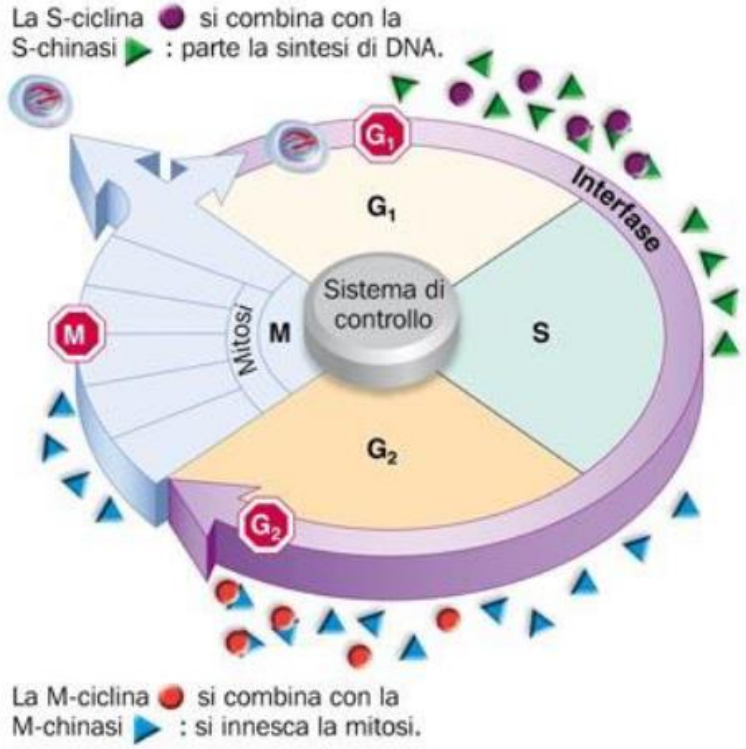
Punto di controllo del fuso mitotico. Se i cromosomi non sono allineati correttamente lungo le fibre del fuso, la mitosi si arresta.

2. Punto di controllo G_2

Punto di controllo della mitosi. La divisione mitotica avviene solo se il DNA è duplicato correttamente, altrimenti, se il DNA risulta danneggiato in modo irreparabile, la cellula va incontro al processo di apoptosi.

CONTROLLO del CICLO CELLULARE

Il ciclo cellulare non procede in modo casuale: esistono diversi punti di controllo che rispondono a molecole segnale che stimolano o inibiscono un preciso evento del ciclo.



Le molecole segnale interne del ciclo cellulare sono le chinasi e le cicline.

Cicline

La concentrazione delle cicline varia nel corso del ciclo cellulare

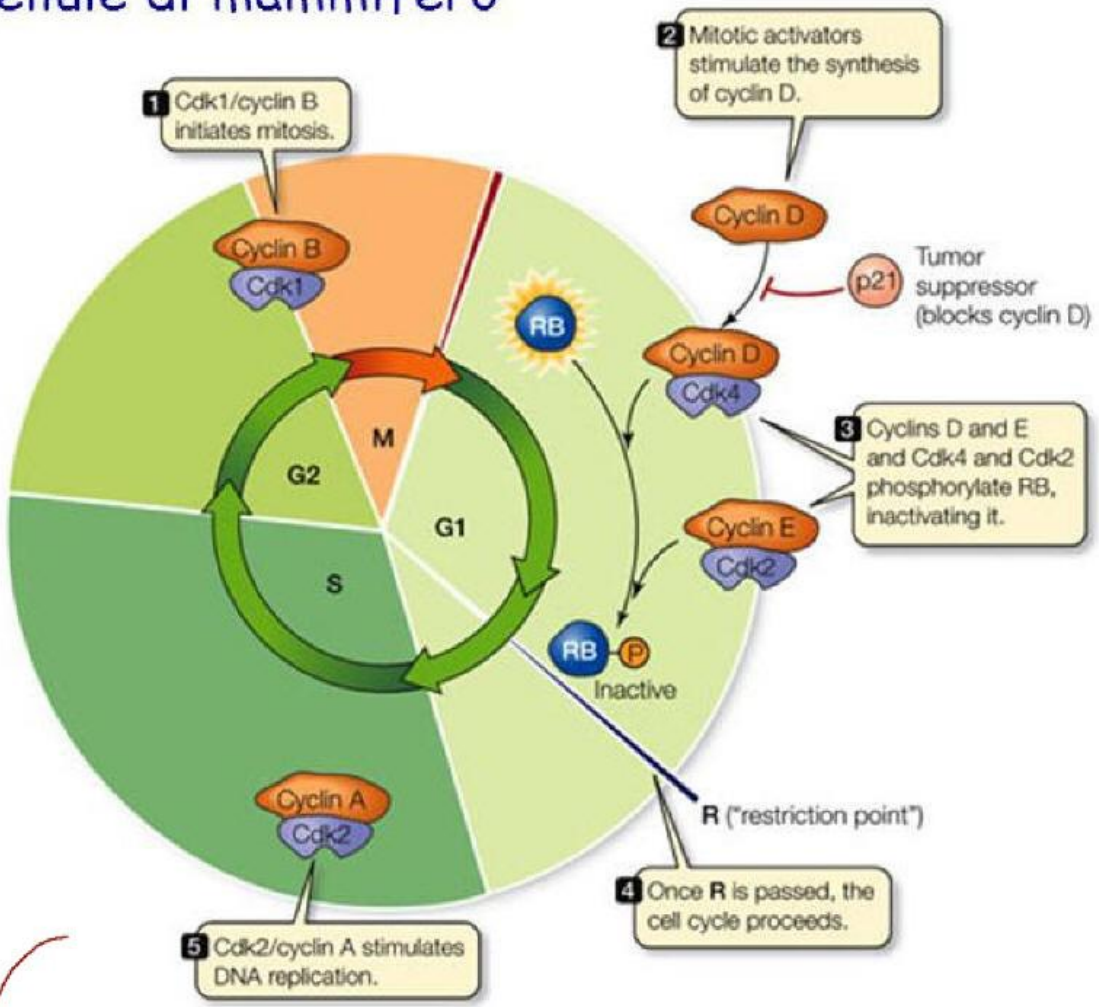
Esse attivano o inattivano le protein chinasi.

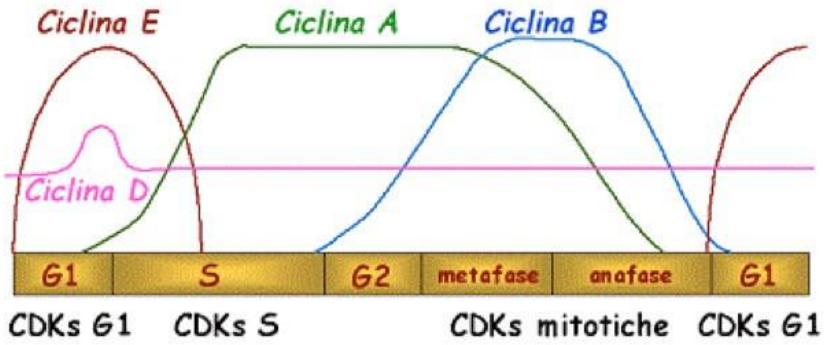
Chinasi–ciclina-dipendenti (CdK)

Le protein chinasi sono presenti durante tutte le fasi, ma vengono attivate dalle cicline solo in particolari momenti del ciclo cellulare, per essere, poi, disattivate rapidamente.

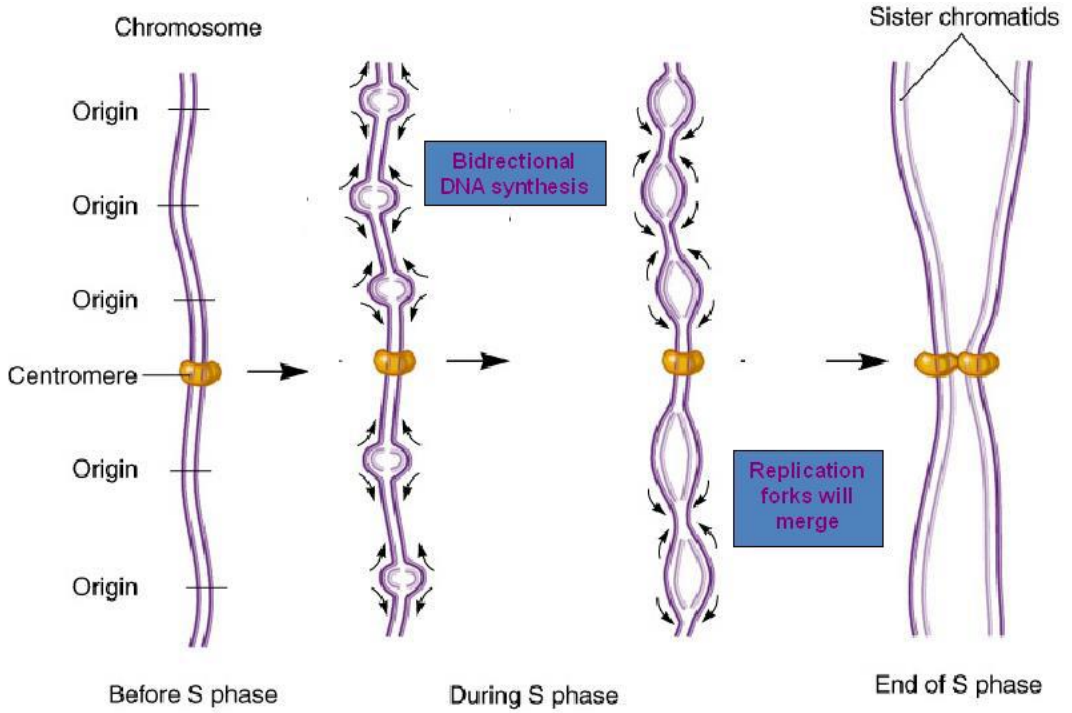
Durante il ciclo cellulare cambia l'attività di queste molecole ma non la concentrazione.

Diverse cdk e diverse cicline regolano il passaggio attraverso le varie fasi del ciclo nelle cellule di mammifero



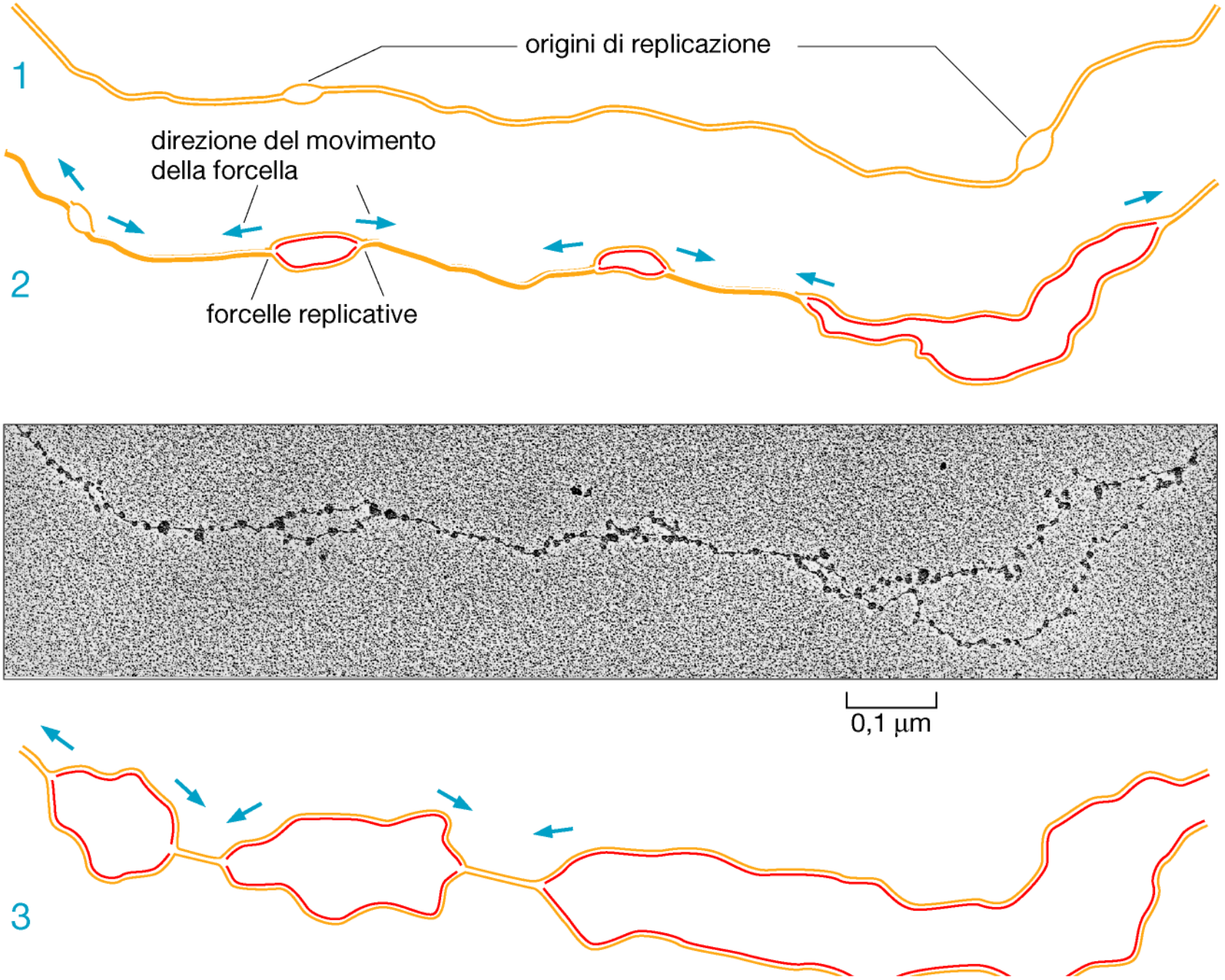


DNA synthesis begins at replication origins

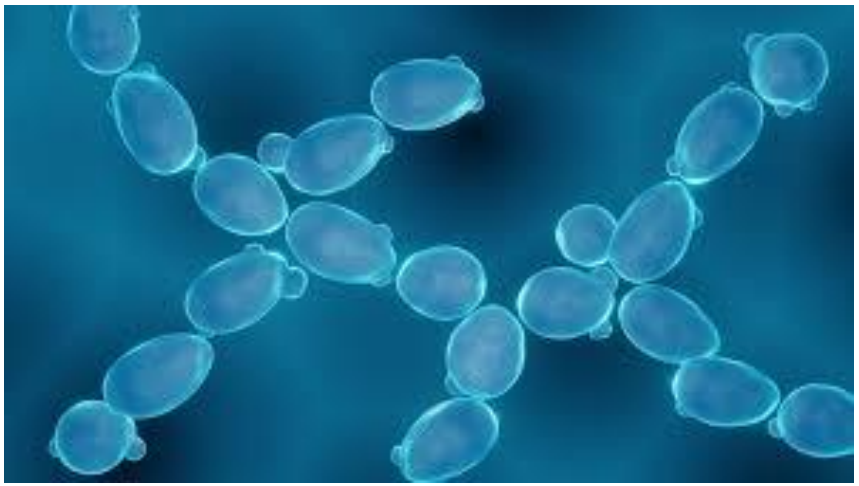


Ciascun cromosoma eucariota e' organizzato in molteplici Unita' di Replicazione, dette **repliconi**, che comprendono 50-80 origini, spaziate da 30.000-300.000 nt.

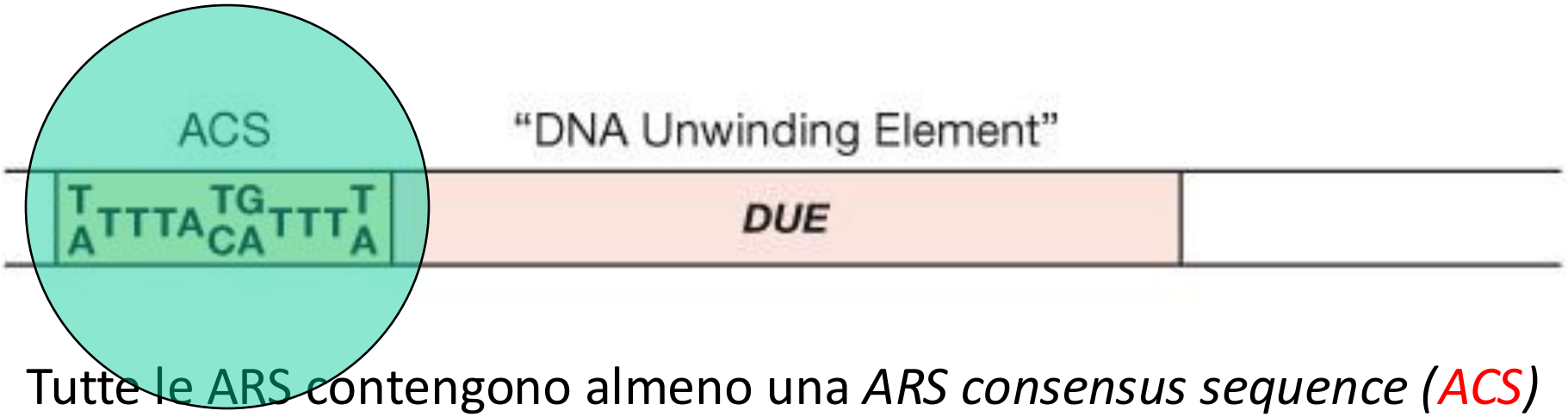
Ognuna di queste e' organizzata con un punto di origine da cui partono due forcelle di replicazione opposte. I punti di terminazione di due repliconi adiacenti coincidono, cosi' che a tempi tardivi di replicazione, repliconi adiacenti si fondono l'uno con l'altro.


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

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

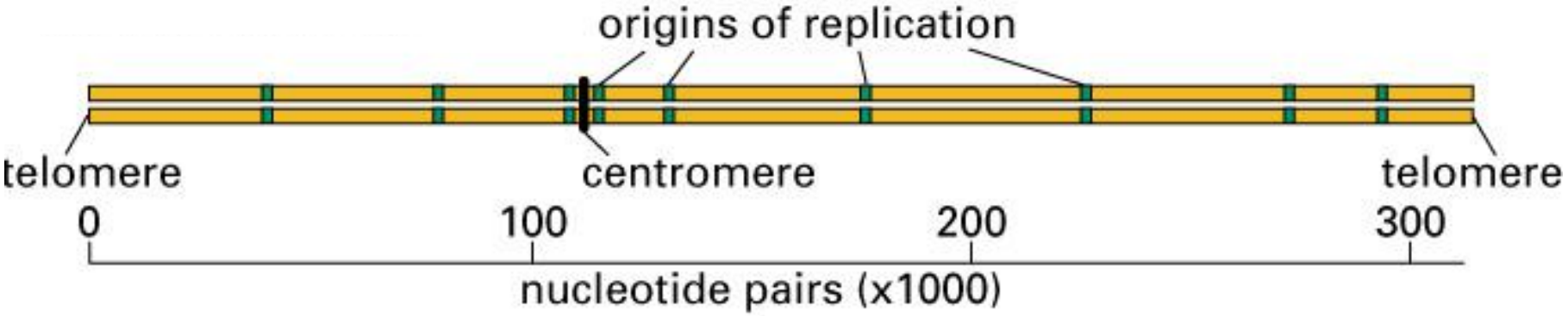


ARS sequences - autonomously replicating sequences are replication origins in yeast.



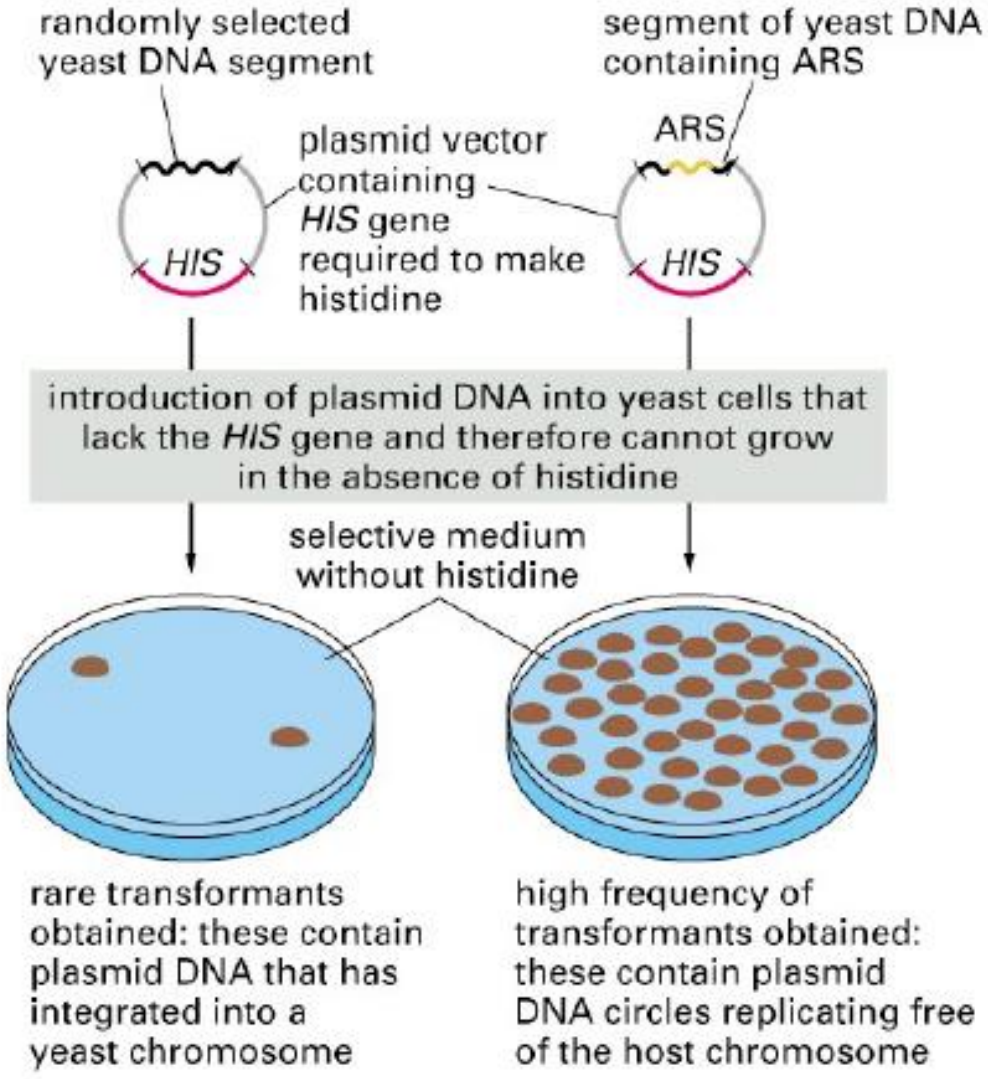
- Tutte le ARS contengono almeno una *ARS consensus sequence (ACS)* **di 11 pb**, ricca di A e T, seguita da altre regioni di lunghezza variabile, *DNA unwinding elements (DUE)*, coinvolte nell'apertura della doppia elica.
- Mutazioni nelle ACS aboliscono la funzione della ARS

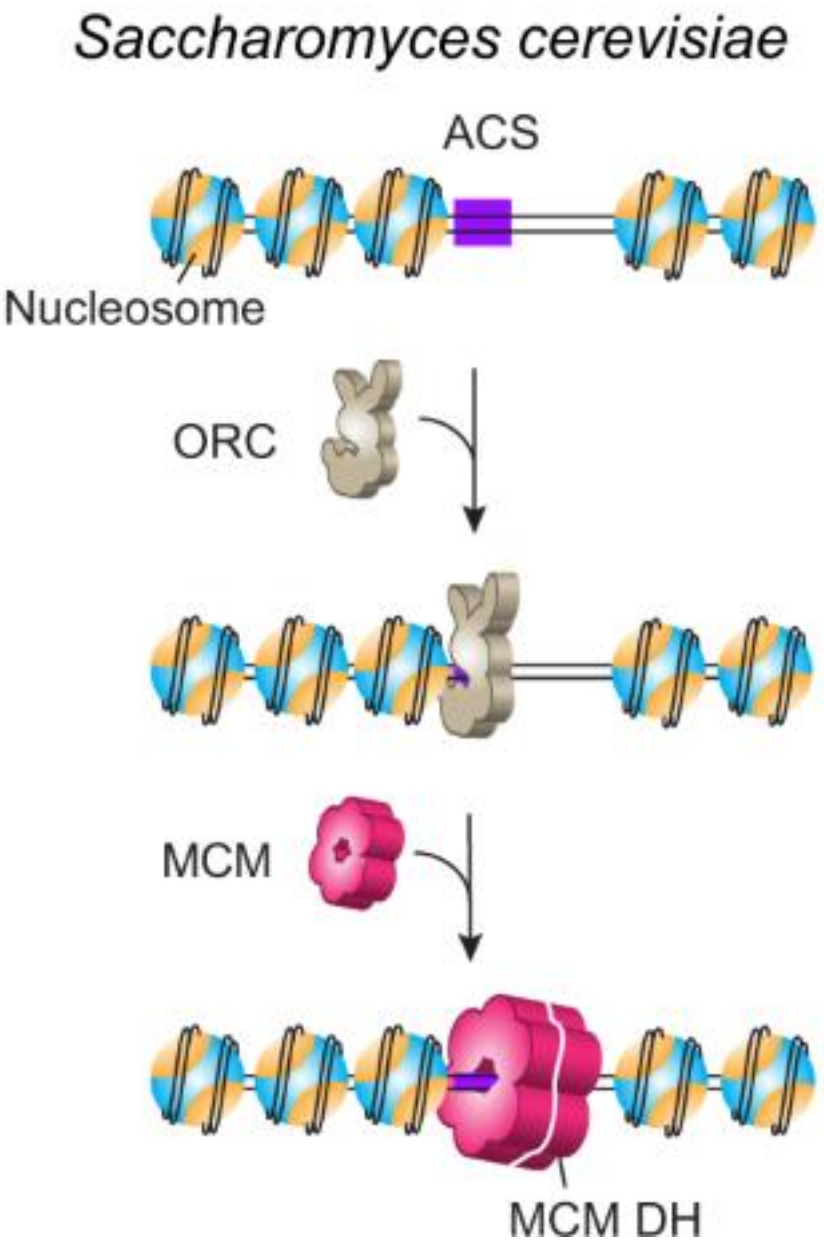
La perdita di origini di replicazioni (per danni, rotture dei cromosomi) e' tollerabile entro certi limiti; se la perdita diventa massiccia, il cromosoma interessato alla delezione viene gradualmente perso, probabilmente perche' e' duplicato troppo lentamente.



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.





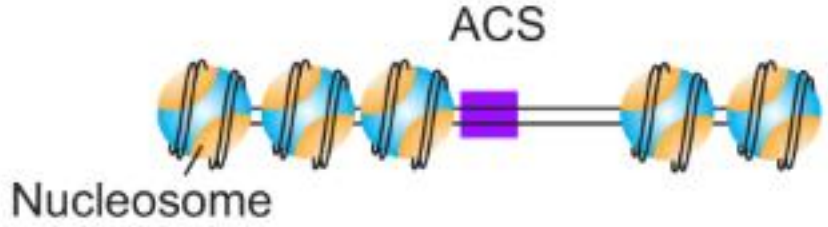
The ACS region is the main binding site for a large, multisubunit initiator protein called **ORC** (origin recognition complex).

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

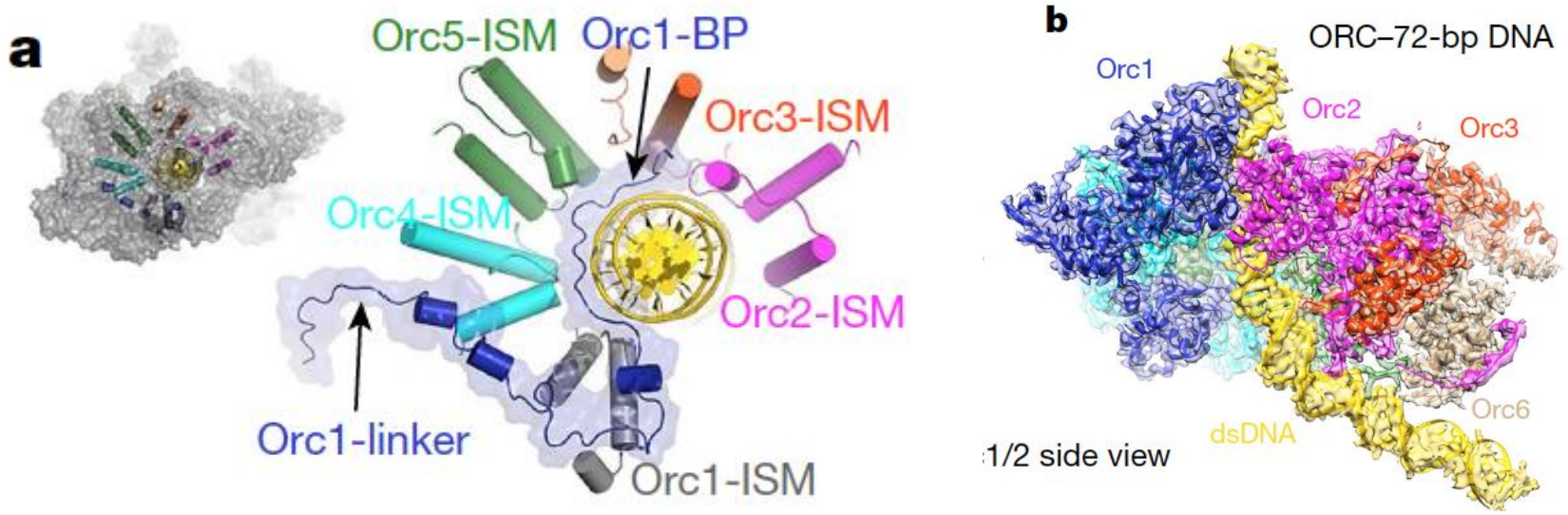
- Absence of any biochemical activity besides origin binding

- 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

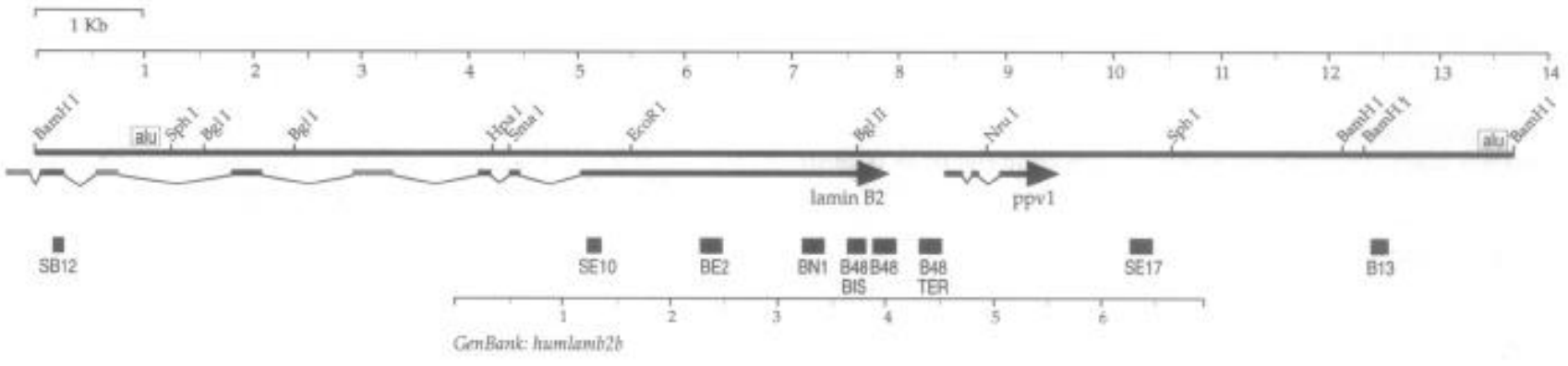
Fine mapping of a replication origin of human DNA

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

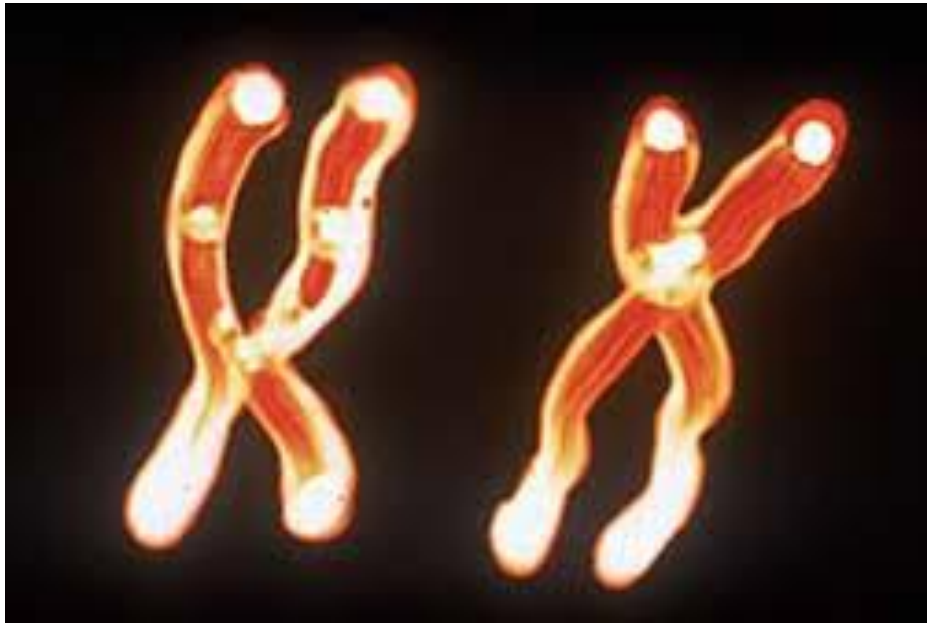
MAURO GIACCA*, LORENA ZENTILIN*, PAOLO NORIO*, SILVIA DIVIACCO*, DANIELA DIMITROVA*, GIOVANNA CONTREAS*, GIUSEPPE BIAMONTI†, GIOVANNI PERINI†, FLORIAN WEIGHARDT†, SILVANO RIVA†, AND ARTURO FALASCHI*‡

*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

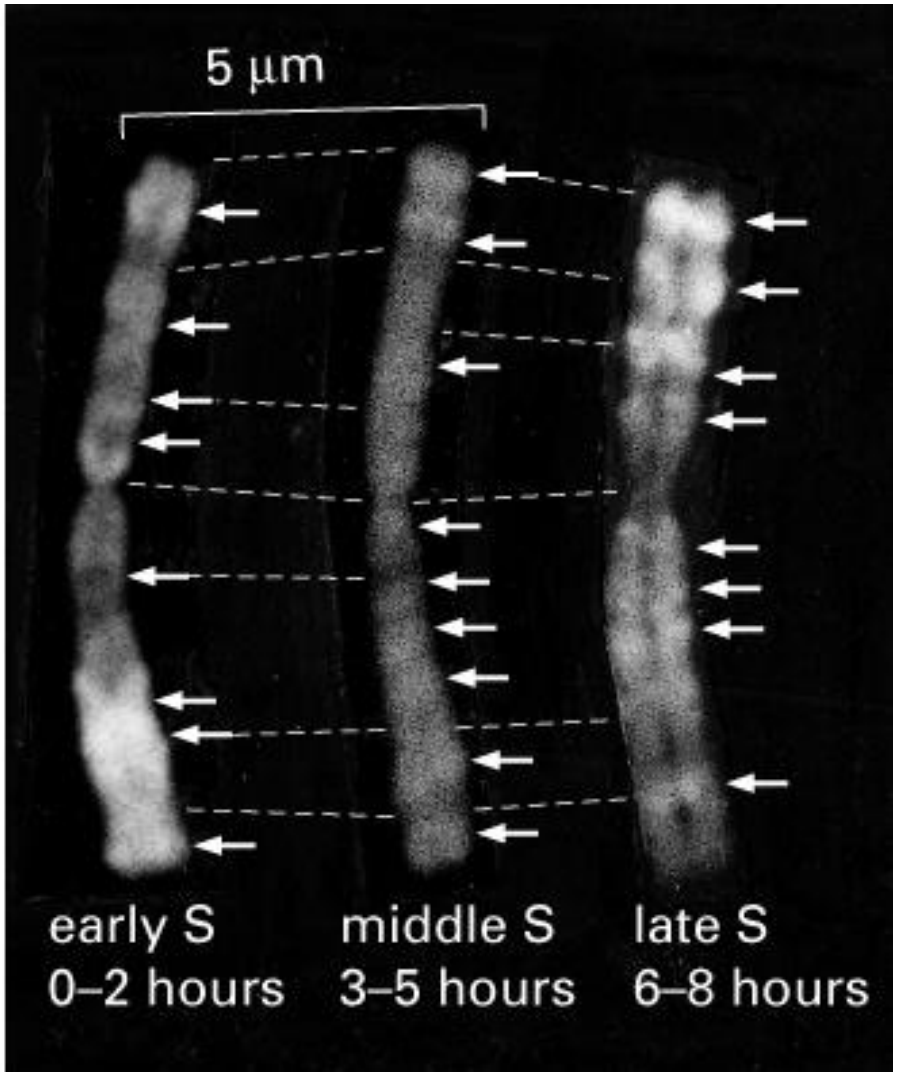


- the two X chromosomes contain the same DNA sequence

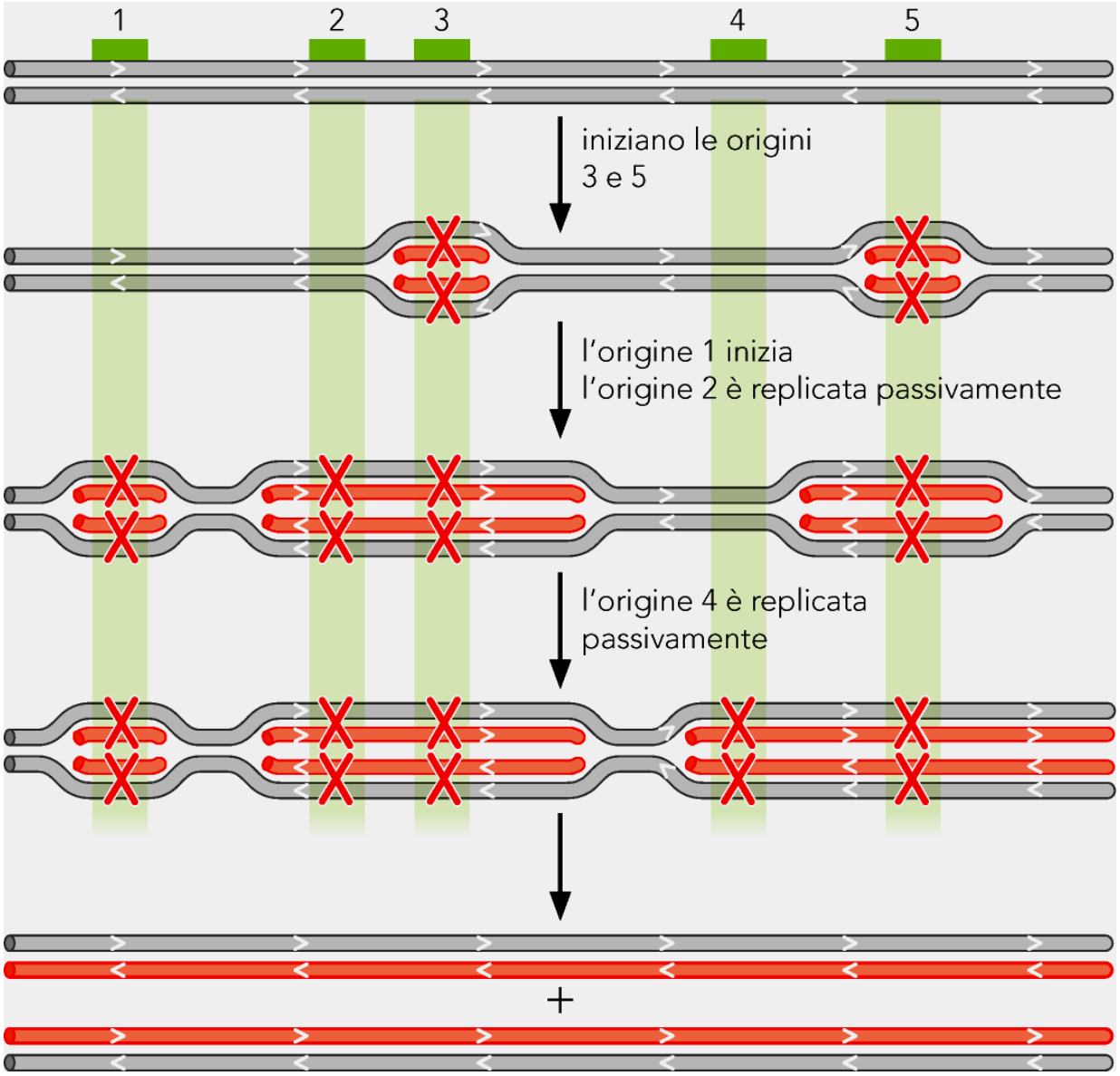
- one is inactive for transcription and is condensed into heterochromatin --> its DNA replicates late in S phase

- 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)

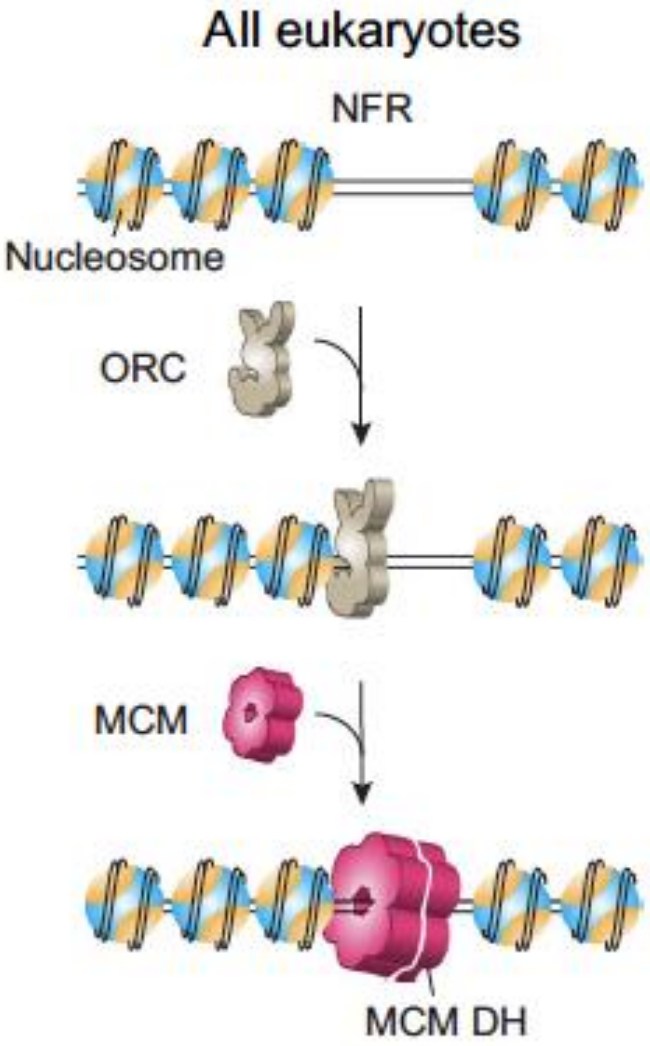
Alcune origini sono replicate passivamente (per estensione della/e bolla/e adiacenti); e non verranno quindi attivate poiché "contengono" DNA neosintetizzato

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

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

Received: 12 April 2022

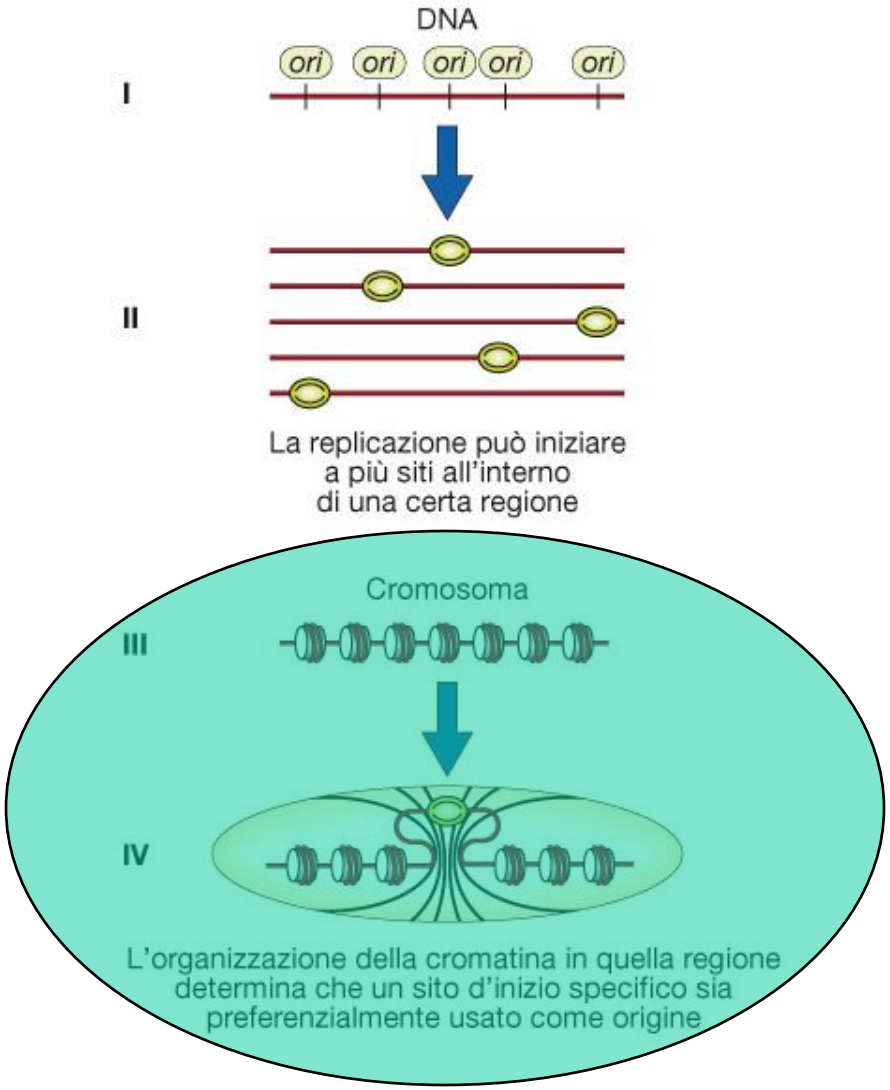
Accepted: 9 August 2022



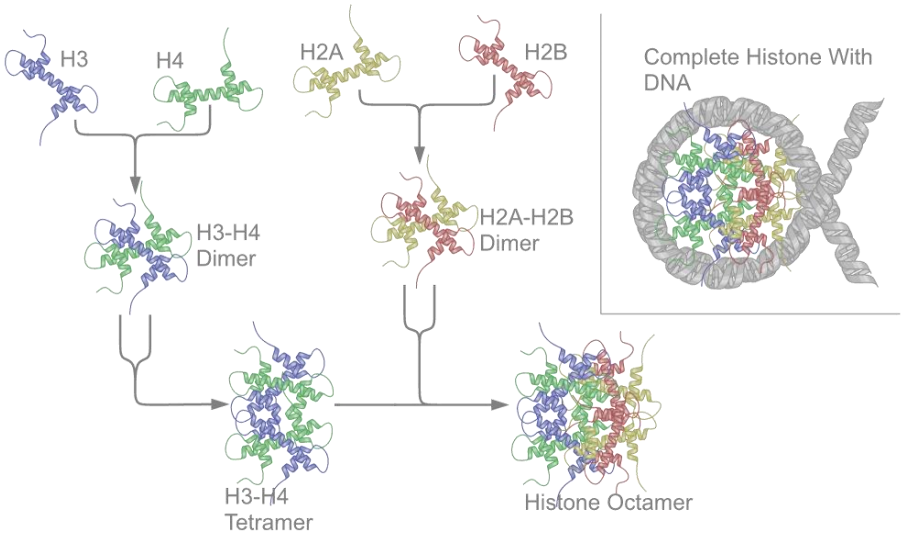
Origins in most eukaryotes lack a consensus ACS motif, but still utilize ORC which is known to bind nucleosomes.

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?



HISTONES



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

Article

576 | Nature | Vol 577 | 23 January 2020

H2A.Z facilitates licensing and activation of early replication origins

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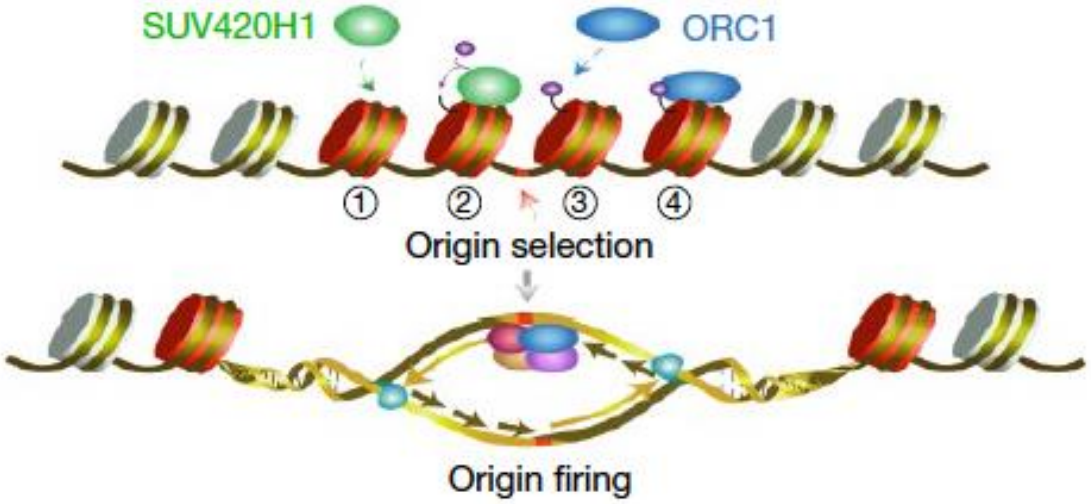
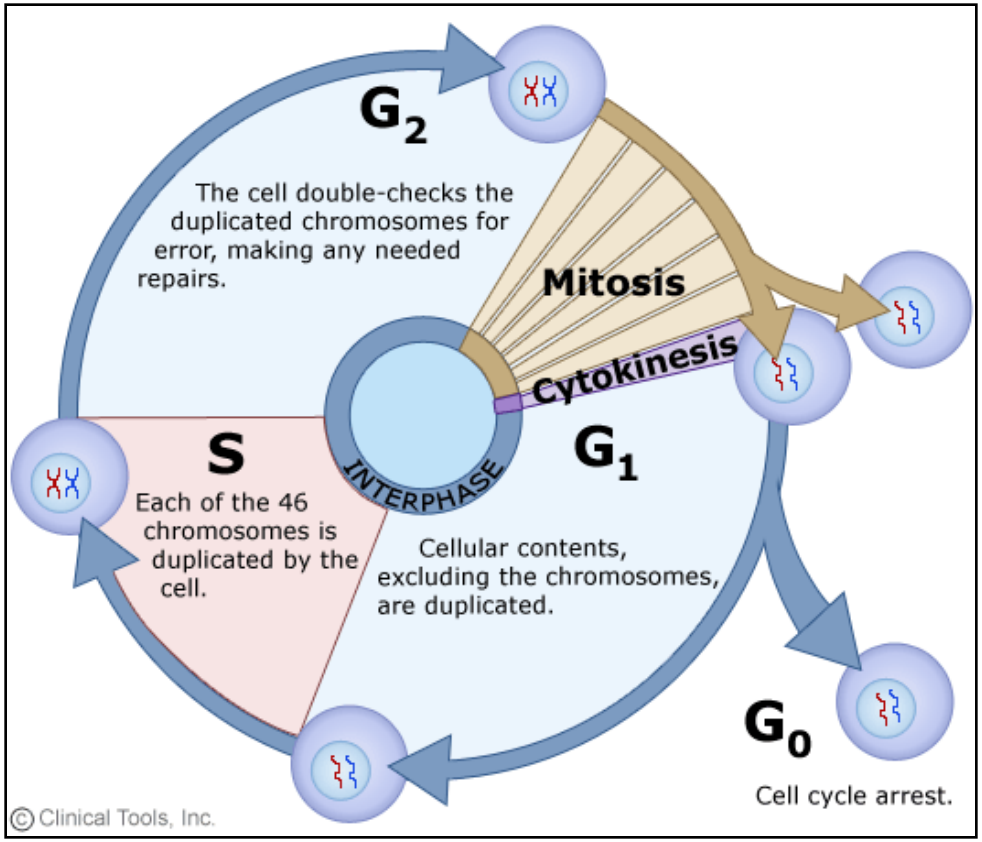
Haizhen Long[1,2,10], Liwei Zhang[1,10], Mengjie Lv[3,10], Zengqi Wen[1,2,10], Wenhao Zhang[4], Xiulan Chen[2,5], Peitao Zhang[6], Tongqing Li[7], Luyuan Chang[1,2], Caiwei Jin[2,3], Guozhao Wu[1,2], Xi Wang[8], Fuquan Yang[2,5], Jianfeng Pei[7], Ping Chen[1], Raphael Margueron[9], Halteng Deng[4], Mingzhao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



- ORC1 and nascent DNA strands co-localize with H2A.Z histone variant
- 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.
- Nucleosomes containing the histone variant H2A.Z are enriched with histone H4 that is dimethylated on its lysine 20 residue this methylation is required for ORC1 binding.

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).

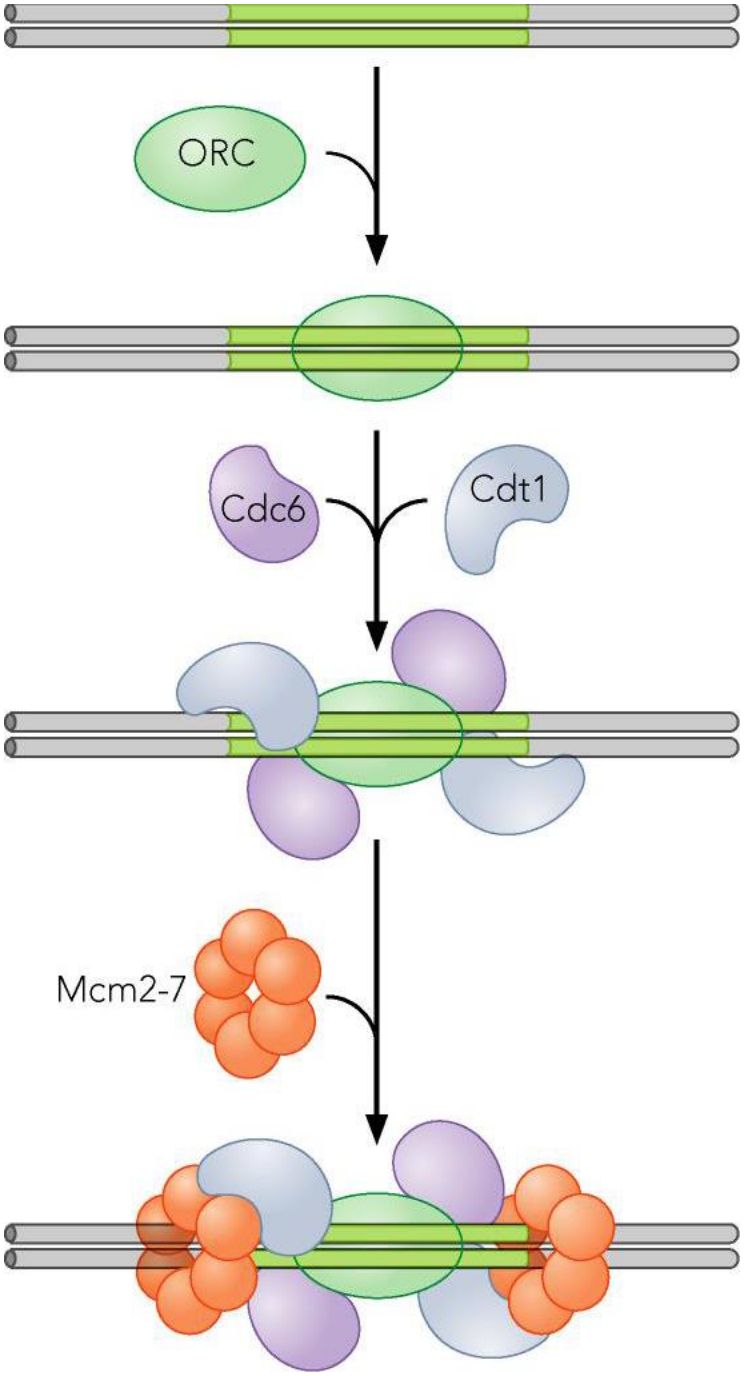


I- The Pre-RC **assembly** (G1)

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

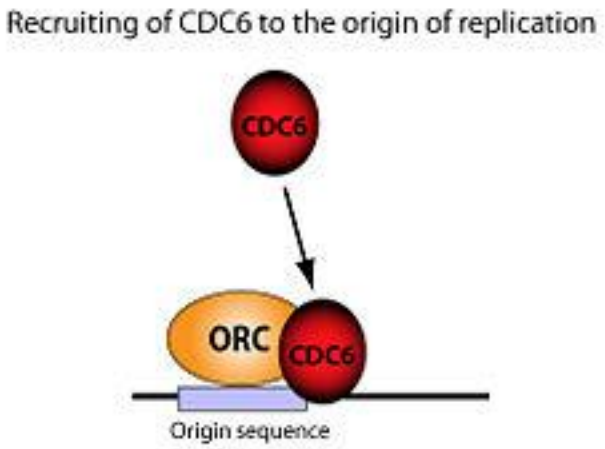
Ad ORC si associano due proteine chiave del controllo replicativo, **cdc6** e **cdt1**, richieste per l'attivazione dell'elicasi, nota come **Mcm2-7.**

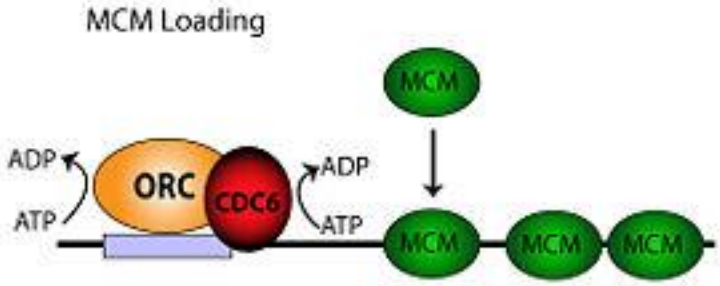
A questo punto l'origine e' "**licenced**" per la replicazione

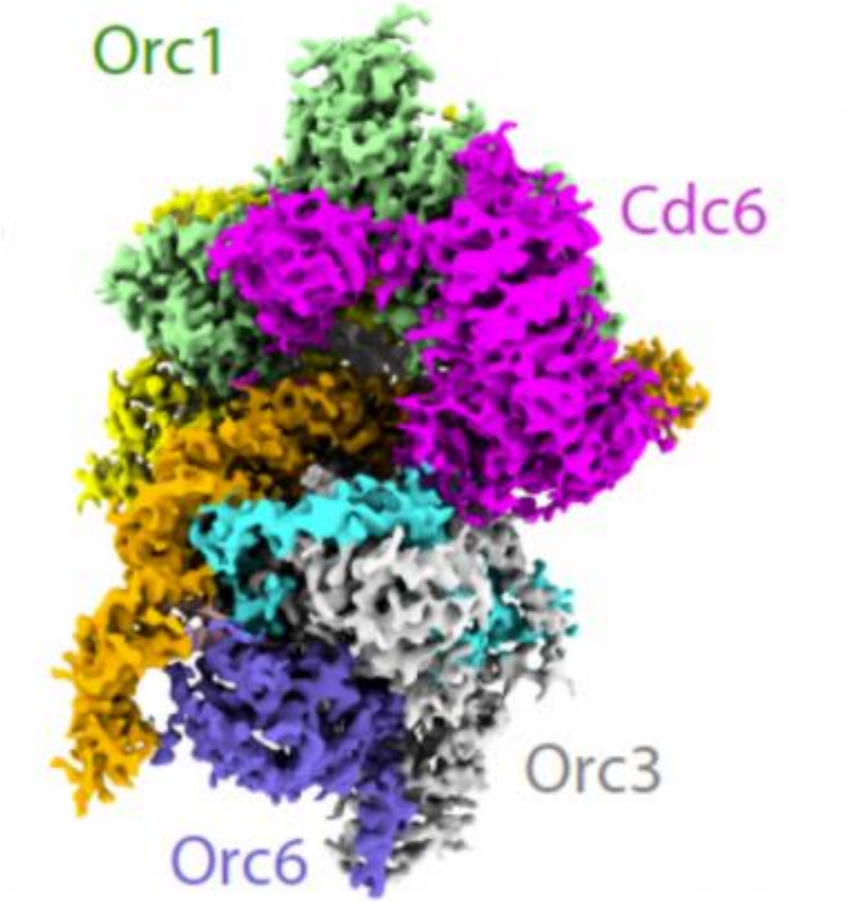


cdc-6

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.

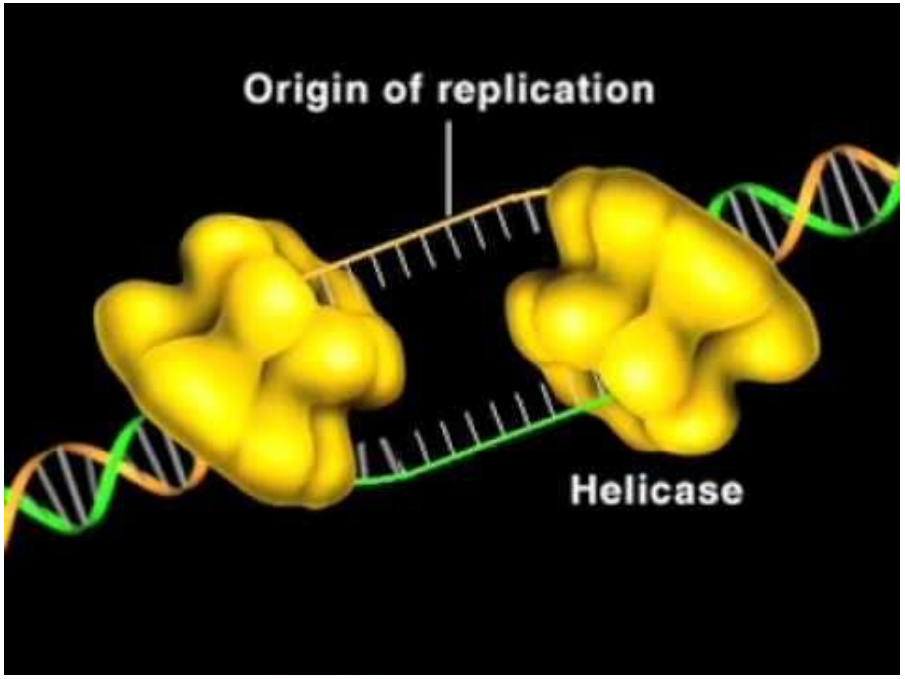


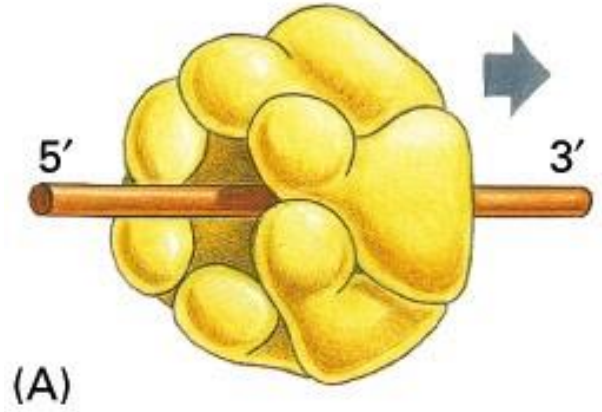




Mcm2-7

MCM2-7 helicase function arises from its architecture.



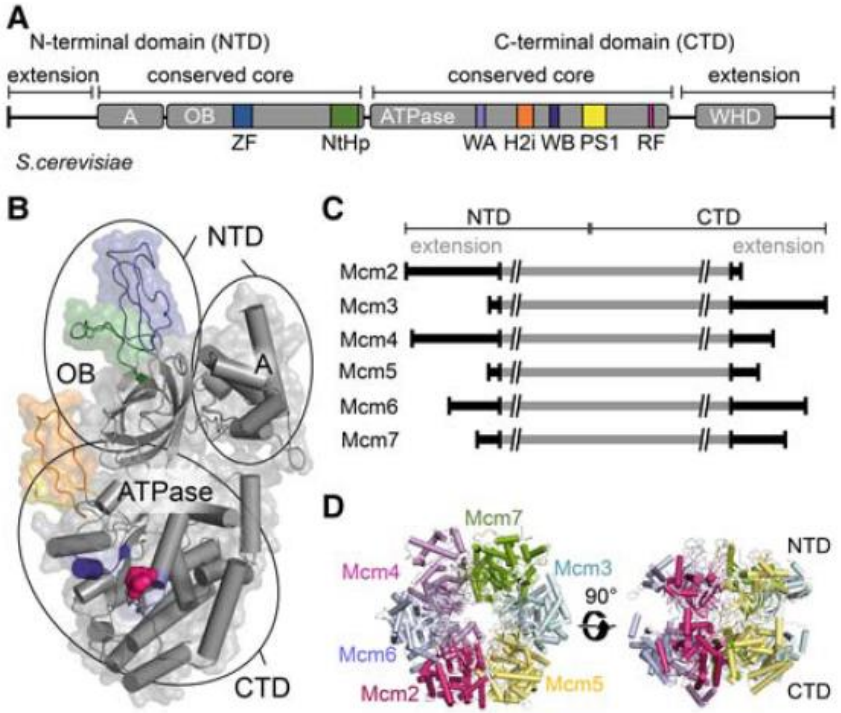


REVIEW

From structure to mechanism—understanding initiation of DNA replication

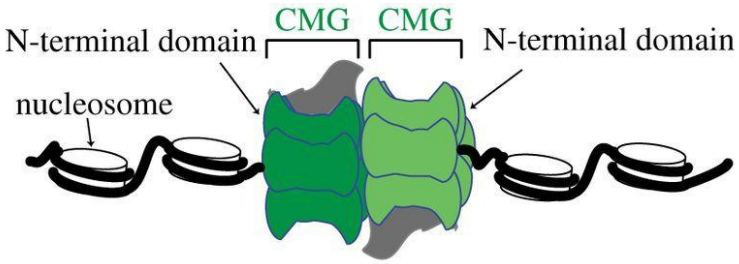
Alberto Riera,[1] Marta Barbon,[1,2,3] Yasunori Noguchi,[1,3] L. Maximilian Reuter,[1,3] Sarah Schneider,[1,3] and Christian Speck[1,2]

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

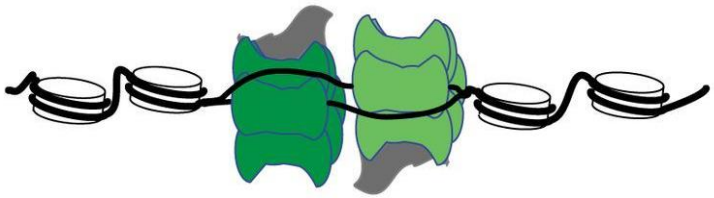


Mechanism of action of Mcm2-7

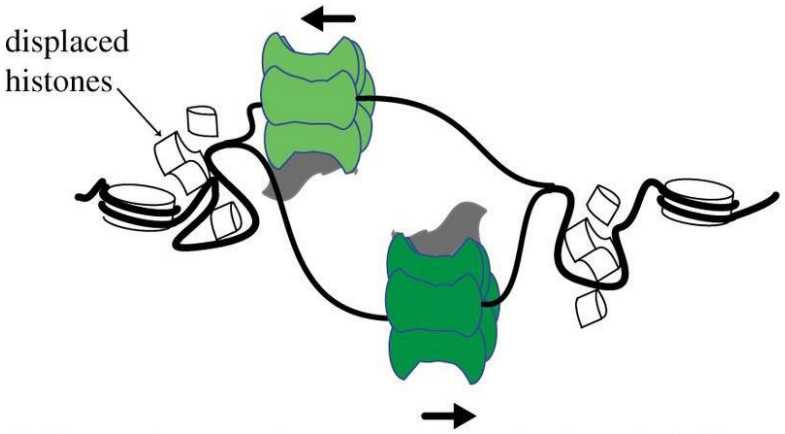
Il complesso elicasico “abbraccia” il DNA a doppia elica



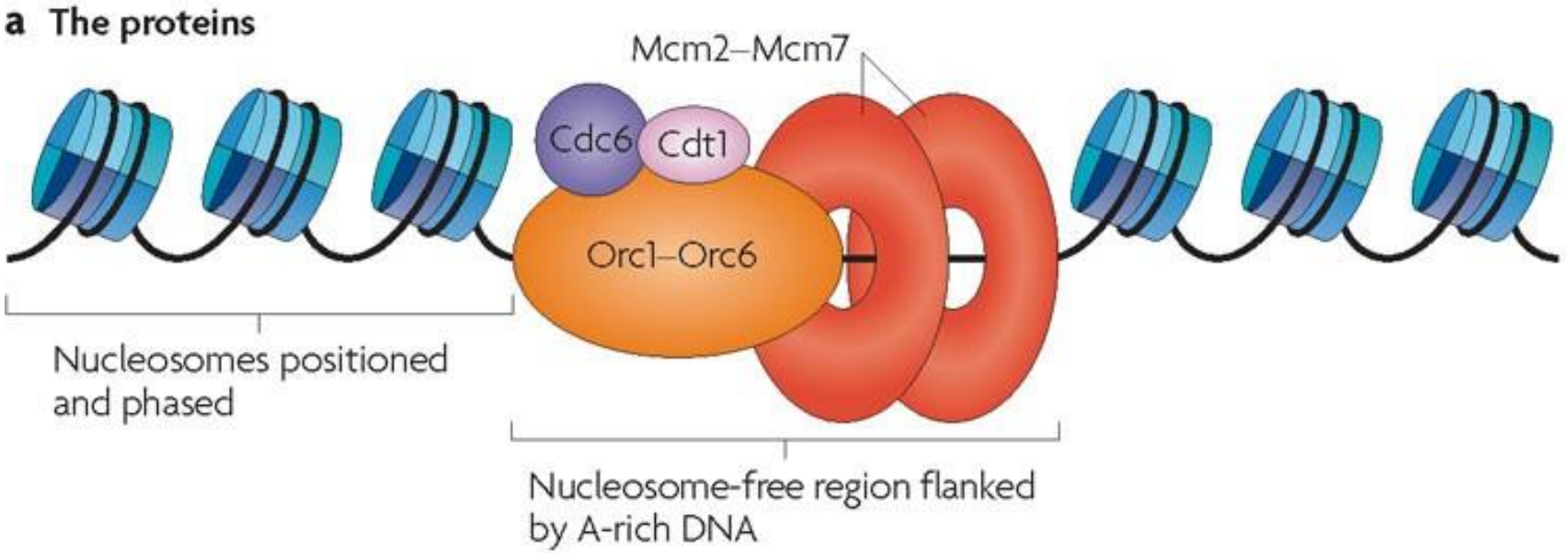
I due filamenti sono separati e uno dei due è incanalato nello spacio centrale del complesso proteico



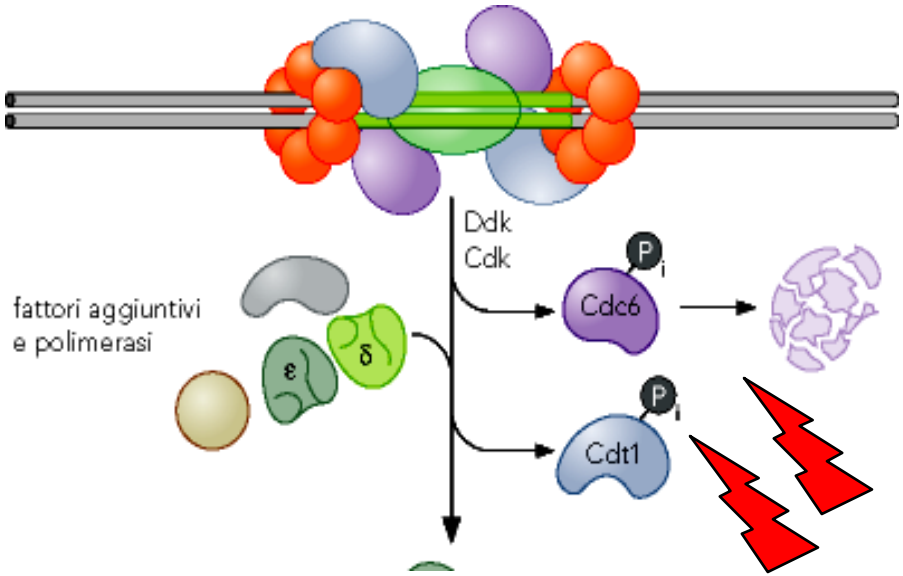
Successivamente le due elicasi si allontanano in direzione opposta, creando la bolla di replicazione



I- The Pre-Replication Complex



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

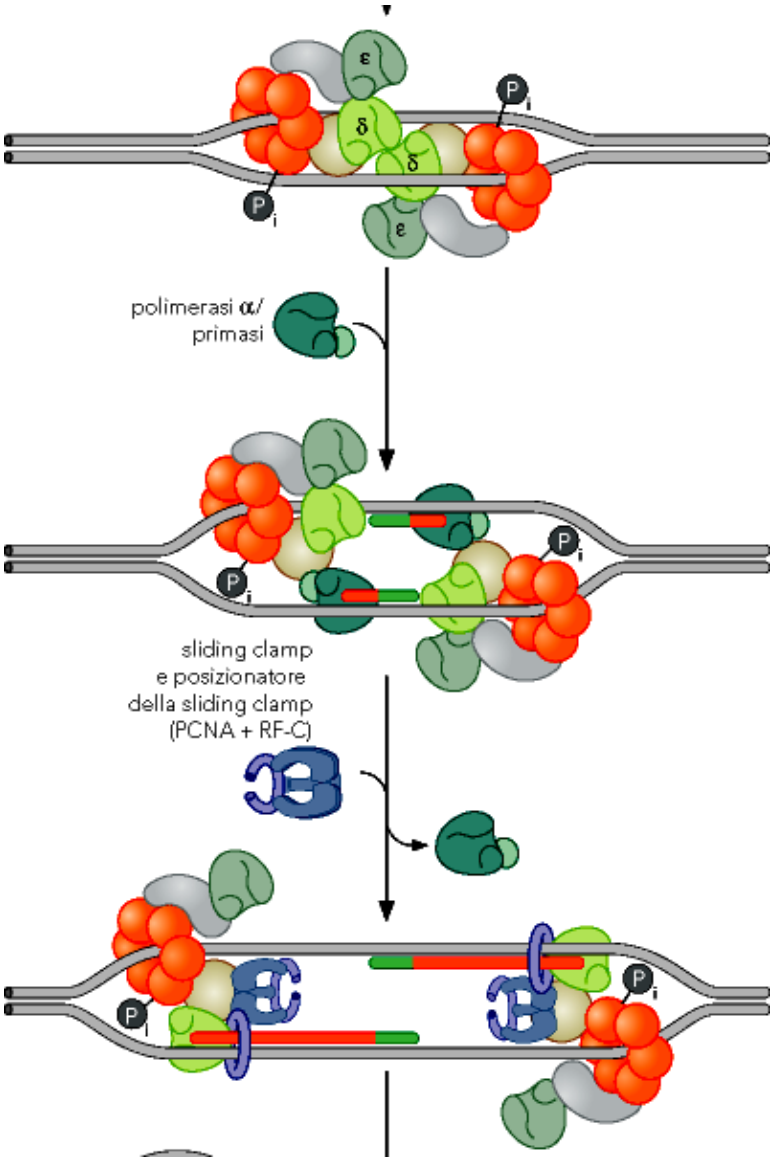


- Il passaggio da Pre-RC a Pre-IC richiede l'attivita' chinasica delle CDKs (chinasi ciclino-dipendenti).

- 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.

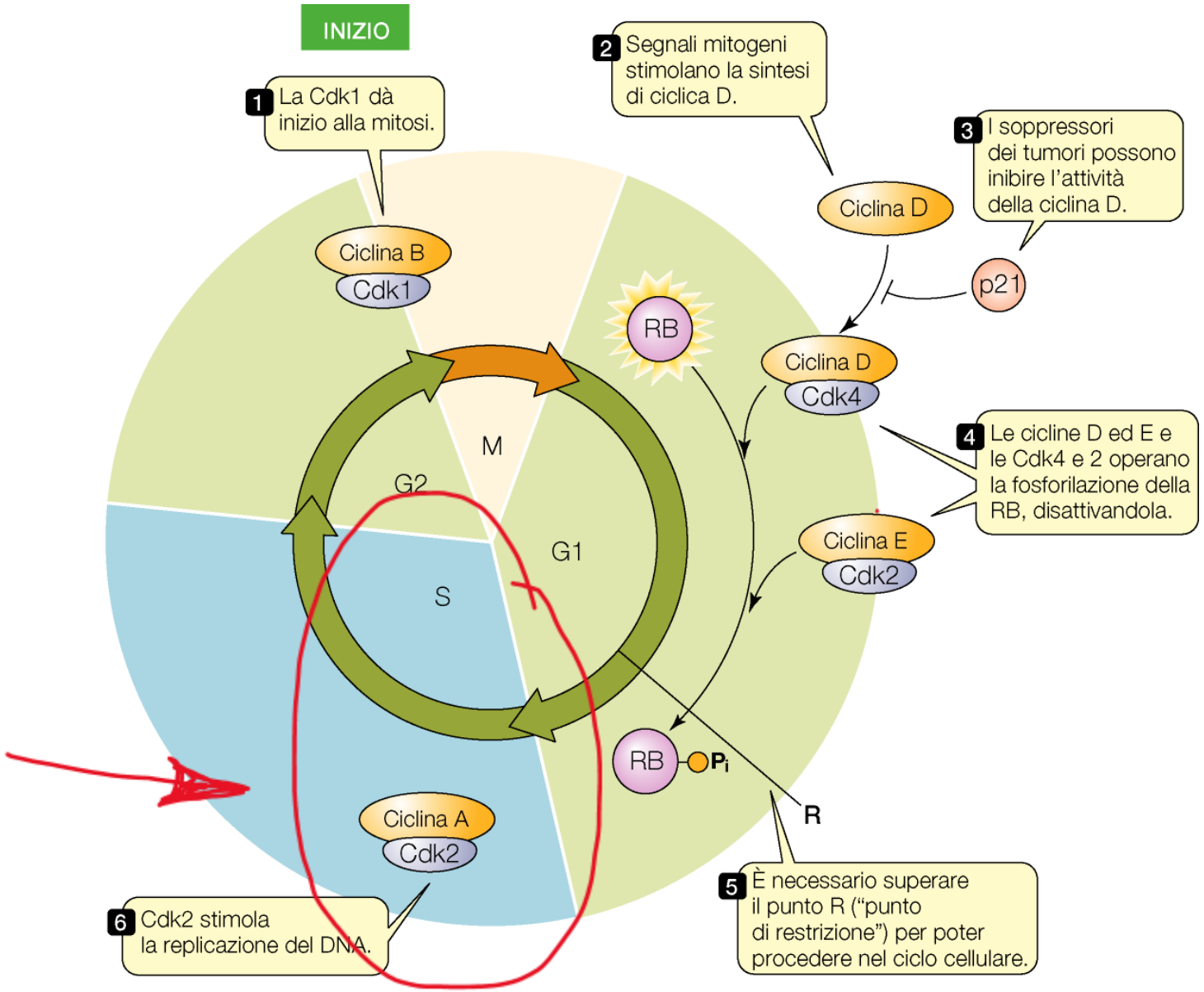
Il Pre-IC (G1-S)

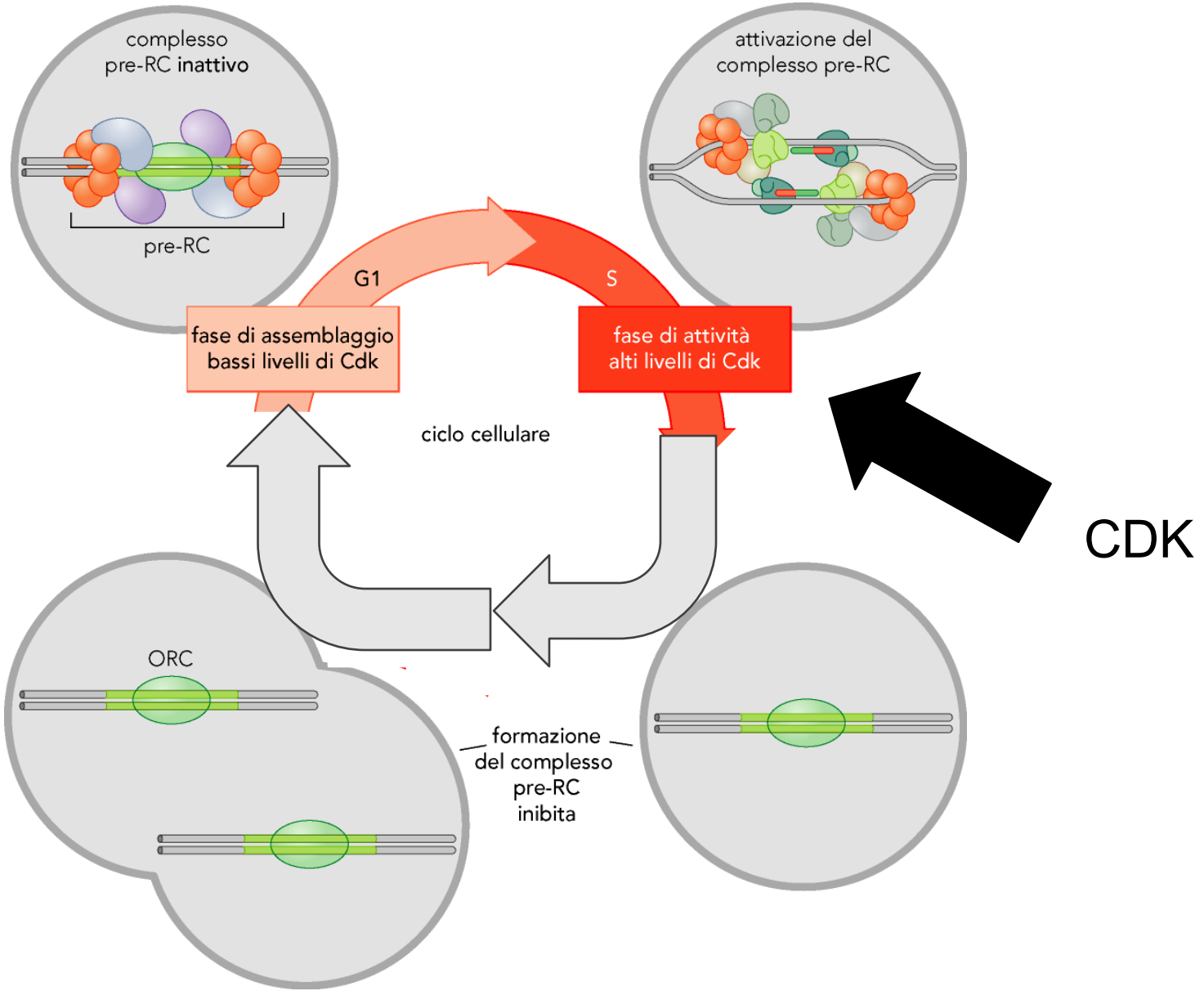


- L'elicasi Mcm2-7 denatura il DNA sull'origine
- Vengono sequenzialmente caricate le proteine implicate nella replicazione: la polimerasi alfa con attività primasica; le polimerasi delta ed epsilon che interagendo con la PCNA=pinza procedono alla replicazione dello stampo.

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

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

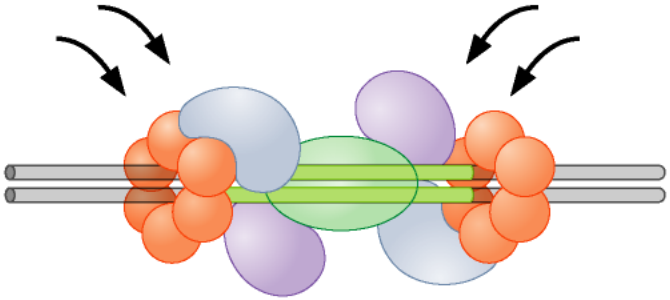



complesso
pre-RC inattivo
pre-RC
attivazione del
complesso pre-RC
G1
S
fase di assemblaggio
bassi livelli di Cdk
fase di attività
alti livelli di Cdk
ciclo cellulare
CDK
ORC
formazione
del complesso
pre-RC
inibita

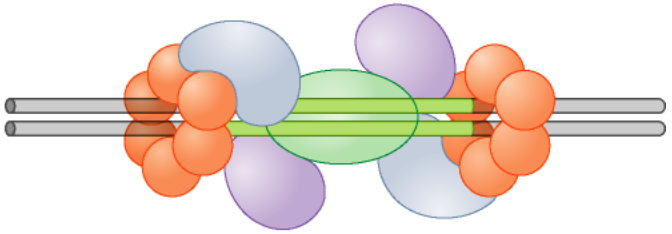
FASE G1

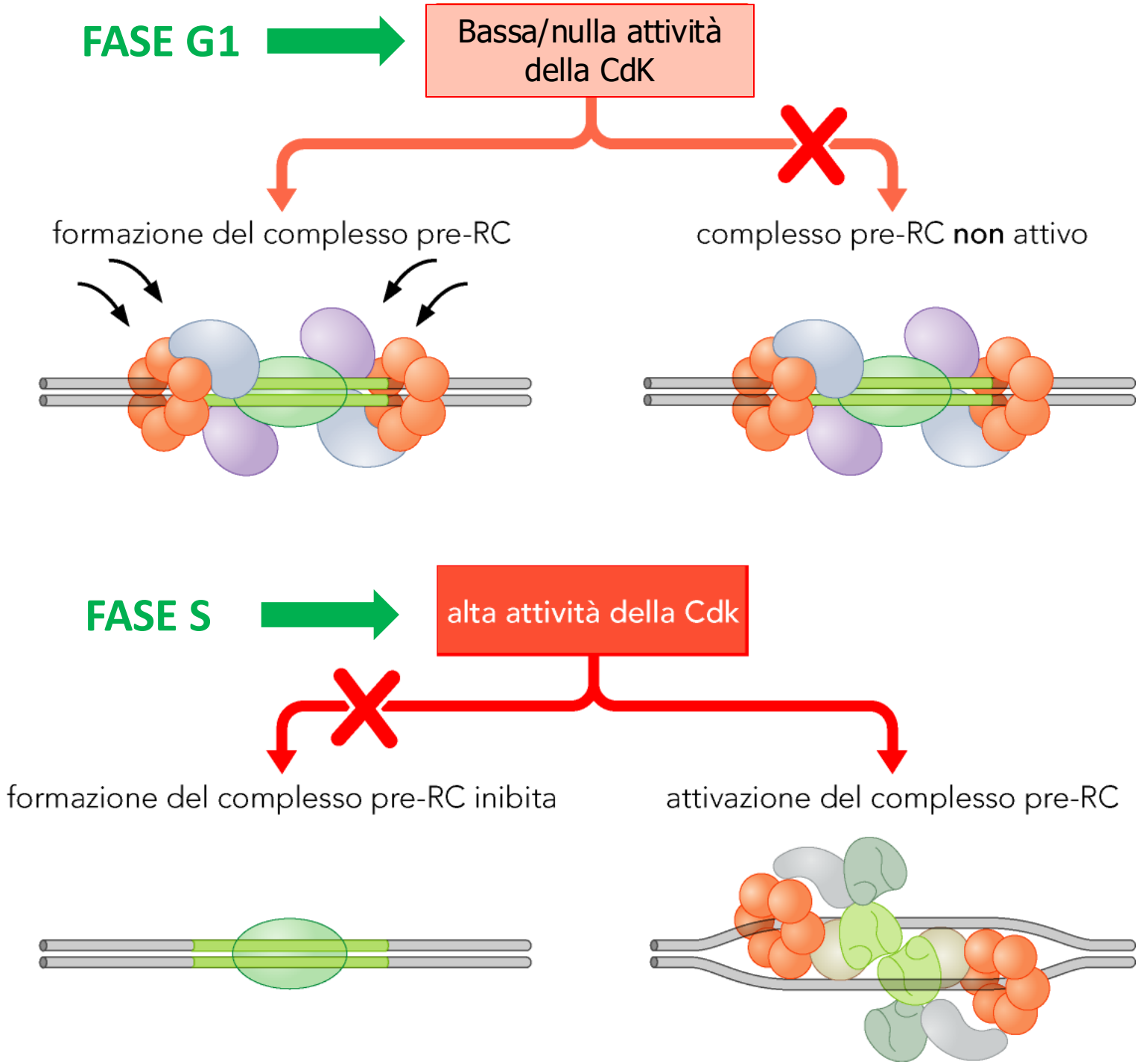
Bassa/nulla attività della CdK

formazione del complesso pre-RC

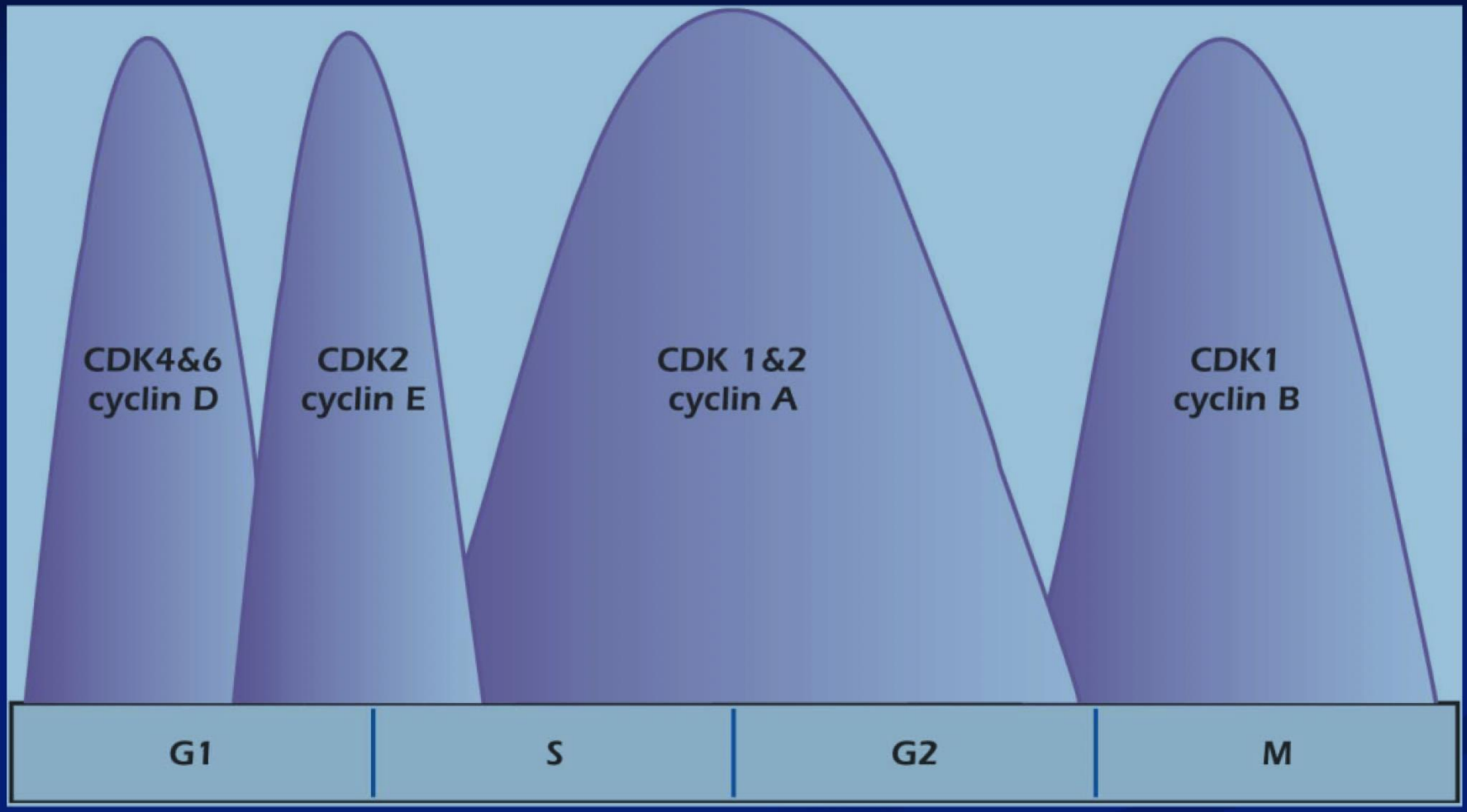


complesso pre-RC **non** attivo




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

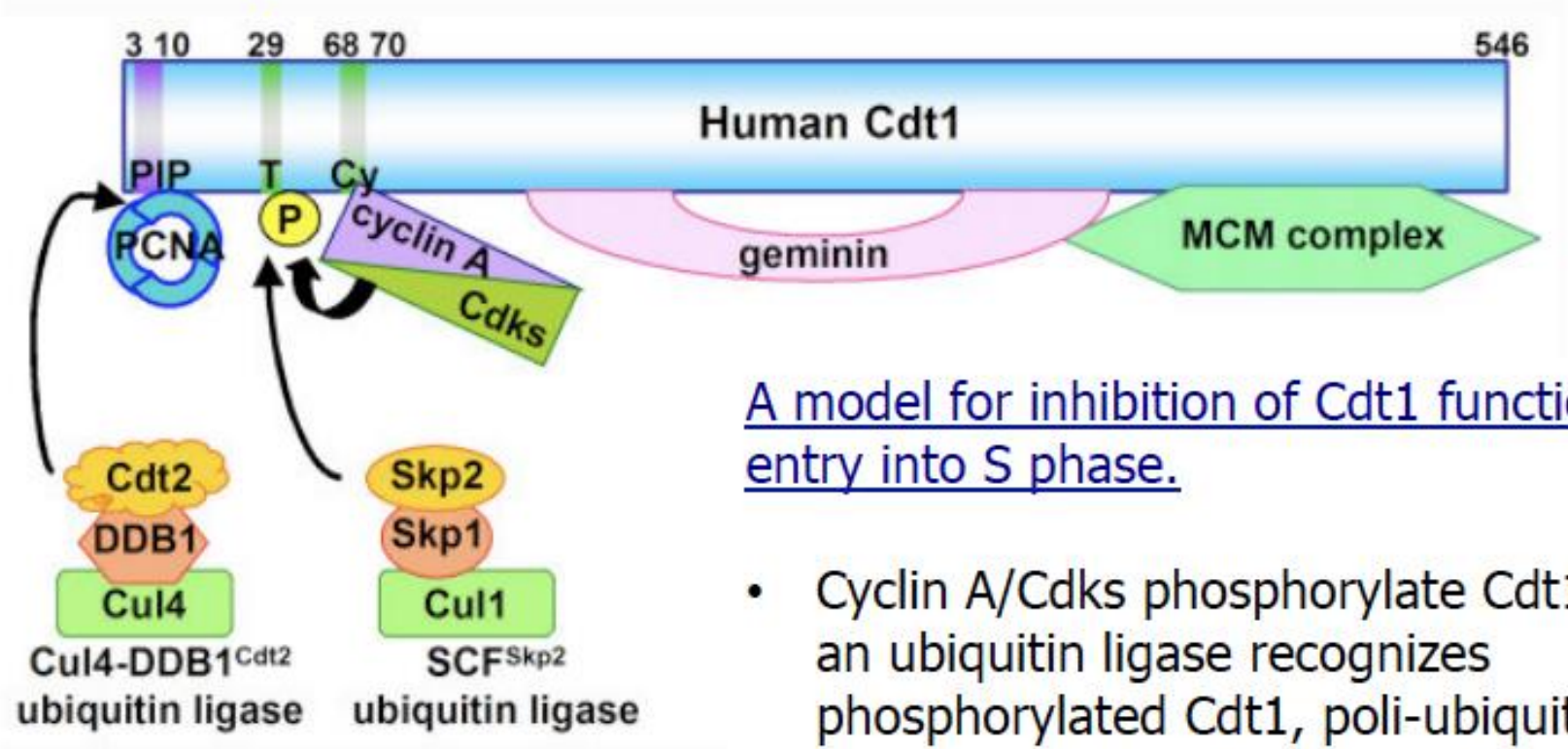
Le diverse cicline nelle cellule di Mammifero



Cdk activities through the cell cycle

from CELL BIOLOGY A short course
SR Bolsover et al

CDT1 (Chromatin licensing and DNA replication factor 1)



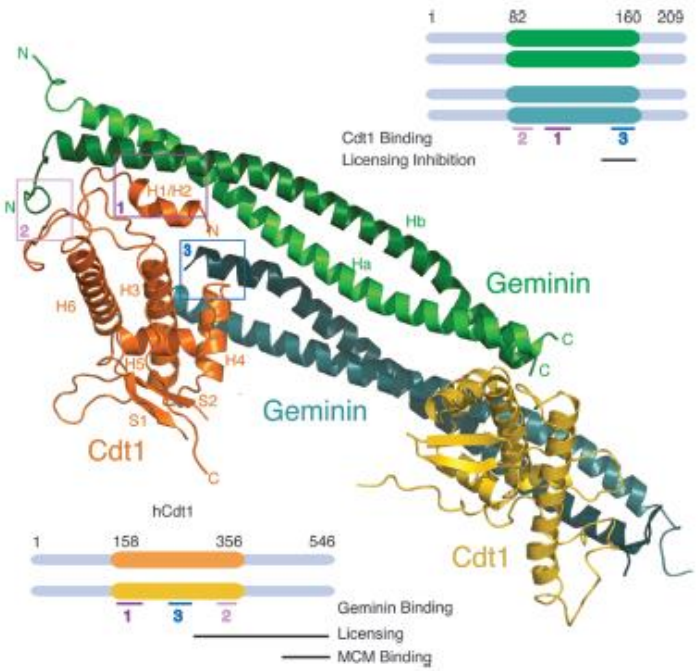
A model for inhibition of Cdt1 function after entry into S phase.

- 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.

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]



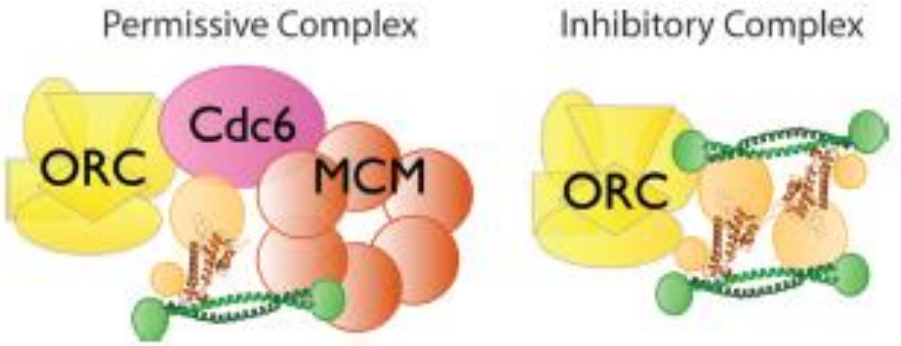
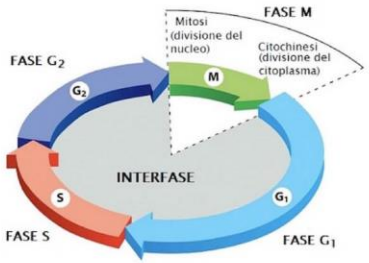


Fig. 6. Proposed model for the mechanism of DNA licensing inhibition by Geminin.

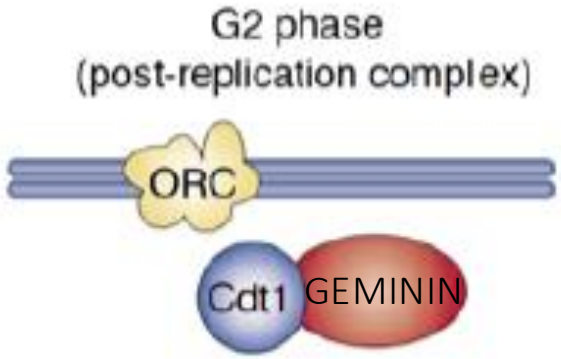
The molecular model for Geminin activity involves an equilibrium between a heterotrimer and a heterohexamer, whose relative abundance is regulated during the cell cycle. The heterohexamer represents an inhibitory complex, which prohibits DNA licensing.

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.

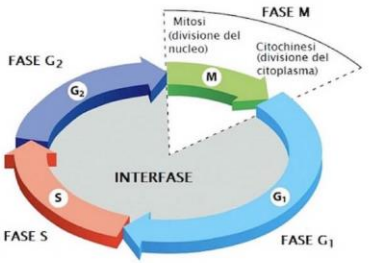
Come viene assicurato che la replicazione avvenga una sola volta/ciclo cellulare?



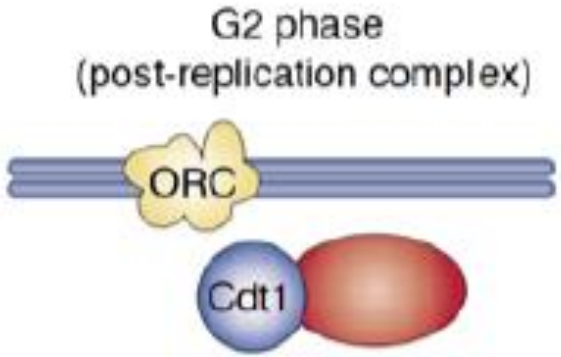
Durante la fase G2 ed M, Cdt1 e' sequestrato dalla proteina GEMININ.



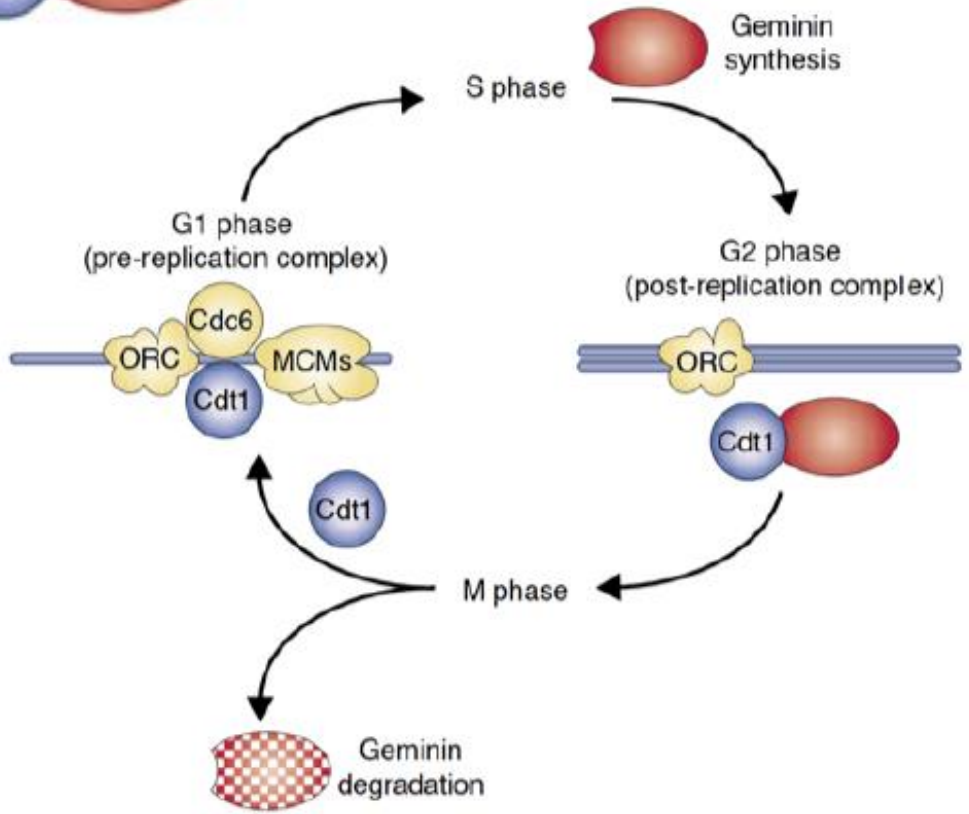
Come viene assicurato che la replicazione avvenga una sola volta/ciclo cellulare?

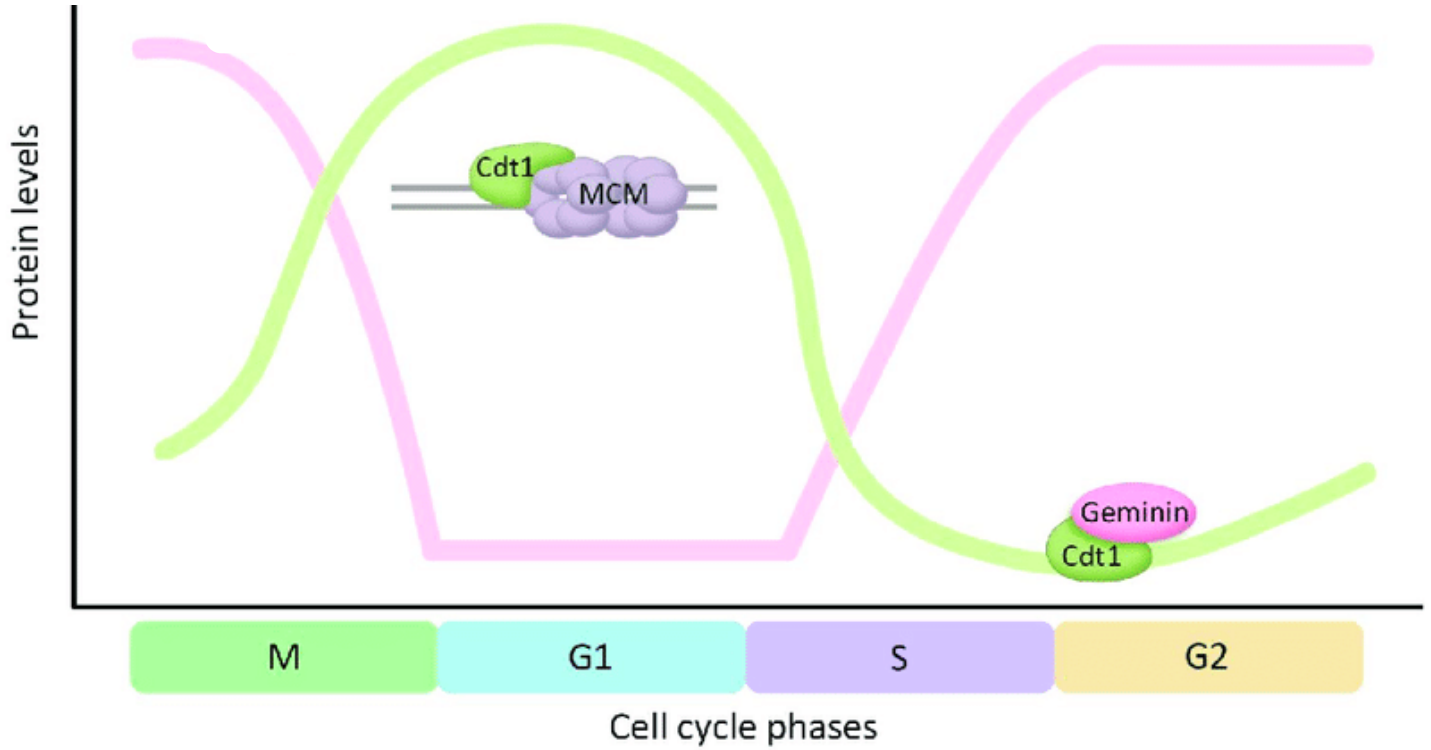


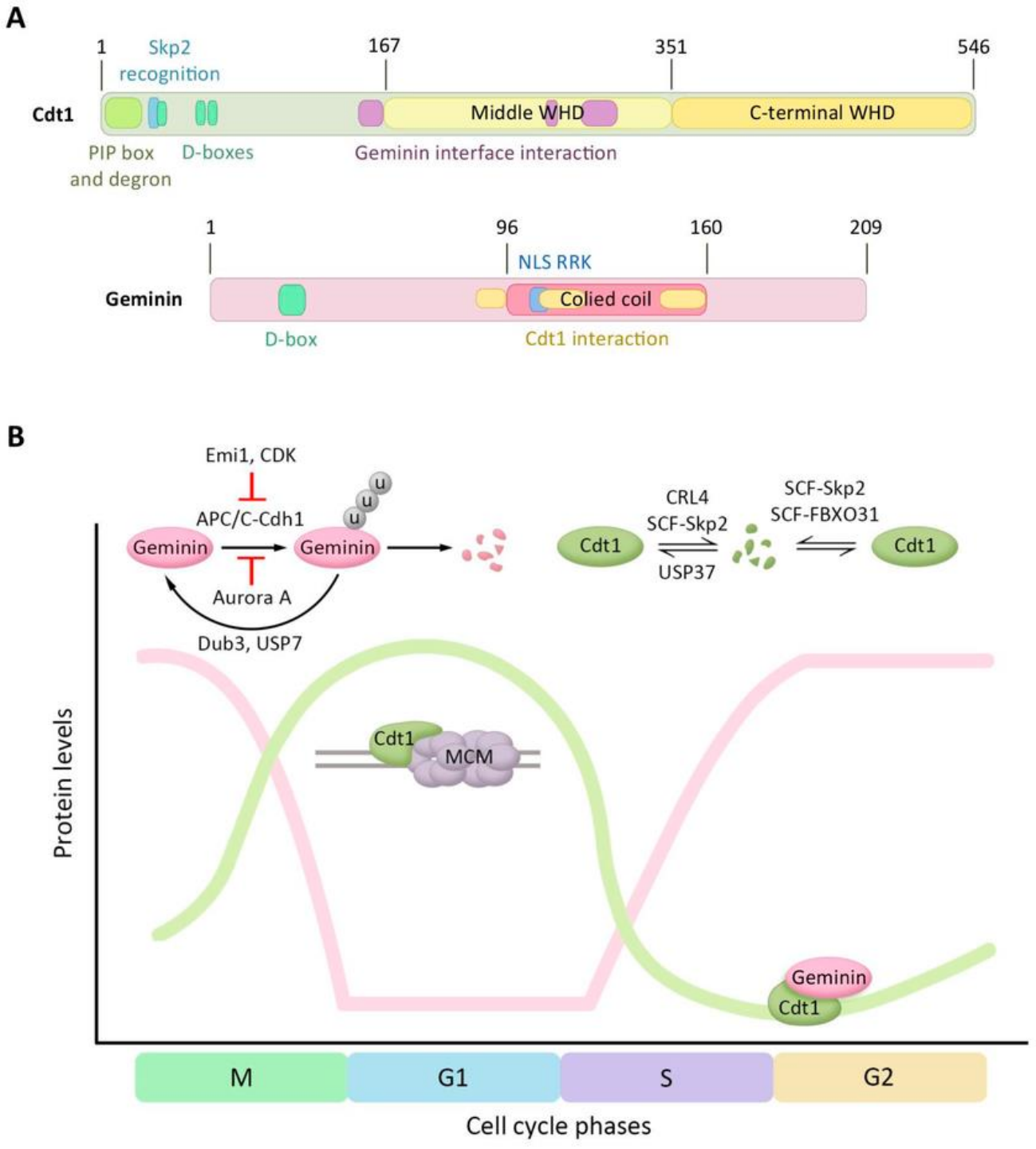
Durante la fase G2 ed M, Cdt1 e' sequestrato dalla proteina GEMININ.



A fine M, early 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


A
1
Skp2
recognition
167
351
546
Cdt1
Middle WHD
C-terminal WHD
PIP box
and degron
D-boxes
Geminin interface interaction
1
96
160
209
NLS RRK
Geminin
Colied coil
D-box
Cdt1 interaction
B
Emi1, CDK
APC/C-Cdh1
Geminin
Geminin
u
u
u
Aurora A
Dub3, USP7
CRL4
SCF-Skp2
USP37
SCF-Skp2
SCF-FBXO31
Cdt1
Cdt1
Protein levels
Cdt1
MCM
Geminin
Cdt1
M
G1
S
G2
Cell cycle phases

Review

Control of DNA Replication Initiation by Ubiquitin

Esperanza Hernández-Carralero [1], Elisa Cabrera [1], Ignacio Alonso-de Vega [1], Santiago Hernández-Pérez [1,2], Veronique A. J. Smits [1] and Raimundo Freire [1,*]

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