Cell cycle

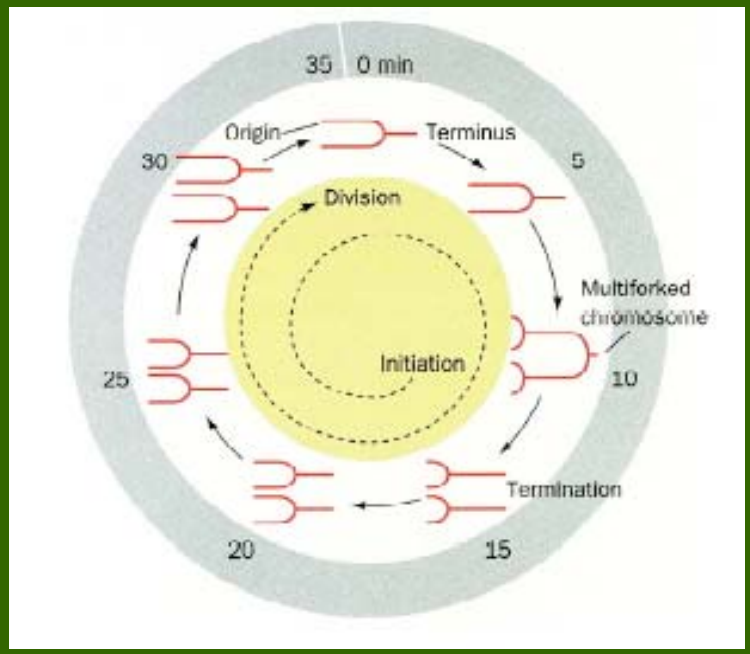
DNA replication and cell division

Regulation of cell division

- cell cycle:
  - cell division
  - cell growth

- control of the progeny (daughter cells) quality
  - transfer of incompletely replicated genomes to daughter cells prevented –„checkpoints“

E. coli cell cycle

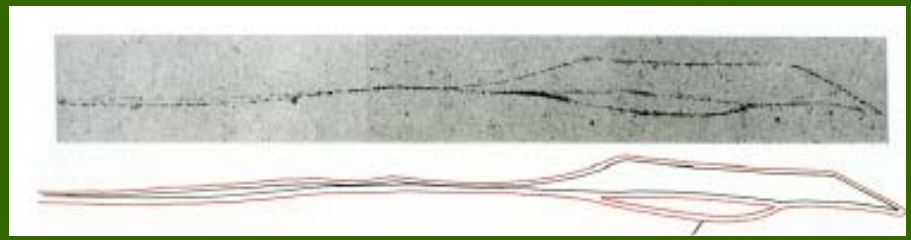


DNA synthesis (genome replication)

40 min

cell division

20 min after completion of replication



„doubling time" can be less than 60 min (40+20)

Cell cycle

in steps

specialized protein kinases, phosphatases, proteases function as switches, couple cell cycle with environmental conditions, facilitate quality control

Saccharomyces, Schizosaccharomyces, Drosophila models

cellular machinery of the cell cycle highly conserved in eukaryots:

- protein kinases
- enzymes of DNA replication
- cytoskeletar struktures (mitosis – chromosome movements)
- components of ubiquitin-dependent pathway for protein degradation

Cell division

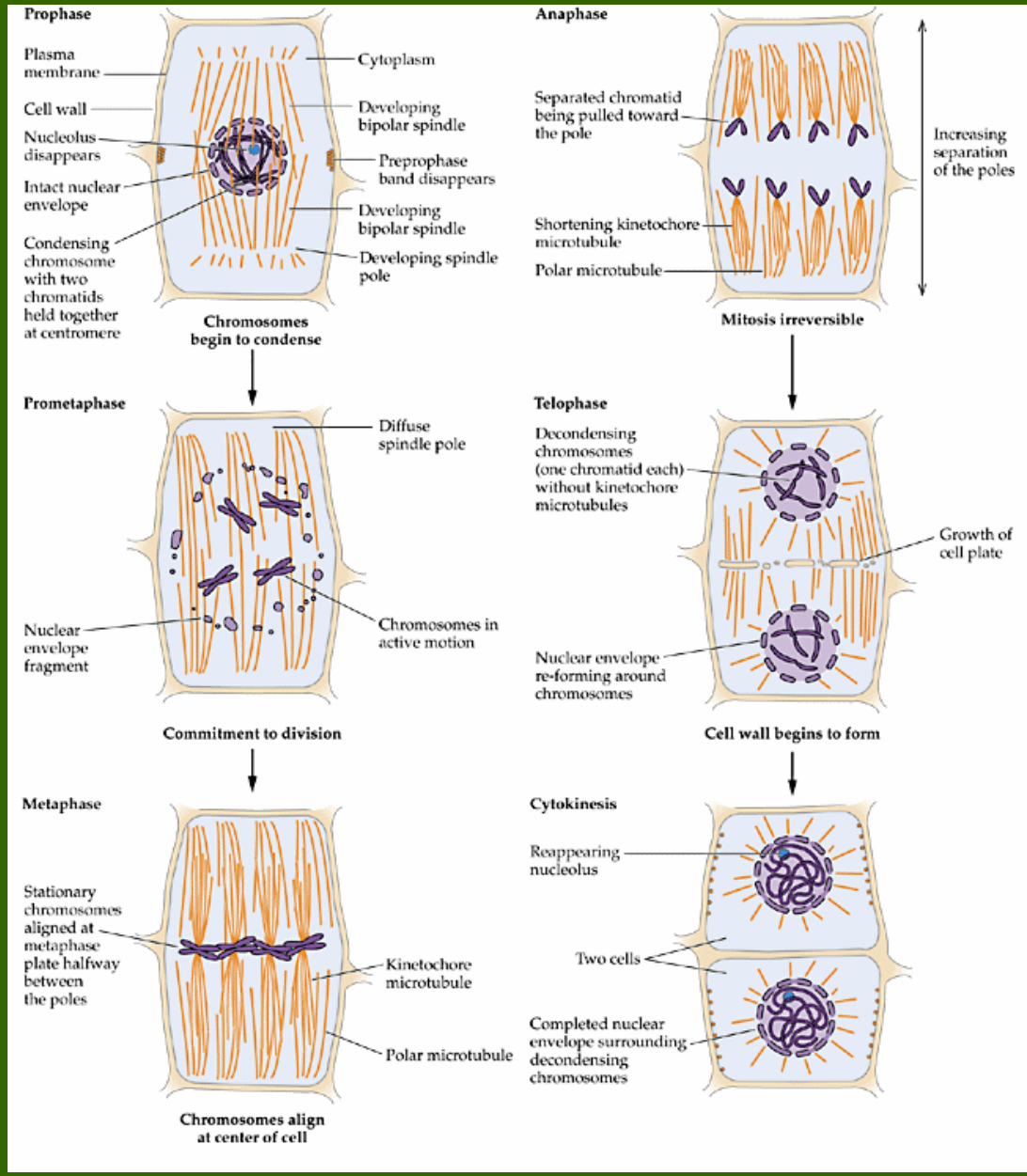
specifics in plants:

separation by cell walls – plate formation at the equator (not constriction)

replicaton of 3 genomes (nuclear and organellar)

repeated organ formation, demaged or dead organs replaced (controlled cell proliferation)

Mitosis


Prophase
Plasma membrane
Cell wall
Nucleolus disappears
Intact nuclear envelope
Condensing chromosome with two chromatids held together at centromere
Cytoplasm
Developing bipolar spindle
Preprophase band disappears
Developing bipolar spindle
Developing spindle pole
Chromosomes begin to condense
Prometaphase
Diffuse spindle pole
Nuclear envelope fragment
Chromosomes in active motion
Commitment to division
Metaphase
Stationary chromosomes aligned at metaphase plate halfway between the poles
Kinetochore microtubule
Polar microtubule
Chromosomes align at center of cell
Anaphase
Separated chromatid being pulled toward the pole
Shortening kinetochore microtubule
Polar microtubule
Increasing separation of the poles
Mitosis irreversible
Telophase
Decondensing chromosomes (one chromatid each) without kinetochore microtubules
Growth of cell plate
Nuclear envelope re-forming around chromosomes
Cell wall begins to form
Cytokinesis
Reappearing nucleolus
Two cells
Completed nuclear envelope surrounding decondensing chromosomes

protoplas fusion at different stages of the cell cycle

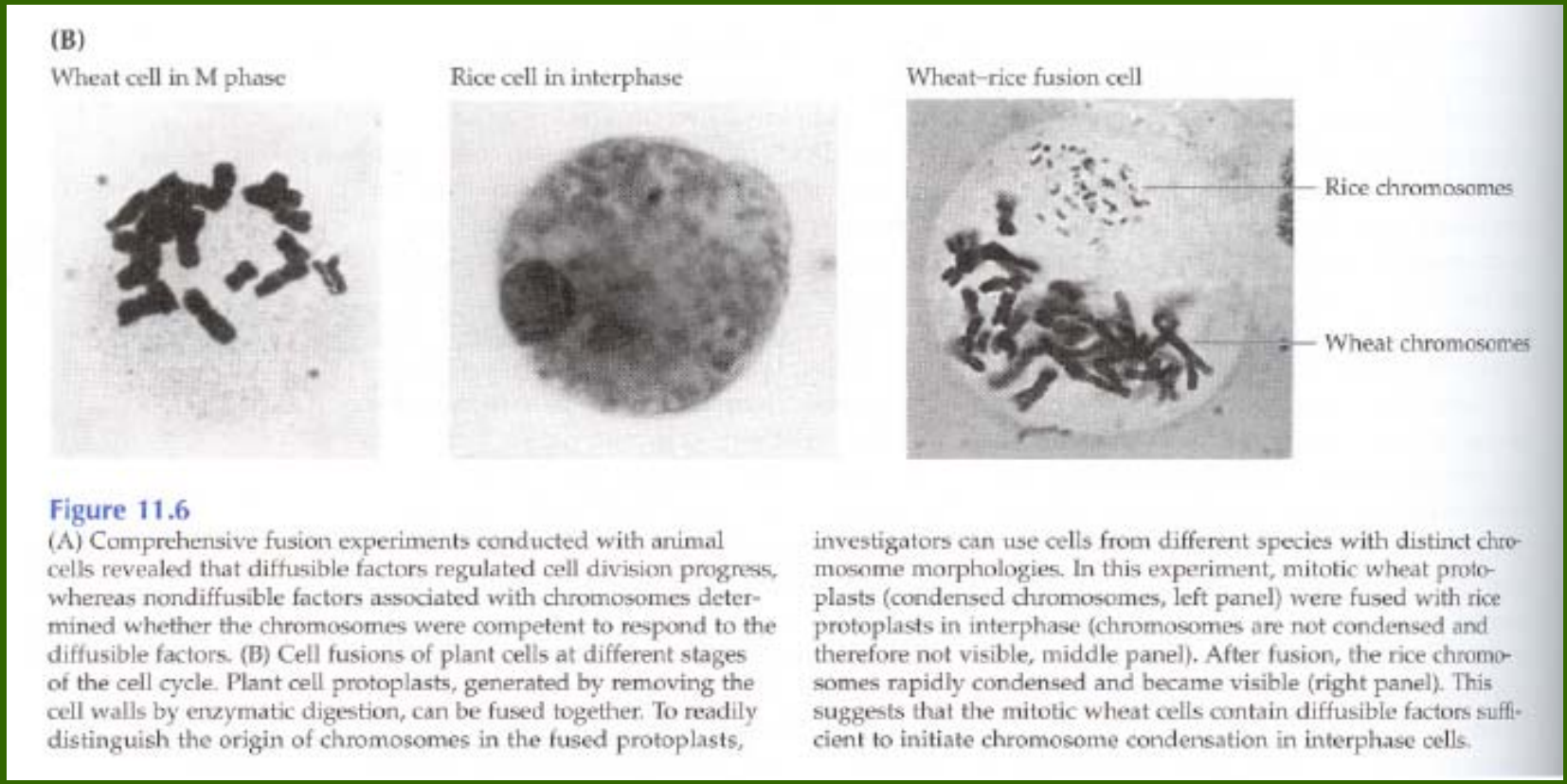
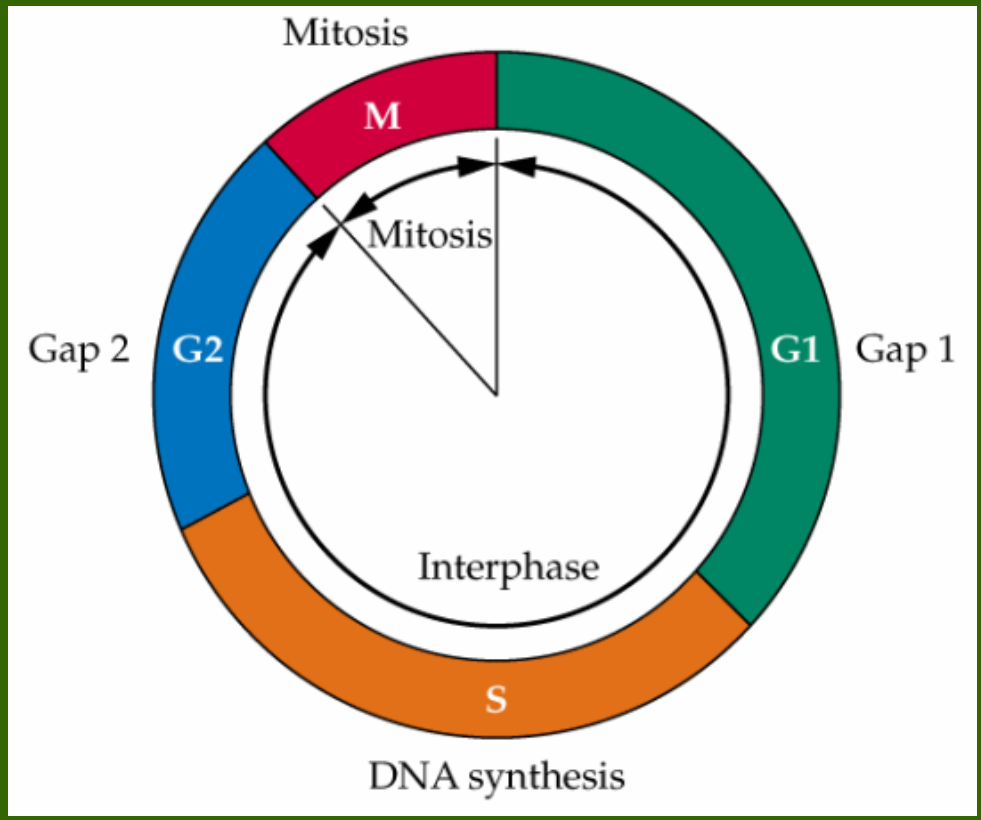


Figure 11.6
(A) Comprehensive fusion experiments conducted with animal cells revealed that diffusible factors regulated cell division progress, whereas nondiffusible factors associated with chromosomes determined whether the chromosomes were competent to respond to the diffusible factors. (B) Cell fusions of plant cells at different stages of the cell cycle. Plant cell protoplasts, generated by removing the cell walls by enzymatic digestion, can be fused together. To readily distinguish the origin of chromosomes in the fused protoplasts, investigators can use cells from different species with distinct chromosome morphologies. In this experiment, mitotic wheat protoplasts (condensed chromosomes, left panel) were fused with rice protoplasts in interphase (chromosomes are not condensed and therefore not visible, middle panel). After fusion, the rice chromosomes rapidly condensed and became visible (right panel). This suggests that the mitotic wheat cells contain diffusible factors sufficient to initiate chromosome condensation in interphase cells.

Cell cycle

interphase x mitosis

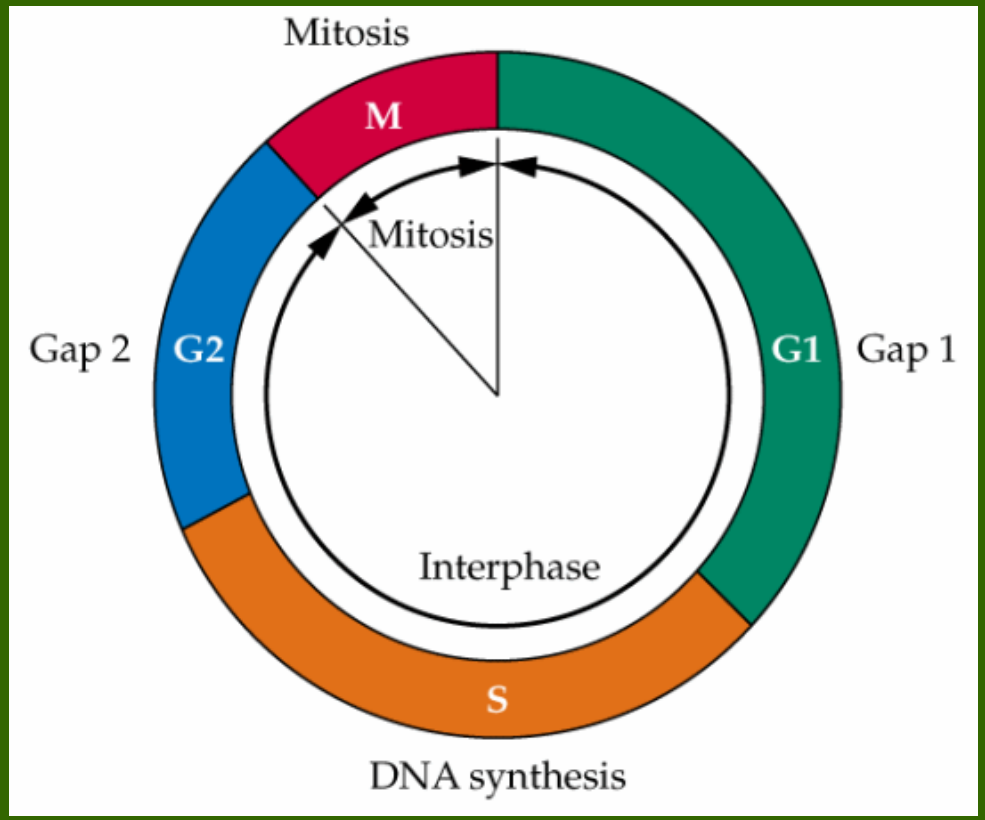


Control of DNA replication

very strict

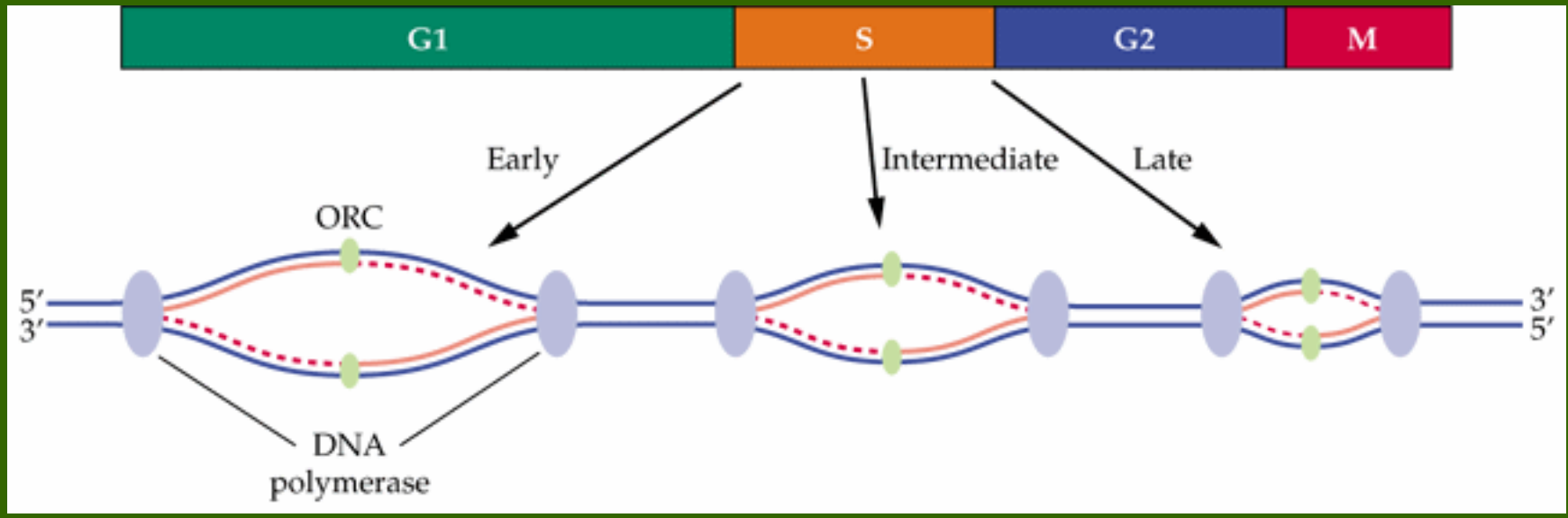
iniciation of DNA synthesis inhibited in G2, M and G1

synthesis from discreet origins (dicots á 66 kb, monocots á 47 kb)



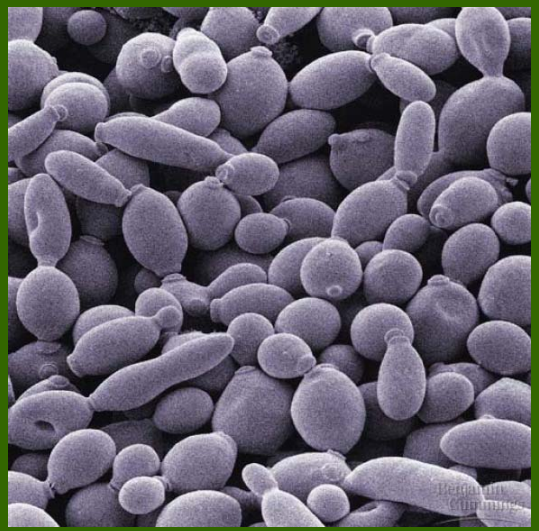
origins of replication

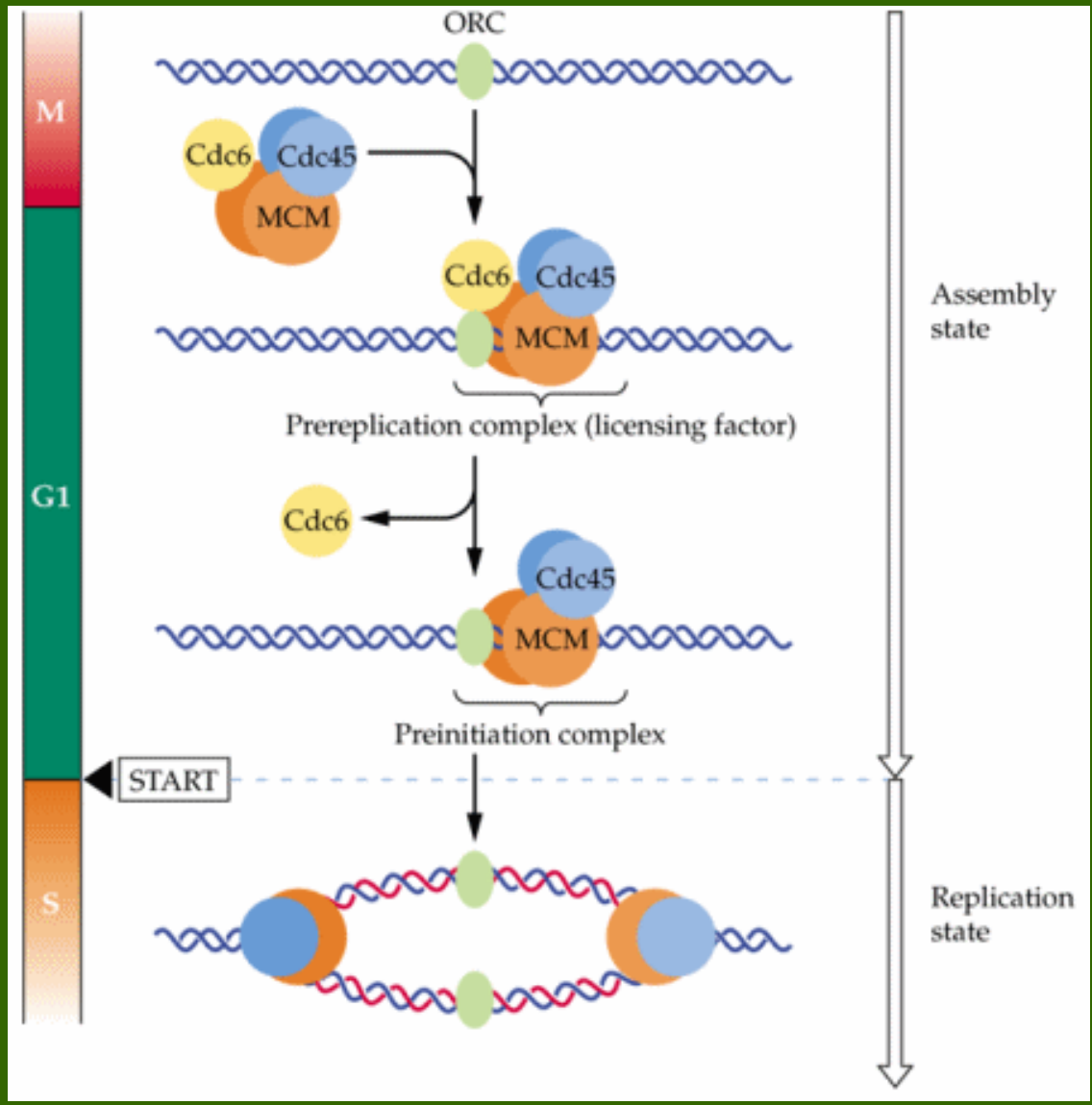
- throughout CC bound by ORC (origin recognition complex)
- Orc proteins interact with other proteins:
  - Cdc (cell division control)
  - MCM (initiation of replication only once per cycle –„licencing factor")
  - DNA polymerase



Initiation of replication

budding yeast





Gene *PROLIFERA*

Arabidopsis, product Mcm7, mutation lethal

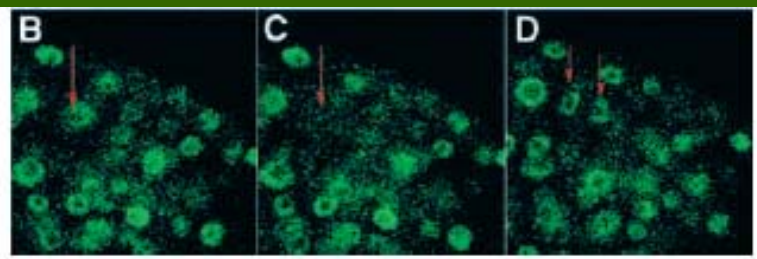


Fig. 3. PROLIFERA is localized in the nucleus during the G_1 phase of the cell cycle. (A) Whole mount of root tip from *prl*/+ plant stained with X-Gluc, showing GUS localized in the nucleus of individual files of cells. (B-D) Accumulation of PRL::GFP fusion in root tip nuclei. The time between images in B and C is 4 minutes. The time between the images in C and D is 28 minutes. The arrow in B marks a cell that has PRL::GFP localized to the nucleus. Localization is then lost (C), and returns in daughter nuclei (D) following mitosis.

Springer et al. (2000) Development 127; 1815.

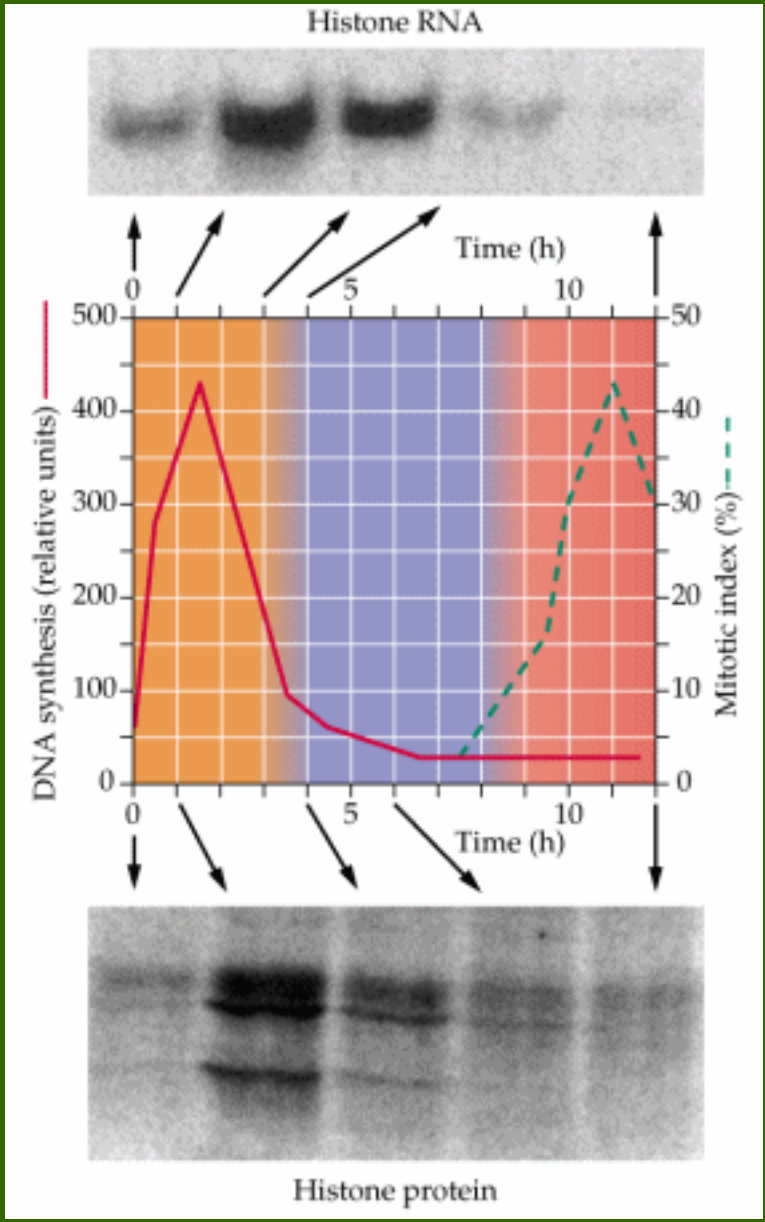
Other replication proteins

establish conditions favorable for DNA replication

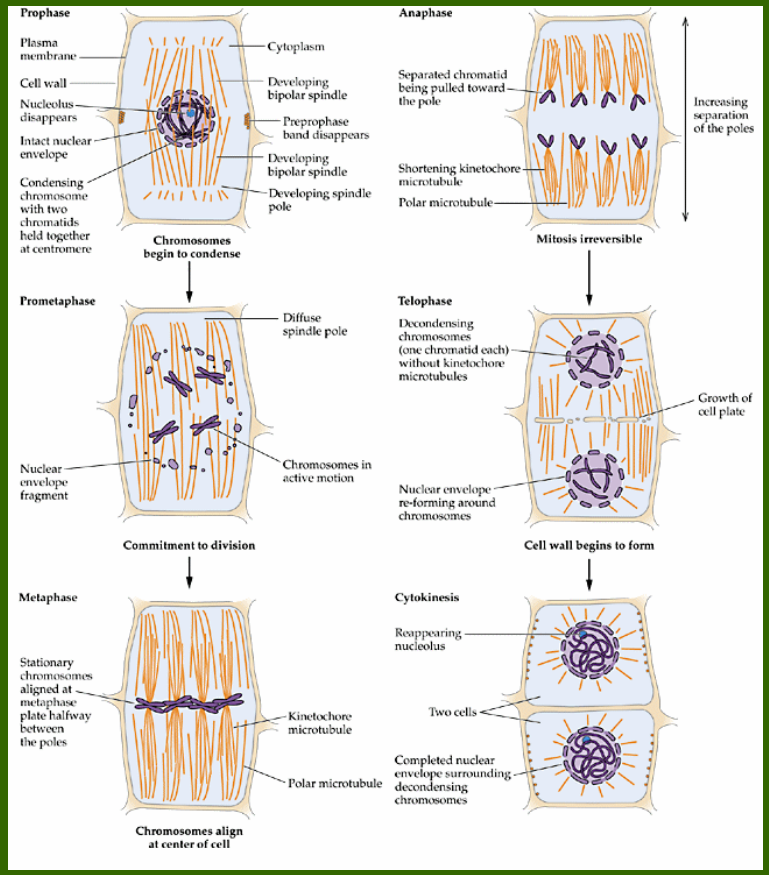
e.g. pathways required for synthesis of dNTPs (DNA synthesis substrates) – stimulated just before the onset of S-phase

early S-phase – new histones synthesized (chromatin)

Histone synthesis in S-phase



APC (anaphase-promoting complex)



-destruction of specific proteins

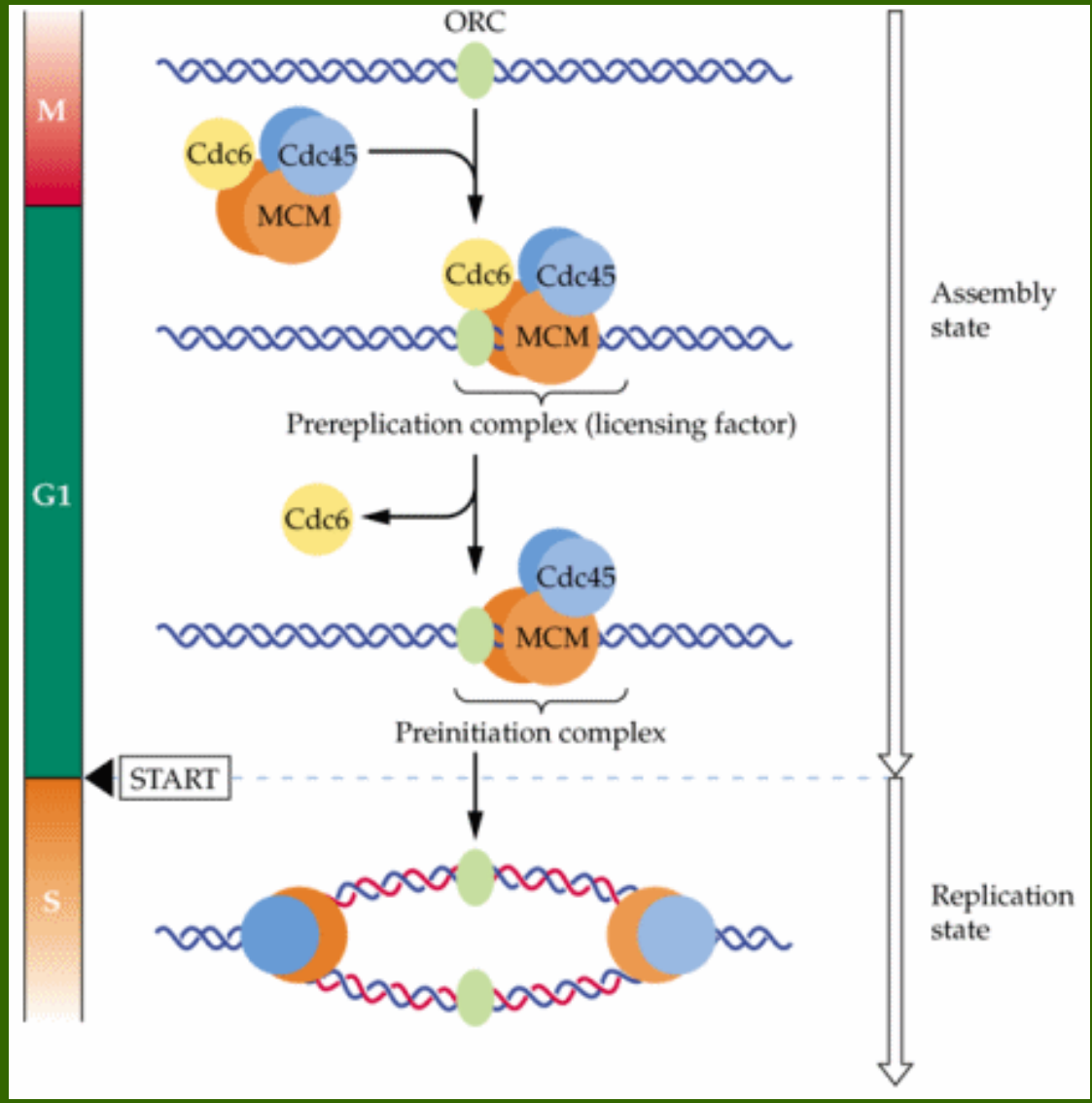
-degradation of mitotic cyklins (exit from M-phase)

end of M-phase onset of G1

budding yeast

Cdc6 interacts with ORC complex

- only in the absence of mitotic kinase activity



Iniciace mitózy

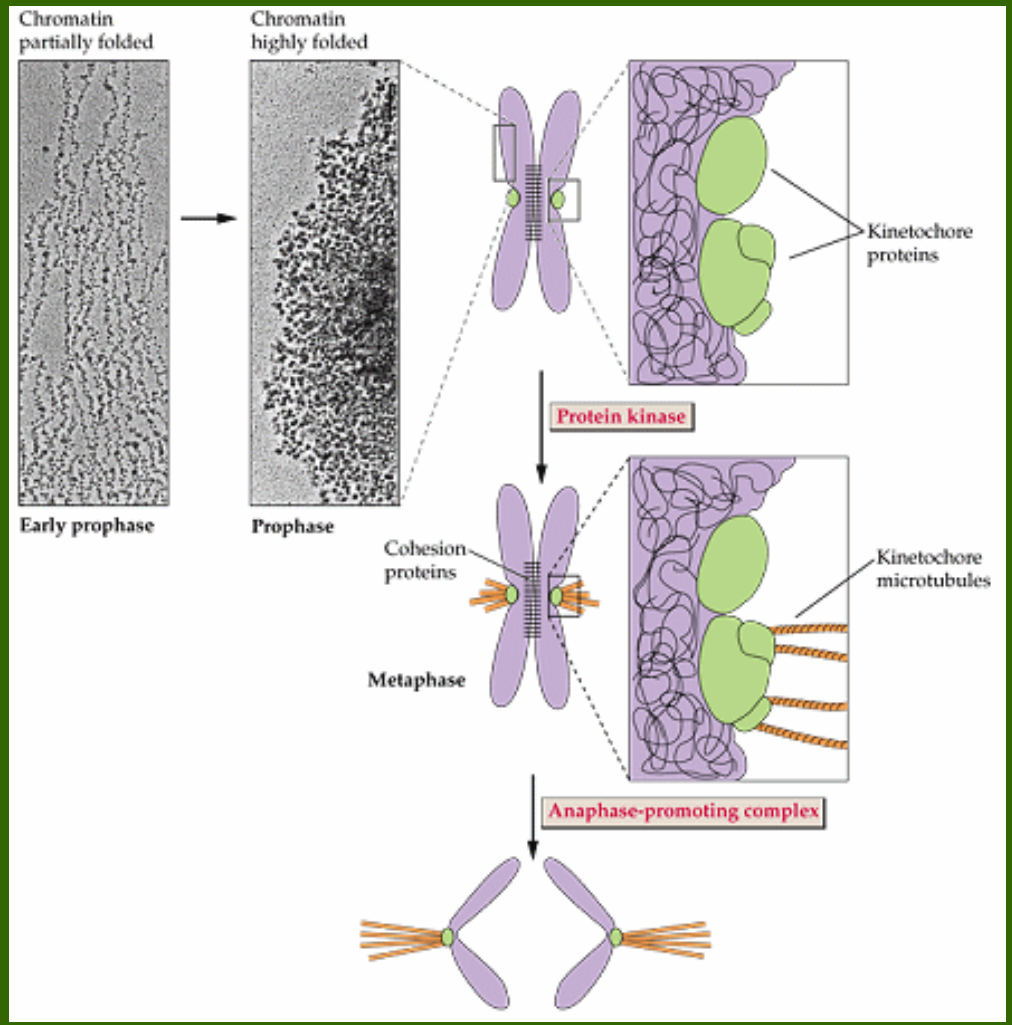
mitosis initiated in S-phase

full condensation of chromatin in prophase

kinetochoes –protein complexes bound to centromeres, attachment points for spindle microtubules

APC (anaphase-promoting complex) tags mitosis inhibitor (Pds1p) for proteolysis

destruction of **cohesion proteins** joining sister chromatids



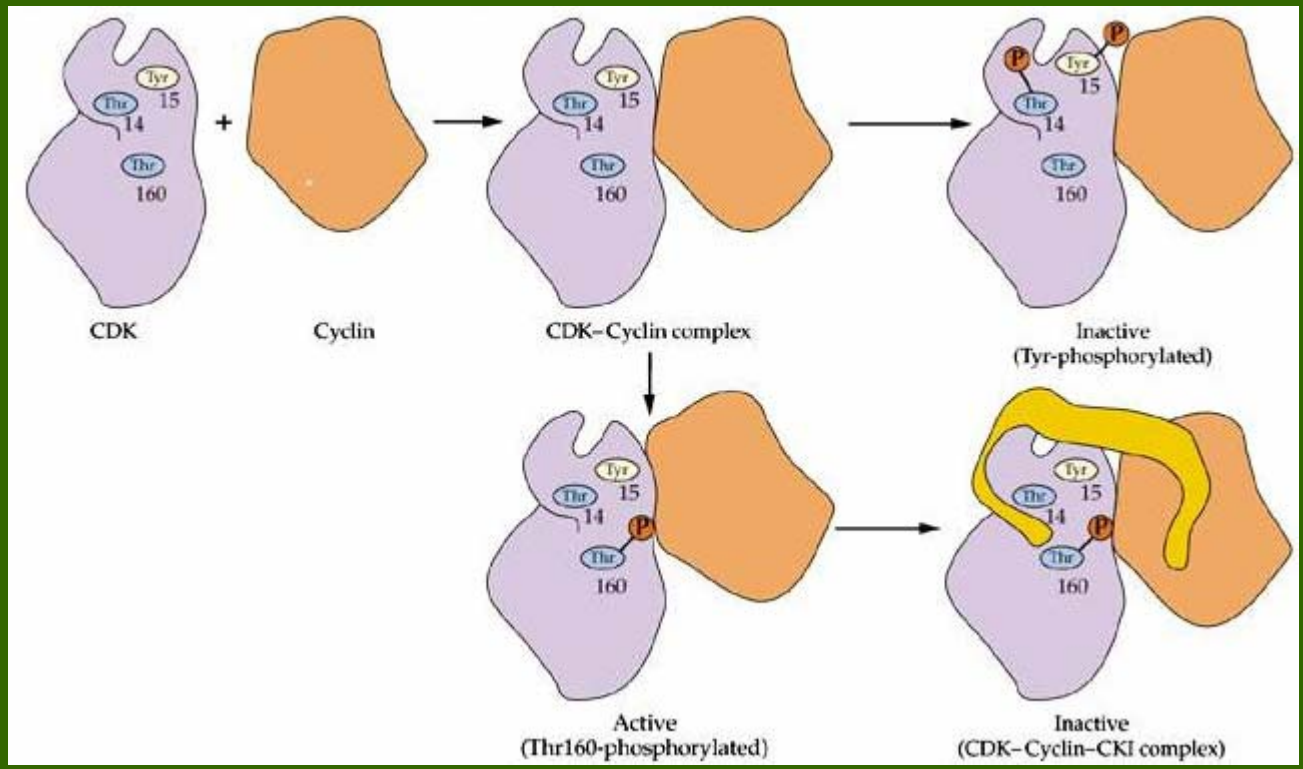
cell cycle control

by activity changes of **CDKs** (cyclin-dependent kinases)

CDK:

catalytic subunit activated by association with cyclin

CDK complexes:



cyclins

small group of proteins which accumulate and disappear during the cell cycle,

required for CDK activity

confer substrate specifity to the cyclin-CDK complexes

determine subcellular localization of CDKs

cyclins

1. mitotic

B-type/M ……G2 to M

A-type/S ……S phase

„cyclin box"

„mitotic destruction box"

2. G1 cyclins

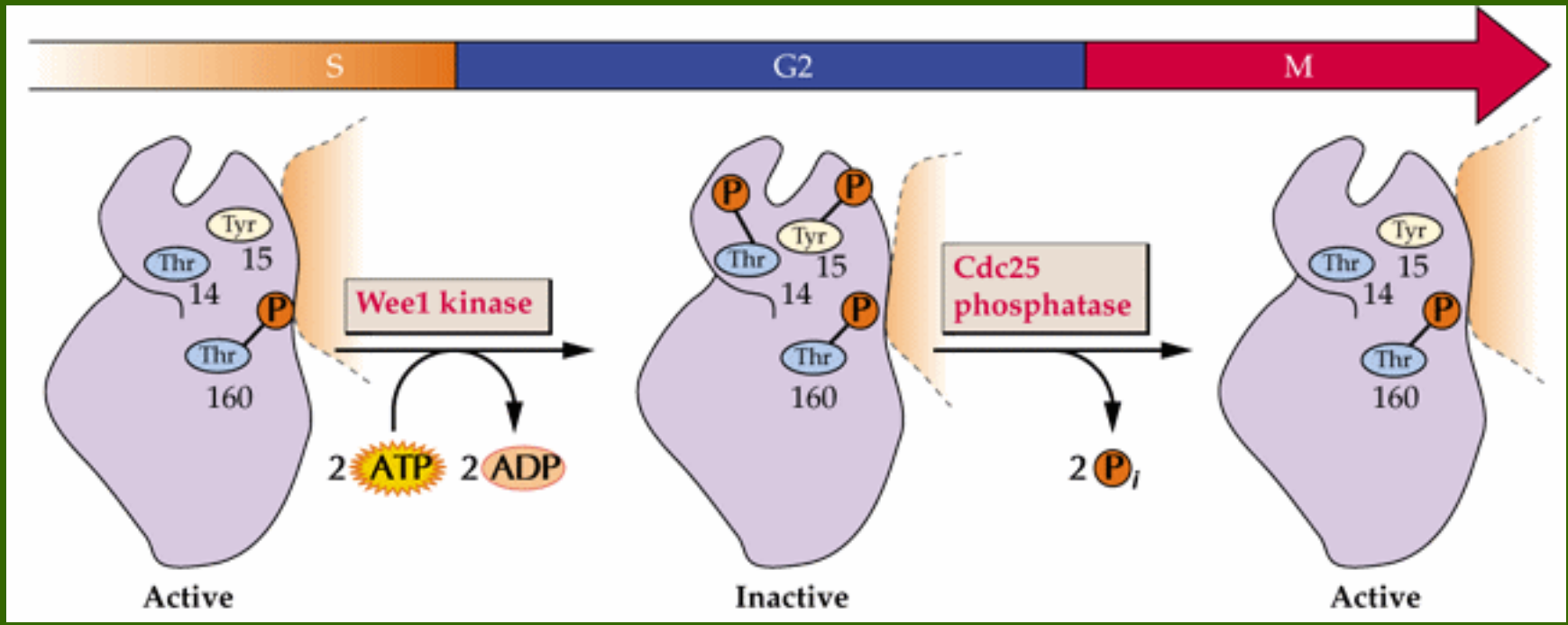
(D-type and E-type)

mainly D-type – in G1, degraded at beginning of S-phase (some in low amounts throughout the CC

central cyclin box less conserved

CDK activity control

kinases, phosphatases, specific inhibitors – example from yeast



CDK phosphorylation inhibits protein kinase activity of the enzyme

CDK activity control by inhibitors

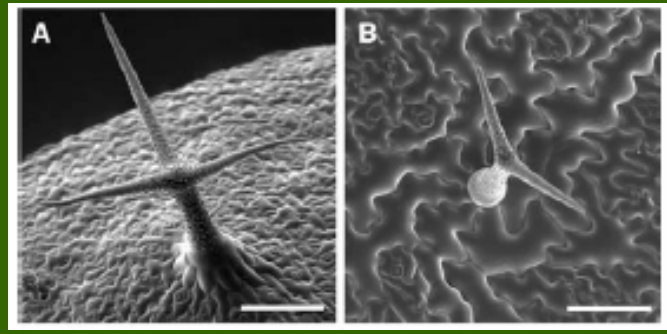
CKIs: inhibitors of CDKs

associate with the activated CDK-cyclin complex to prevent it from phosphorylating substrates

Arabidopsis – gene *ICK1*

encodes a CKI, induced by ABA, probably mediates „cell cycle arrest"

in trichomes acts in concentration-dependent manner: blocks G1-S transition at high conc., G2-M at low conc.



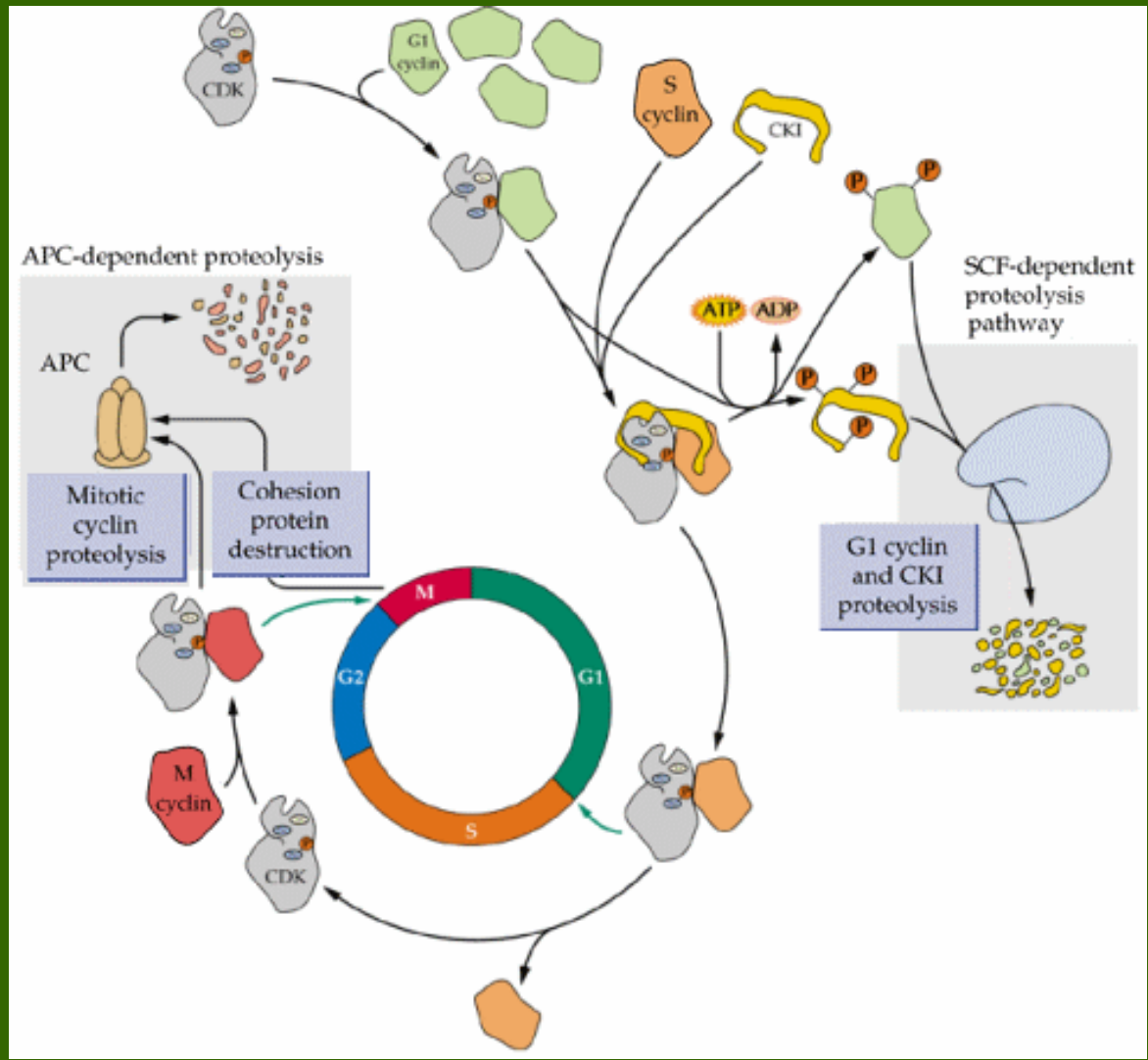
(A) and (B) Scanning electron micrographs of mature Arabidopsis trichomes and their neighboring cells.
(A) Typical wild-type trichome with three branches surrounded by rectangular trichome-neighboring cells, which are polarized toward the trichome.
(B) Trichomes in Pro_{GL2}:*ICK1*/*KRP1*^{109} are smaller and develop fewer branches, whereas trichome-neighboring cells are lobed and greatly enlarged but are still oriented toward the trichome.

Weinl et al. (2005) Plant Cell 17; 1704

Proteolysis in cell cycle

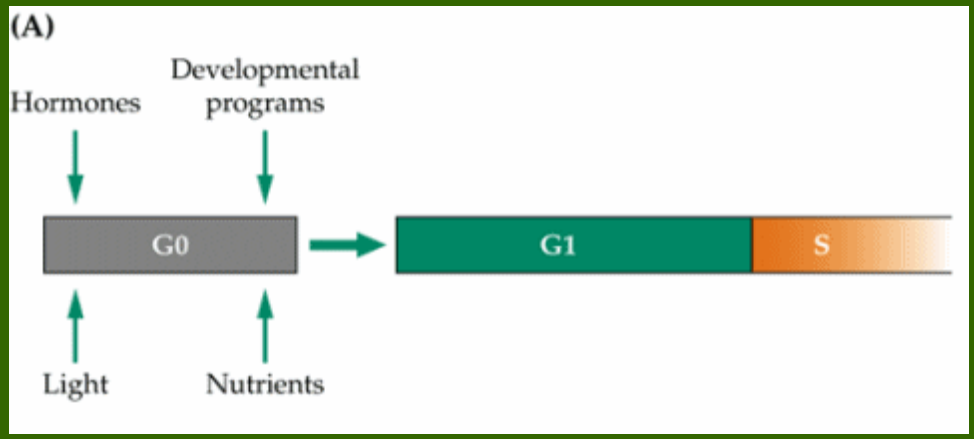
proteolysis of CKIs (G1-S transition)

degradation of cohesion proteins (metaphase-anaphase)

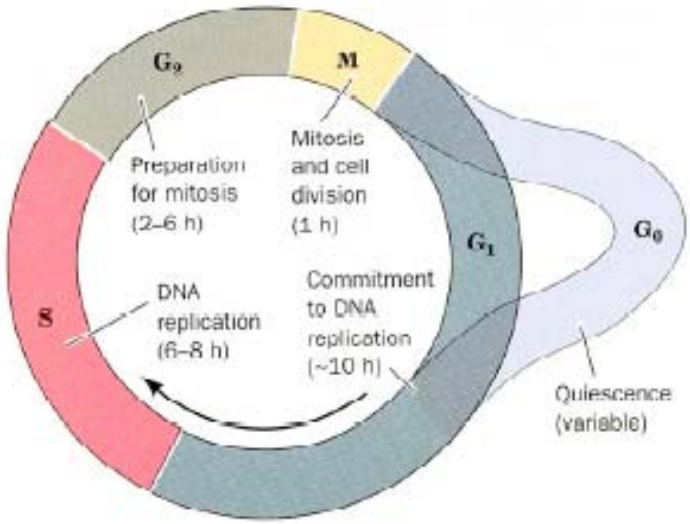


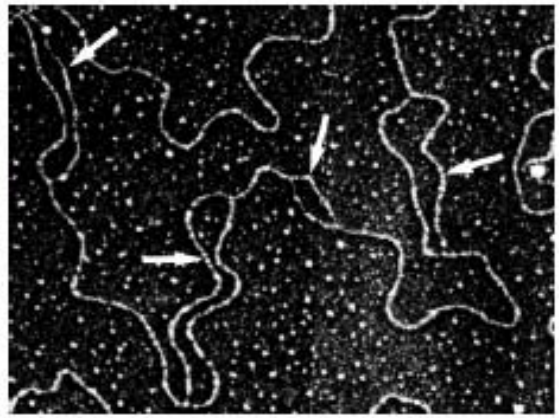
in plants

G0-phase



quiescent cells do not proliferate without signal



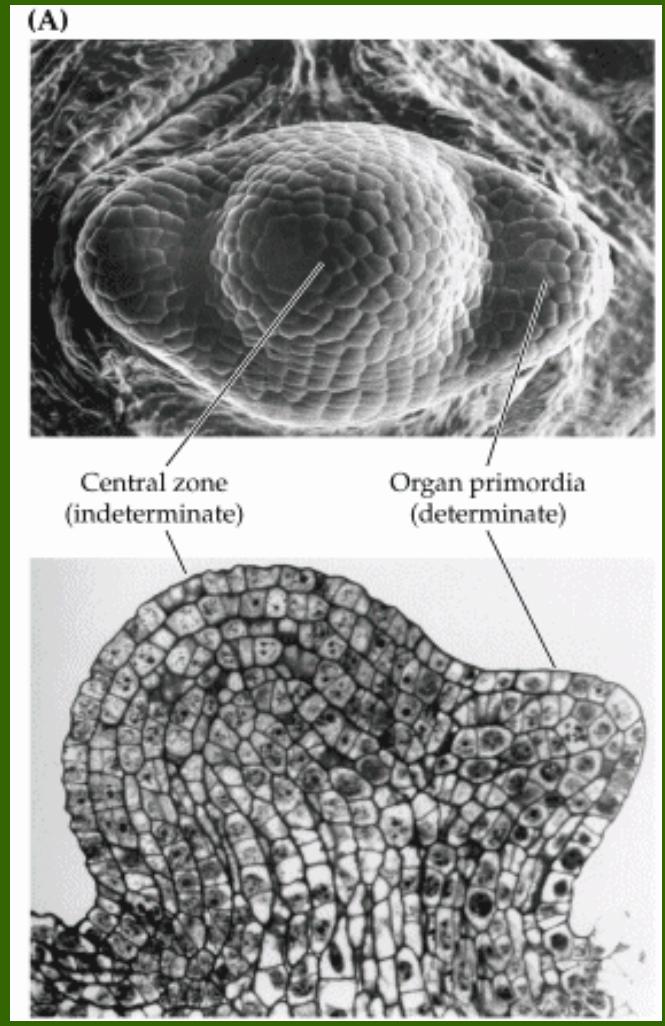


Eukaryotic DNA Replication:
The limited time for DNA replication (6-8 hours) combined to the increased size of the genomes (>10^7 base pairs) explain the requirement for multiple replication origins

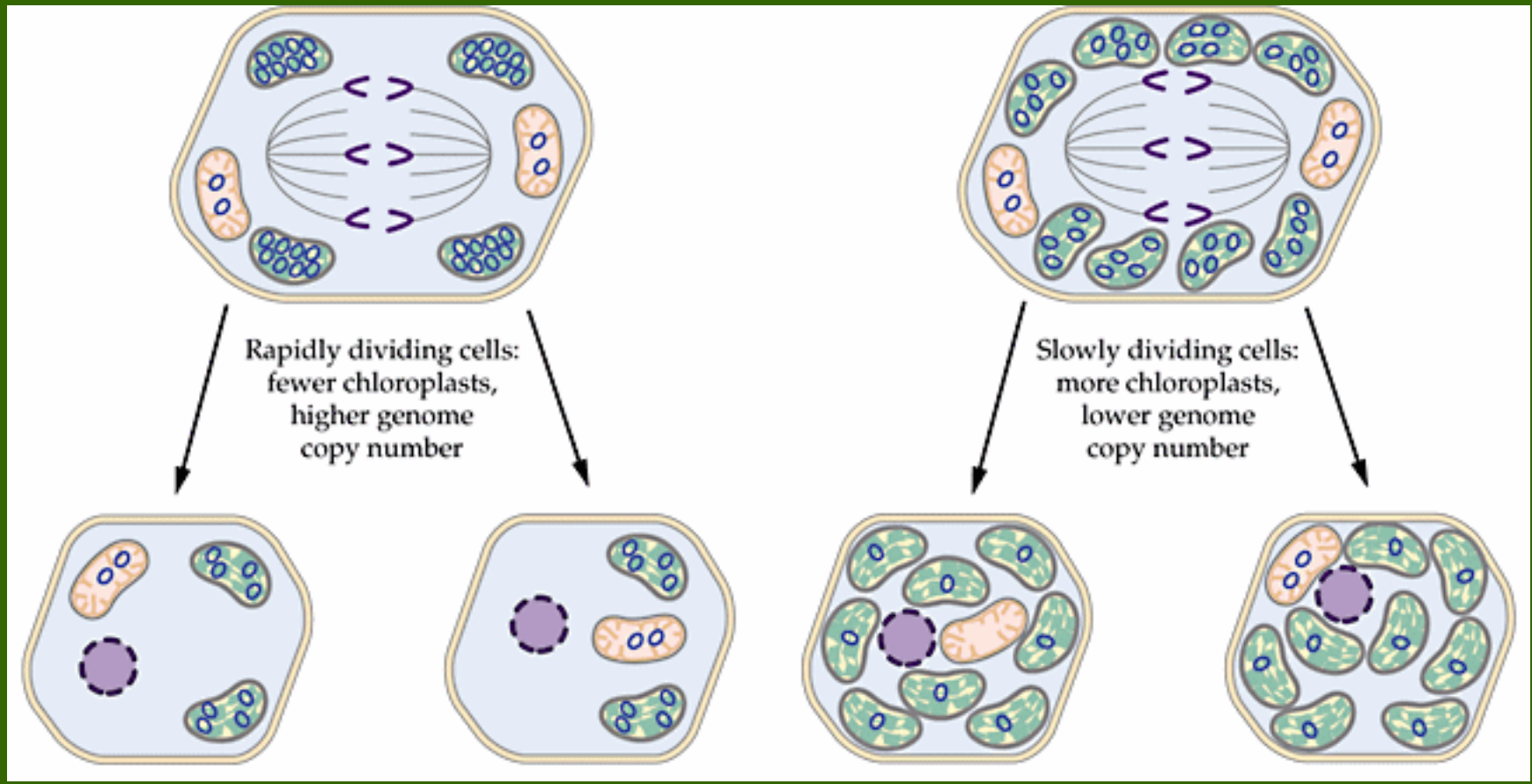
organization of meristems (SAM)

genes for proper function of meristems:

- establish and maintain the indeterminate central zone (self-renewal of meristem)
- direct differentiation in organ primordia
- localize cell division in organ primordia



maintanance of three genomes



Arabidopsis

regulation of cell cycle by plant hormones and other regulators