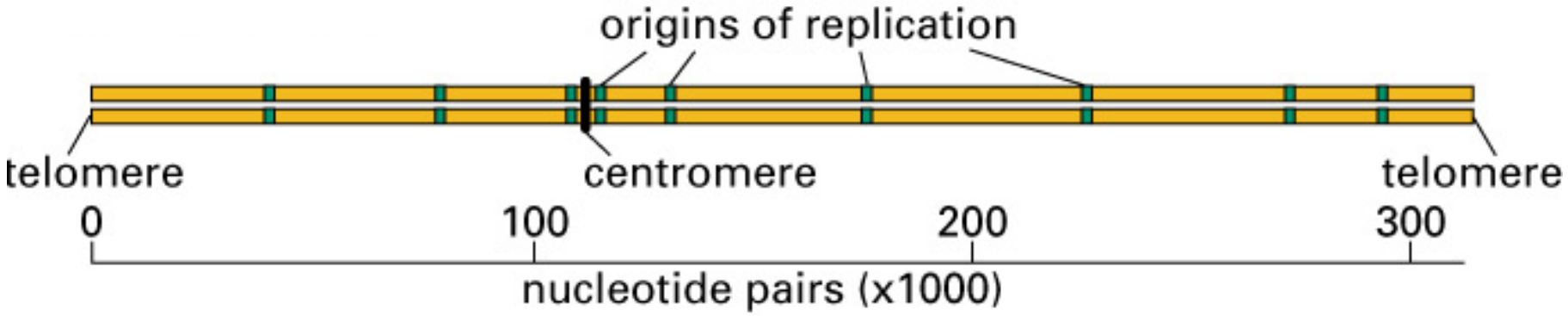
Quali sono le caratteristiche di un’origine di replicazione eucariota?

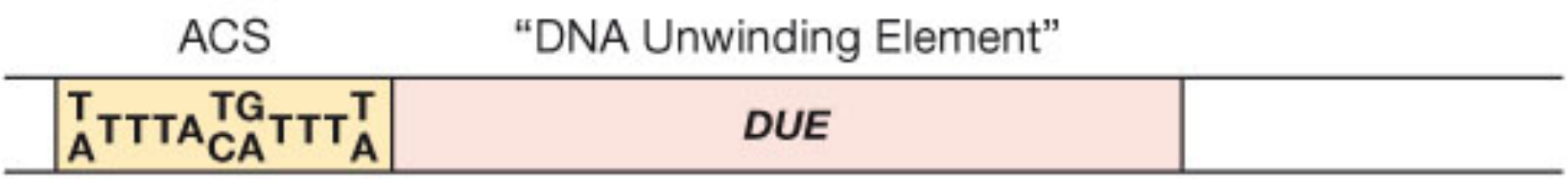
Well-defined DNA sequences serve as replication origins in yeast: the ARS sequences (autonomously replicating sequences)

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.

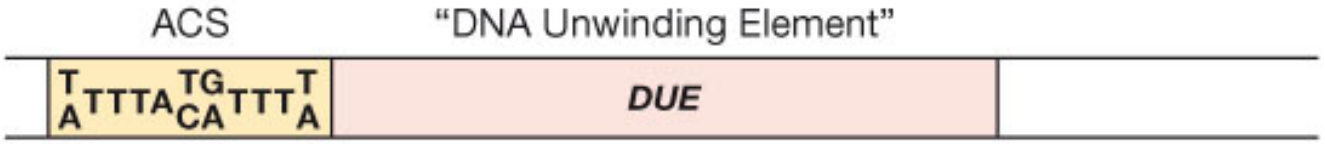
Quali sono le caratteristiche di una Autonomous Replicating Sequence (ARS) in *S. Cerevisiae?*



- 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

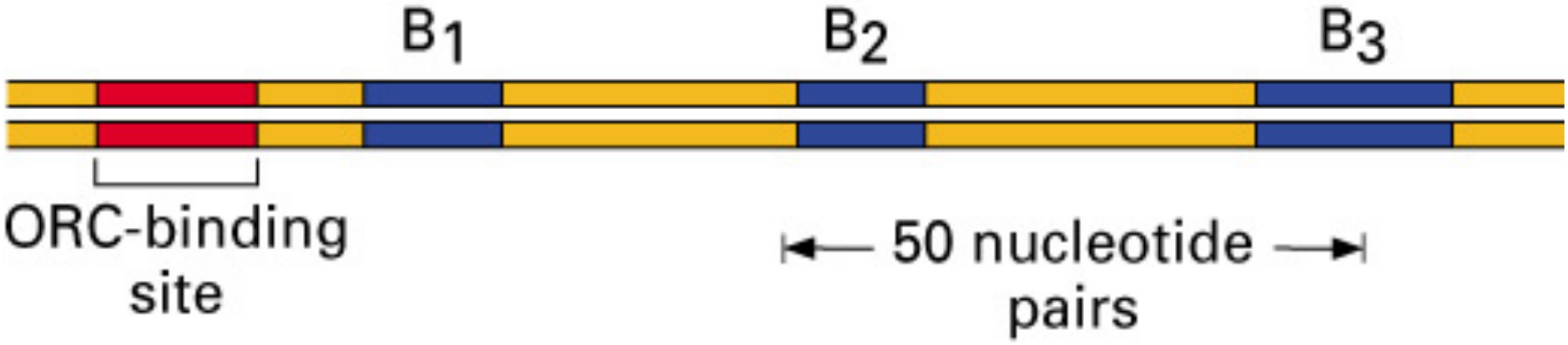
Origin Recognition Complex (ORC)



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

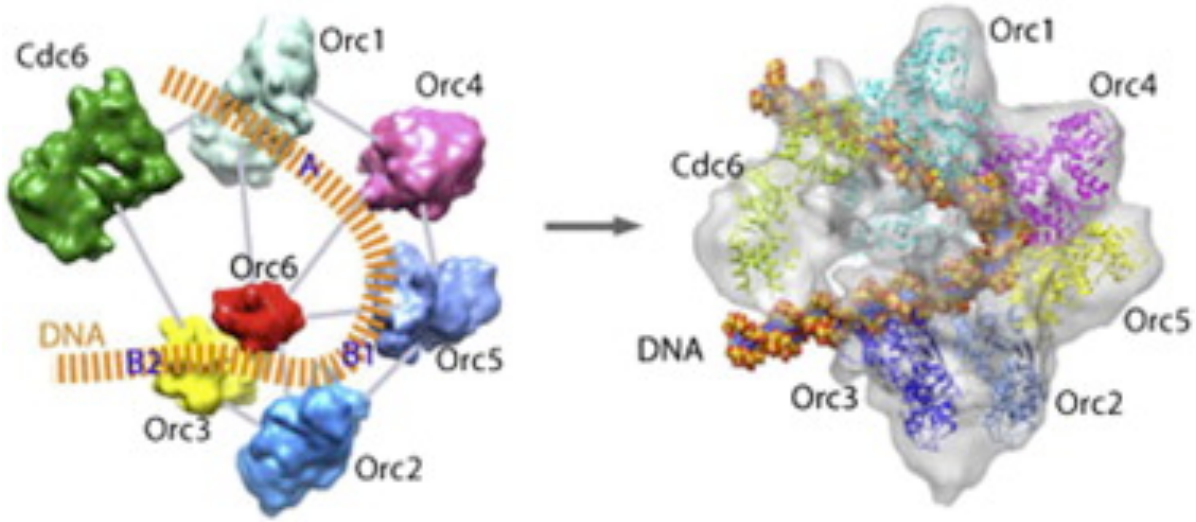
Several auxiliary binding sites (B1, B2 etc) exist for ORC subunits on the ARS in yeast.

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



Origin Recognition Complex (ORC)

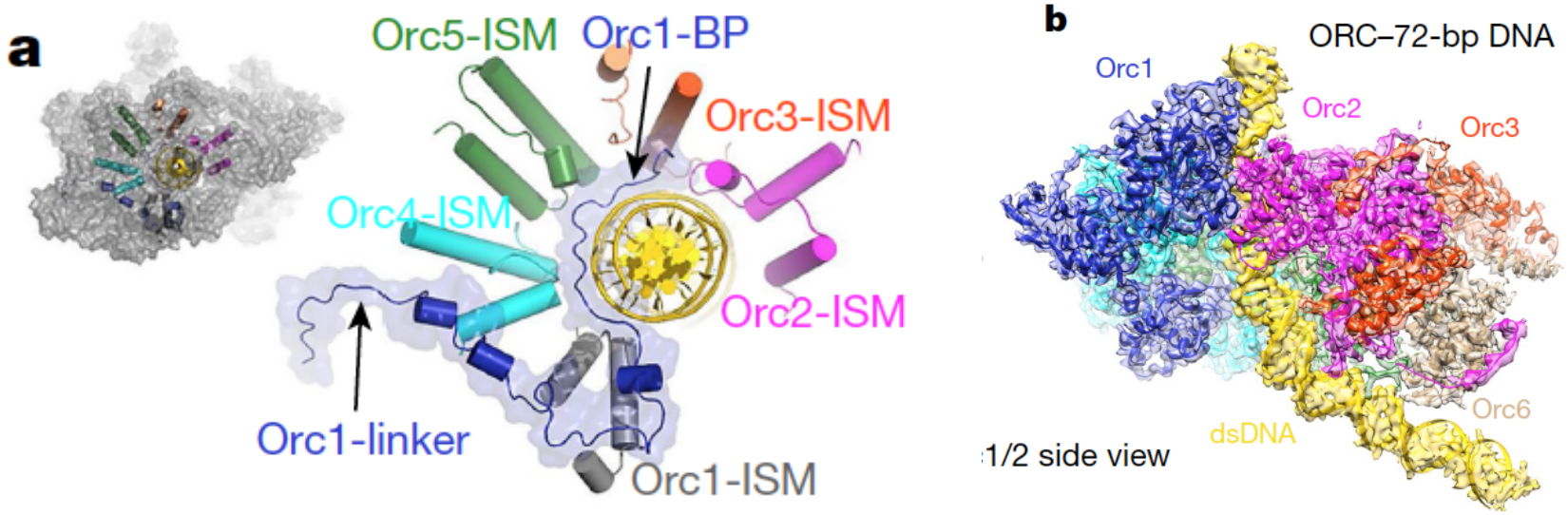
- Six subunits (Orc1p-6p); 120, 72, 62, 56, 53, 50 kDa)
- Essential for viability
- Binds to ACS in an ATP-dependent manner
- Orc 1-2-and 5 have ATP binding motifs
- Mutantions that disrupt ORC binding to DNA also disrupt origin function in vivo
- Conserved in different species
- Absence of any biochemical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



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.

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)

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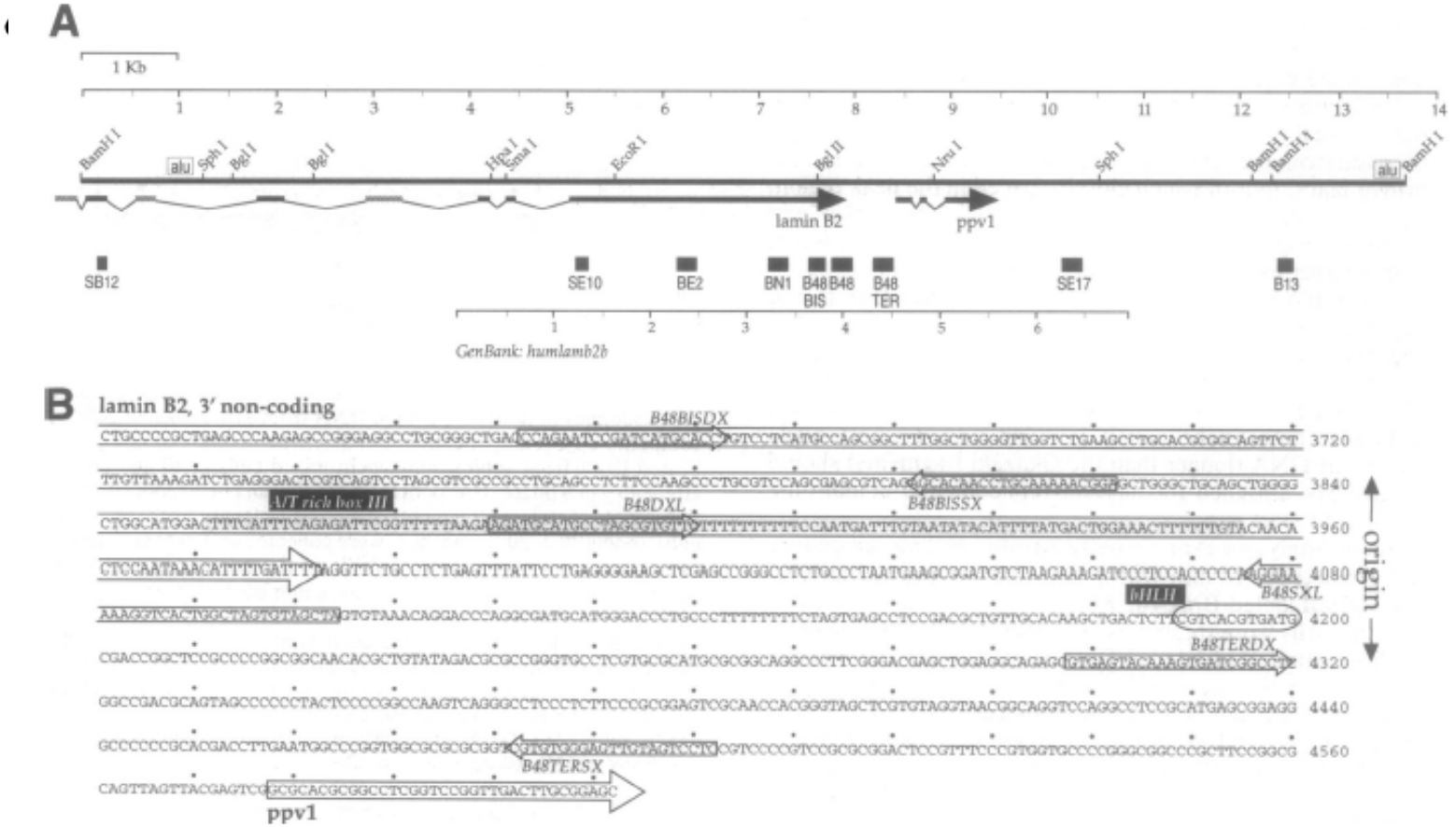
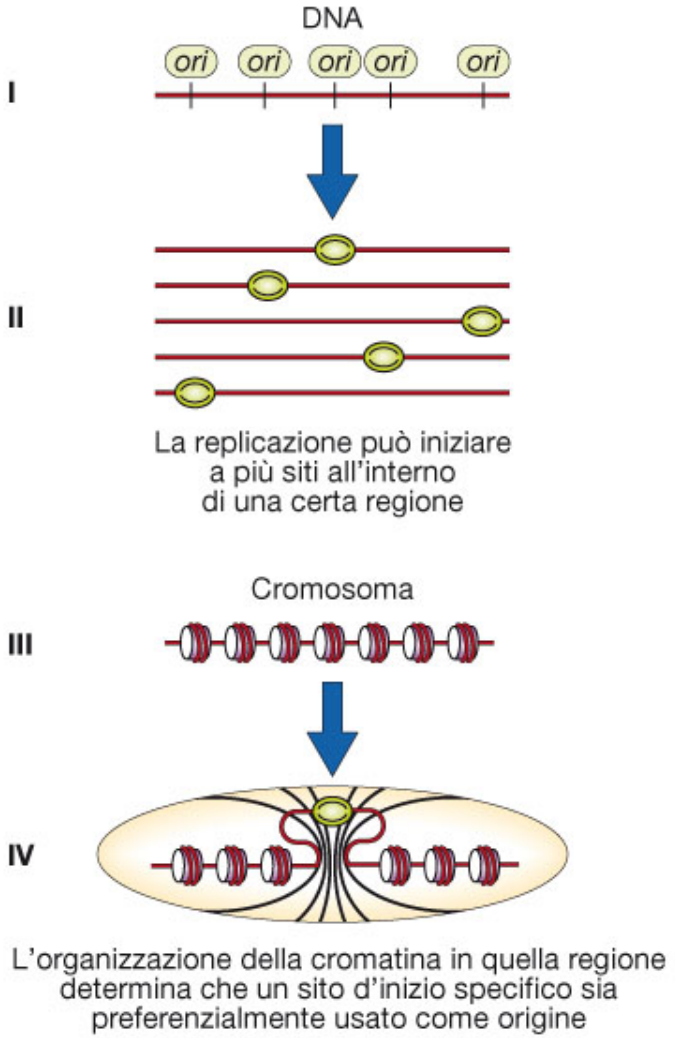
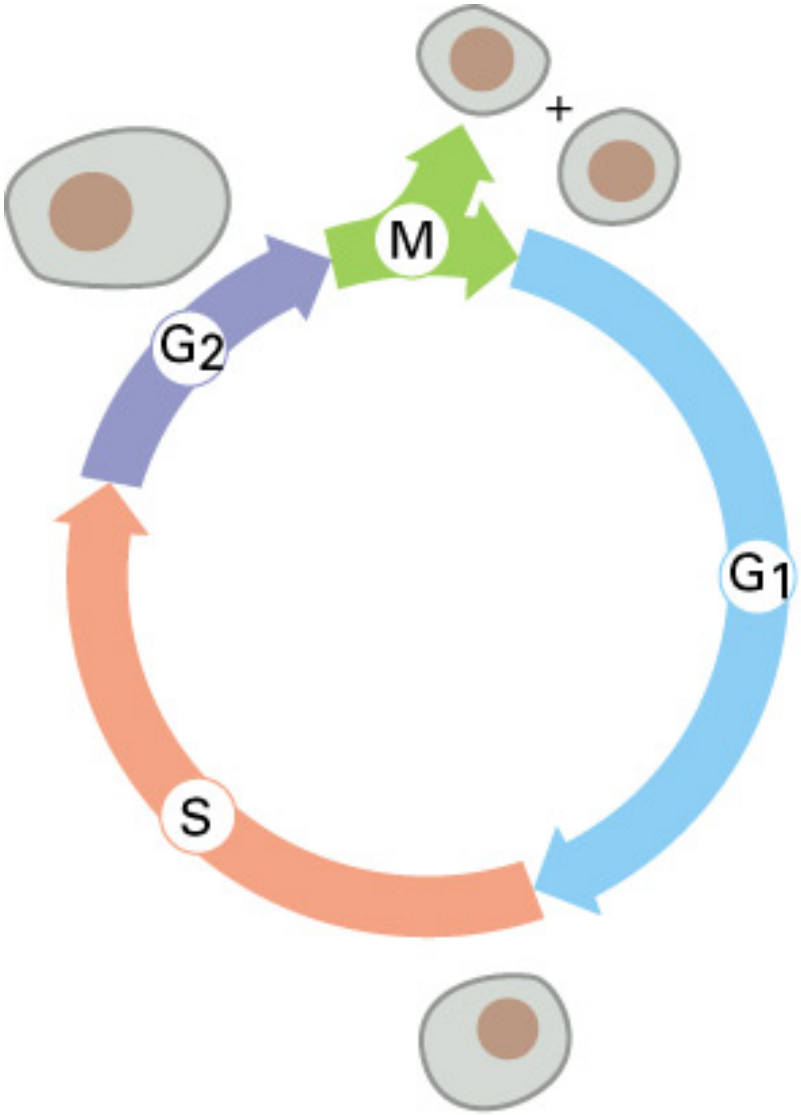


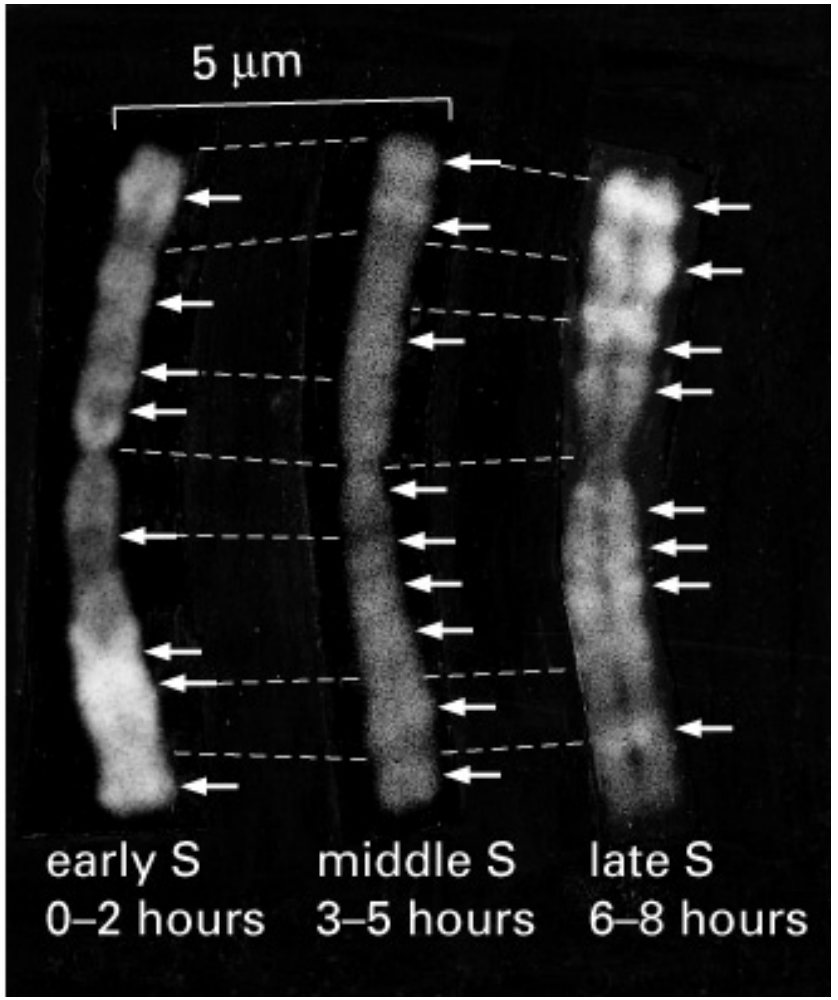
In homo sapiens…

- 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*.



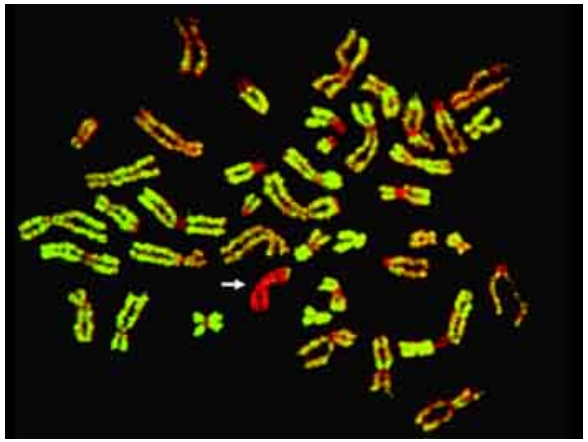
DNA replication takes place during S phase





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

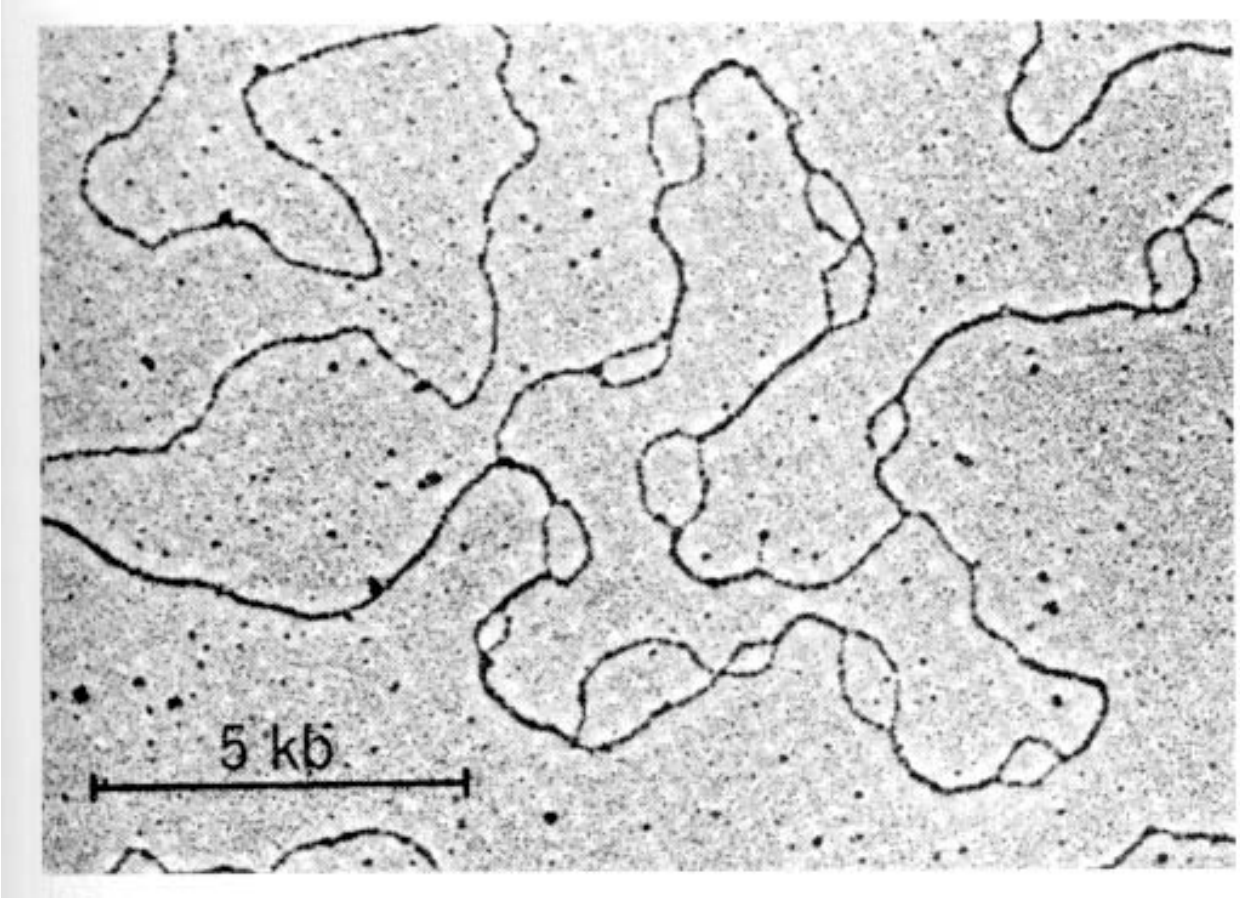
- the two X chromosomes contain the same DNA sequence

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

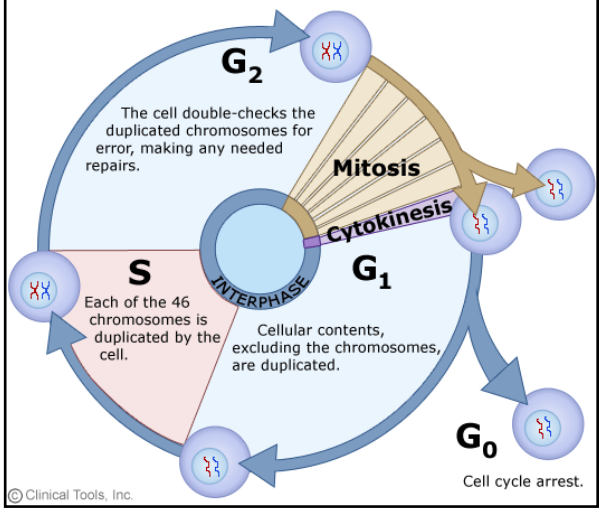
- one is active for transcription and is less condensed --> it replicates throughout S phase

ETEROCROMATINA: replicazione tardiva
EUCROMATINA: replicazione precoce

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



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

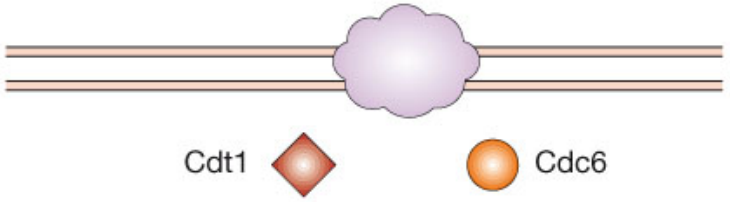


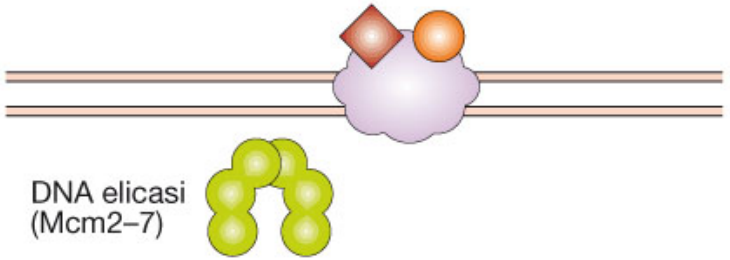
La preparazione delle origini alla replicazione avviene quando le cellule *escono dalla mitosi* e *proseguono nella fase G1* del ciclo cellulare (late M-early G1).

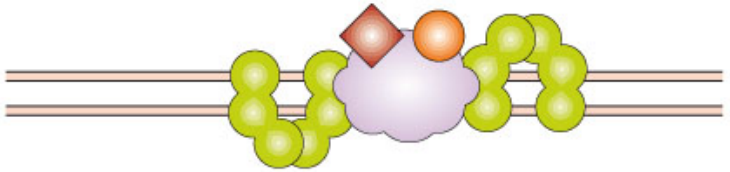
In questo momento si forma su ciascuna origine il **complesso di pre-replicazione** (pre-RC), il cui costituente principale e' il **COMPLESSO ORC**.

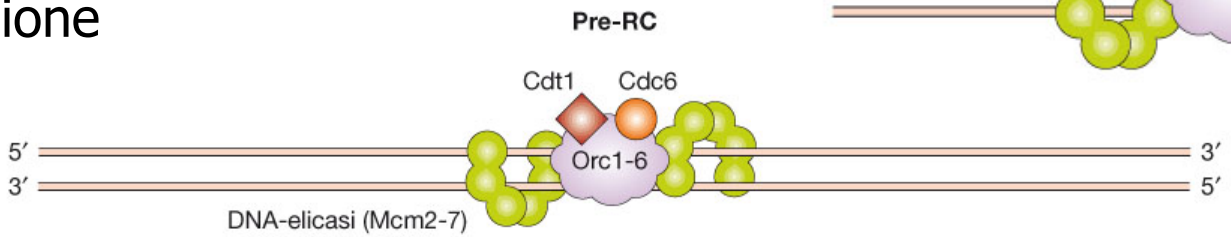
Ad ORC si associano due proteine chiave del controllo replicativo, **cdc6** e **cdt1**, richieste per l'attivazione dell'elicasi, nota come **Mcm2-7.maintenance protein 2,3,4,5,6,7).**

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



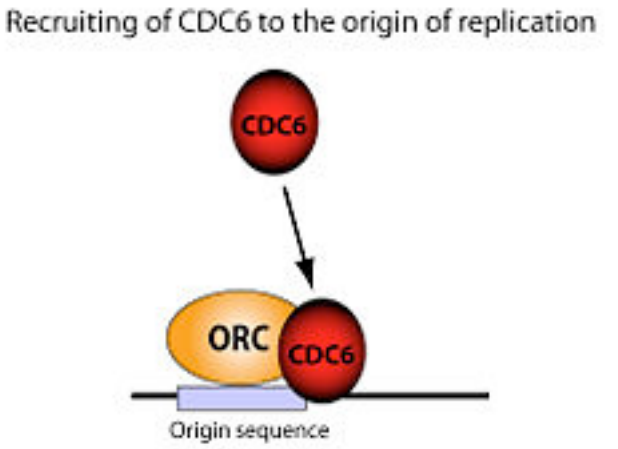


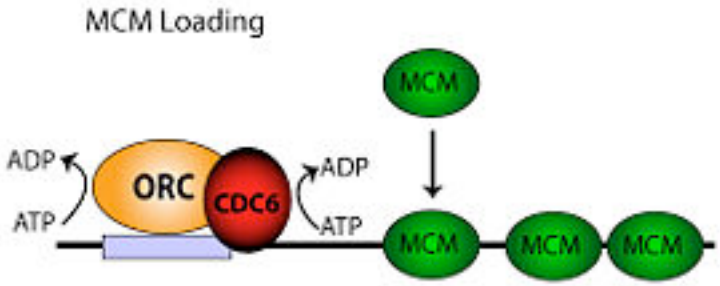


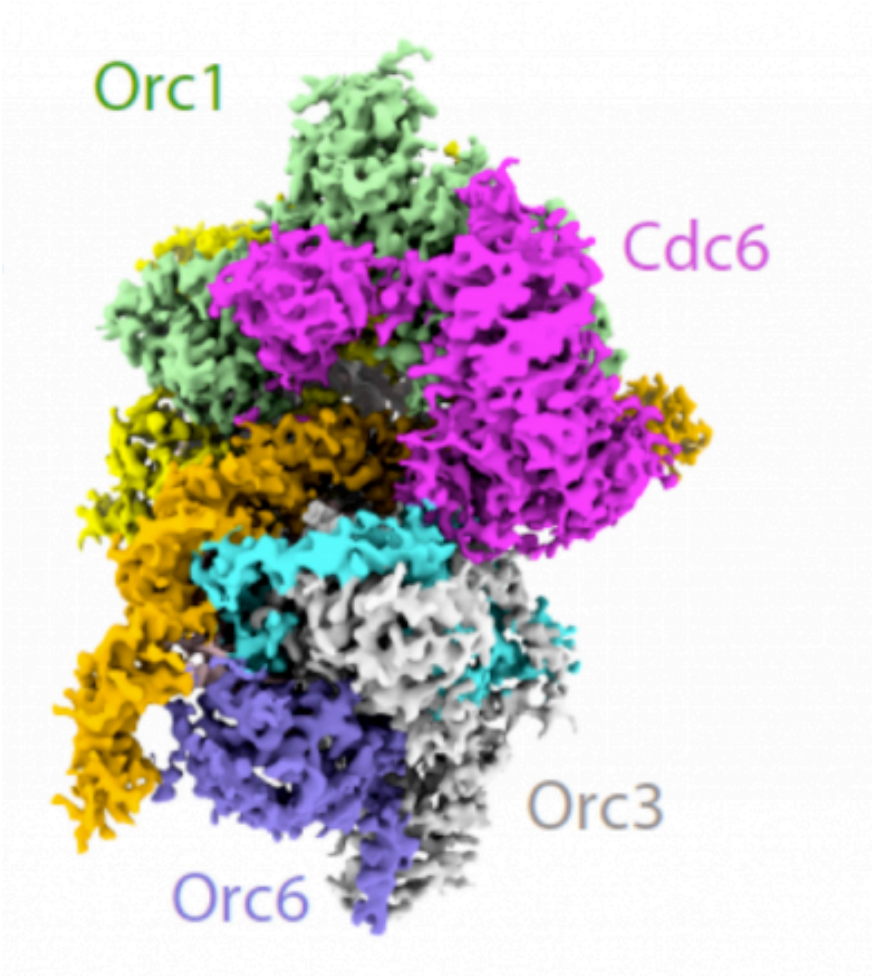


cdc-6

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.

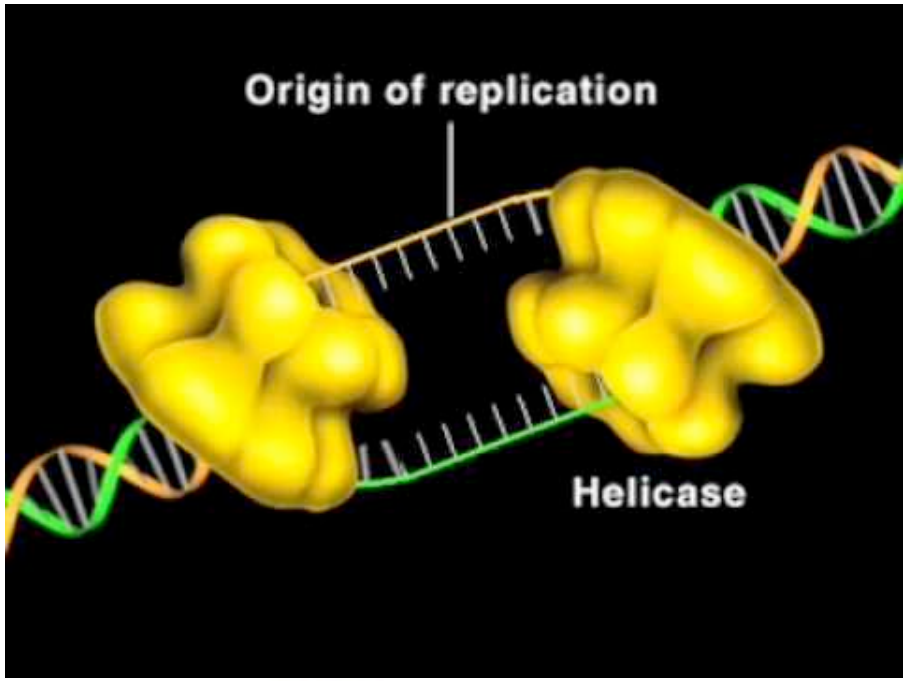


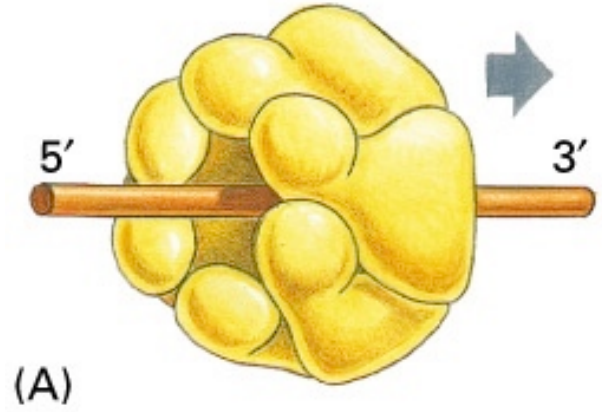




Mcm2-7

MCM2-7 helicase function arises from its architecture.



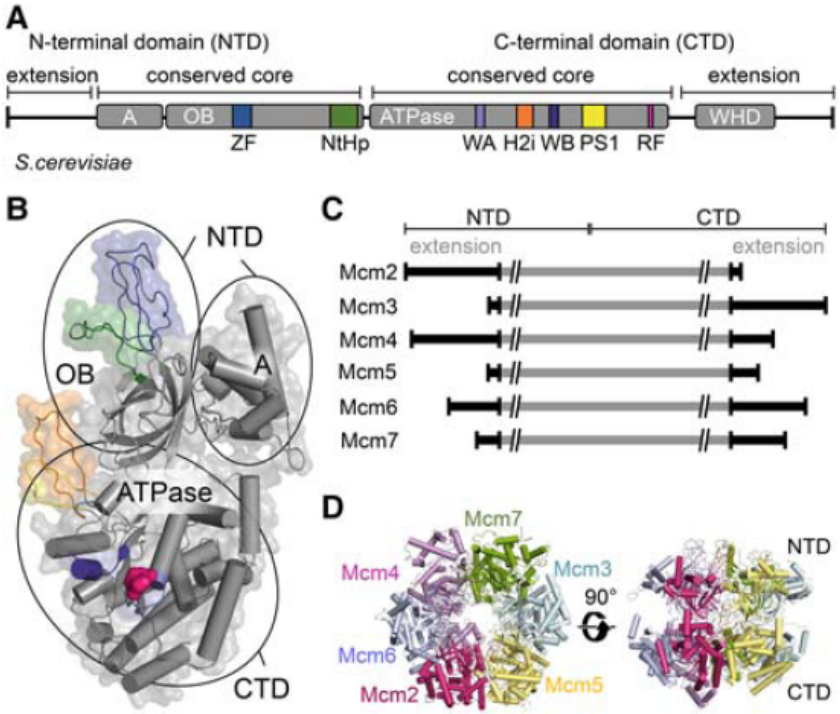


REVIEW

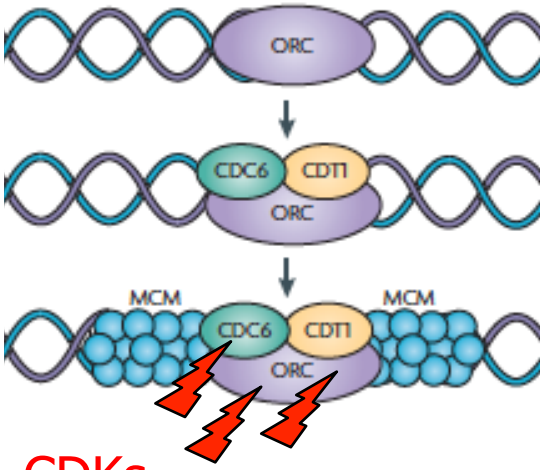
From structure to mechanism—understanding initiation of DNA replication

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]

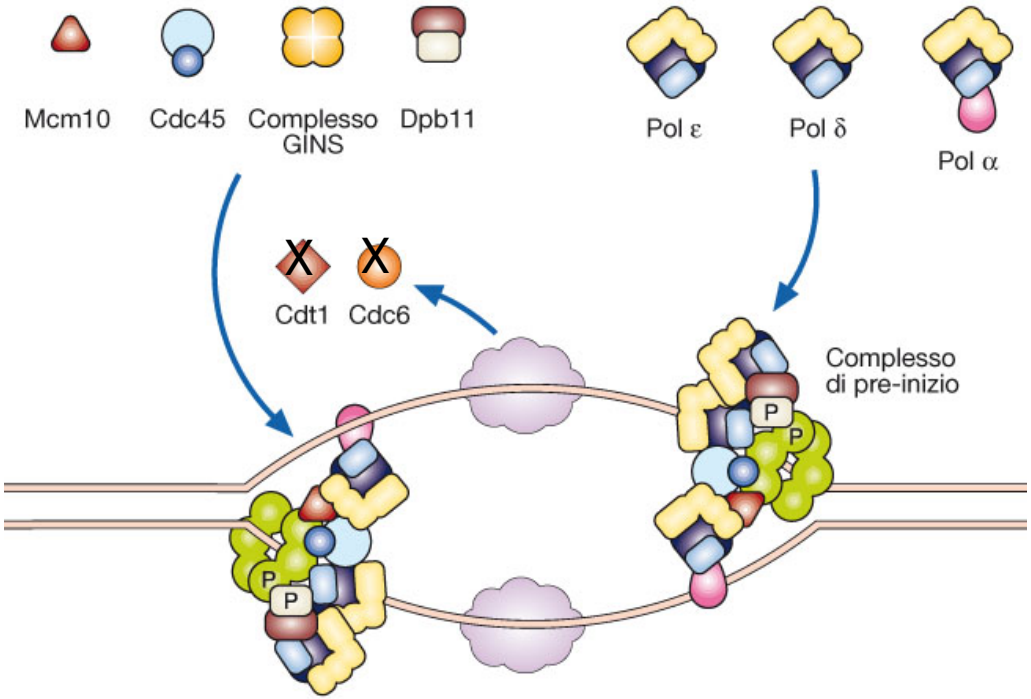
GENES & DEVELOPMENT 31:1073–1088 Published by Cold Spring Harbor Laboratory Press; ISSN 0890-9369/17; www.genesdev.org



I- The Pre-RC (early G1)



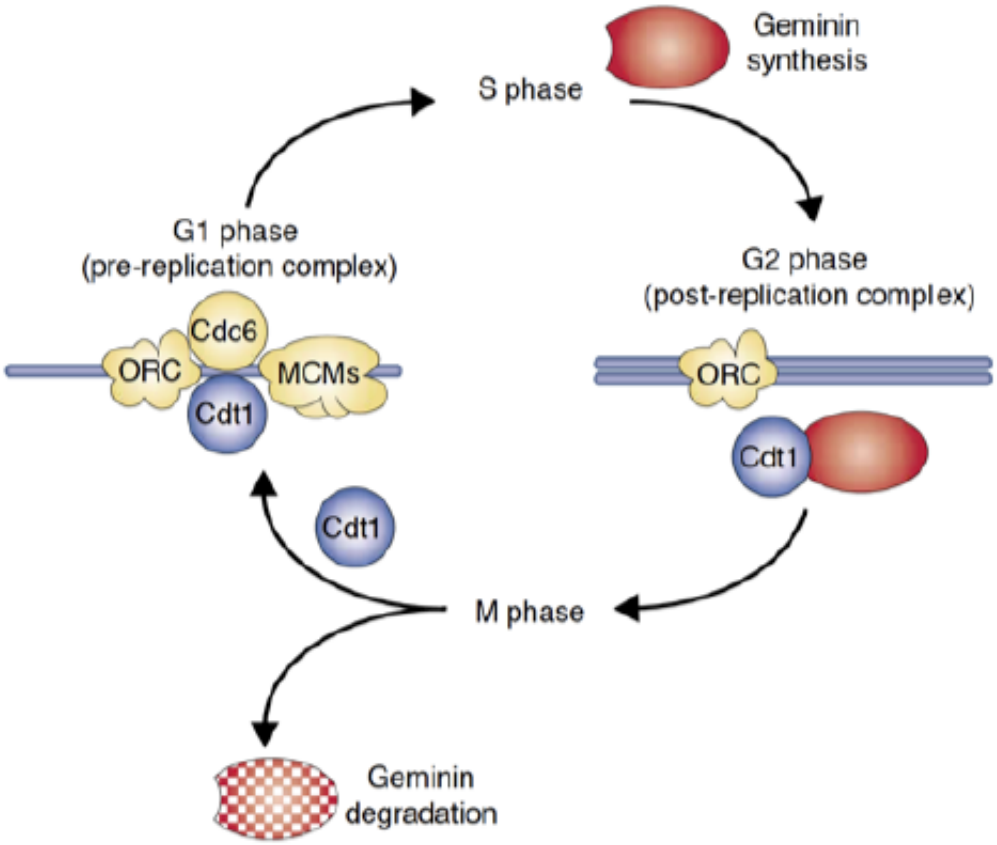
II- The Pre-IC (G1)

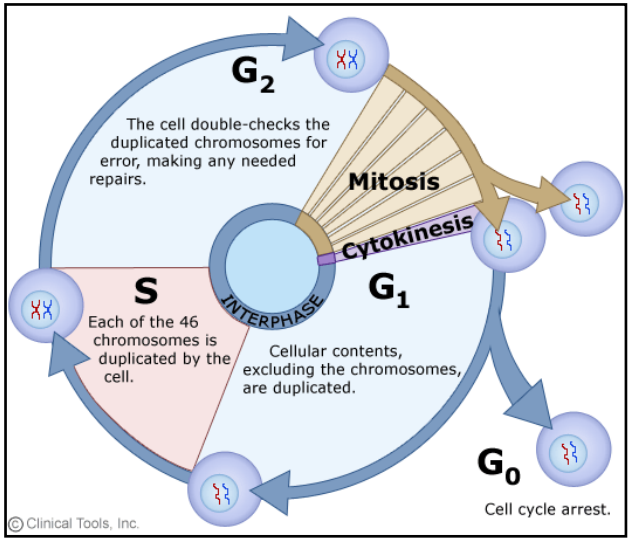


- Il passaggio da Pre-RC a Pre-IC richiede l'attivita' chinasica delle CDKs (chinasi ciclino-dipendenti).

- 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.

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





- ✓ Durante le fasi M e 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, G2 e M, impedendo cosi' che il DNA subisca piu' rounds di replicazione all'interno dello stesso ciclo cellulare.

Replication in context: dynamic regulation of DNA replication patterns in metazoans

Mirit I. Aladjem