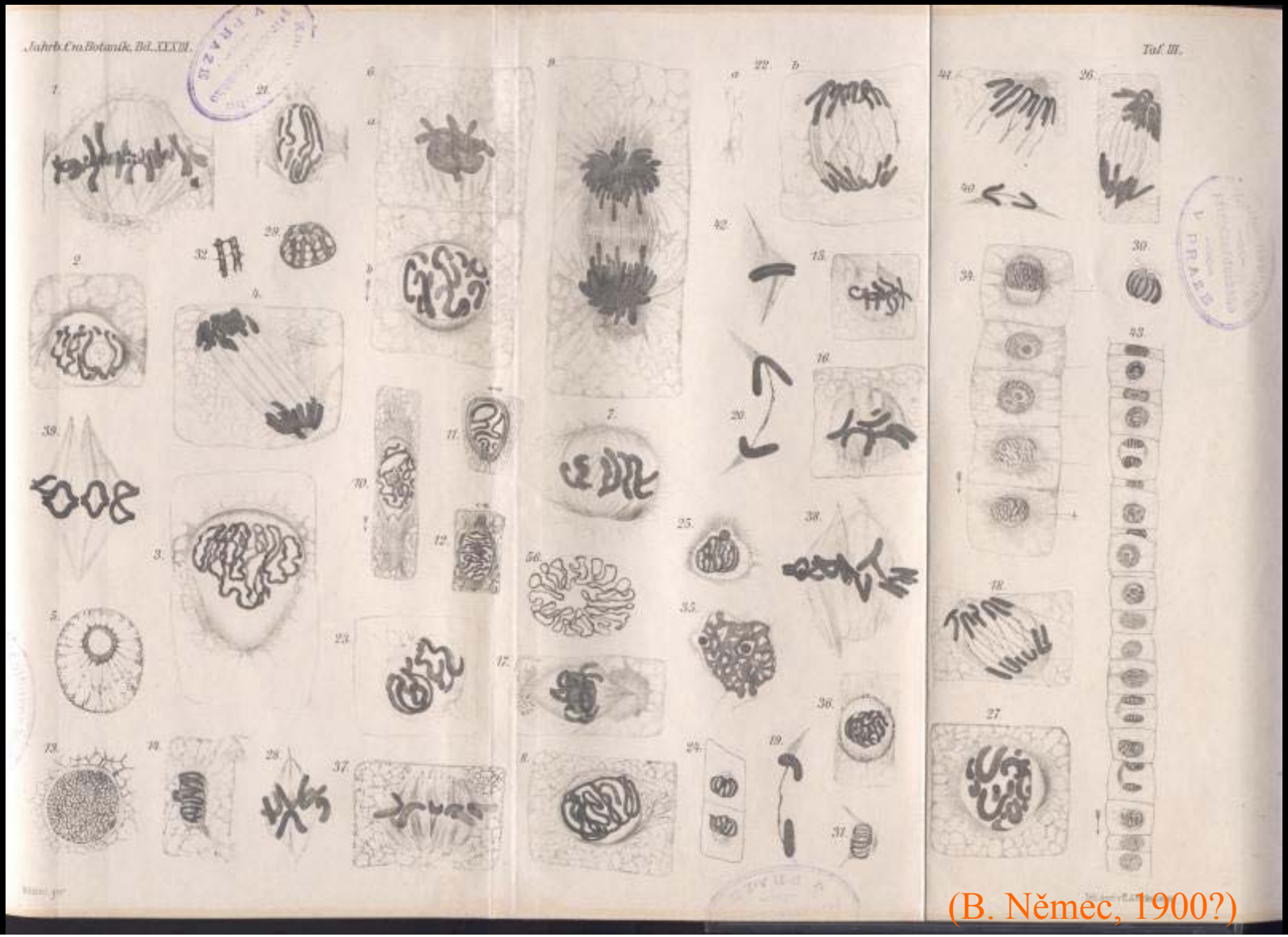
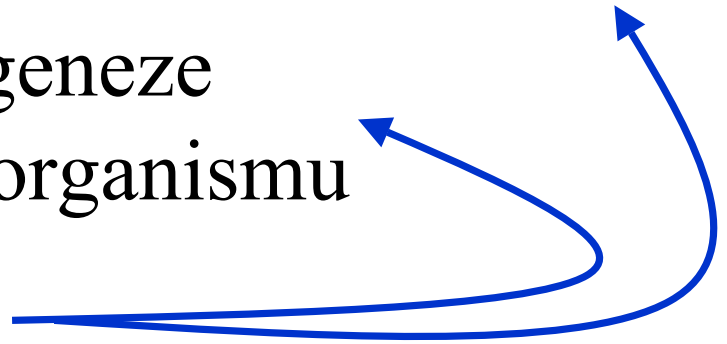
Buněčný cyklus



(B. Němec, 1900?)

Buněčný cyklus – úhly pohledu

- Strukturní události (replikace DNA, segregace chromozómů, cytokineze)
- Začlenění do ontogeneze mnohobuněčného organismu
- Regulační stránka



Regulace BC: historicko-metodický výlet

The Nobel Prize in Physiology or Medicine 2001

"for their discoveries of key regulators of the cell cycle"

Leland H. Hartwell

1/3 of the prize

USA

Fred Hutchinson Cancer Research Center
Seattle, WA, USA

b. 1939

R. Timothy (Tim) Hunt

1/3 of the prize

United Kingdom

Imperial Cancer Research Fund
London, United Kingdom

b. 1943

Sir Paul M. Nurse

1/3 of the prize

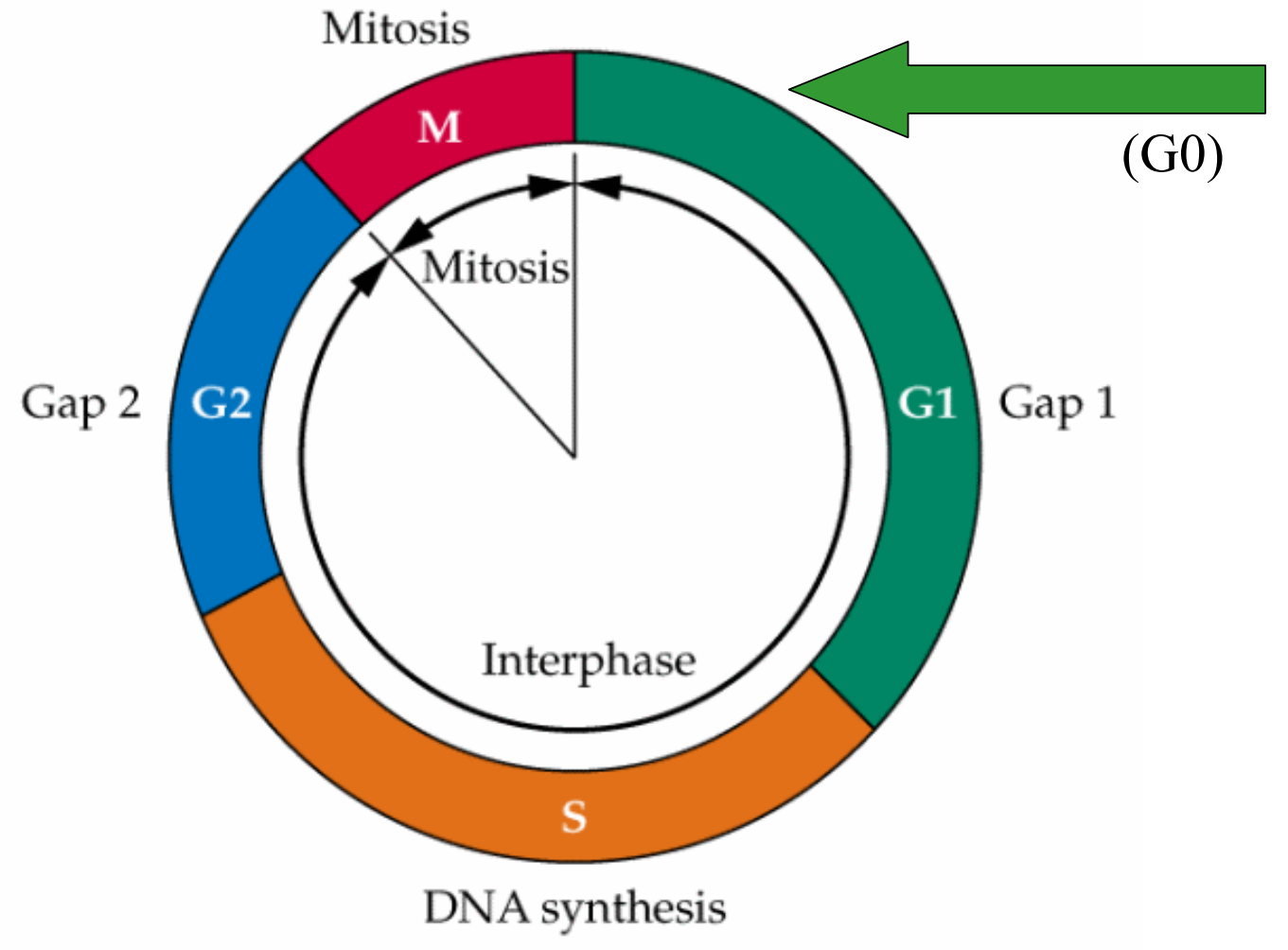
United Kingdom

Imperial Cancer Research Fund
London, United Kingdom

b. 1949

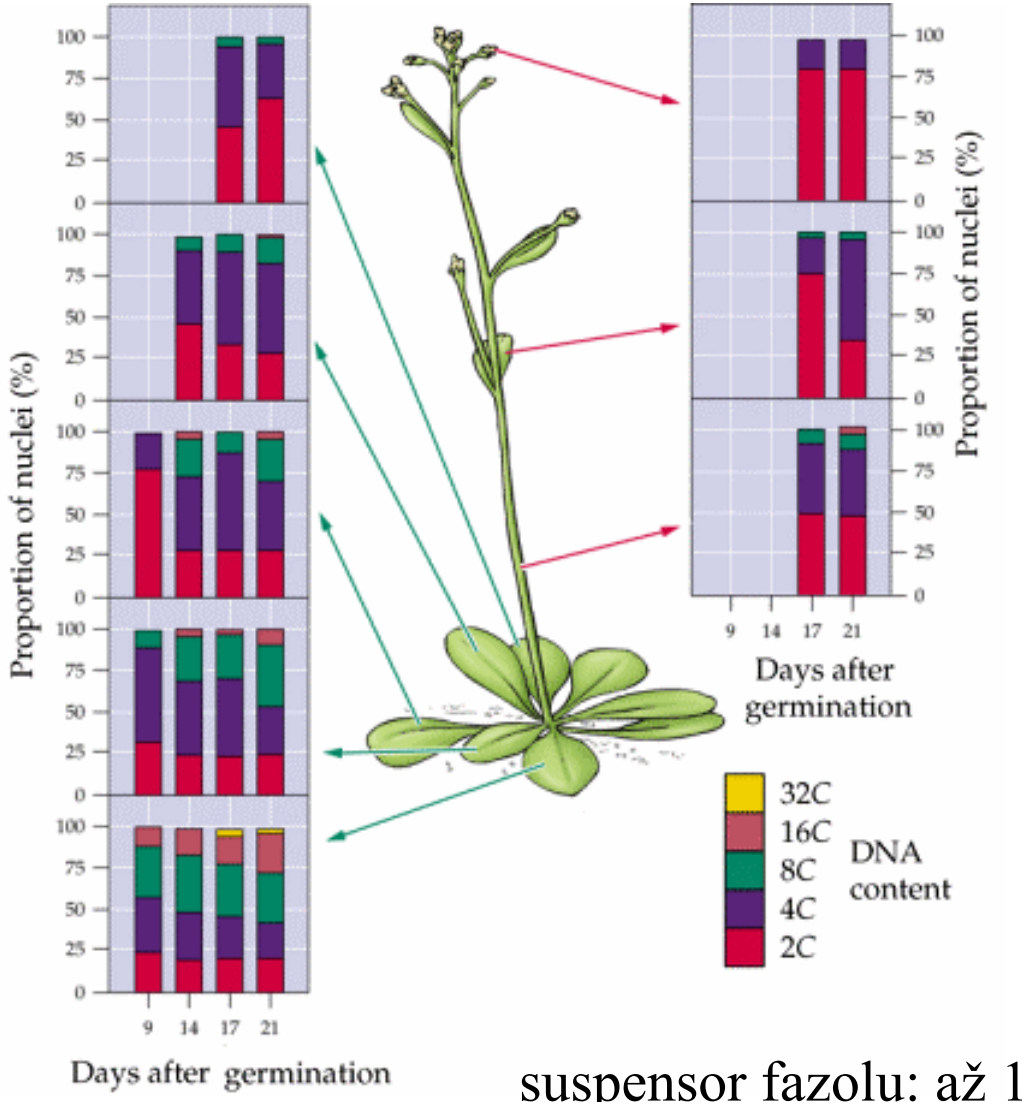
... ale začalo to mnohem dřív ...

Obecné schéma eukaryotního buněčného cyklu

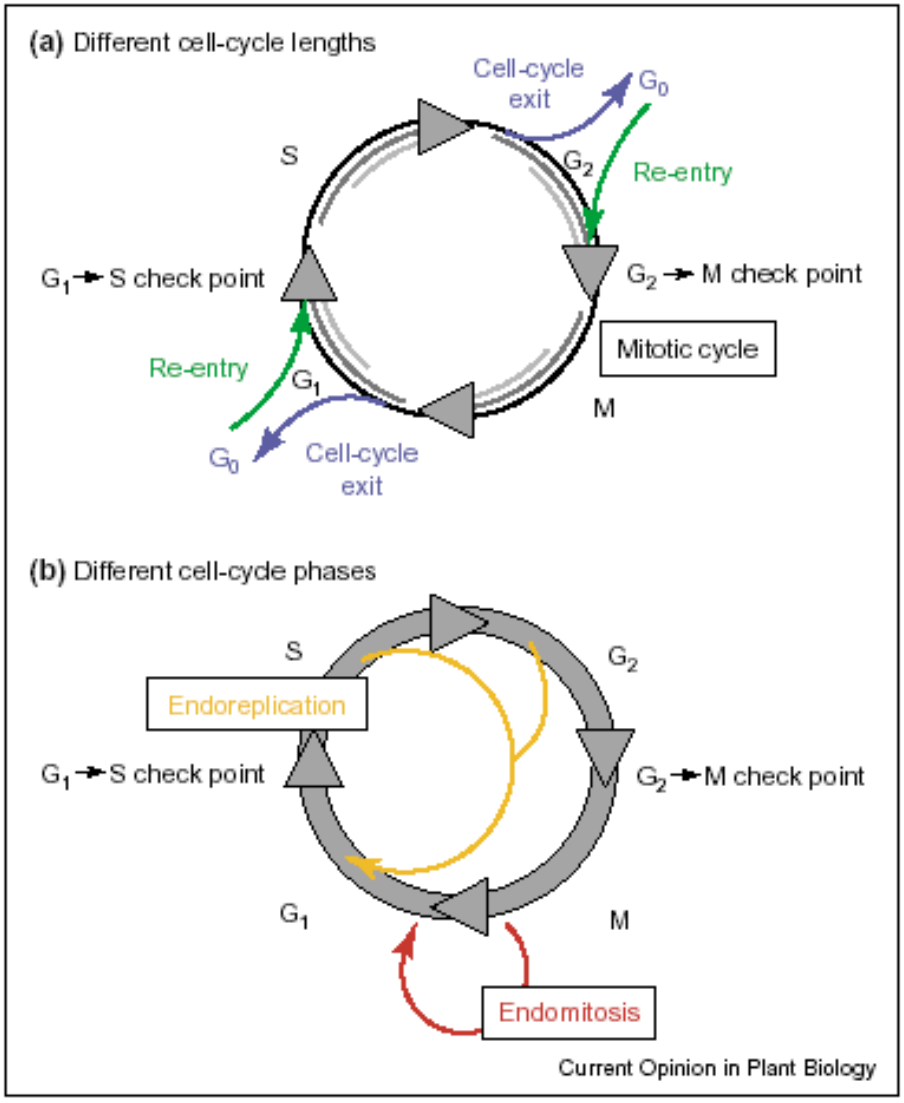


(1. pozorování - **Howard and Pelc, 50. léta, rostliny**!)

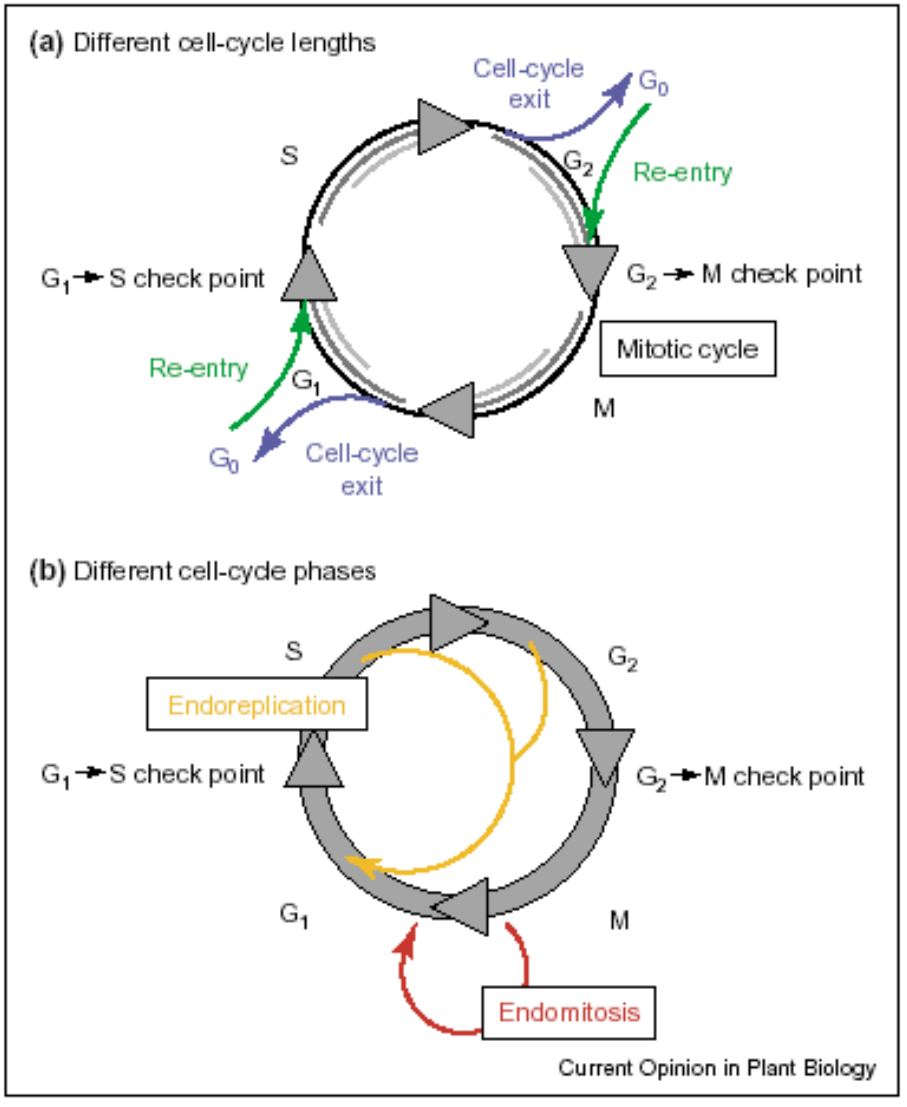
Výjimky z pravidel v rostlině



suspensor fazolu: až 10^3 C



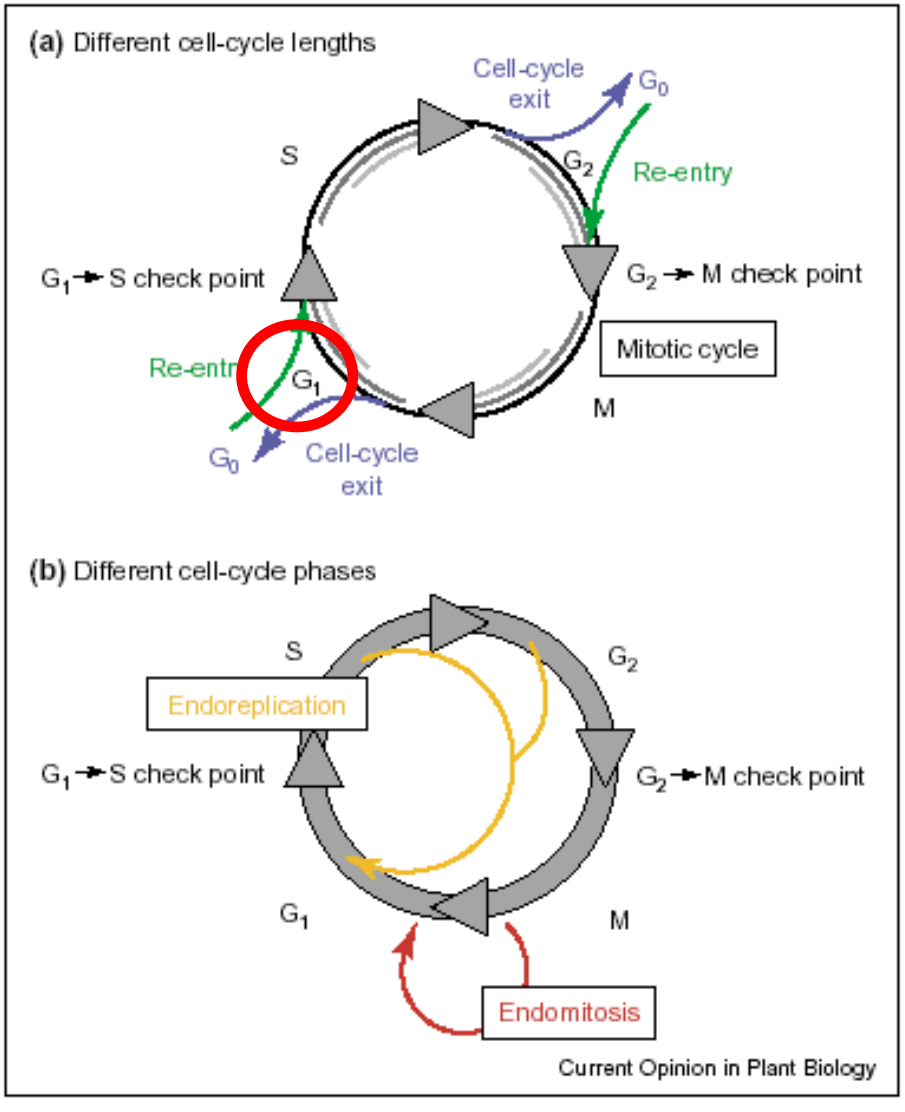
Many different cell-cycle modes are executed in plants. **(a)** The different cell-cycle modes can vary with respect to cell-cycle phase lengths, ranging from a rapid, proliferative mode to an exit from cell cycle in either G_1 or G_2. **(b)** The composition of different cell-cycle modes can also differ; for example, there is no mitosis in an endoreplicating mode.



Endoreplikace = opak. S-fáze bez M.

Endomitosa = opak. M-fáze bez S (po endoreduplikaci). Př. buněčné cykly řas – *Scenedesmus* (Šetlík, Vondrejs 70-léta)

Many different cell-cycle modes are executed in plants. **(a)** The different cell-cycle modes can vary with respect to cell-cycle phase lengths, ranging from a rapid, proliferative mode to an exit from cell cycle in either G_1 or G_2. **(b)** The composition of different cell-cycle modes can also differ; for example, there is no mitosis in an endoreplicating mode.



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Otázky (pro „ideální cyklus“):

- Jak je zajištěno, že ke zdvojení struktur dochází právě jednou za cyklus?
- Co udržuje pořadí a vzájemnou koordinaci zdánlivě nepříbuzných procesů?
- Co zajišťuje koordinaci růstu a dělení?
- Jak buňka ví, kam má dát nové struktury?

(Wheals, 1976)

Metabolicko-energetické podmínky postupu buněčného cyklu

The Journal of Cell Biology, Vol 37, 773-780, Copyright © 1968 by Rockefeller University Press

CONTROL OF CELL PROGRESSION THROUGH THE MITOTIC CYCLE BY CARBOHYDRATE PROVISION

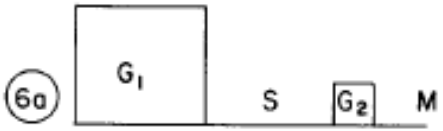
I. Regulation of Cell Division in Excised Plant Tissue*

JACK VAN'T HOF

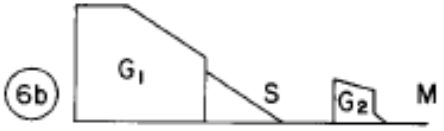
From the Biology Department, Brookhaven National Laboratory, Upton, New York 11973

ABSTRACT

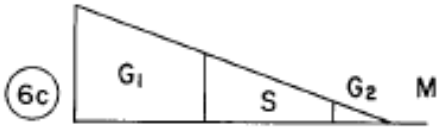
A stationary phase in the root meristem of excised pea roots was established by prolonged carbohydrate deprivation in sterile culture medium. When the stationary phase had been established, **cells that had collected in the G1 period of the mitotic cycle were induced to enter the S stage by subjection to relatively short intervals of carbohydrate provision (sucrose** spurts). Progression and cycle location of the G1 cells induced to enter S were measured with tritiated thymidine and radioautography. The results indicated that the number of G1 cells induced to enter S increased directly with the spurt duration and that cells could be positioned and retained in the S and/or G2 periods by varying the duration of the spurt. The data support the hypothesis that **S and maybe M stages have a relatively larger dependence on carbohydrate availability, and presumably a greater energy requirement, than G1 and G2.**



0 sacharosa



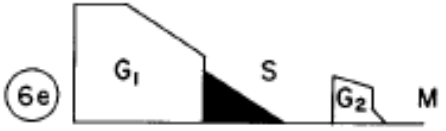
6h sacharosa



12h sacharosa

Znač. Thym.

0 sacharosa



6h sacharosa

12h sacharosa

FIGURE 6 Schematic representation of postulated cell distributions in the mitotic cycle of stationary phase root tips that received no sucrose spurt, *a*, a 6-hr spurt *b*, and a 12 hr spurt of sucrose, *c*, during the 72 hr stationary phase. *d–f* represent cell distributions of tritiated thymidine-labeled interphase cells: after no sucrose spurt and a 12 hr labeling period, *d*; after a 6 hr spurt with sucrose and simultaneous labeling with tritiated thymidine, *e*; and after a 12 hr sucrose spurt and tritiated thymidine labeling, *f*. The shaded portions in *d–f* represent tritiated thymidine-labeled interphase cells.

Otázky (pro „ideální cyklus“):

- Jak je zajištěno, že ke zdvojení struktur dochází právě jednou za cyklus?
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- Co zajišťuje koordinaci růstu a dělení?
- Jak buňka ví, kam má dát nové struktury?

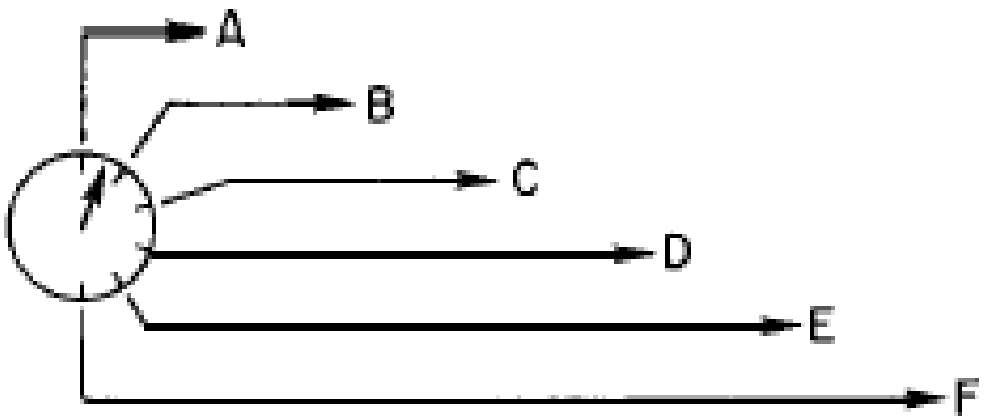
(Wheals, 1976)

Odpověď: 2 alternativní modely!

dependent pathway model (domino)

A - B - C - D - E - F

independent pathways model (hodiny)



(Hartwell 1974)

Model typu „domino“ (L. Hartwell)

Východisko: mutace buň. cyklu Saccharomyces

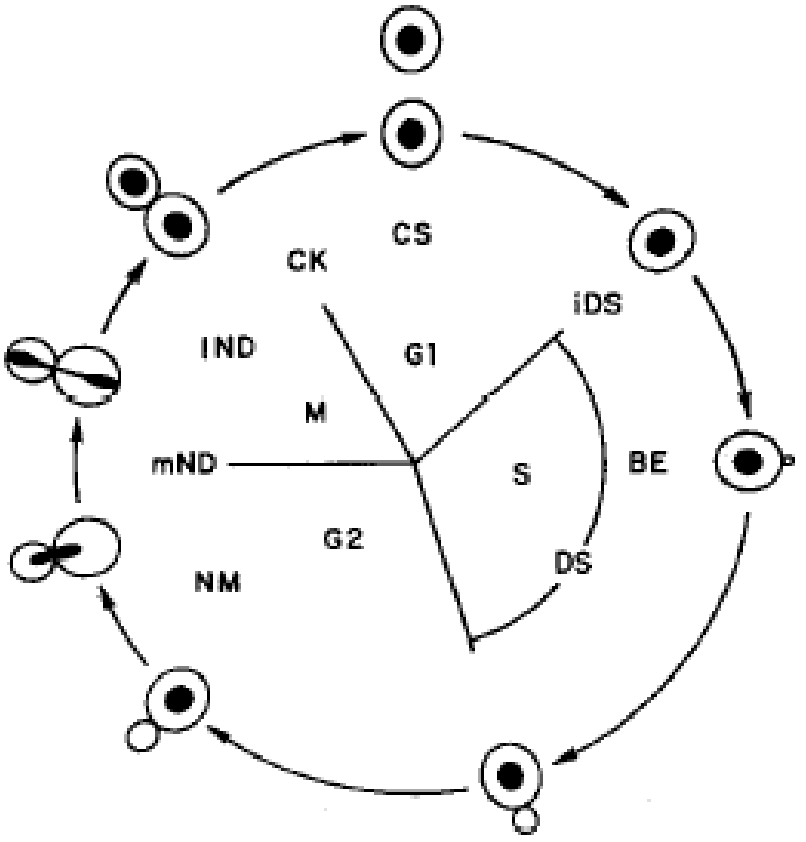
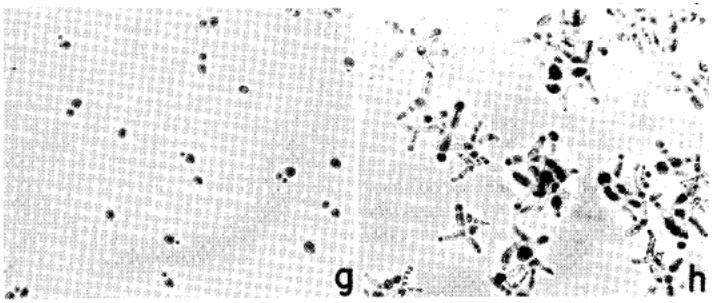


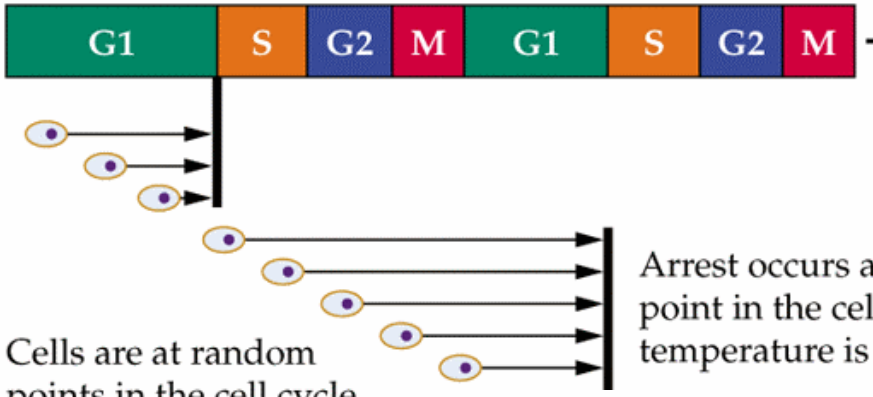
Fig. 1. The sequence of events in the cell division cycle of yeast: ***iDS,*** initiation of DNA synthesis; ***BE,*** bud emergence; ***DS,*** DNA synthesis; ***NM,*** nuclear migration; ***mND,*** medical nuclear division; ***lND,*** late nuclear division; ***CK,*** cytokinesis; CS, cell separation. Other abbreviations: ***G1,*** time interval between previous cytokinesis and initiation of DNA synthesis; ***S,*** period of DNA synthesis; ***G2,*** time between DNA synthesis and onset of mitosis; and ***M,*** the period of mitosis.

cdc mutace

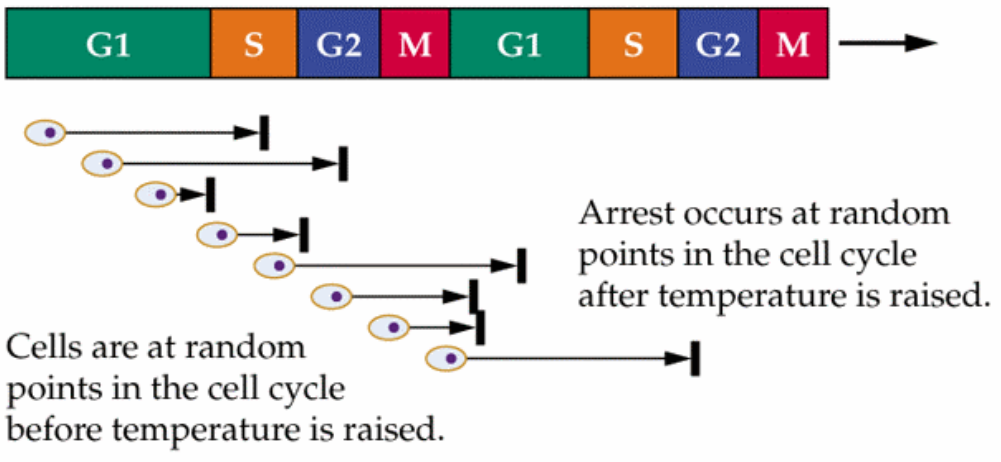
cdc4, kont. **ts** mut. v rest. tepl.



(A) Cell division cycle *(cdc)* mutants arrest uniformly after temperature is raised.

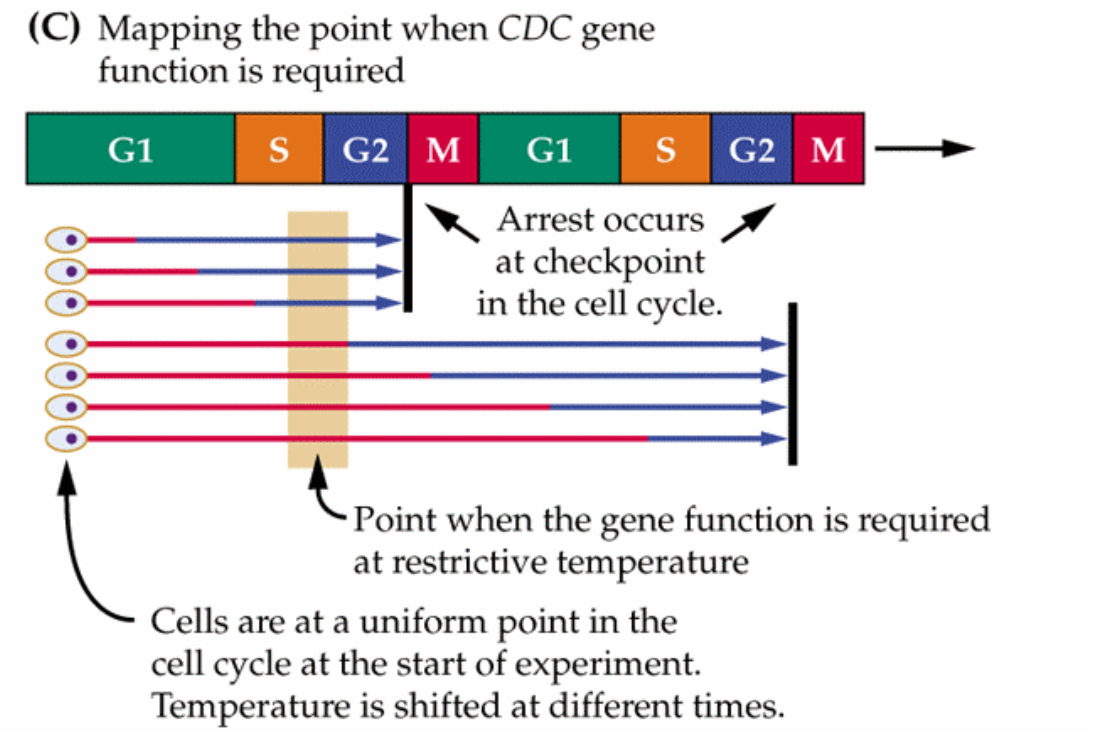


(B) Other temperature-sensitive mutants arrest randomly after temperature is raised.

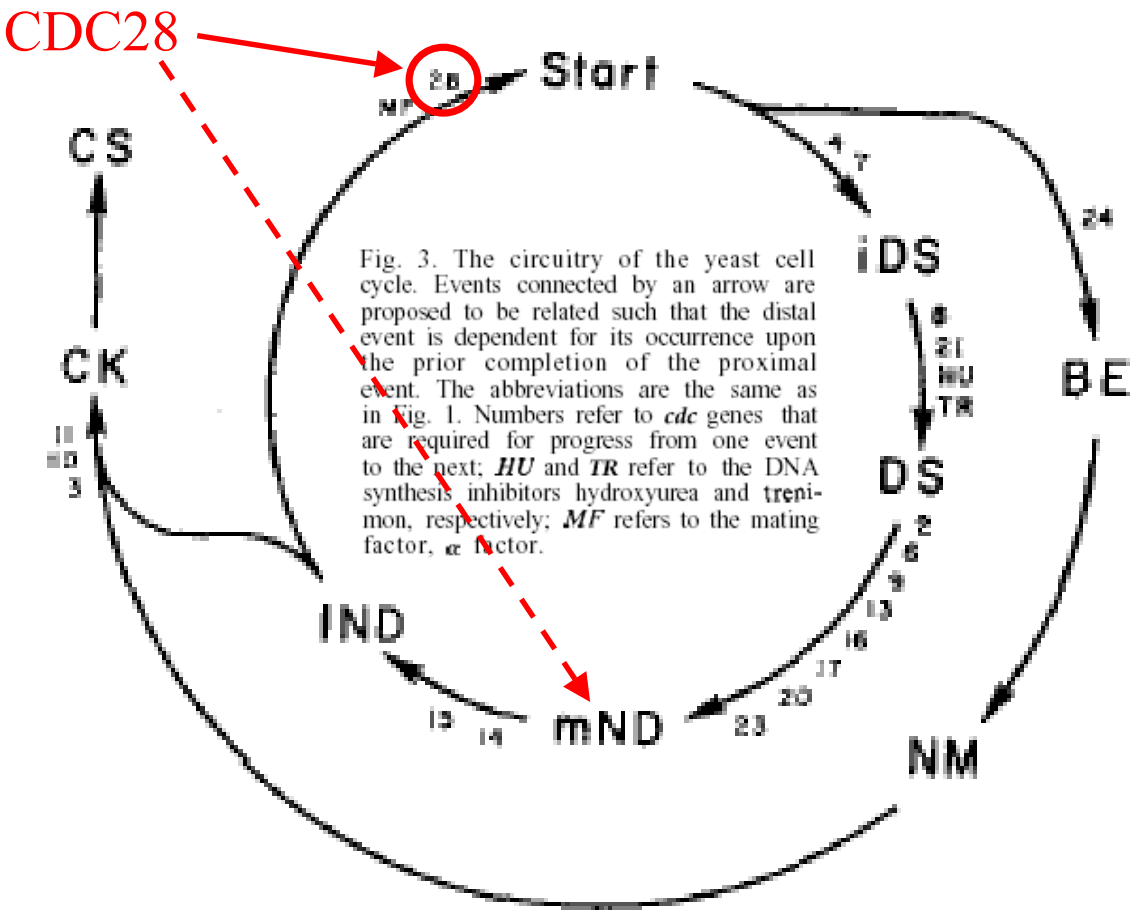


Pořadí funkce CDC genů

- Vzájemná závislost a pořadí
- Závislost - pořadí vůči místu účinku inhibitorů
- Synchronní kultury výhodou (ne podmínkou)

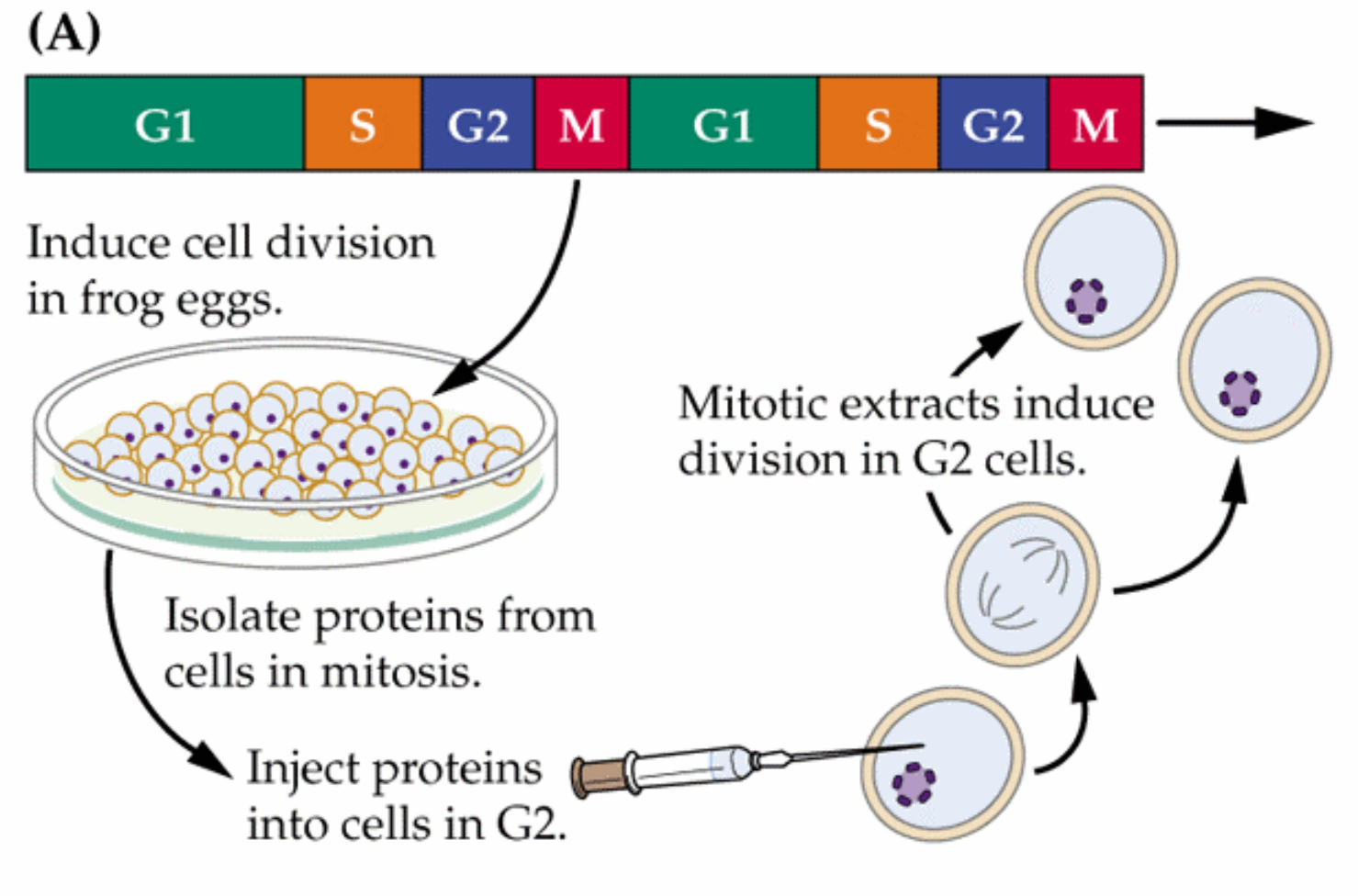


Mapa funkcí CDC genů



(Hartwell 1974)

Modely typu „hodiny“ (T. Hunt, M. Kirschner, A. Murray)



MPF - maturation promoting factor

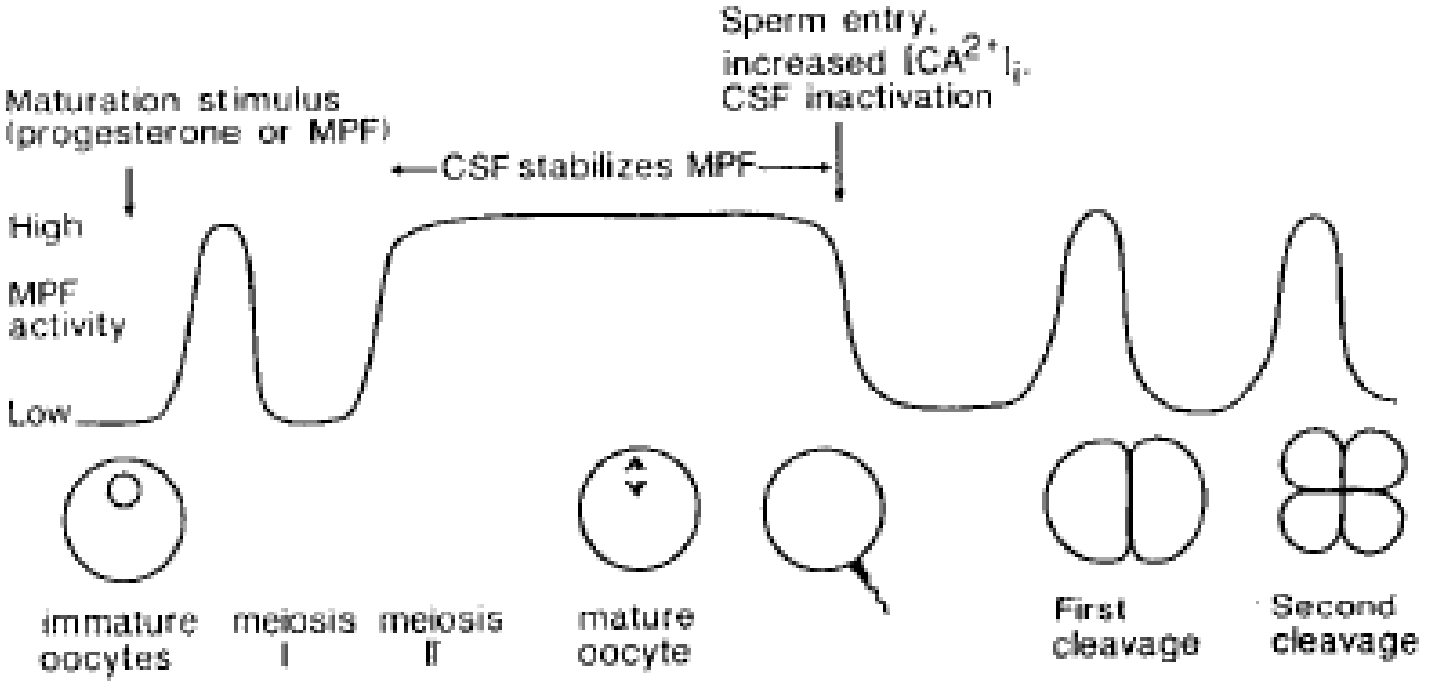
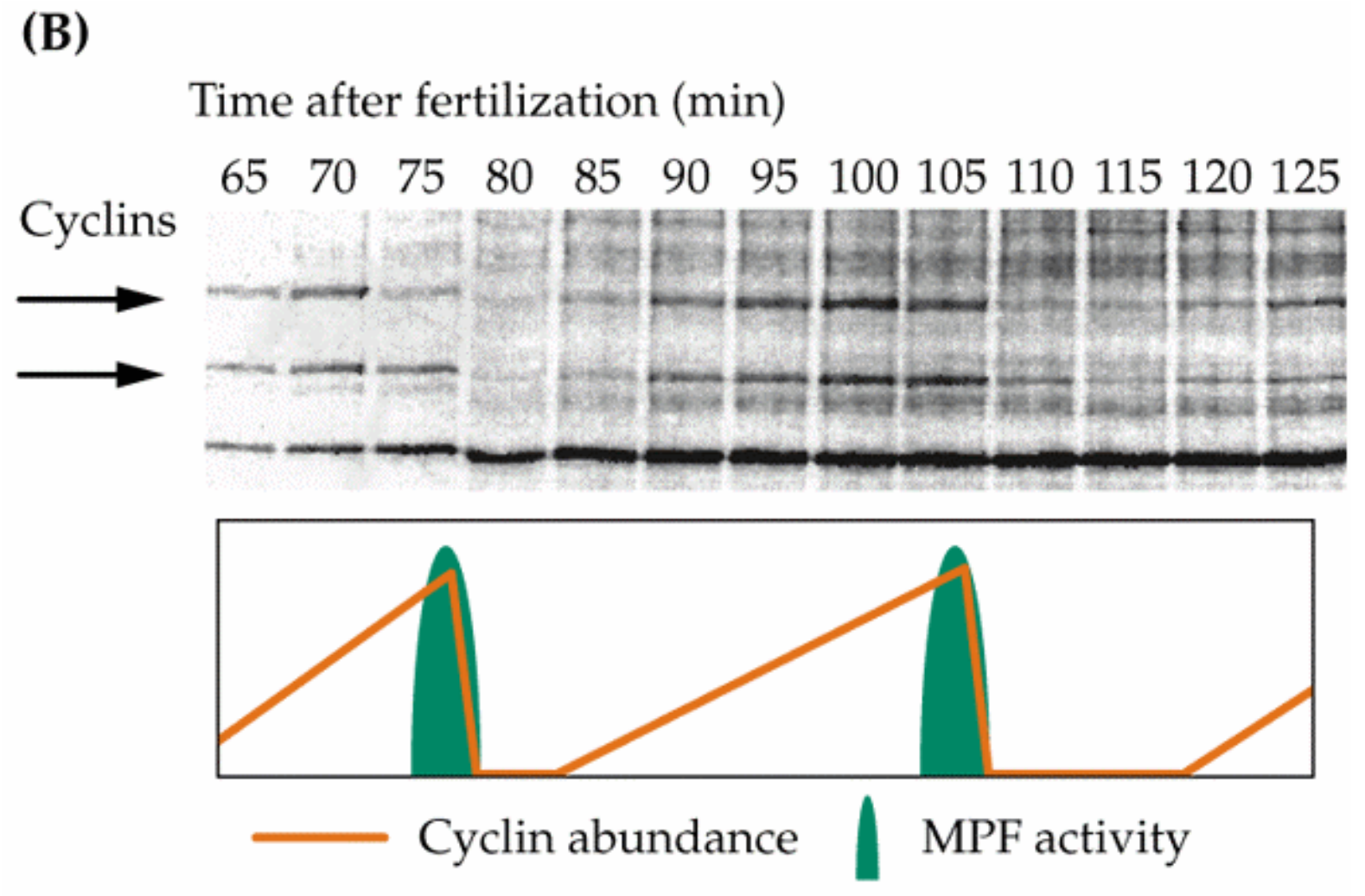
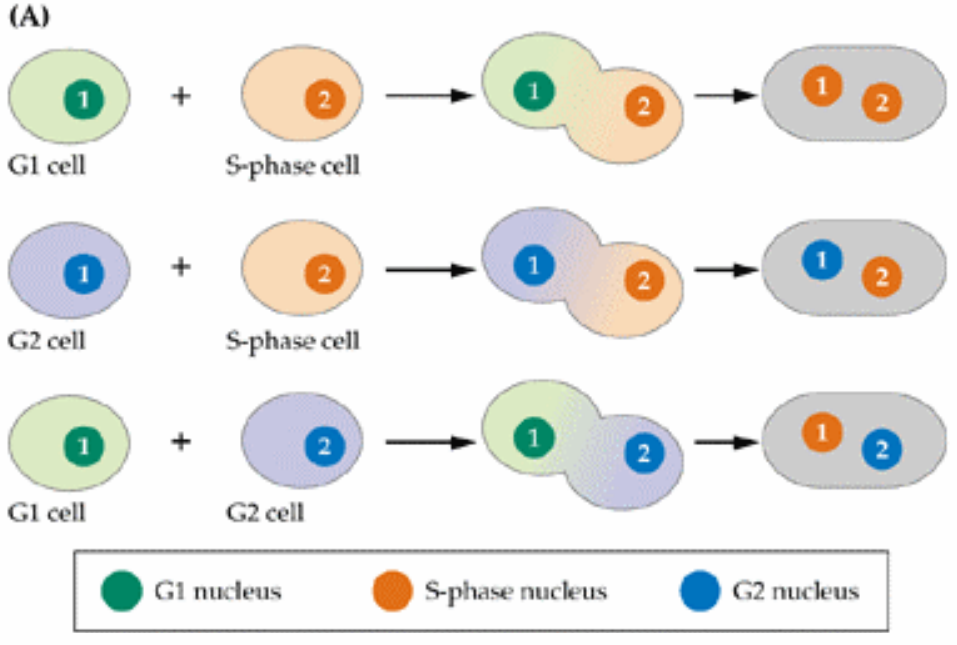


FIG. 1 MPF levels during early *Xenopus* embryonic development. The fluctuation in MPF levels as an immature oocyte passes through meiotic maturation, fertilization and the first two mitotic cell cycles is shown. For further details see the text.

MPF = p34 + „cyklin“

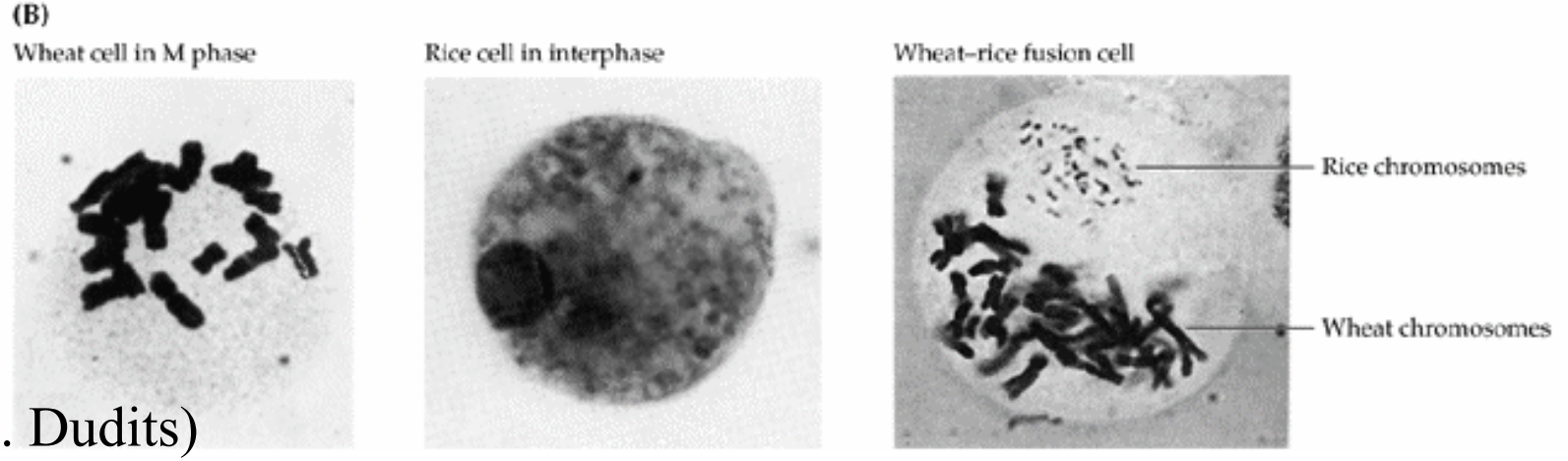


Další doklad pro „hodiny“: fúze buněk



Observations	Conclusions
G1 nucleus replicates. Early S-phase nucleus continues replication.	G1 nucleus is competent to replicate. S-phase cells contain activator.
G2 nucleus does not replicate. S-phase nucleus continues replication. G2 nucleus does not progress into M phase.	G2 nuclei are not competent and do not re-replicate. G2 cells do not inhibit replication. S-phase nuclei retard mitosis in G2 nuclei.
G1 nucleus replicates at normal time; G2 nucleus does not replicate.	G2 cells do not suppress S-phase entry of G1-phase nuclei.

(Rao and Johnson)



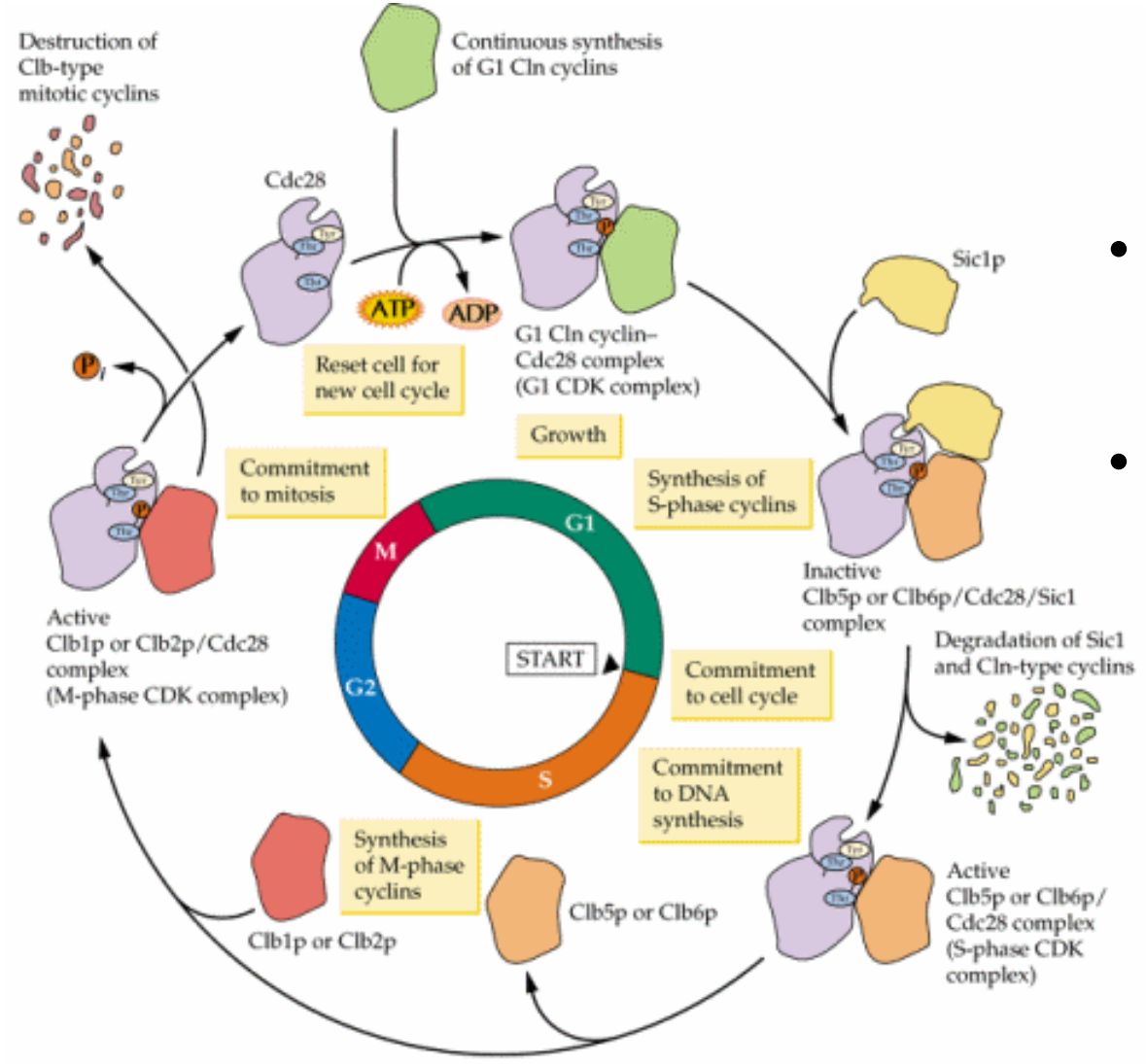
(D. Dudits)

Sjednocení modelů (P. Nurse):

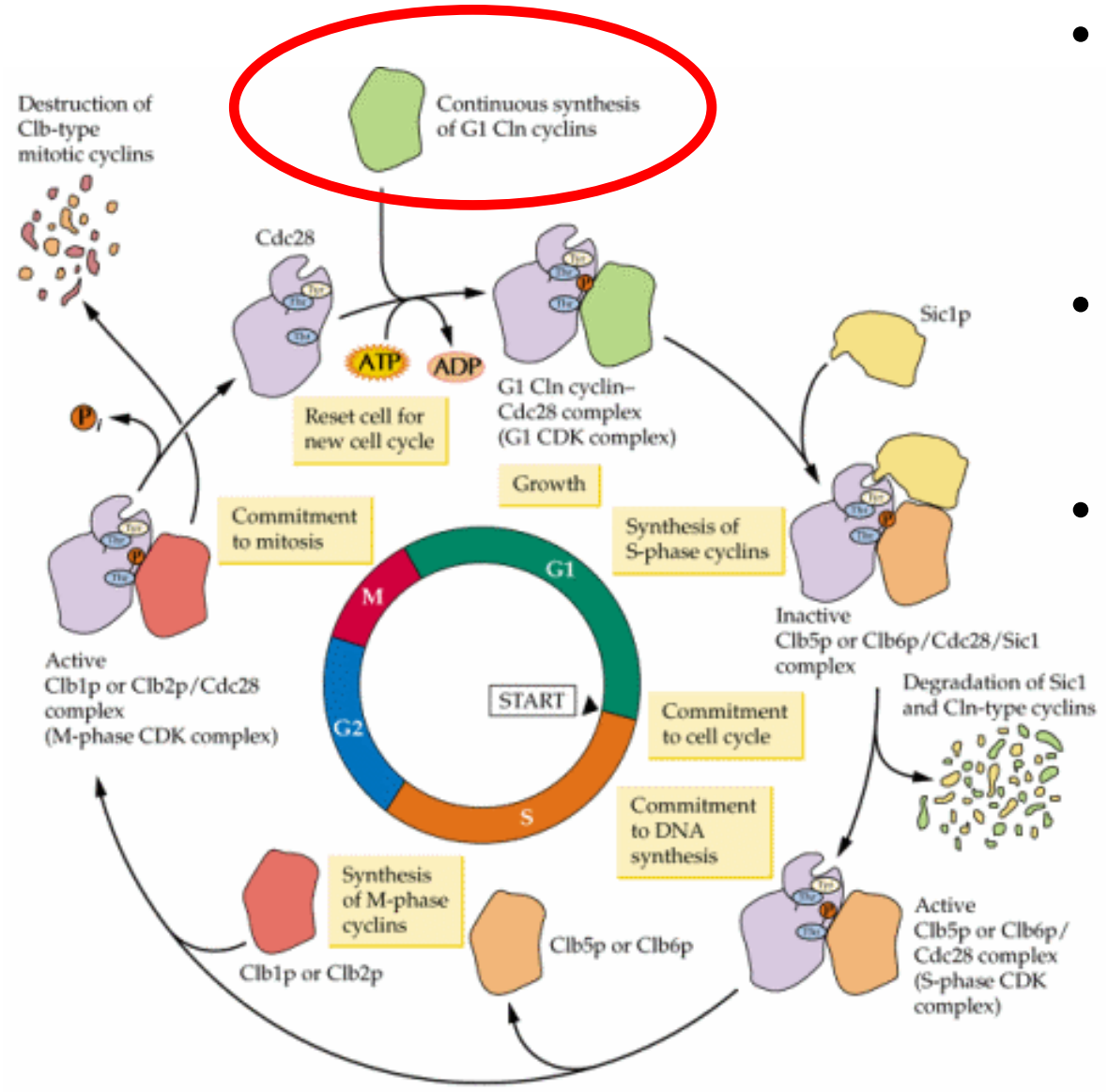
CDC28 kóduje p34!

„cyclin-dependent kinase“, CDK

(dílčí procesy mohou běžet podle modelu „domino)

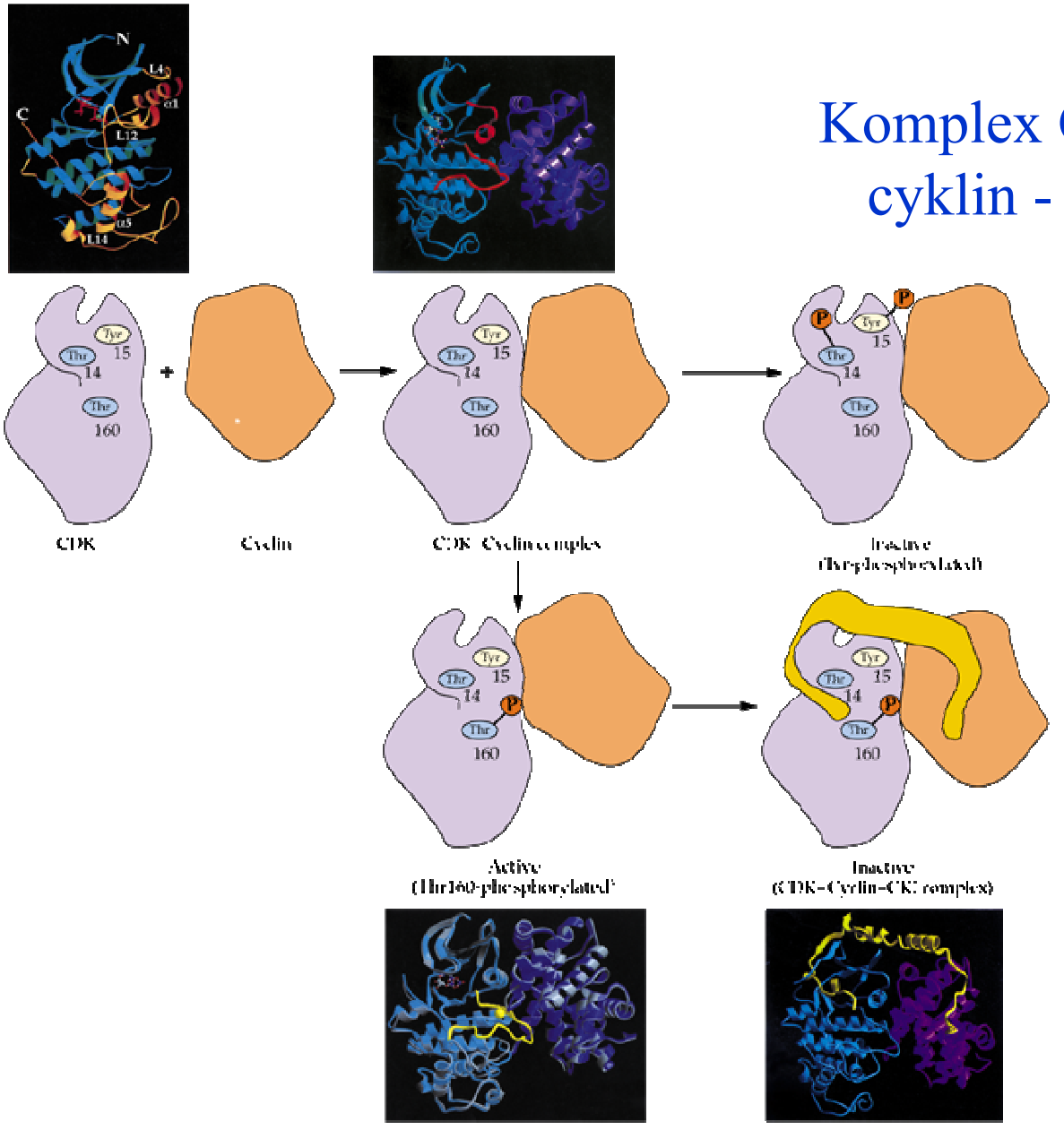


- Vlny
  - CDK
  - cyklinů
  - CDK inhibitorů
- Regulace:
  - transkripce
  - proteolýza
- Modulace aktivity CDK



- Vlny
  - CDK
  - cyklinů
  - CDK inhibitorů
- Regulace:
  - transkripce
  - proteolýza
- Modulace aktivity CDK

Komplex CDK - cyklin - CKI



Diversita CDK a cyklinů

(živočišná terminologie)

	CDK1	CDK2	CDK3	CDK4	CDK5	CDK6	CDK7	CDK8	CDK9
PSTAIRE motif	PSTAIRE	PSTAIRE	PSTAIRE	PISTVRE	PSSALRE	PLSTIRE	NRTALRE	MSACRE	PITALRE
Activator	cyclin A cyclin B1-3	cyclin A cyclin E	Ik3-1	cyclin D1-3	p35 / p25 p39 cyclin D1	cyclin D1-3	cyclin H	cyclin C	cyclin K cyclin T1
Cellular functions	cell cycle (G2/M)	cell cycle (G1/S, S, G2)	cell cycle ?	cell cycle (G1 & G2/M)	Neurite outgrowth Rac signalling	cell cycle (G1)	Transcription cell cycle	transcription	transcription

exocytosis via NSF
(PCTAIRE! – Liu et al. 2006)

(Doerner lab 2005)

Diversita CDK a cyklinů

(živočišná terminologie)

	CDK1	CDK2	CDK3	CDK4	CDK5	CDK6	CDK7	CDK8	CDK9
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exocytosis via NSF
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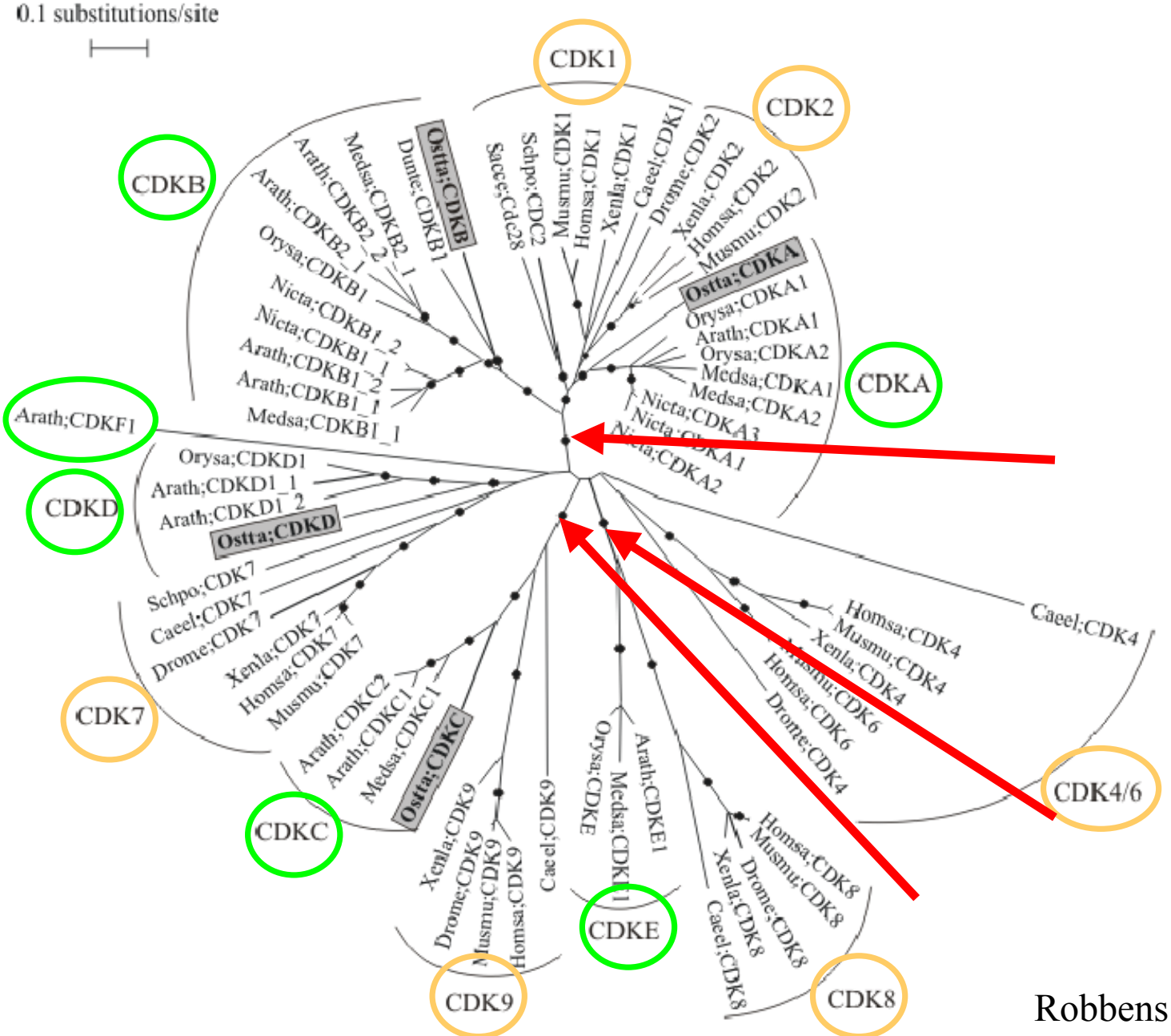
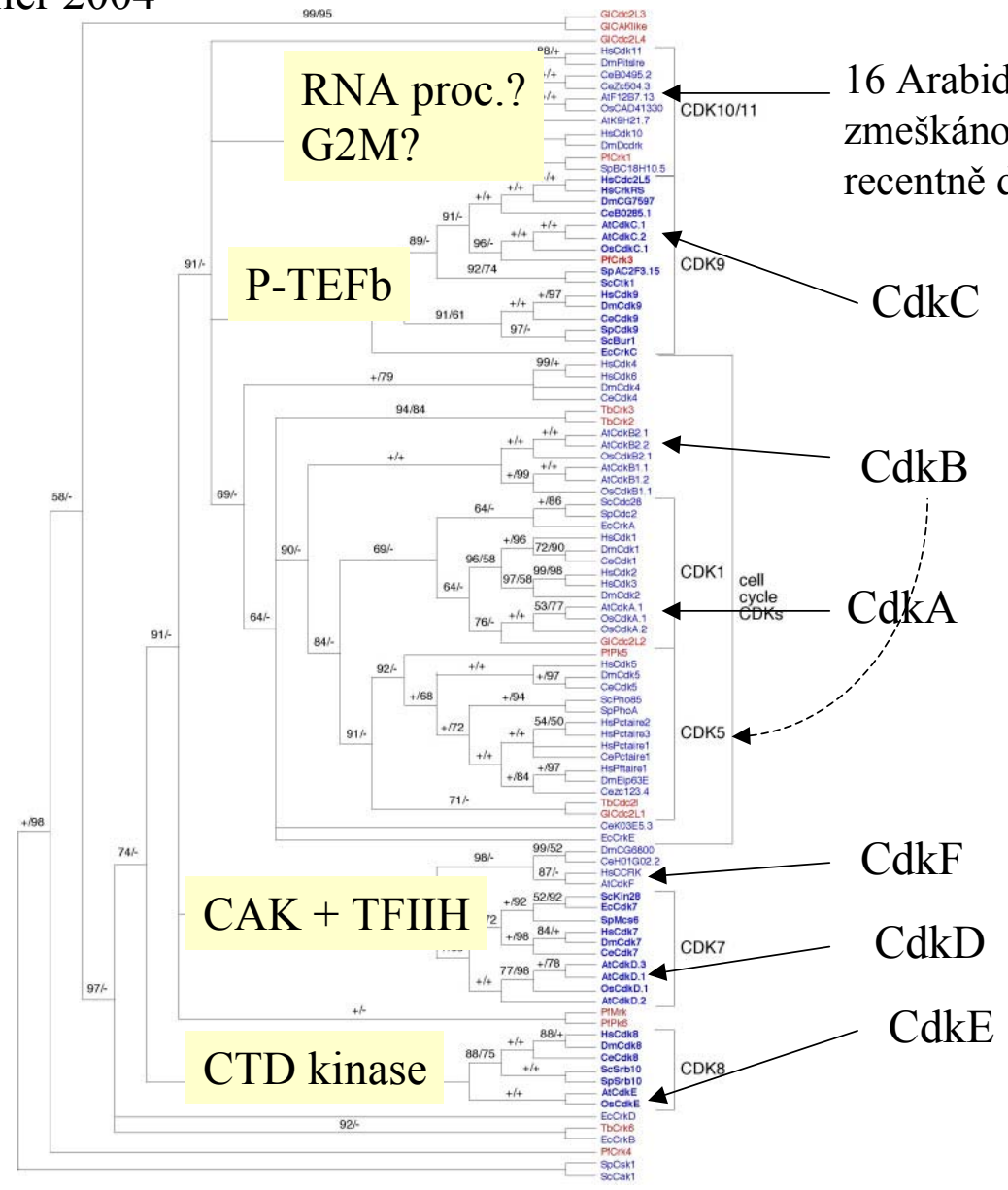


Evoluce CDK

Robbens et al. 2004

Guo and Stiller 2004

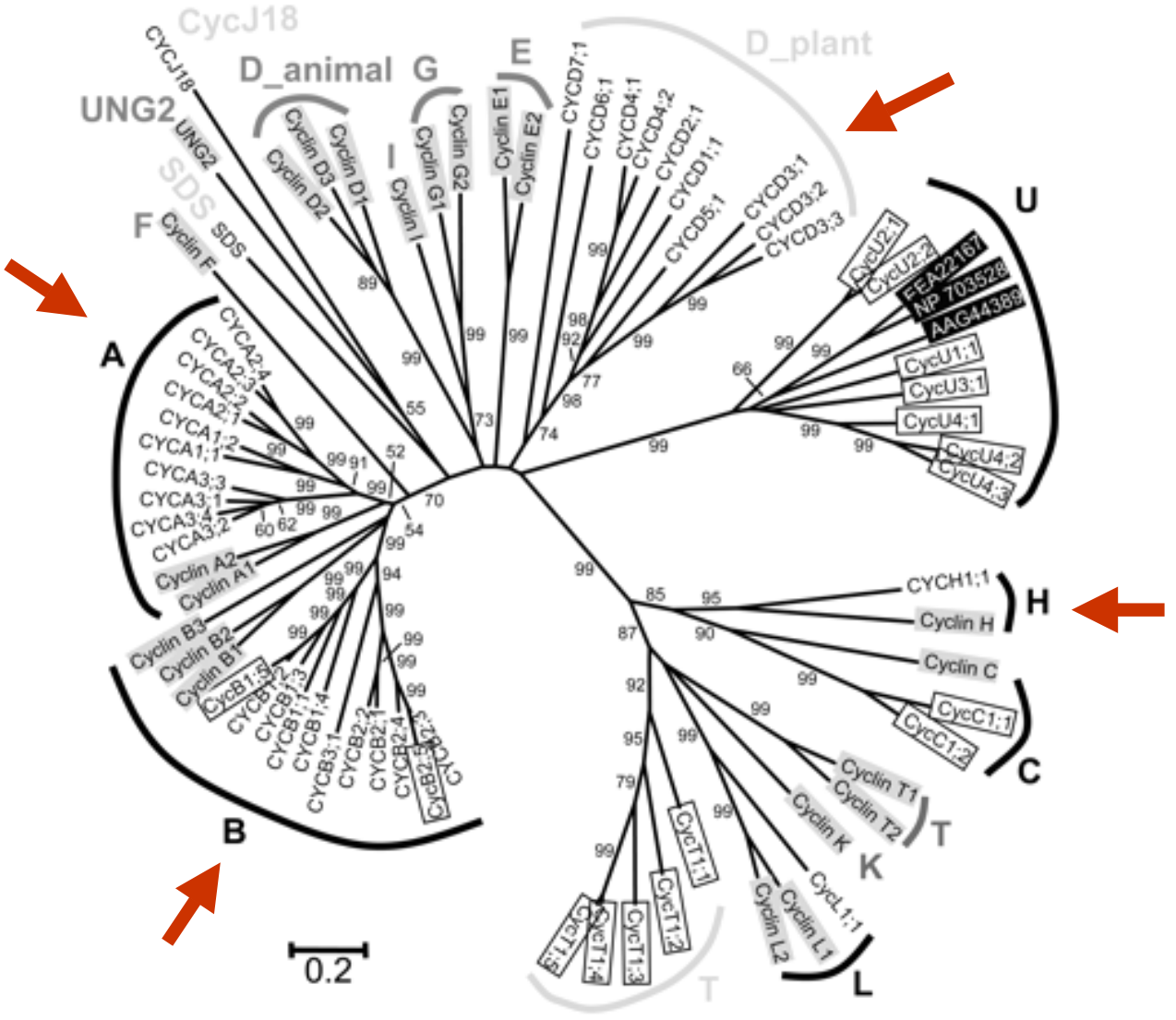


CDK: rozmanitost sekvencí i funkcí

- Kromě „jádrové“ funkce i další (CLN, meiotické ...)
- Spřízněnost s transkripčním aparátem (TFIIH, CTD kinázy)
- ALE rodina CCC (cell cycle control) kináz stará, CDK jsou mladá větev, divergence až v eukaryotech ... a větve dosti slušně konzervovány.

(Guo a Stiller 2004; Krylov et al., Curr. Biol. 13:173-177, 2004)

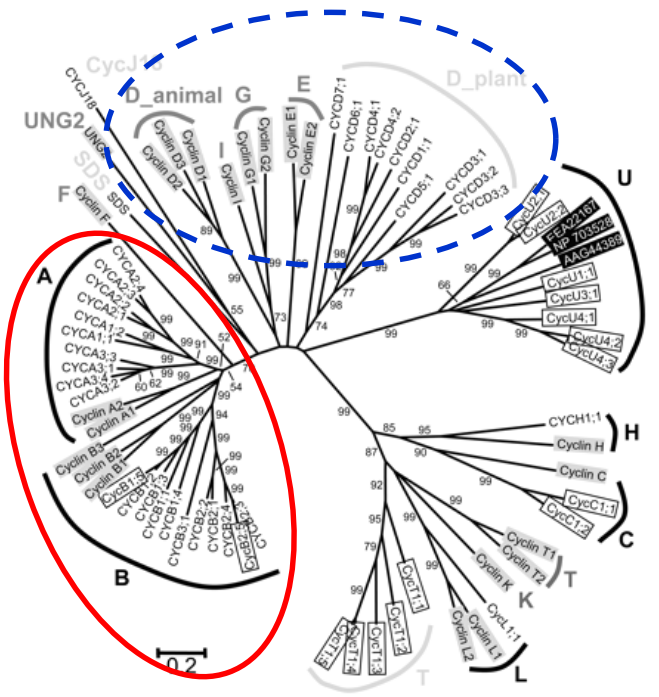
Evoluce cyklinů



Cykliny z Arabidopsis – Wang et al. 2004

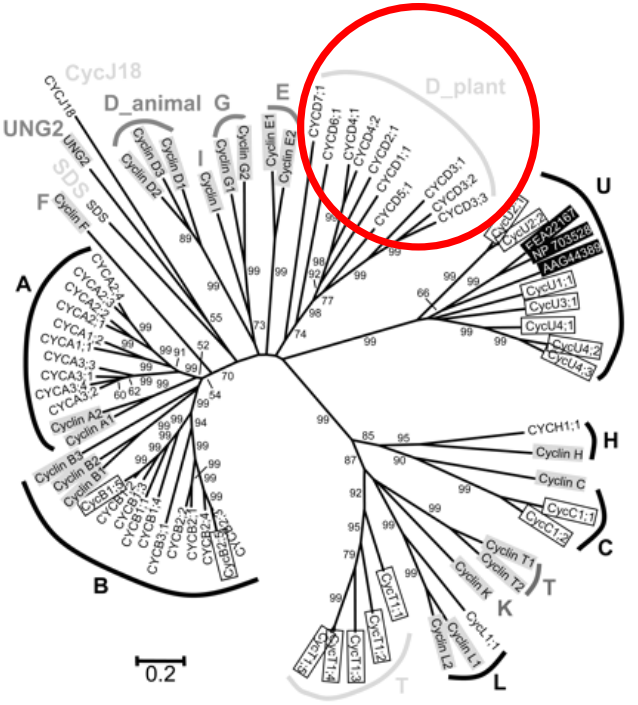
Cykliny - dělba práce

- „Mitotické“ - klasické:
  - A - S fáze
  - B - mitosa
- „G1“ - heterogenní skupina; cell cycle commitment a ???



Cykliny - dělba práce

- „Mitotické“ - klasické:
  - A - S fáze
  - B - mitosa
- „G1“ - heterogenní skupina; cell cycle commitment …
- U rost. **Cyc D** v G1



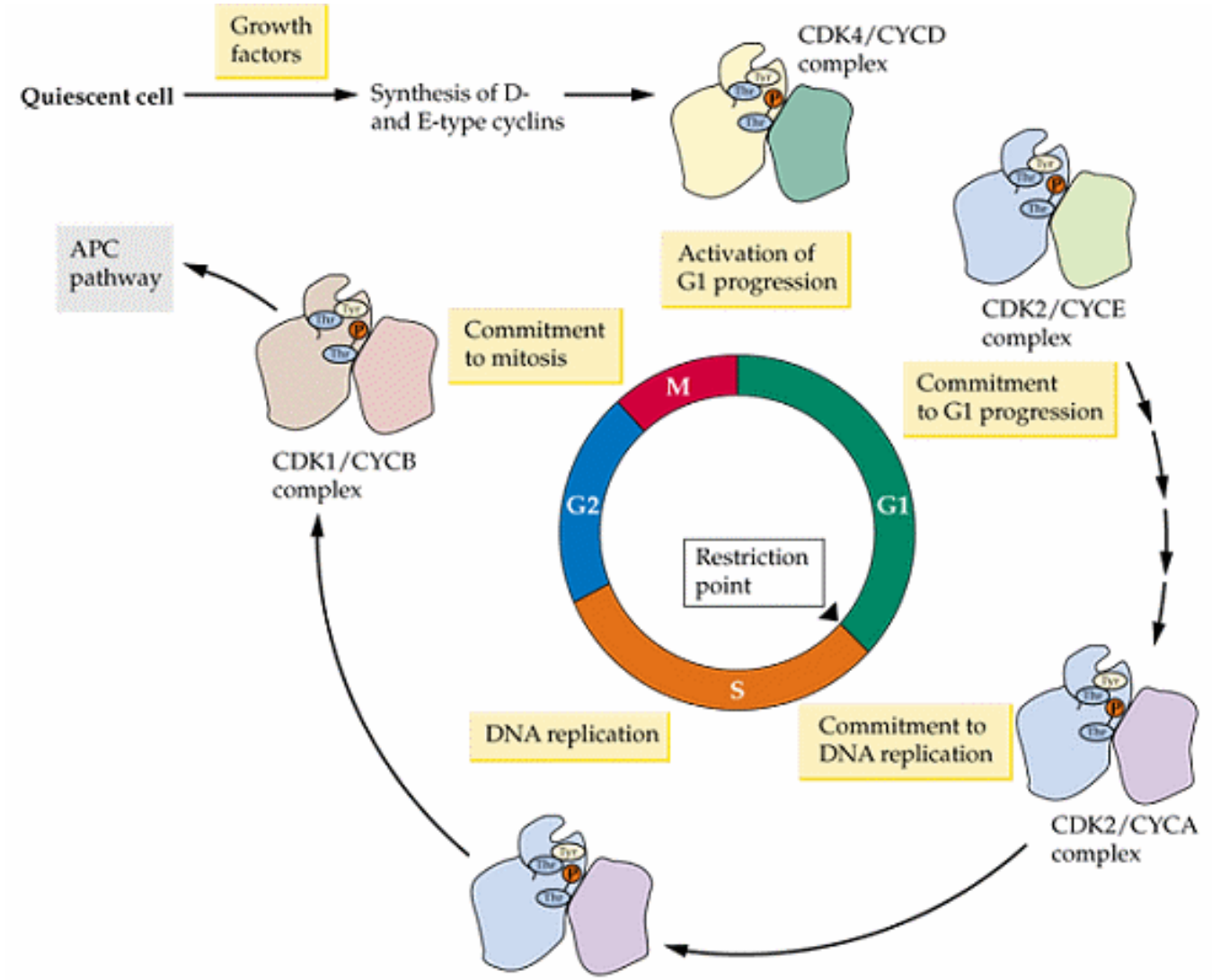
Současný stav u rostlin

(Francis 2007)

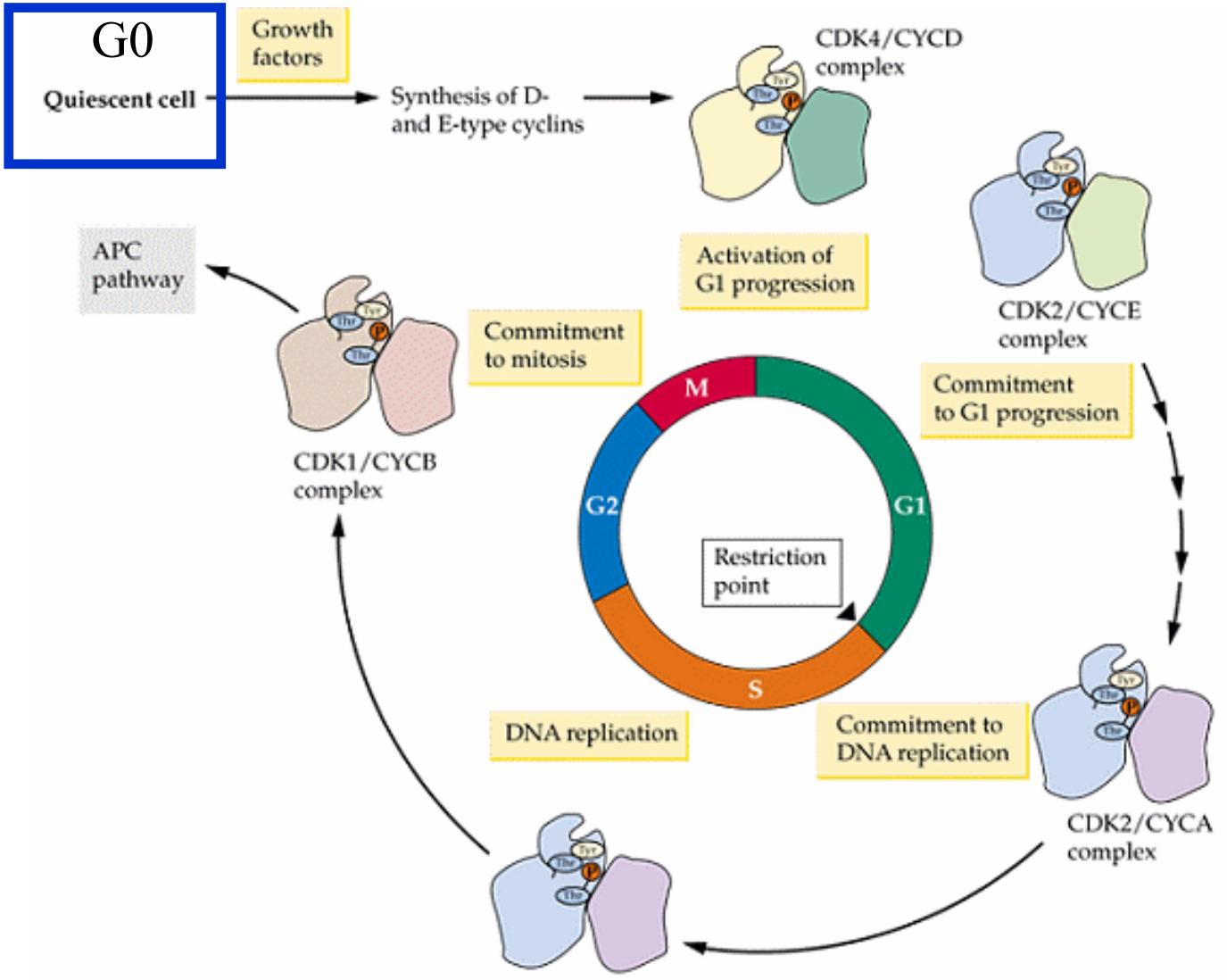
CDKs		
A	;1	G1/S and G2
B	1;1	G2/M
B	1;2	G2/M
B	2;1	G2
B	2;2	G2
C	;1	*Regulation of transcription*
D	;1;2;3	CAK
E		*Regulates RNA polymerase II*
F		CAK
G		?

D	1;1	G0/G1/S
D	2;1	G0/G1/S
D	3;1	G0/G1/S
D	3;2	G0/G1/S
D	3.3	G0/G1/S
D	4;1	G2/M
D	4;2	G2/M
D	5;1	G0/G1/S
D	6;1	G0/G1/S
D	7;1	G0/G1/S

Cyclins		
A	1;1	G1/S (G2/M)
A	1;2	G1/S (G2/M)
A	2;1	G1/S (G2/M)
A	2;2	G1/S (G2/M)
A	2;3	G1/S (G2/M)
A	2;4	G1/S (G2/M)
A	3;1	G1/S (G2/M)
A	3;2	G1/S (G2/M)
A	3;3	G1/S (G2/M)
A	3;4	G1/S (G2/M)
B	1;1	G2 or G2/M
B	1;2	G2 or G2/M
B	1;3	G2 or G2/M
B	1;4	G2 or G2/M
B	2;1	G2 or G2/M
B	2;2	G2 or G2/M
B	2;3	G2 or G2/M
B	2;4	G2 or G2/M
B	3;1	G2 or G2/M



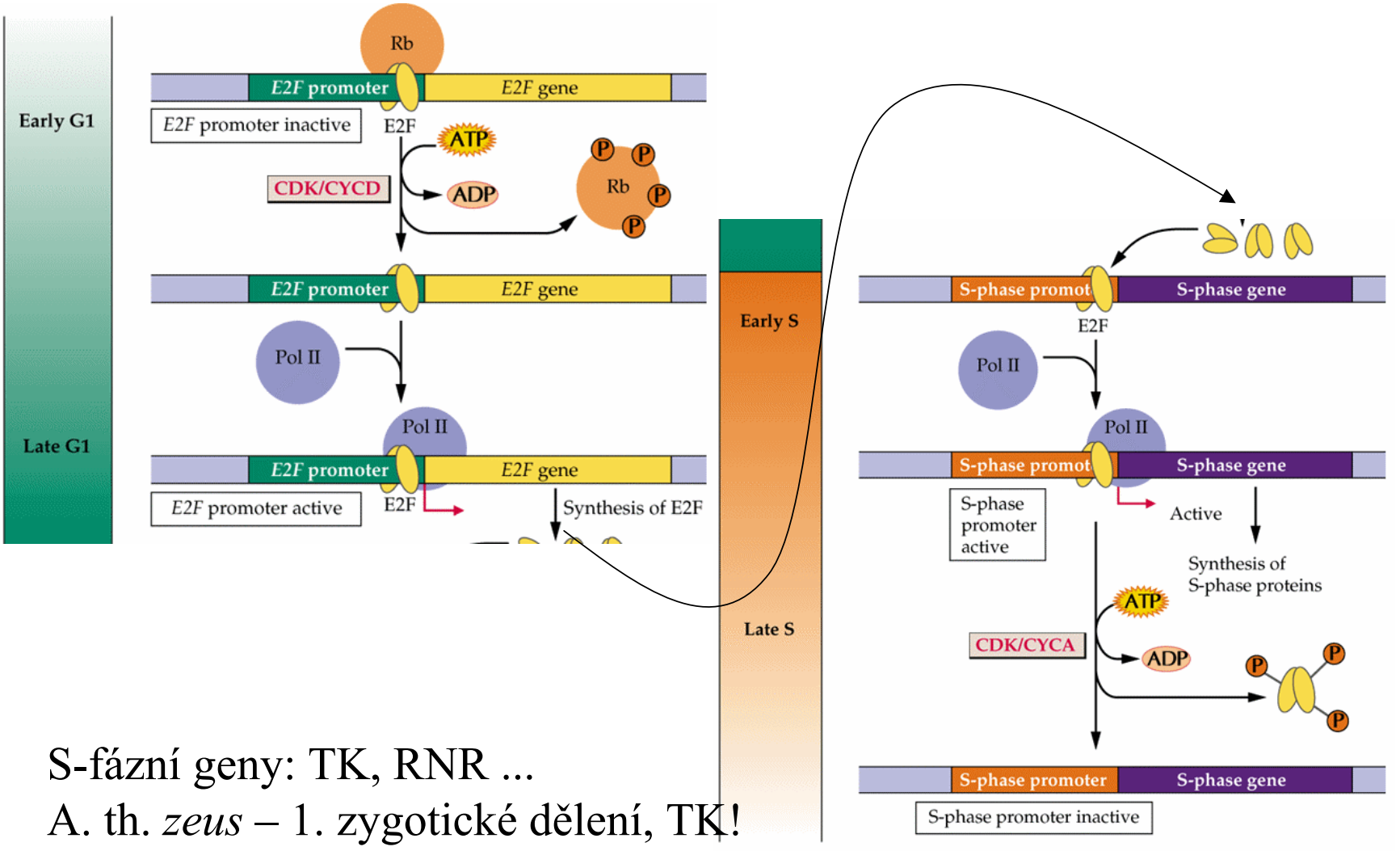
(příklad ovšem živočišný ...)



(příklad ovšem živočišný ...)

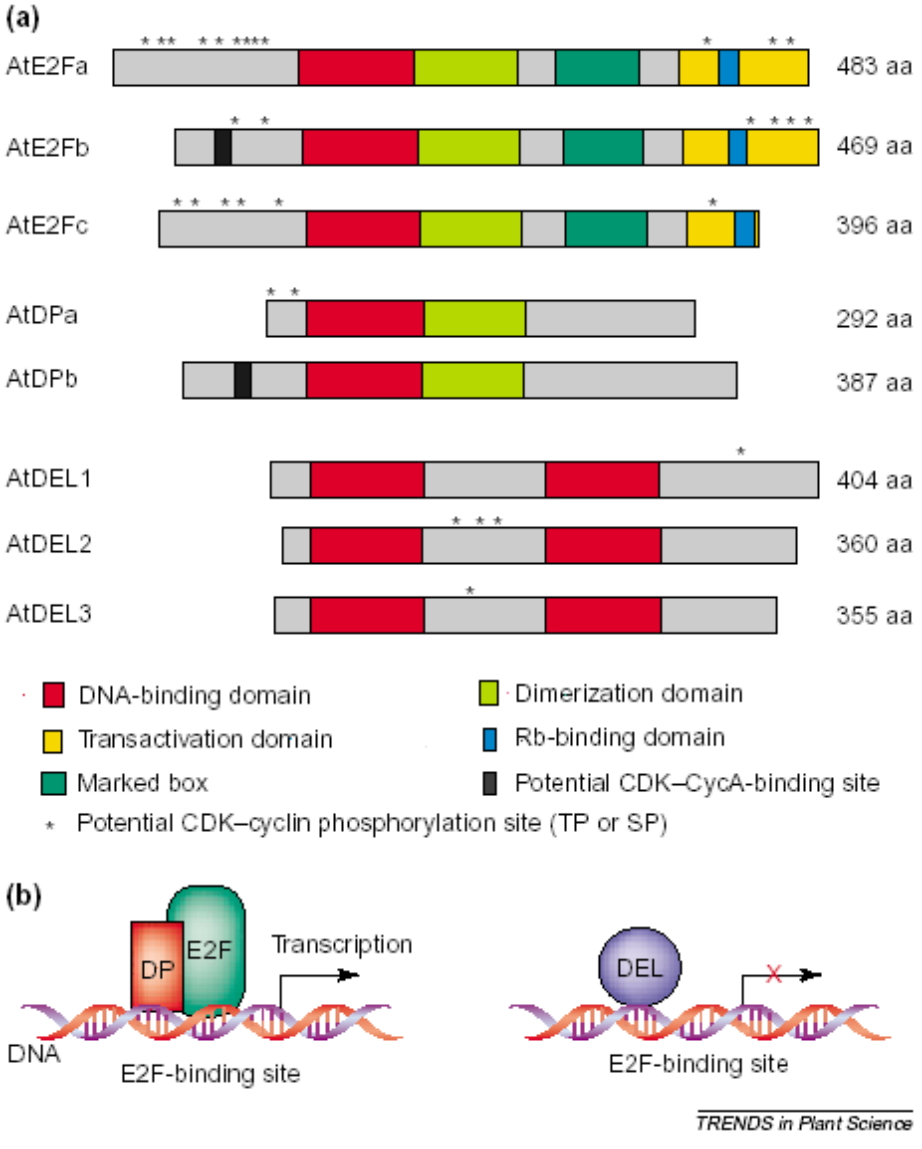
„Pocket proteins“ - příbuzenstvo cyklinů: pRB a spol.

Transkripční regulace - E2F, pRB



S-fázní geny: TK, RNR ...

A. th. *zeus* – 1. zygotické dělení, TK!



Dim.Prot.

Komp. inh. Jako monom.

Fig. 1. Structural organization and DNA-binding properties of the *Arabidopsis* E2F-family proteins. (a) The DNA-binding, dimerization, Marked-box and transactivation domains of the E2F-family proteins. The Rb-binding domain and the potential cyclin-dependent-kinase-cyclin-A (CDK-CycA)-binding domain and CDK-cyclin phosphorylation site are also indicated. Based on the conservation of different domains, the eight *Arabidopsis* proteins are classified into E2F, DP and DEL groups (nomenclature according to Ref. [17]). (b) DNA-binding properties of *Arabidopsis* E2F, DP and DEL proteins. E2F-group proteins bind DNA as heterodimers with DP-group proteins, whereas DEL-group proteins bind DNA as monomers. The DNA sequences specifically recognized by E2F-DP dimers and by DEL monomers are similar and match the animal E2F-binding sites, with the consensus sequence TTT(C/G)(C/G)CGC. Because of the lack of a transactivation domain, the DEL proteins are unable to activate transcription. Their co-production inhibits E2F-DP-mediated transcription, probably through titration of the E2F-binding site [14].

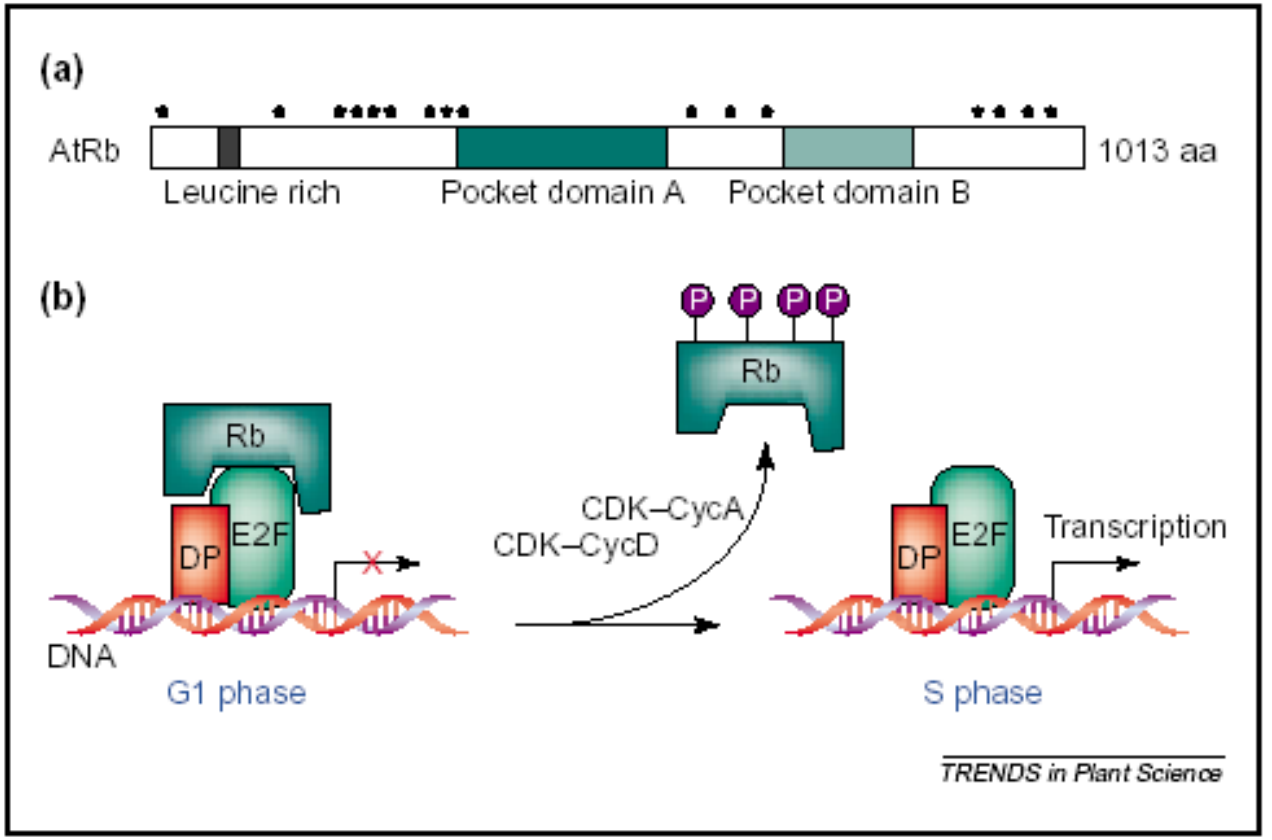
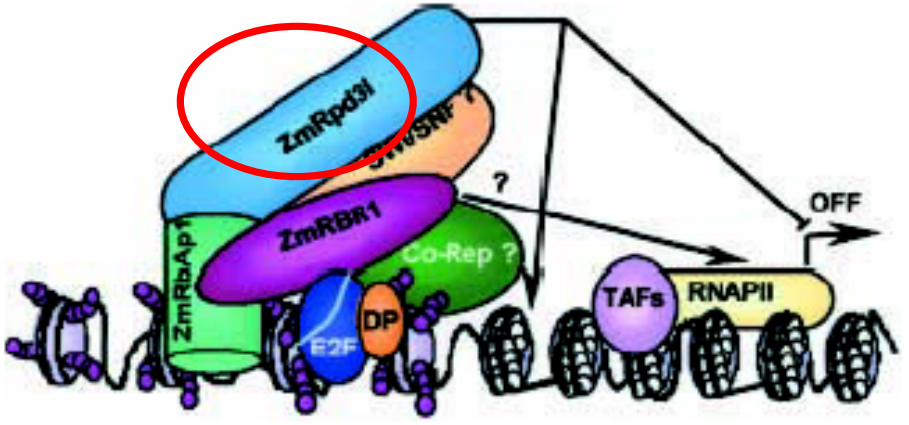


Fig. 2. (a) Structural organization of the *Arabidopsis* retinoblastoma (Rb) protein, showing the conserved pocket domains, the leucine-rich region and the potential cyclin-dependent-kinase-cyclin phosphorylation sites (asterisks). (b) Model for activation of the plant E2F-Rb pathway at the G1-to-S-phase transition. The model is based on results obtained in plants and on parallels with the mammalian E2F-Rb pathway. In growth-arrested cells and during early G1 phase, hypophosphorylated Rb binds E2F-DP dimers and consequently inhibits the E2F transcriptional activity. During late G1 and early S phase, Rb is (hyper)phosphorylated, first by CDK-cyclin-D (CycD) and then by CDK-cyclin-A (CycA) kinases, resulting in the dissociation of Rb from the Rb-E2F-DP complex. The released E2F-DP complex actively promotes transcription of E2F-target genes involved in cell-cycle regulation, DNA synthesis and replication, and chromatin assembly.

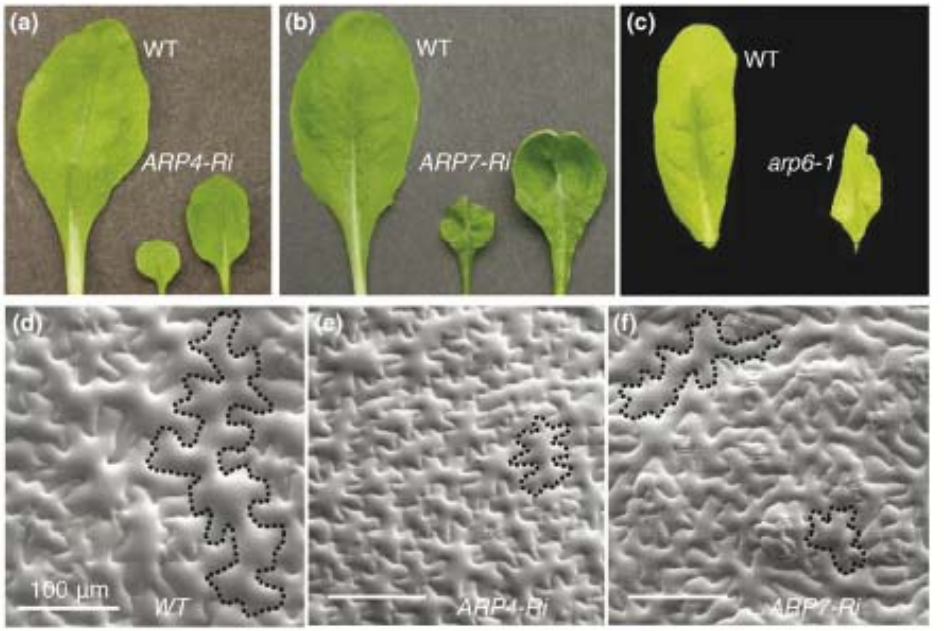
Mechanismus represe je možná trochu složitější ...



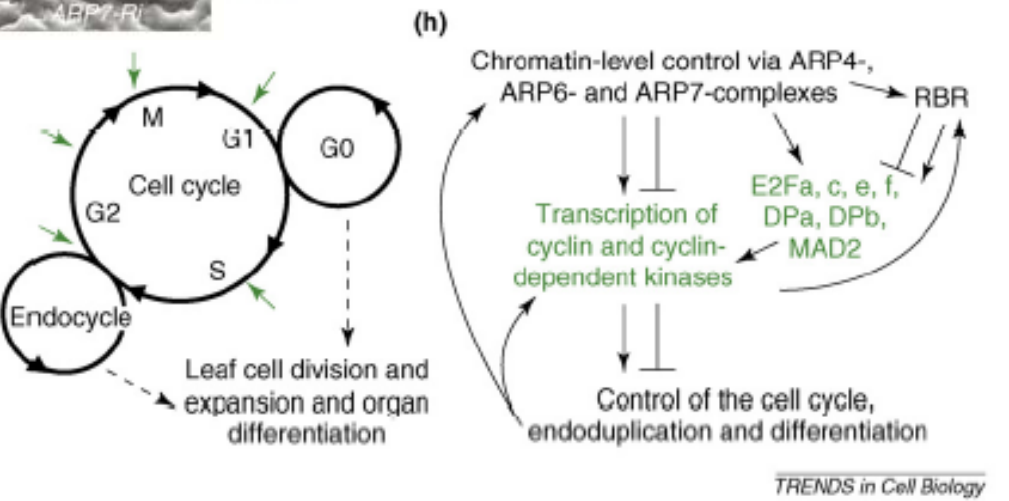
Rb indukuje deacetylaci histonů, což brání transkripc přísluš. oblasti chromatinu

Fig. 2 Molecular model illustrating the mechanism of maize retinoblastoma repression of the G1/S transition by recruiting an Rpd3-type histone deacetylase. This model is based on results obtained in different studies on plant components of the pRb/E2F pathway and on our current research. In this latter research a ZmRBR1 HDAC-independent ability to repress transcription has also emerged, probably due to association of ZmRBR1 with other co-repressors and/or to interference of ZmRBR1with RNA polymerase II holoenzyme (Harbour and Dean 2000). This mechanism and the possible participation of additional components of the RBR/Rpd3 complex (e.g. SWI/SNF-like ATPases) are indicated in the figure by a *question mark*. *Small purple* and *small white circles* represent acetylated and deacetylated histone tails, respectively. The *light blue line* wrapped around E2F depicts the promoter E2F-site. *RNAPII* RNA polymerase II, *TAFs* components of the general transcriptional machinery, *Co-rep* putative ZmRBR1 co-repressors. The description of the chromatin structure and the amino acid regions involved in protein interactions is schematic; no attempt has been made to accurately portray these structures

Na kontrole transkripce a cyklu se podílejí ARP4,6,7!

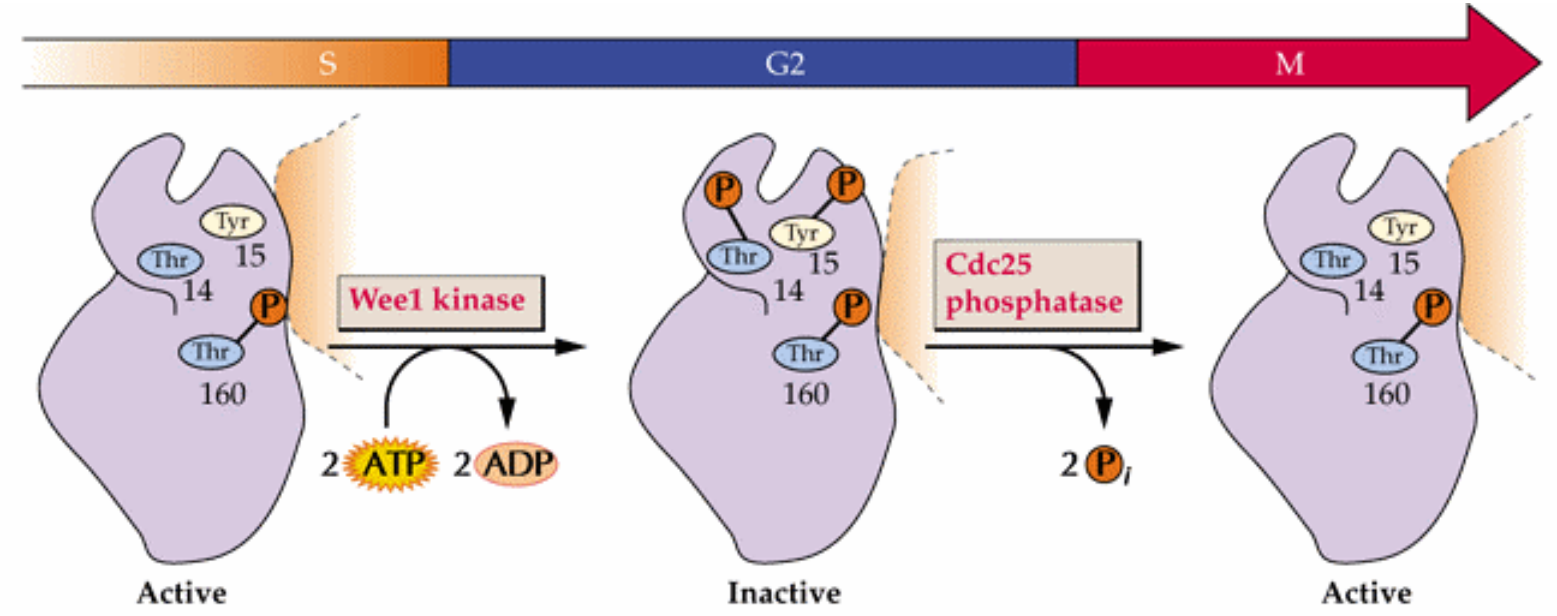


(přes kondensaci chromatinu – pleiotropní, vliv též na kvetení a senescenci květů ...)



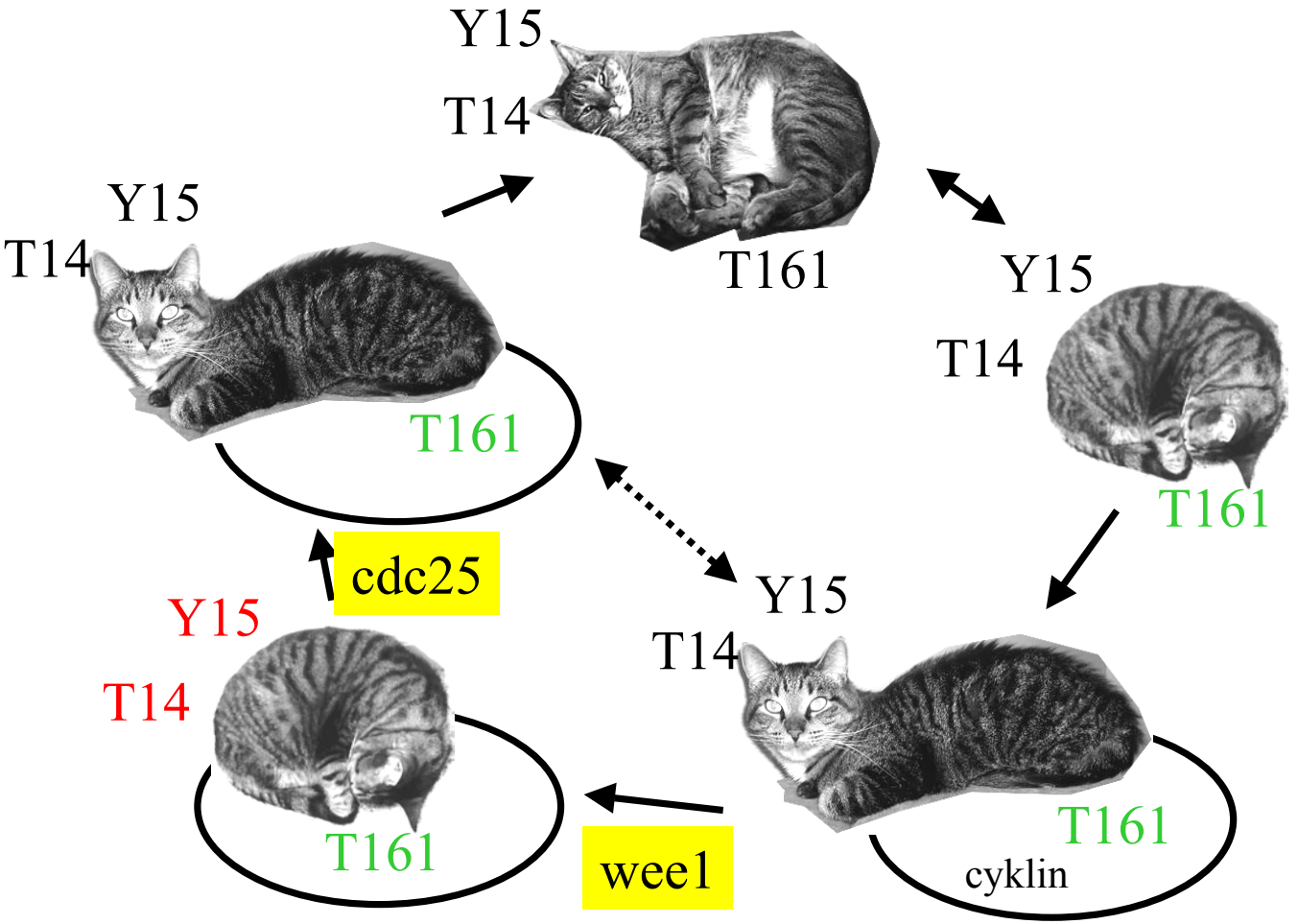
(Meagher et al. 2005, 2007)

Regulace CDK: např. fosforylací

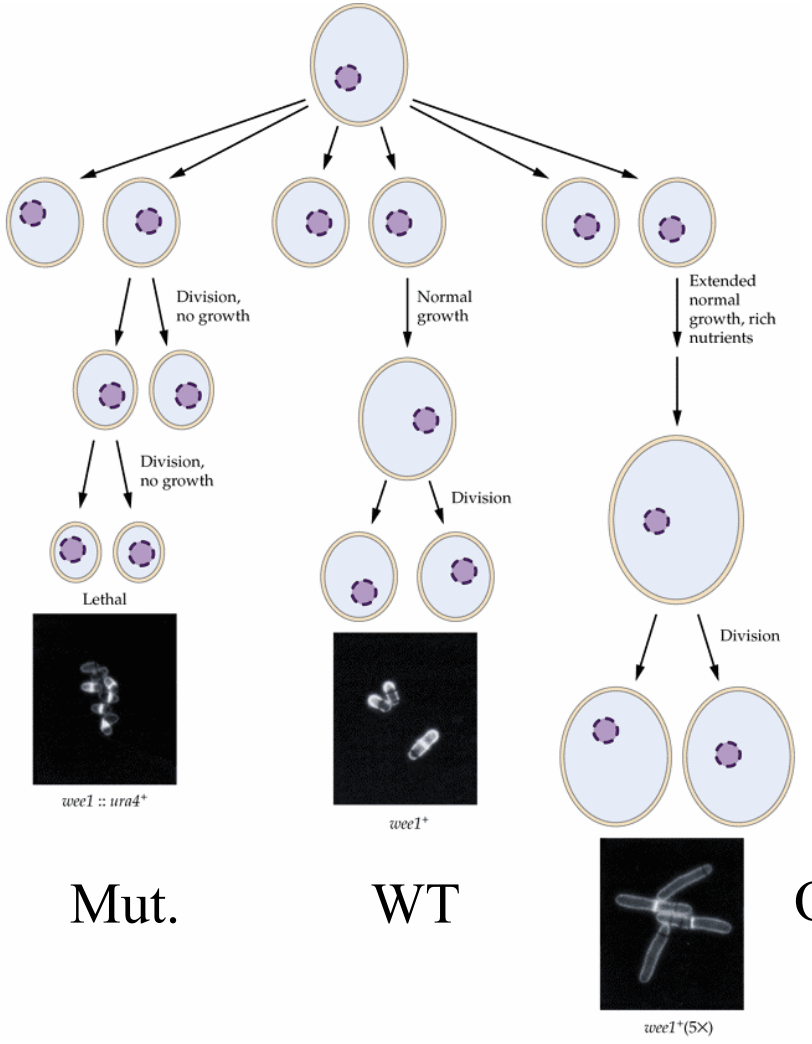


Wee1 kinasa a Cdc25 působí proti sobě.

A to ještě není všechno: CDK mají i další fosforylační místa.



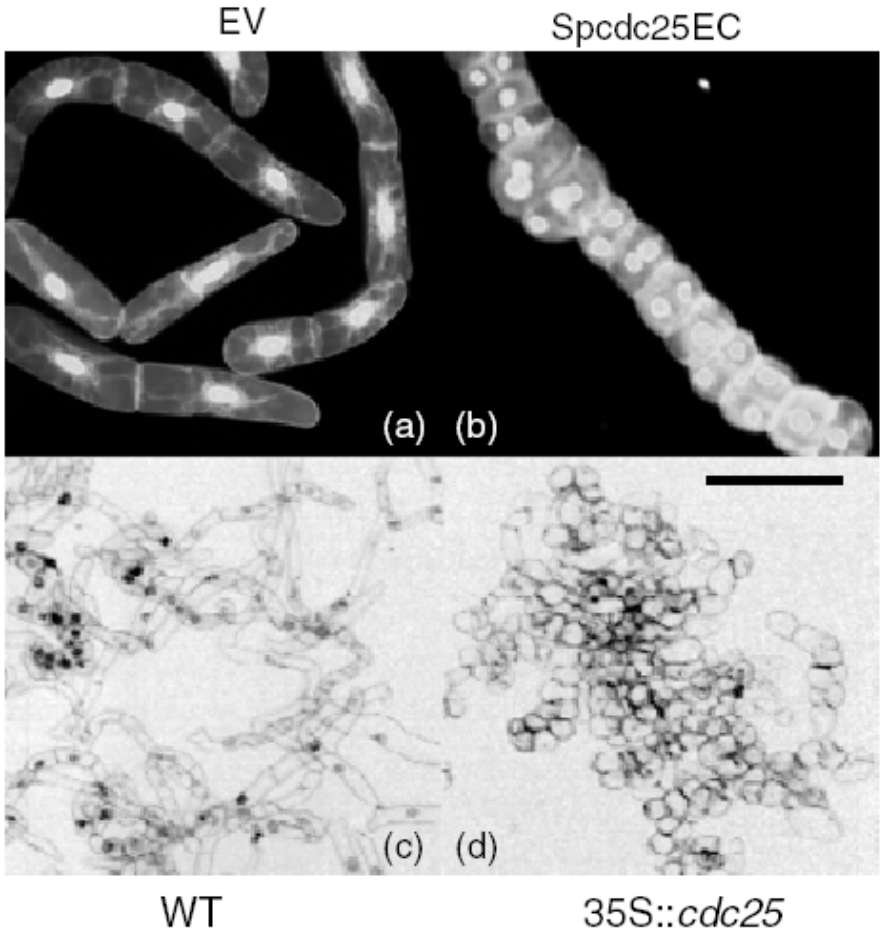
... jakožto jedna z cest ke spřažení cyklu a růstu



Mut. WT OX

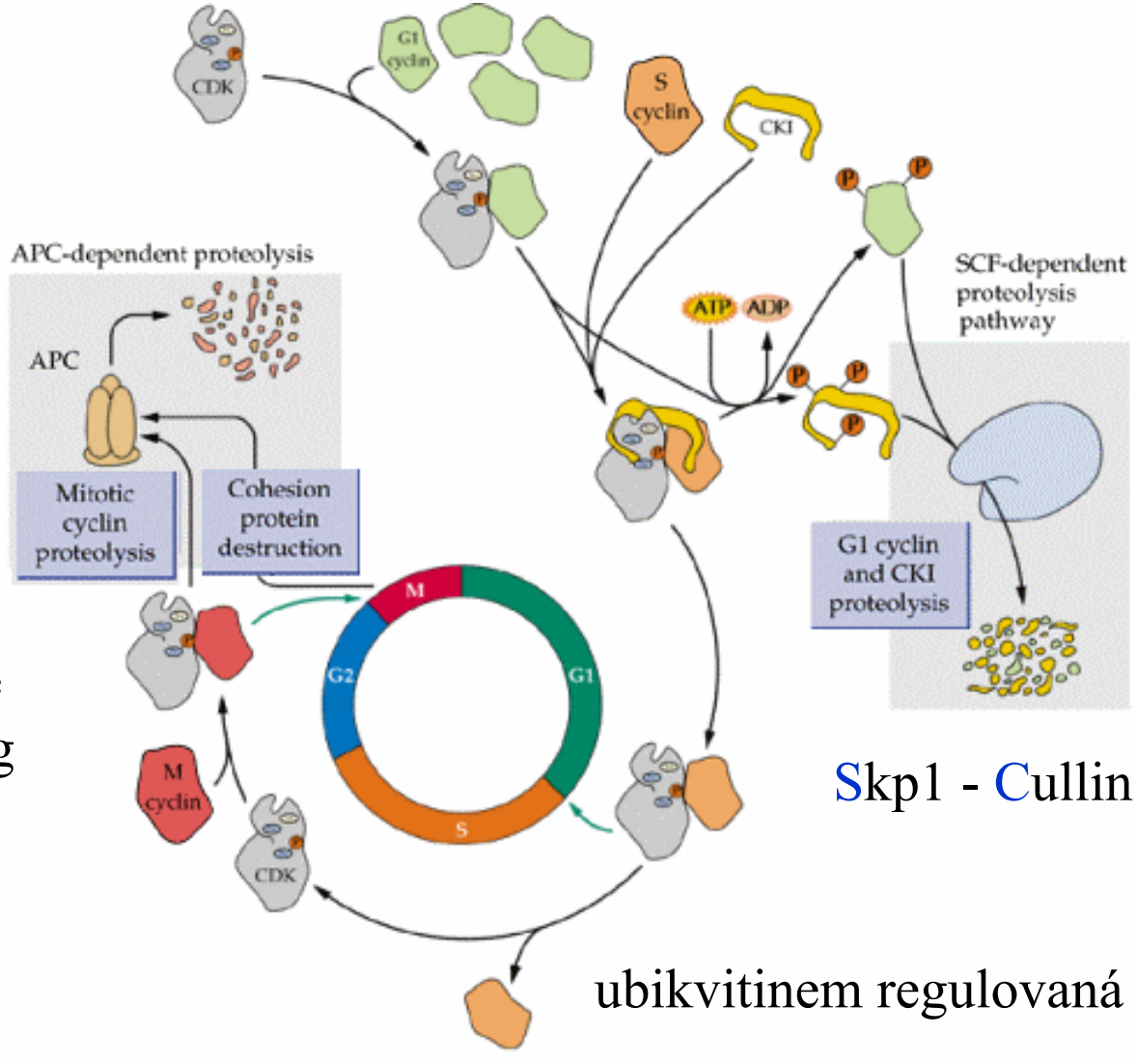
(S. pombe)
wee1

Exprese kvasinkového Cdc25 v rost. buňkách má fenotyp



(Orchard et al. ... Suchomelová, Lipavská ... 2005)

Proteolýza v regulaci BC

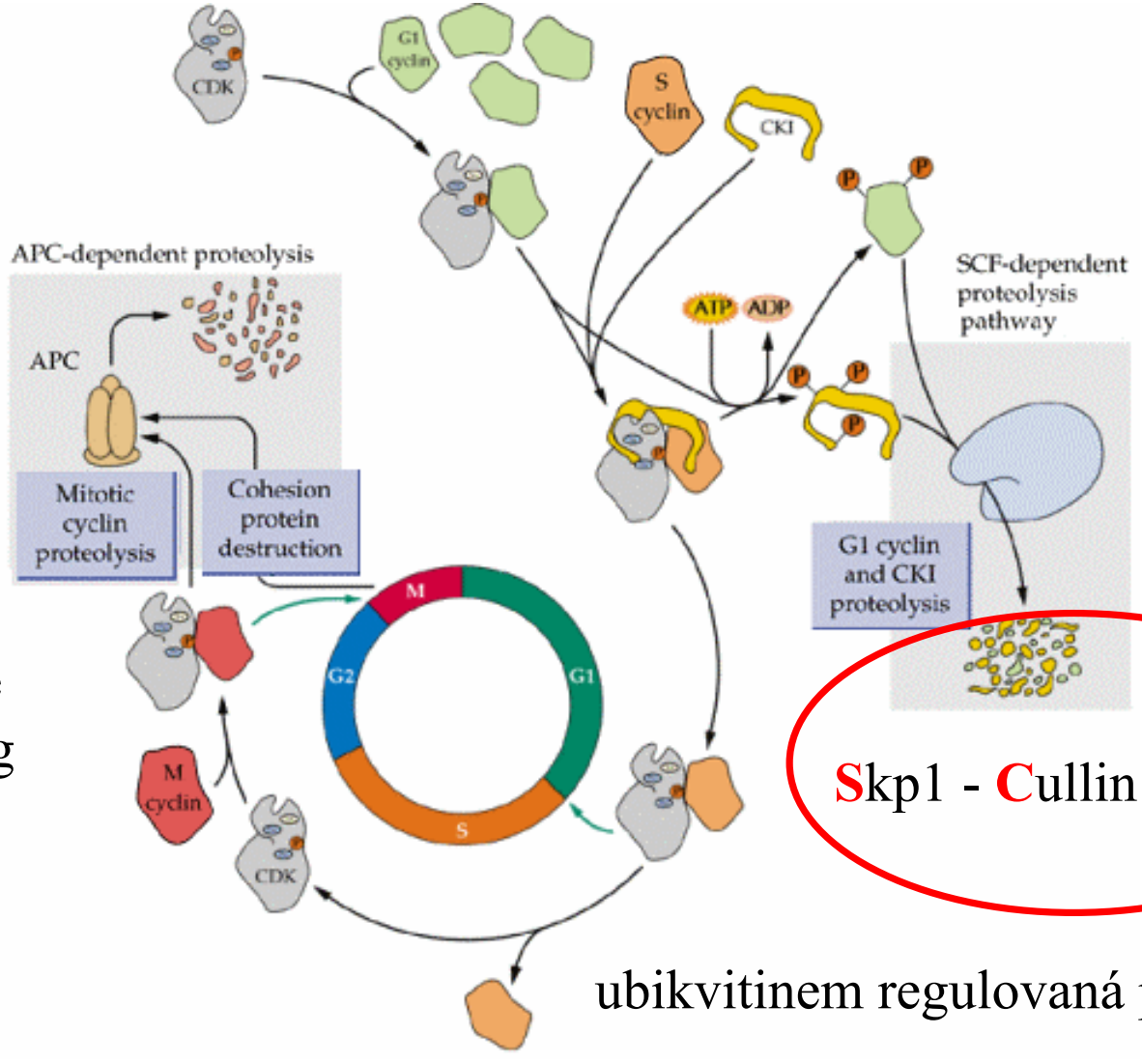


Anaphase promoting complex

Skp1 - Cullin - F-box

ubikvitinem regulovaná proteolýza

Proteolýza v regulaci BC

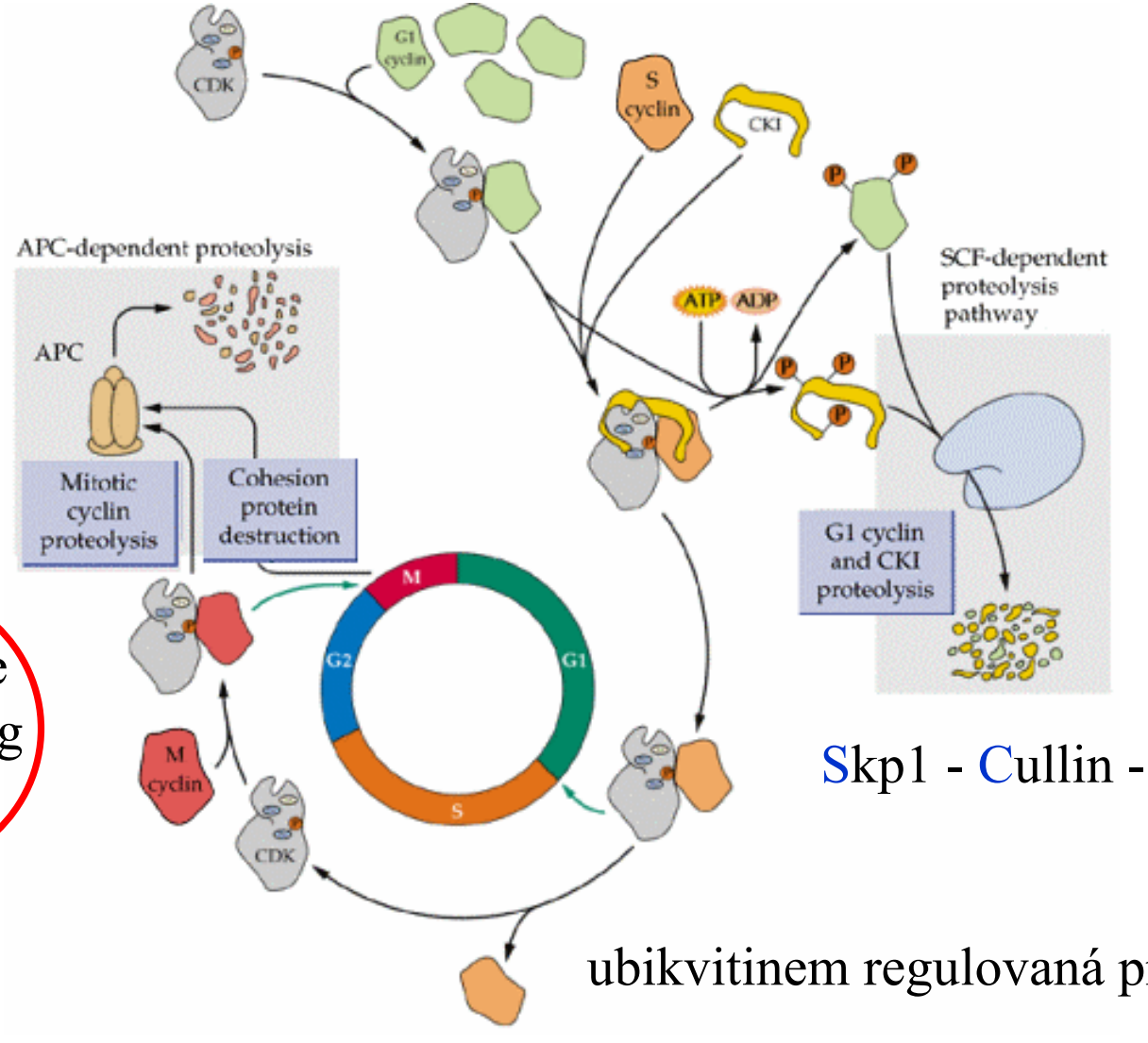


Anaphase promoting complex

Skp1 - **C**ullin - **F**-box

ubikvitinem regulovaná proteolýza

Proteolýza v regulaci BC



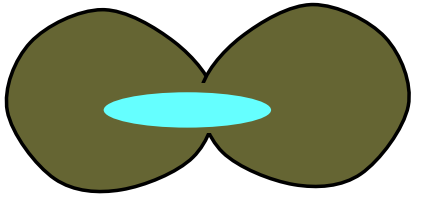
Anaphase
promoting
complex

Skp1 - Cullin - F-box

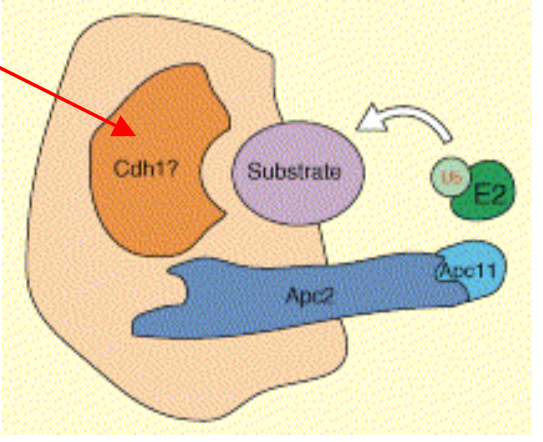
ubikvitinem regulovaná proteolýza

Amatomie APC/cyklosomu (cyclosome)

- E3 Ubi-ligasa
- Podjednotky: Cdc16, Cdc23, Cdc26, Cdc27, BimE + 3 další
- Regulace: Cdc20 nebo Cdh1
  - Cdc20 sám degradován via APC
  - Cdh1 je substrátem CDK (inaktivační Pace!)

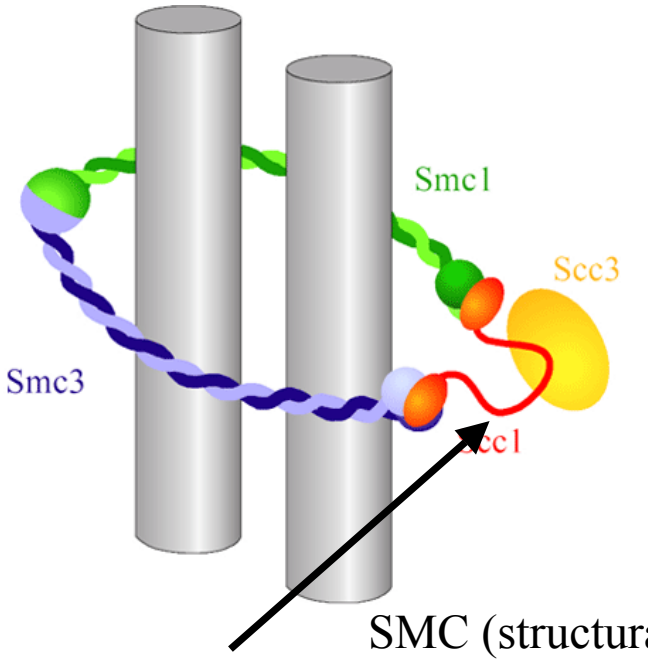


kvasinka: mND fenotyp



Další role APC: separace chromosomů

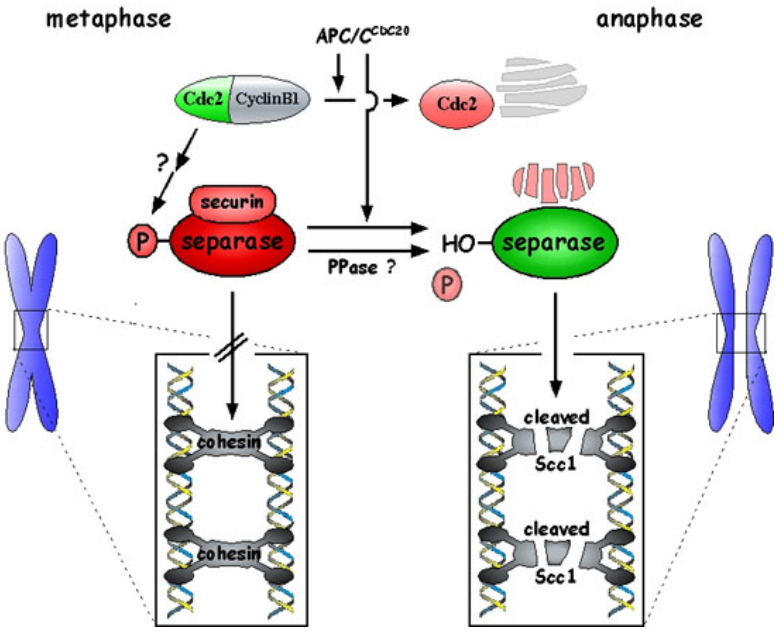
Sesterské chromatidy drží pohromadě **kohesinové komplexy**.



místo proteolyt. štěpení

SMC (structural maintenance of chromosomes)

SCC (sister chromatid cohesion)



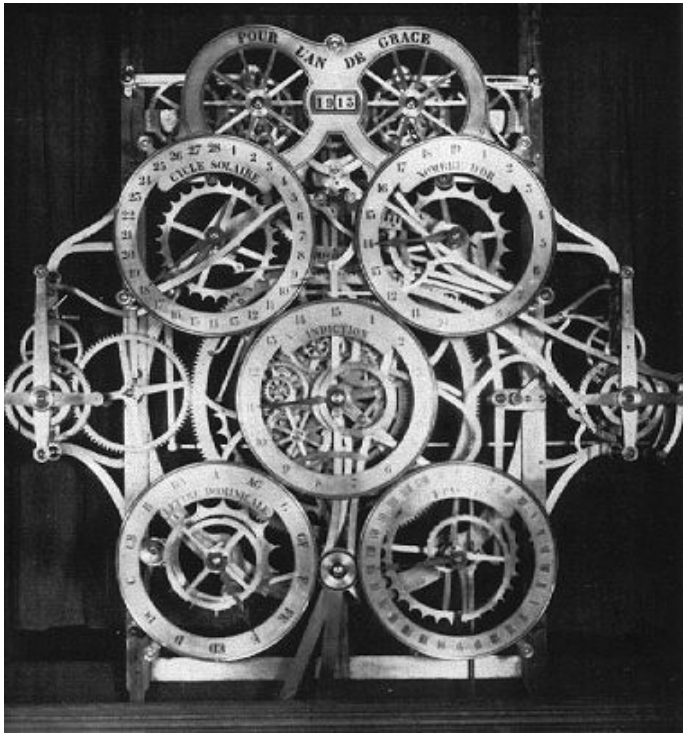
(C. H. Haering)

Ústřední hodiny buněčného cyklu

centrální oscilátor
(„cell cycle engine“)

vstupy

velikost
signály
poškození ...

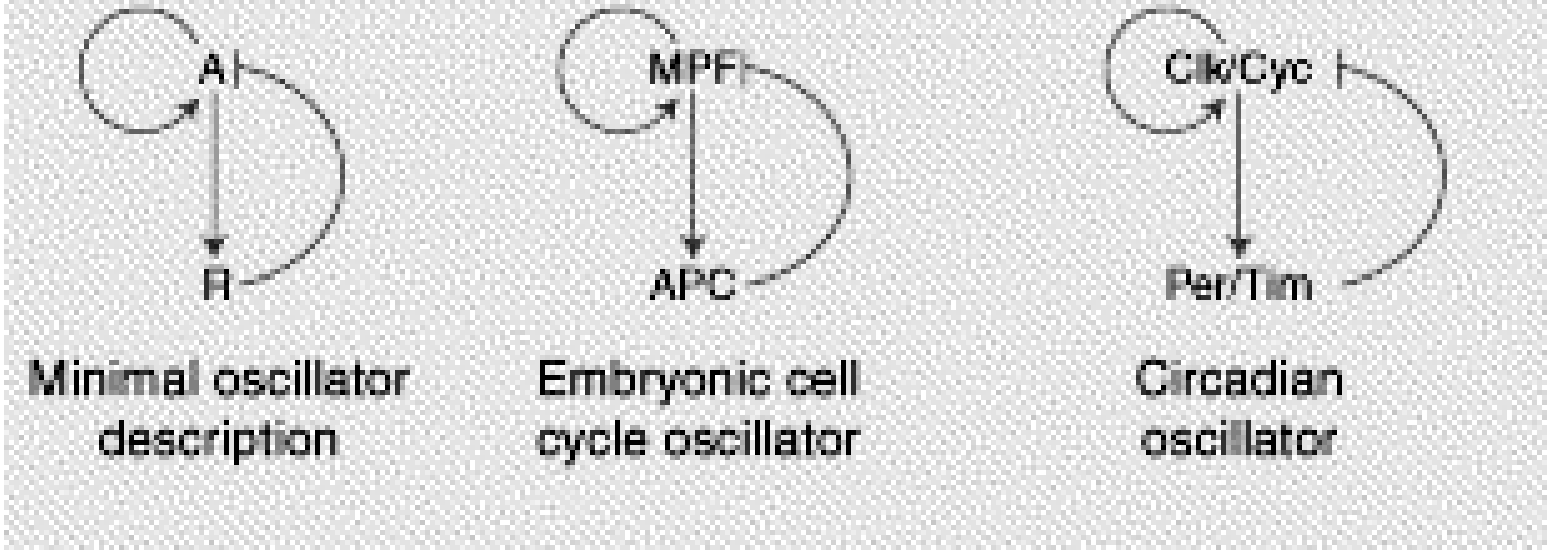


výstupy

gen. exprese
mitosa,
cytokinese ...

Jak vůbec lze zajistit pravidelný chod - oscilace?

Minimální oscilátor (jeden z mnoha)

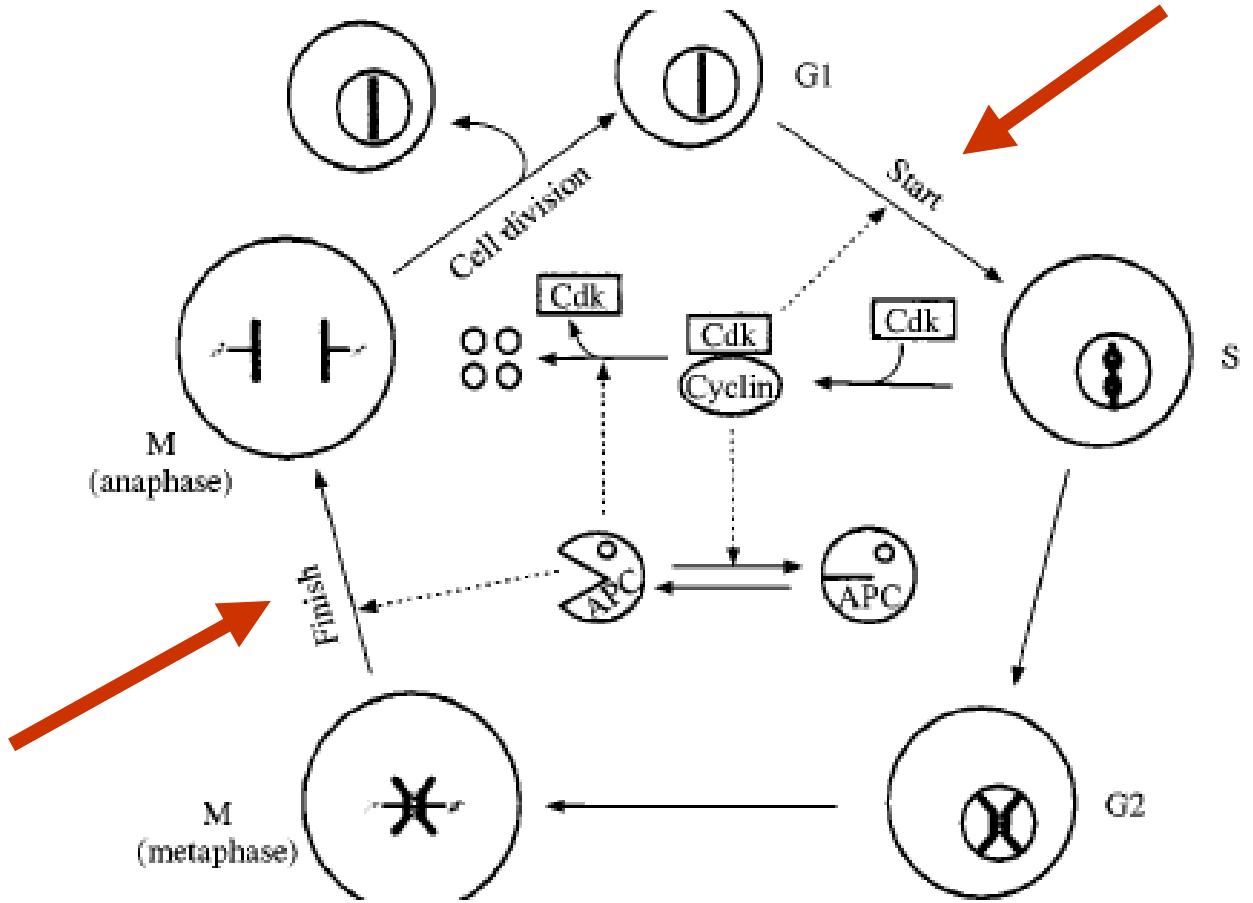


Je lepší než jiné?? Problém robustnosti!

„Turing v čase“

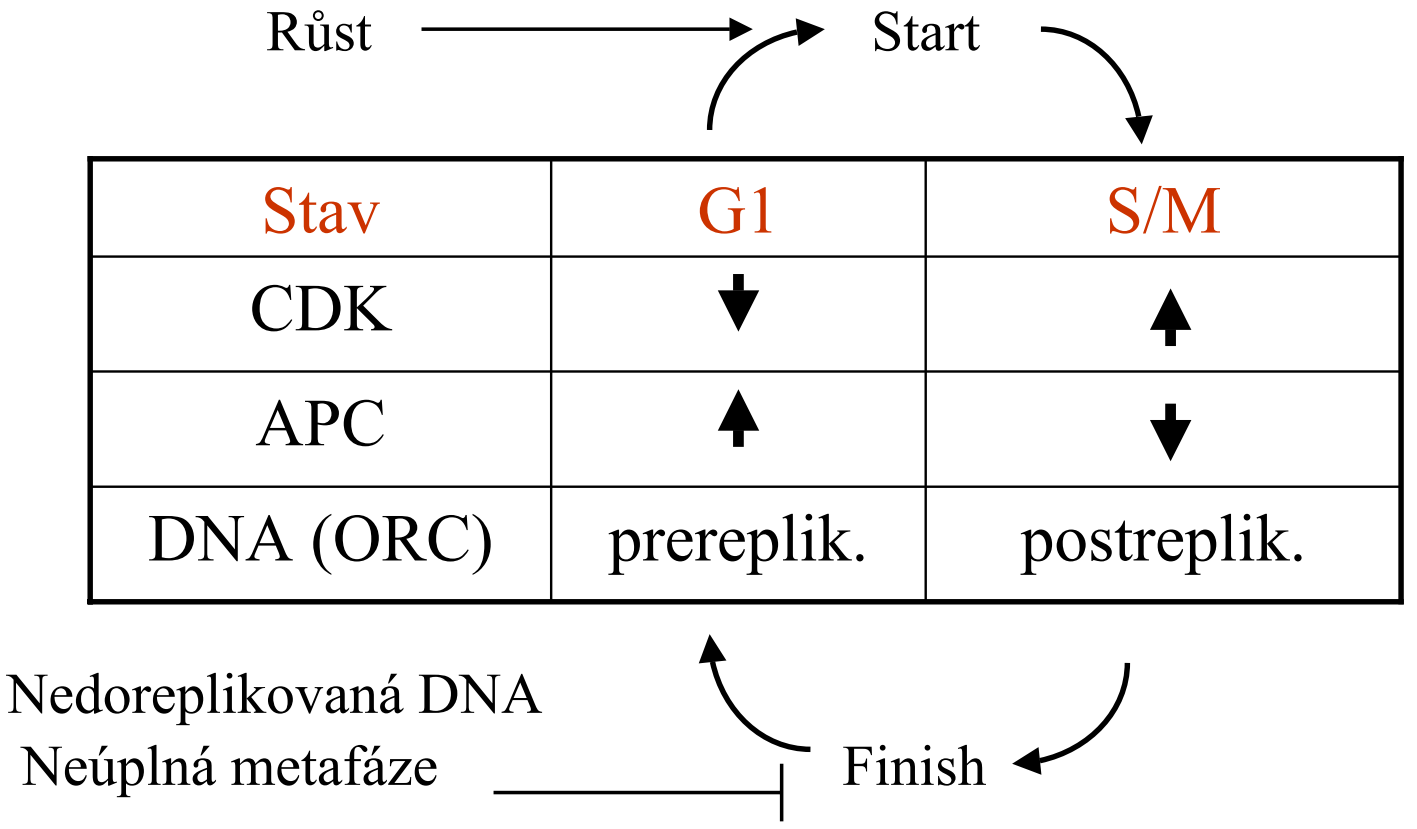
(Ingolia a Murray, Curr. Biol. 2004)

Oscilátor v kontextu tradičního pohledu



Tyson and Novak, J. Theor. Biol. 210:249-263, 2001

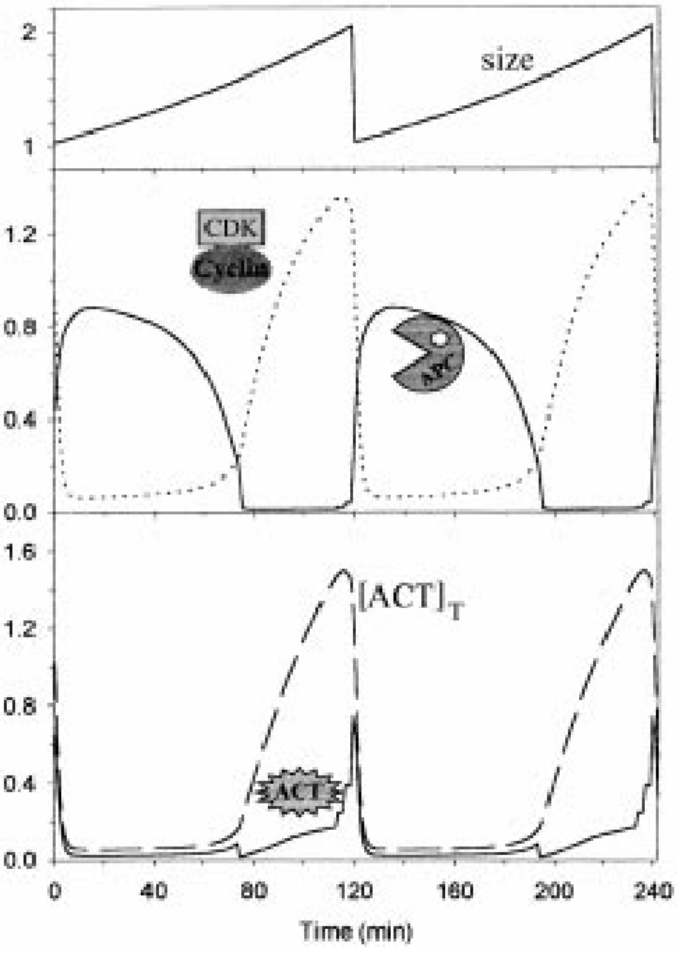
Dva stavy „cyklových hodin“



(Novak et al., Phil.Trans.R.Soc.Lond.B 353:2063-2076, 1998)

Model minimálního cyklu

- Jádro: CDK/cykliny + APC
- Start regul. růstem
- Finish regul. dokončením replikace + vřeténka prostřednictvím „aktivátoru“ APC (ACT)
- Osciluje v širokém rozmezí parametrů!



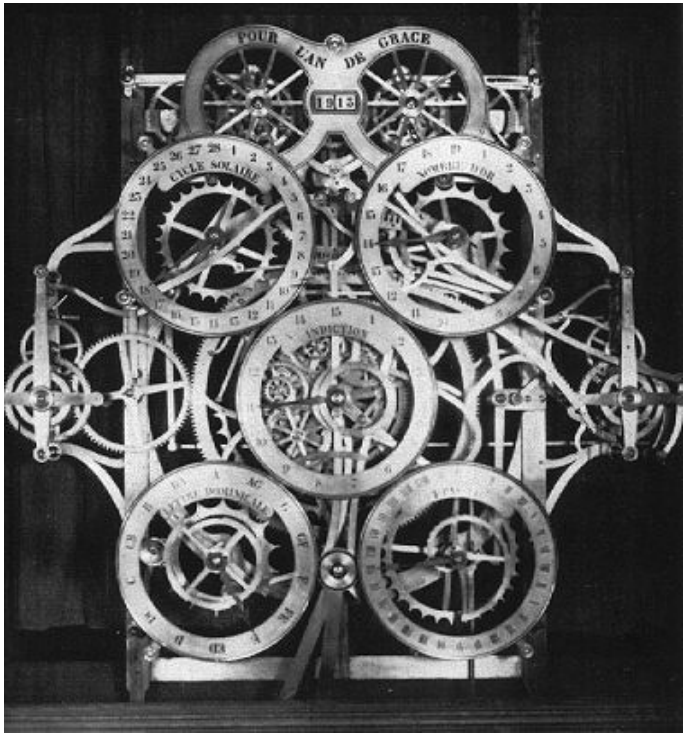
(Novak et al., Phil.Trans.R.Soc.Lond.B 353:2063-2076, 1998)

Vstupy a výstupy - (nejen) „rostlinná specifika“

centrální oscilátor
(„cell cycle engine“)

vstupy

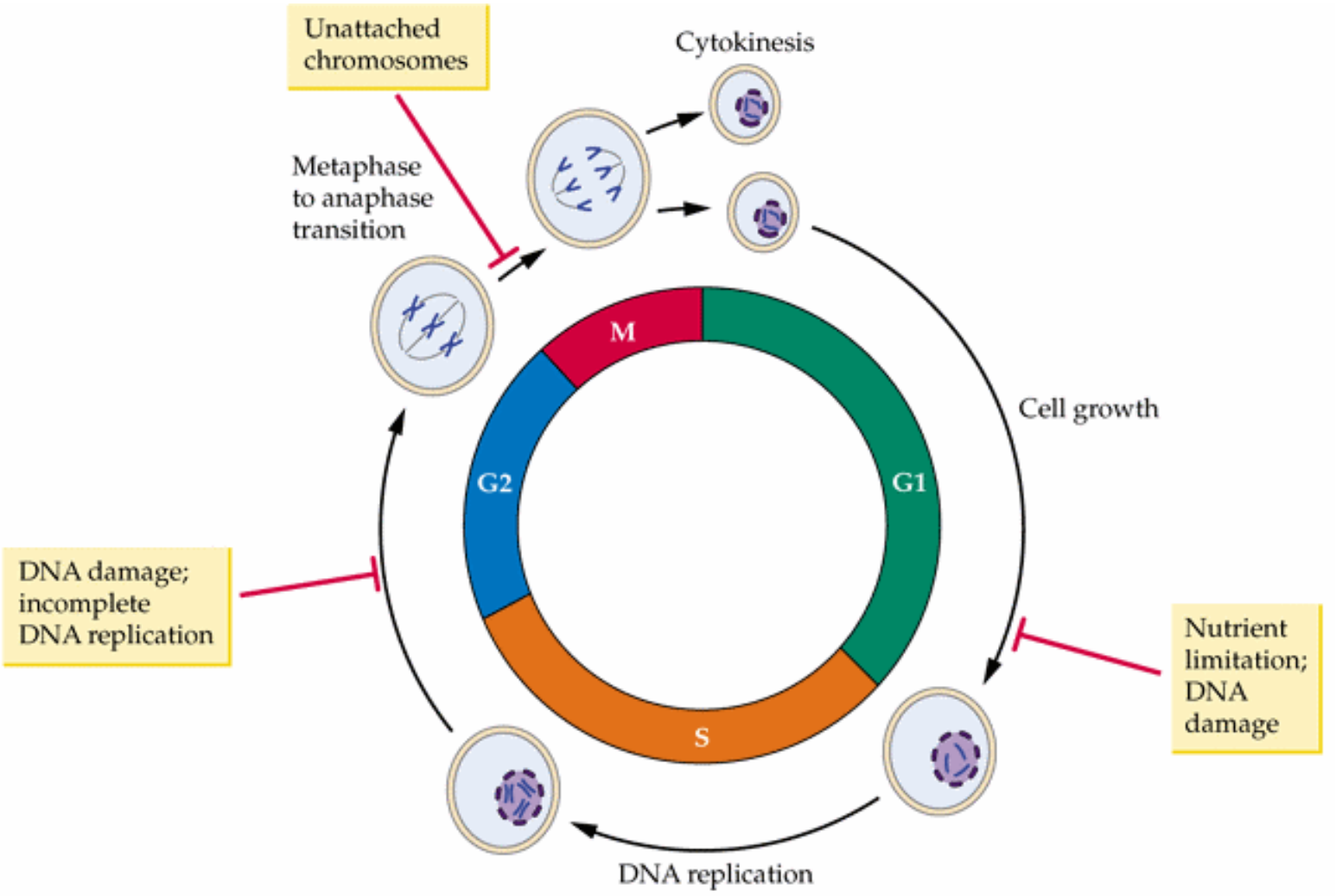
velikost
signály
poškození ...



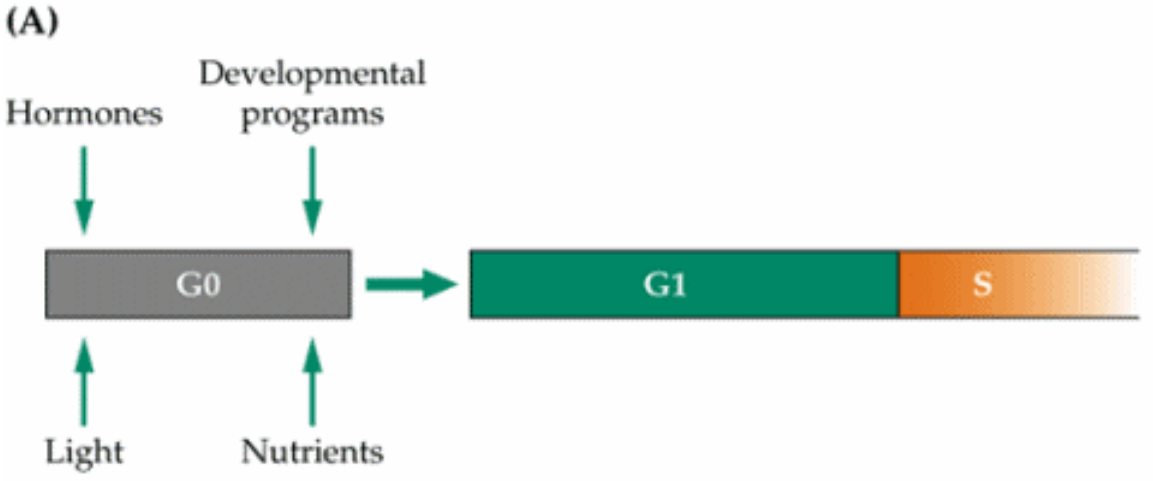
výstupy

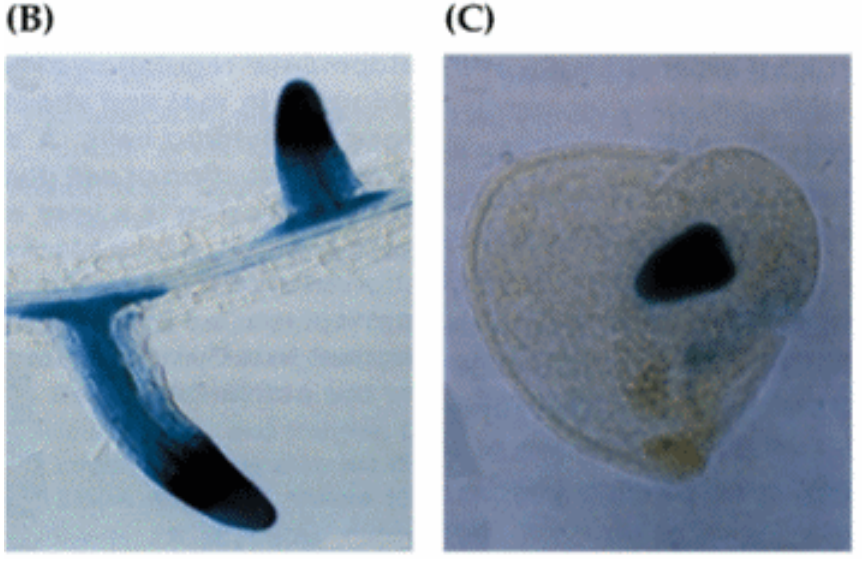
gen. exprese
replikace
mitosa,
cytokinese ...

Obecně cyklus regulován též v závislosti na poškození („checkpoints“)



Ontogenetická kontrola BC - rostlina vládne buňkám





AtCYCB1::GUS

V ranném embryu téměř mizí G1 fáze.

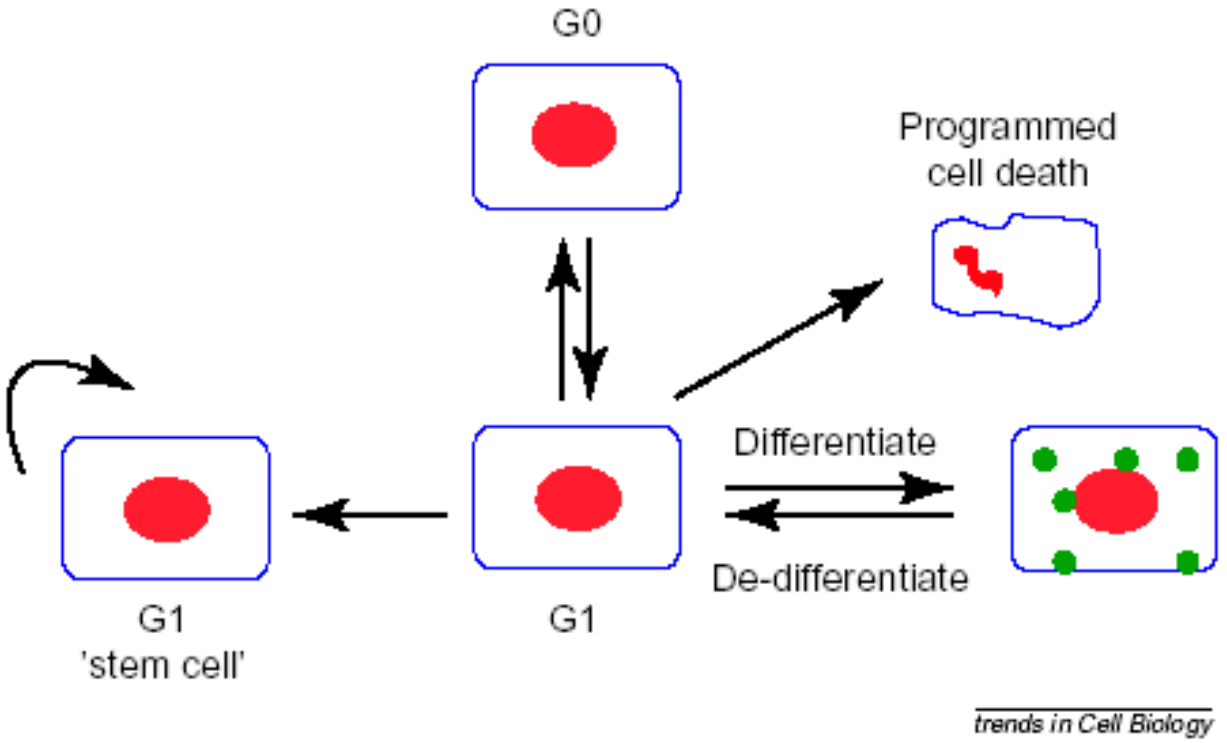


FIGURE 1

Options for G1 cells in plants. Newborn G1 cells can start another round of division ('stem cell') or exit the cycle (non-cycling cells). These cells die (programmed cell death), return into the cell cycle or differentiate. In contrast to animals, differentiated plant cells can more readily de-differentiate and re-enter the cell cycle, given the appropriate signals.

Kontrola cyklu sacharosou a fytohormony

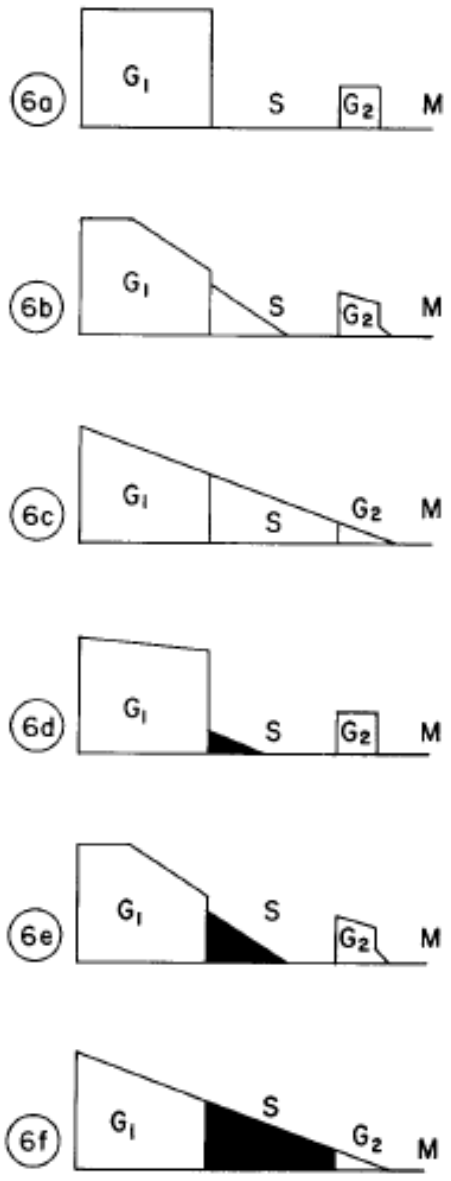
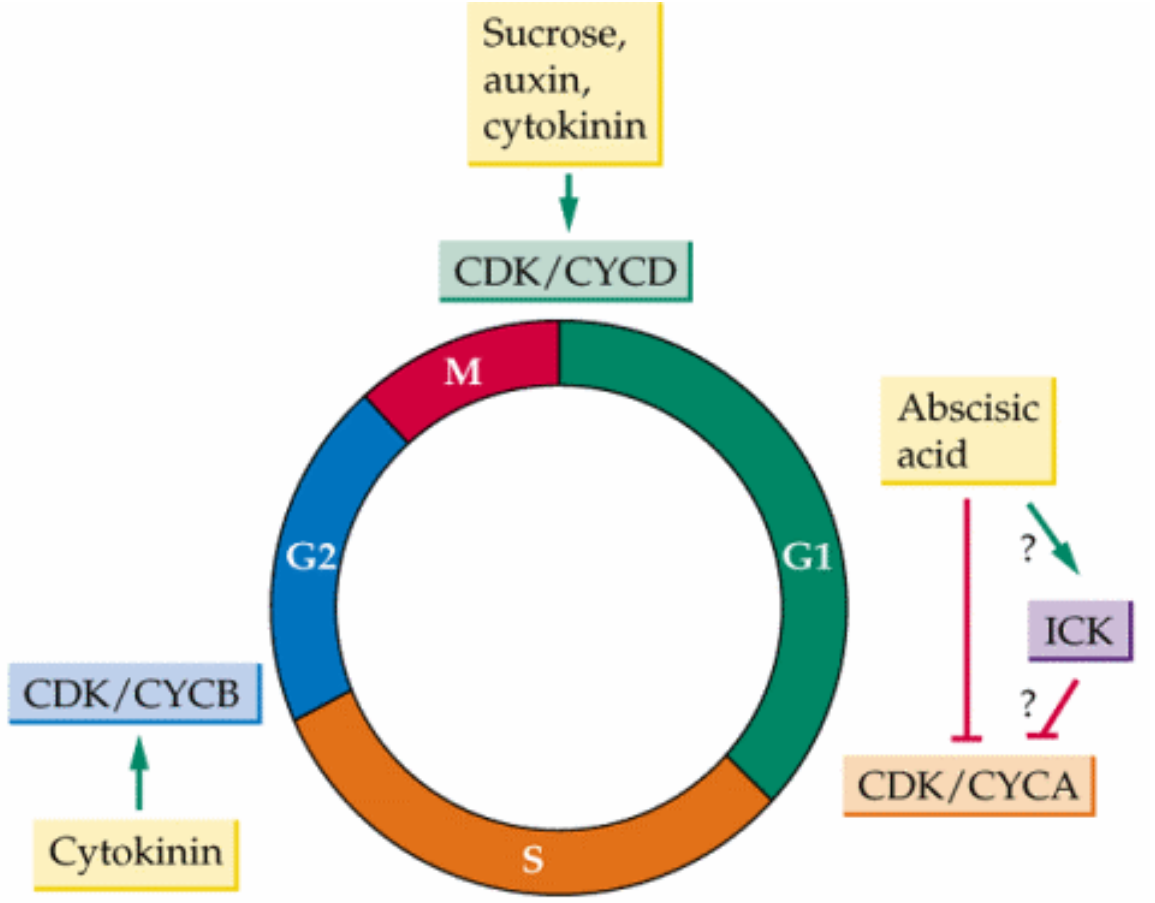


FIGURE 6 Schematic representation of postulated cell distributions in the mitotic cycle of stationary phase root tips that received no sucrose spurt, *a*, a 6-hr spurt *b*, and a 12 hr spurt of sucrose, *c*, during the 72 hr stationary phase. *d–f* represent cell distributions of tritiated thymidine-labeled interphase cells: after no sucrose spurt and a 12 hr labeling period, *d*; after a 6 hr spurt with sucrose and simultaneous labeling with tritiated thymidine, *e*; and after a 12 hr sucrose spurt and tritiated thymidine labeling, *f*. The shaded portions in *d–f* represent tritiated thymidine-labeled interphase cells.

Kontrola cyklu sacharosou a fytohormony



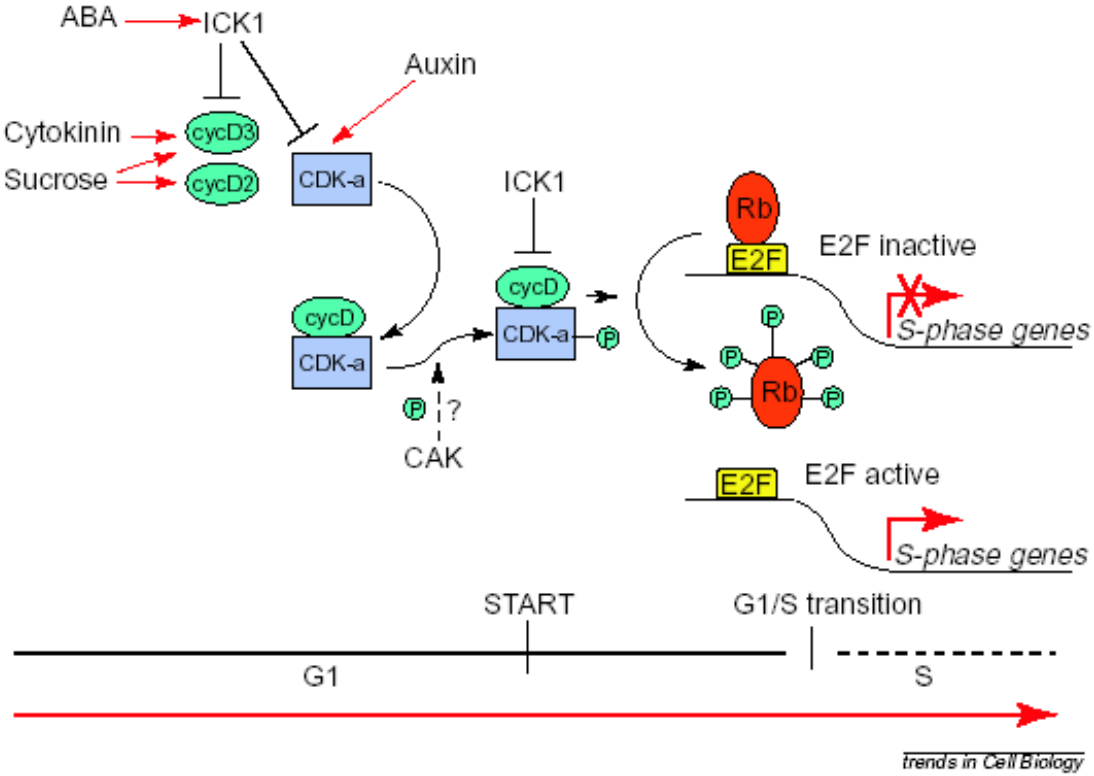
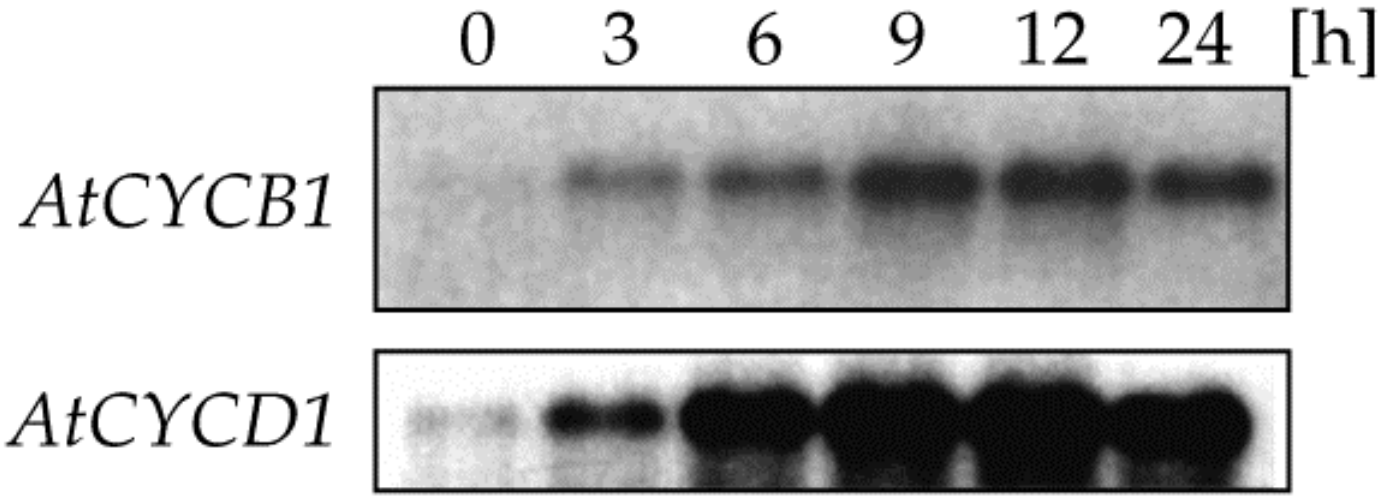


FIGURE 2

Model for G1–S transition in plants. Cytokinin- and sucrose-induced D-type cyclins bind to cyclin-dependent kinase-a (CDK-a) to form inactive heterodimers. Regulation of kinase activity after binding the cyclin might occur either by an inhibitor (ICK1) or by phosphorylation by an activating kinase (CAK). Phosphorylation of the retinoblastoma protein Rb by CDK-a complexes releases the transcription factor E2F, which is the active molecule required to enter S phase. The phosphorylation of plant CDK-a by CAK and the presence of Rb–E2F complexes on the promoters of S-phase genes have not been shown to occur in plants but are based on the mammalian G1–S model.



- odpověď na auxin (A. th. kořeny)

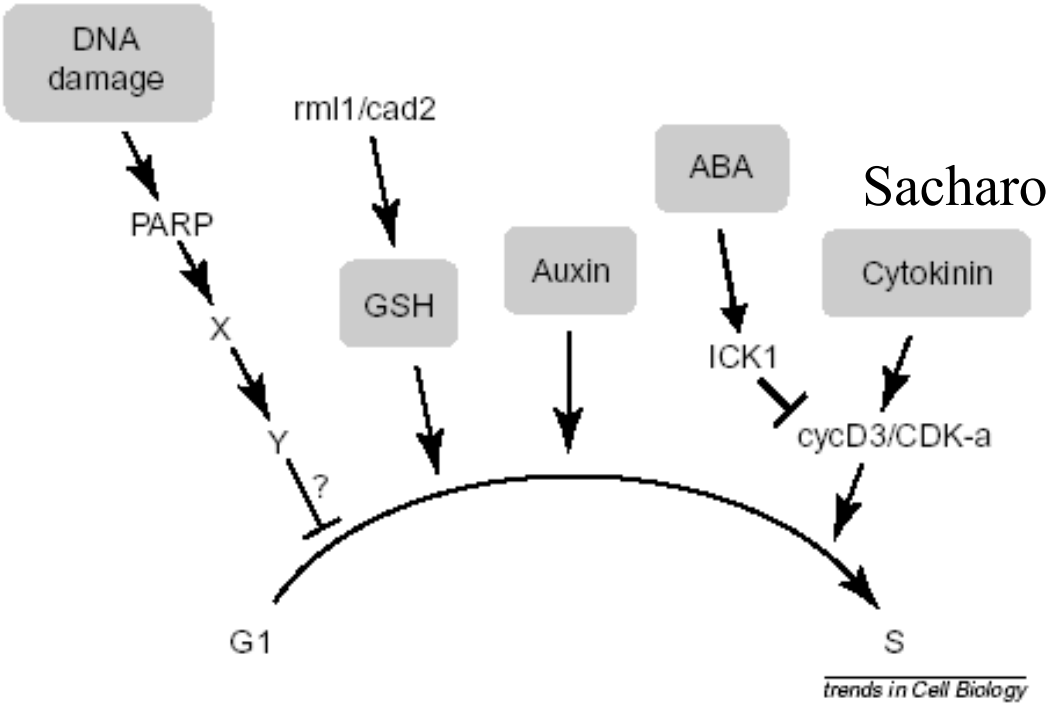


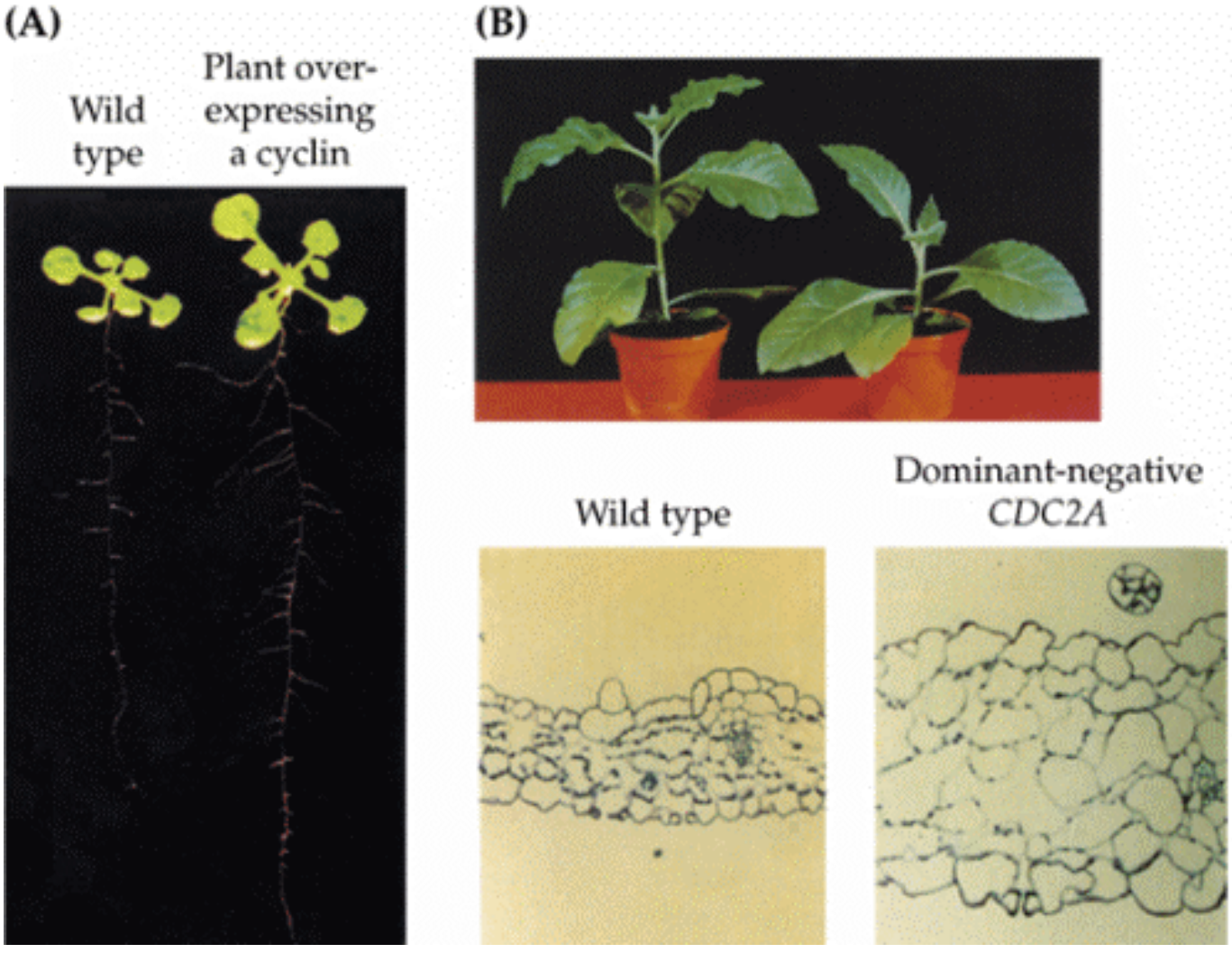
FIGURE 3

Potential signalling pathways feeding into the G1–S transition in plants. Genome instability transcriptionally activates poly(ADP-ribose) polymerase (PARP). In mammalian systems *X* = p53 and *Y* = p21, but their homologues have not been identified in plants. The *rml1/cad2* gene encodes the first enzyme of glutathione (GSH) biosynthesis. When the intracellular GSH concentration falls below a threshold level, the G1–S transition is blocked in dividing root cells. Depletion of auxin arrests cells in G1, and abscisic acid (ABA) induces the inhibitor ICK1 transcriptionally. ICK1 can interact with both cycD3 and CDK-a (cdc2a). Cytokinin activates cycD3 transcription, and constitutive cycD3 expression can rescue the cytokinin requirement of callus.

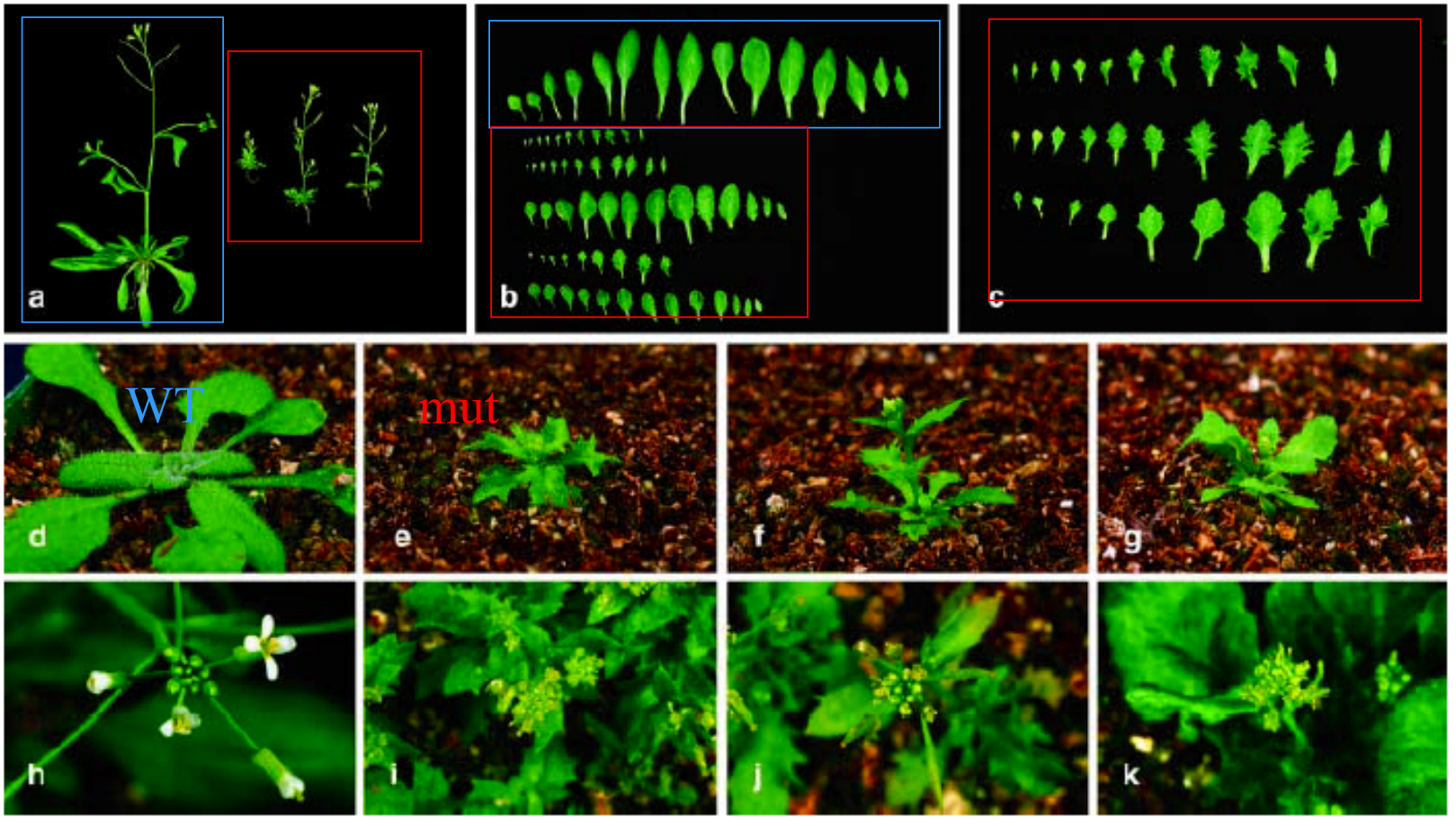
Na úrovni buňky jasné priority…

- Základem je „cell cycle engine“
- Regulace vstupů a výstupů jsou „přívěsky“
- Víme, že rostliny rakovinu nemívají
- ALE...?

Mutace a změny exprese centrálních regulátorů cyklu



OX - Mit. cyclinu
zvyšuje růst kořenů a celé rost.

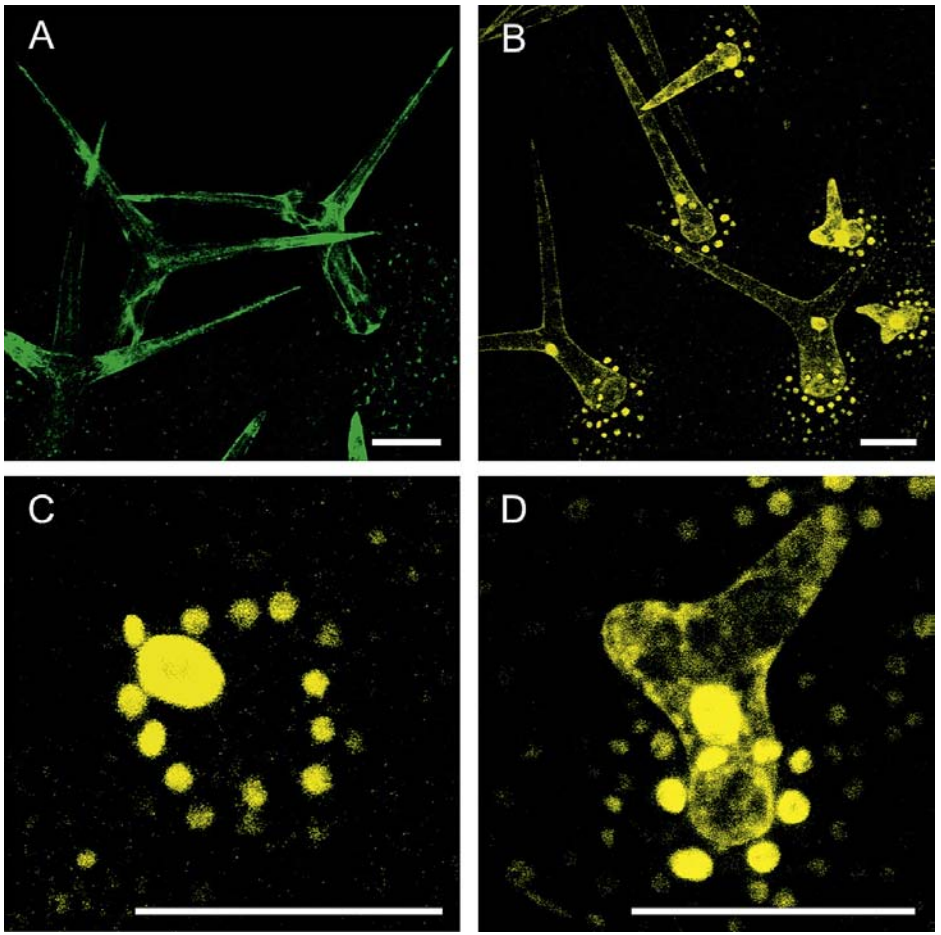


masivní overexprese KRP (inhibitor CDK): malé rostliny, méně buněk

Zhou et al. 2002

Intercellular and subcellular localization of Arath;KRP1

pGL2:GFP



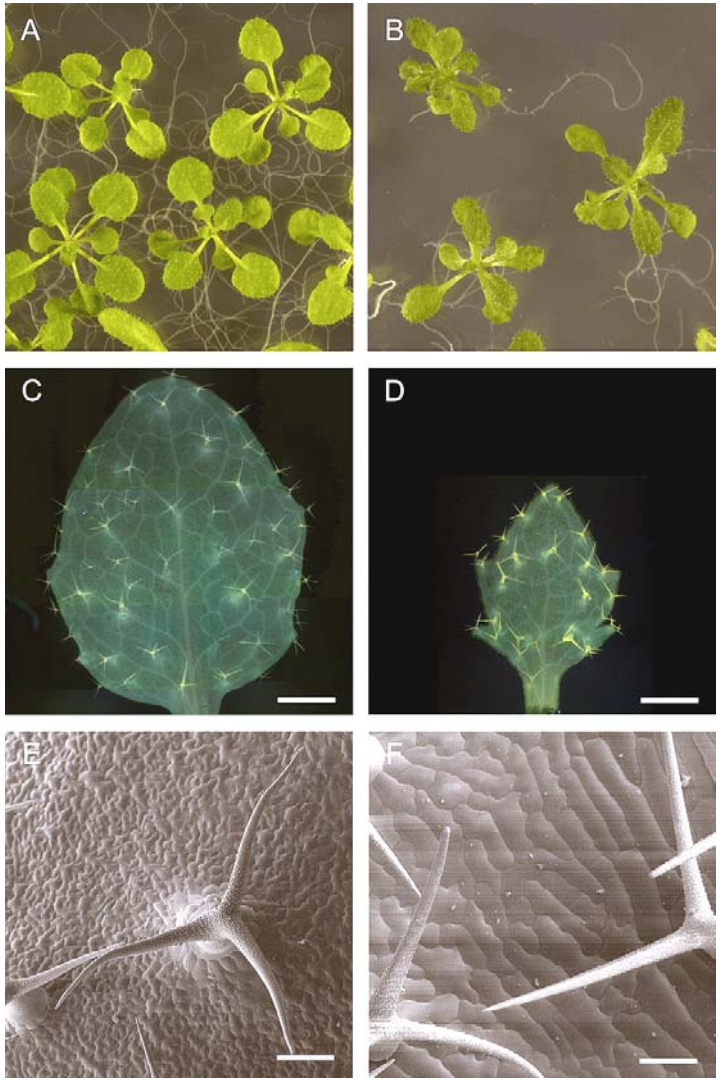
pGL2:KRP1:GFP

Verkest, A., et al. Plant Physiol. 2005;139:1099-1106

(protein je v jádře a leze do sousedních buněk!)

Umírněná overexprese KRP: malá rostlina, velké buňky!

wt



pSTM:KRP

pTMM:KRP

Verkest, A., et al. Plant Physiol. 2005;139:1099-1106

Velké buňky jsou polyploidní!

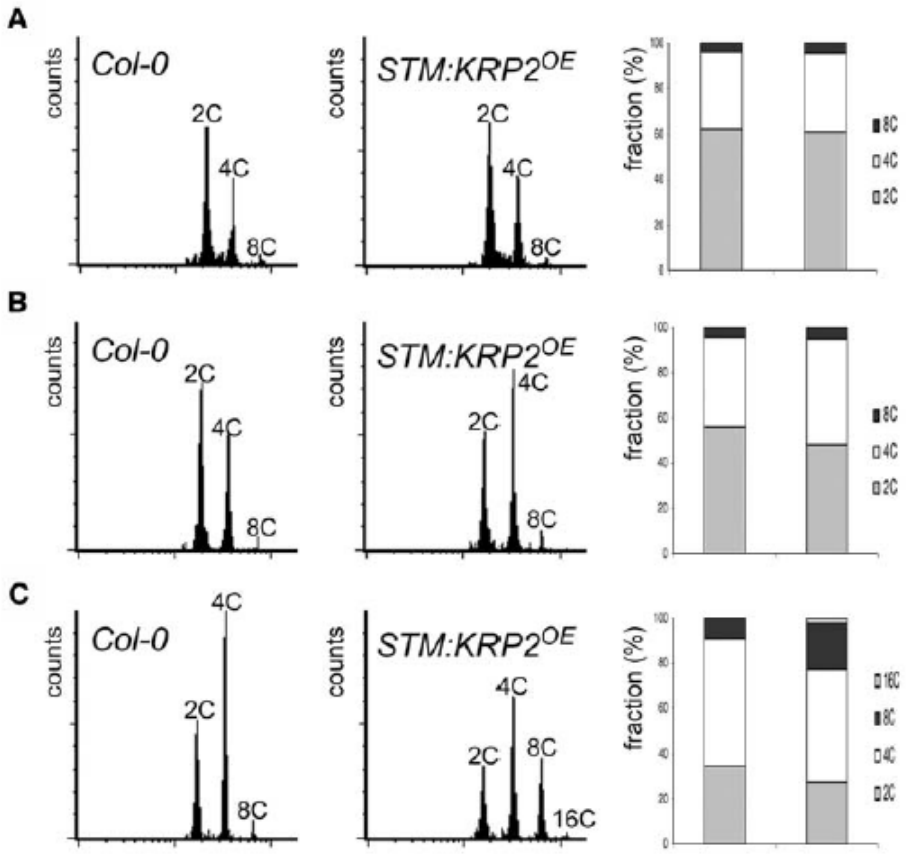


Figure 6. Ploidy Level Distribution of the First Leaves of Wild-Type (Col-0; Left) and *STM:KRP2*^{OE} (Line 5; Right) Plants during Development as Measured by Flow Cytometry.

(A) Eight DAS.
(B) Ten DAS.
(C) Twelve DAS.
Histograms represent average data of two to four independent measurements.

(Verkest et al. 2005)

KRP kontrolují endoreduplikaci

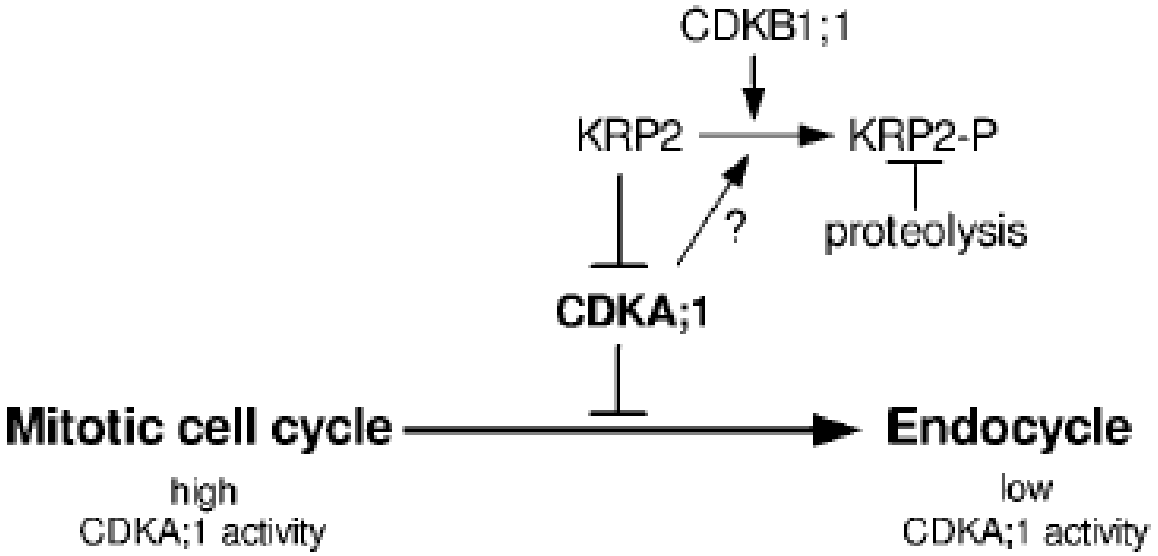


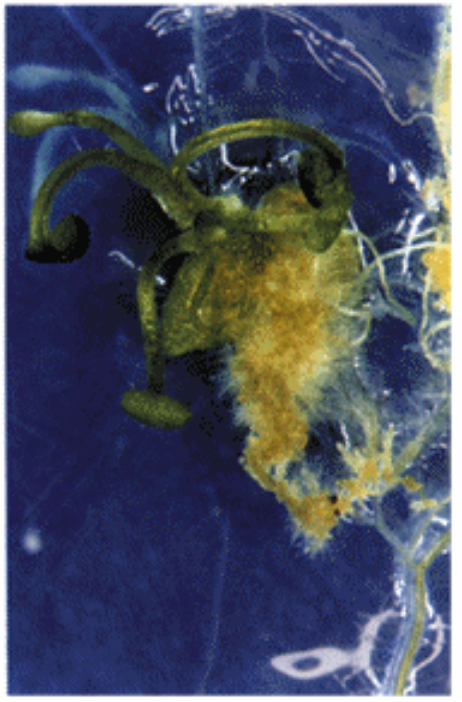
Figure 9. Model Illustrating the Role of CDK Activity in Controlling the Onset of Endoreduplication.

Mutanti v odpovědi na hormony

(A)

brassinosteroid. deficience

(B)



superroot (auxin ++)

(C)

vp1 (no ABA response

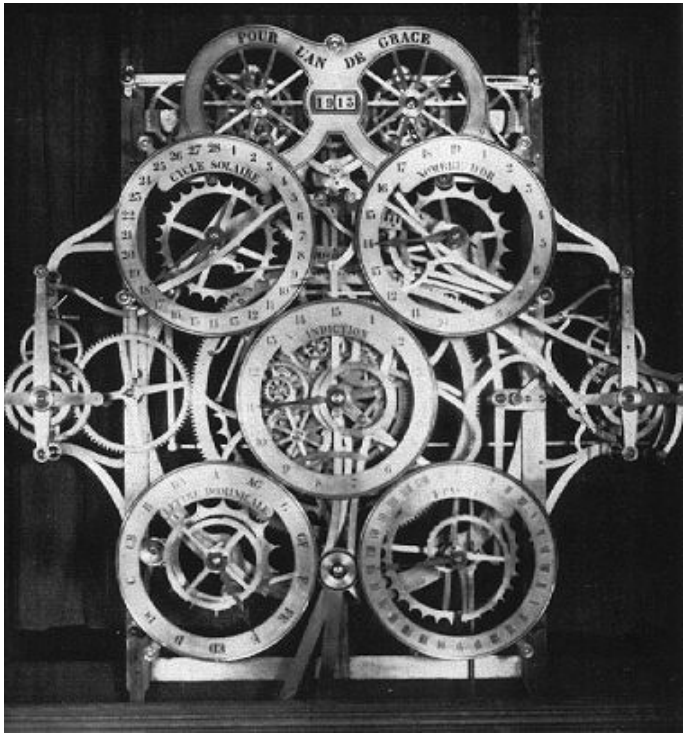
Rostlina vládne buňkám, ne naopak!

Vstupy a výstupy ... zpět k cytoskeletu

centrální oscilátor
(„cell cycle engine“)

vstupy

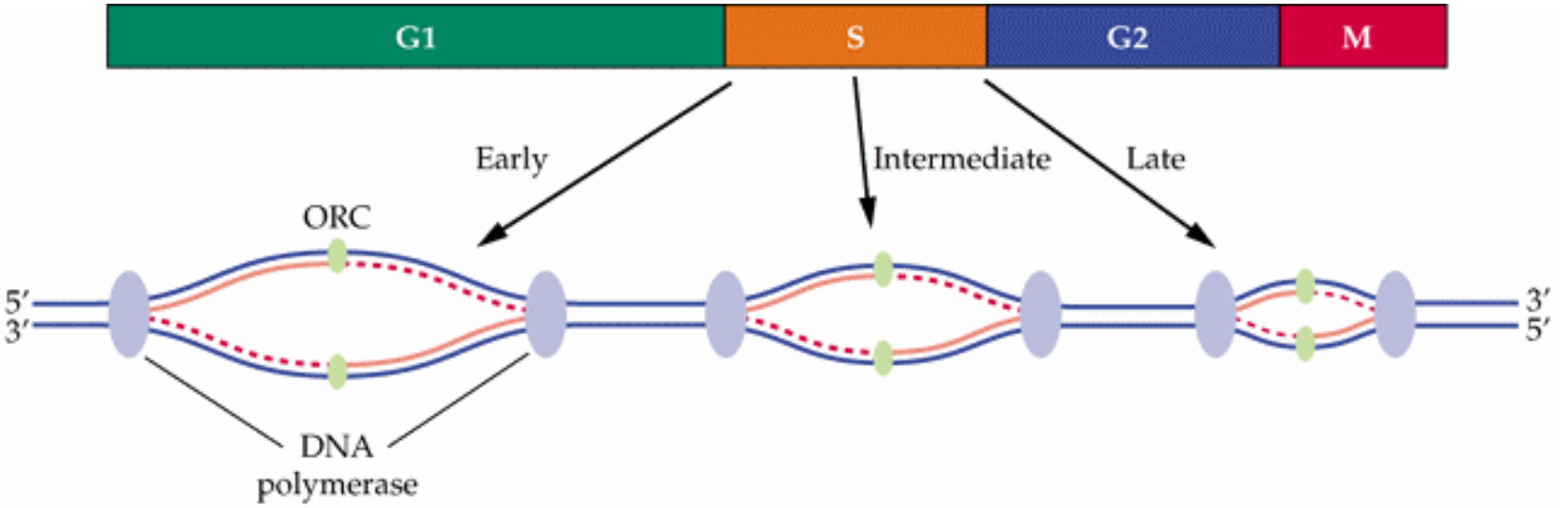
velikost
signály
poškození ...



výstupy

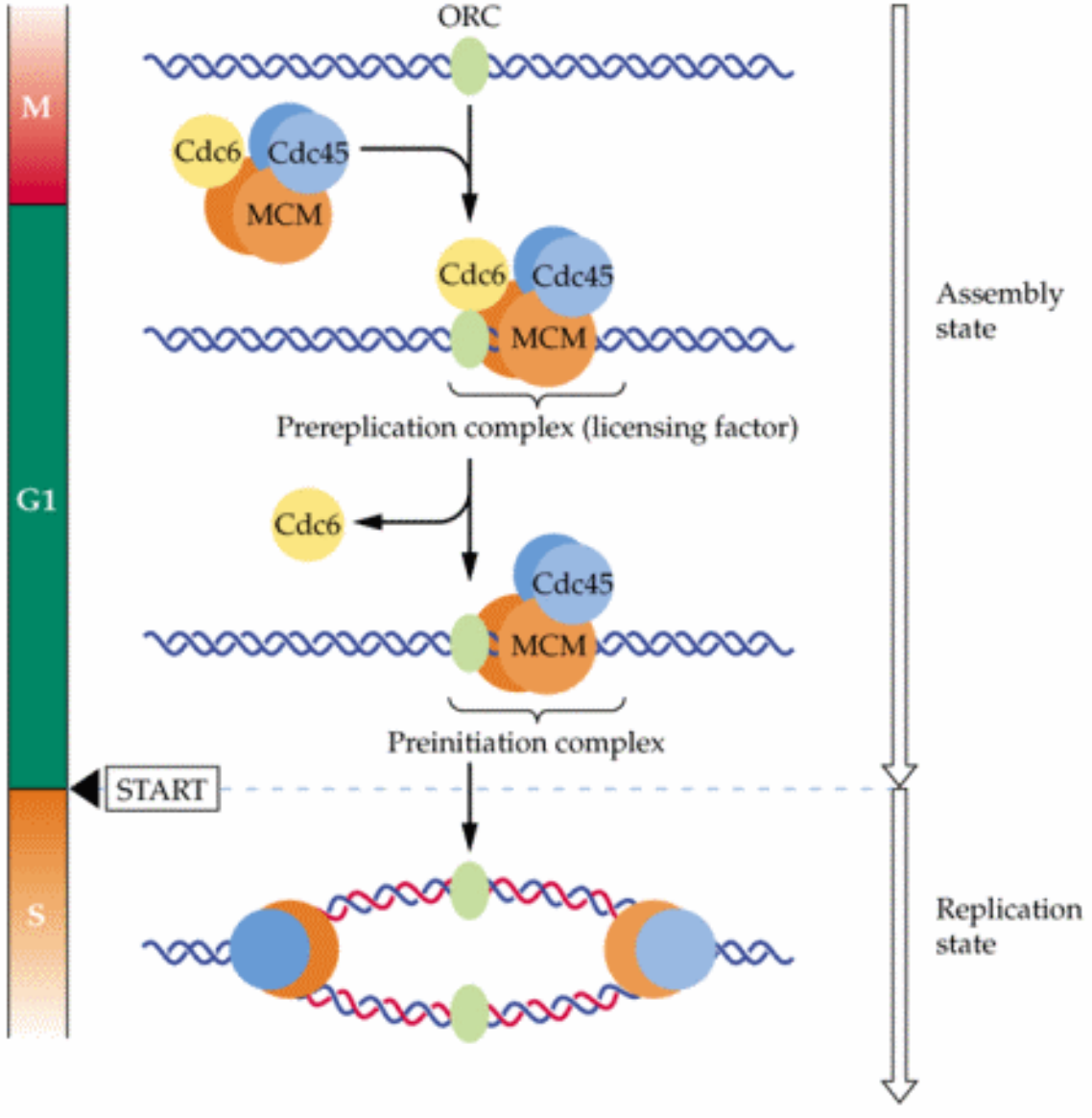
gen. exprese
replikace
mitosa,
cytokinese ...

Replikace genomu

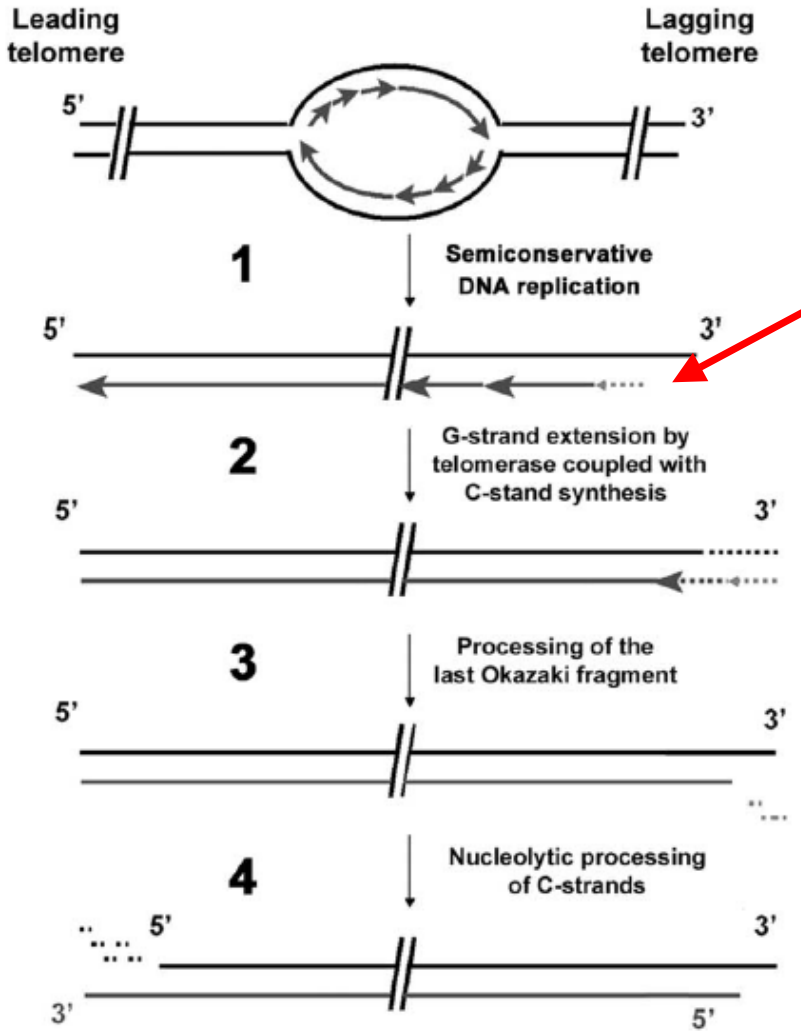


- klíčová úloha replikačních počátků!

Funkce závisí na kontextu: žitné chromosomy v triticale užívají 4x víc počátků než v žitě


ORC
M
Cdc6
Cdc45
MCM
Cdc6
Cdc45
MCM
Assembly state
Prereplication complex (licensing factor)
G1
Cdc6
Cdc45
MCM
Preinitiation complex
START
S
Replication state

Konce - telomery



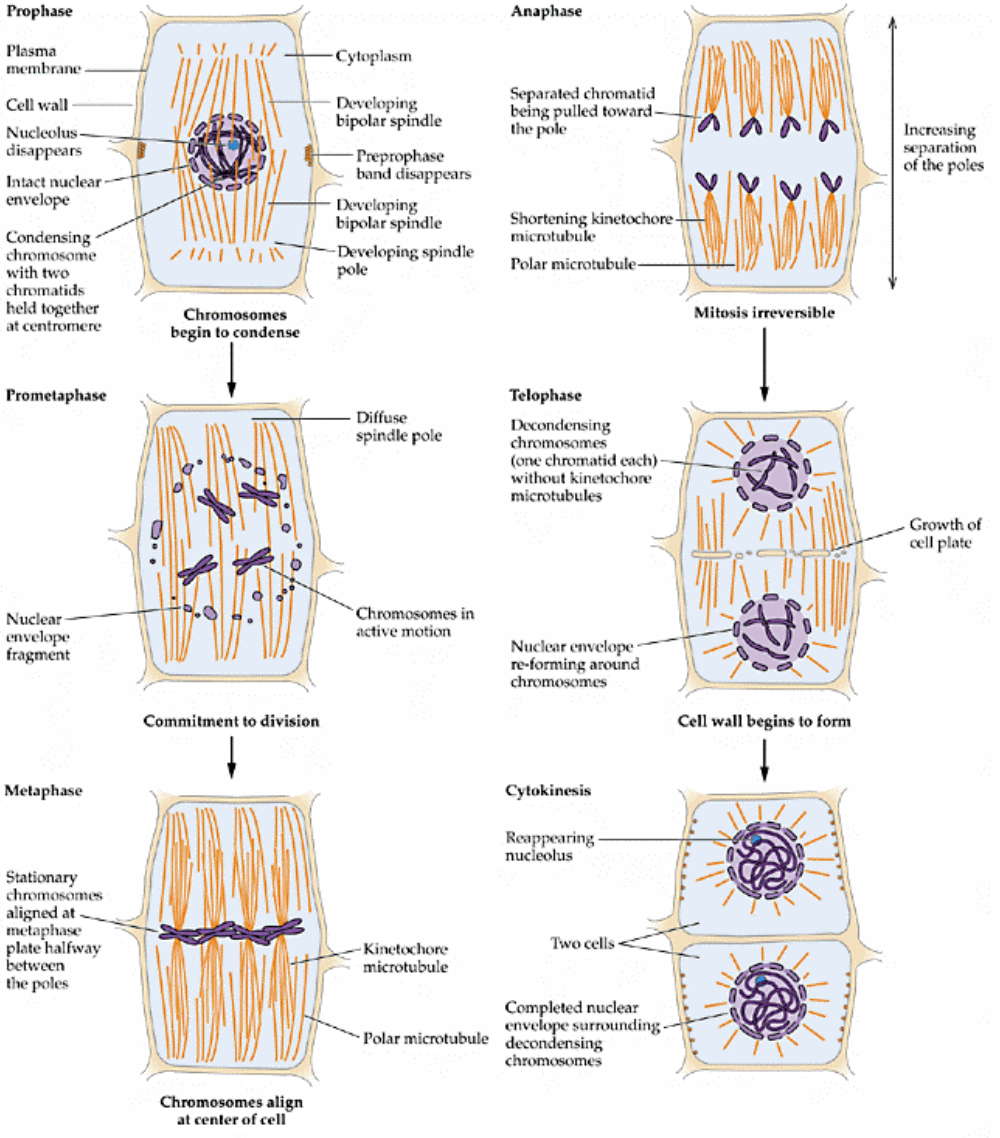
telomeráza

$(TTTAGGG)_n$ 2.7 - 3.5 kb

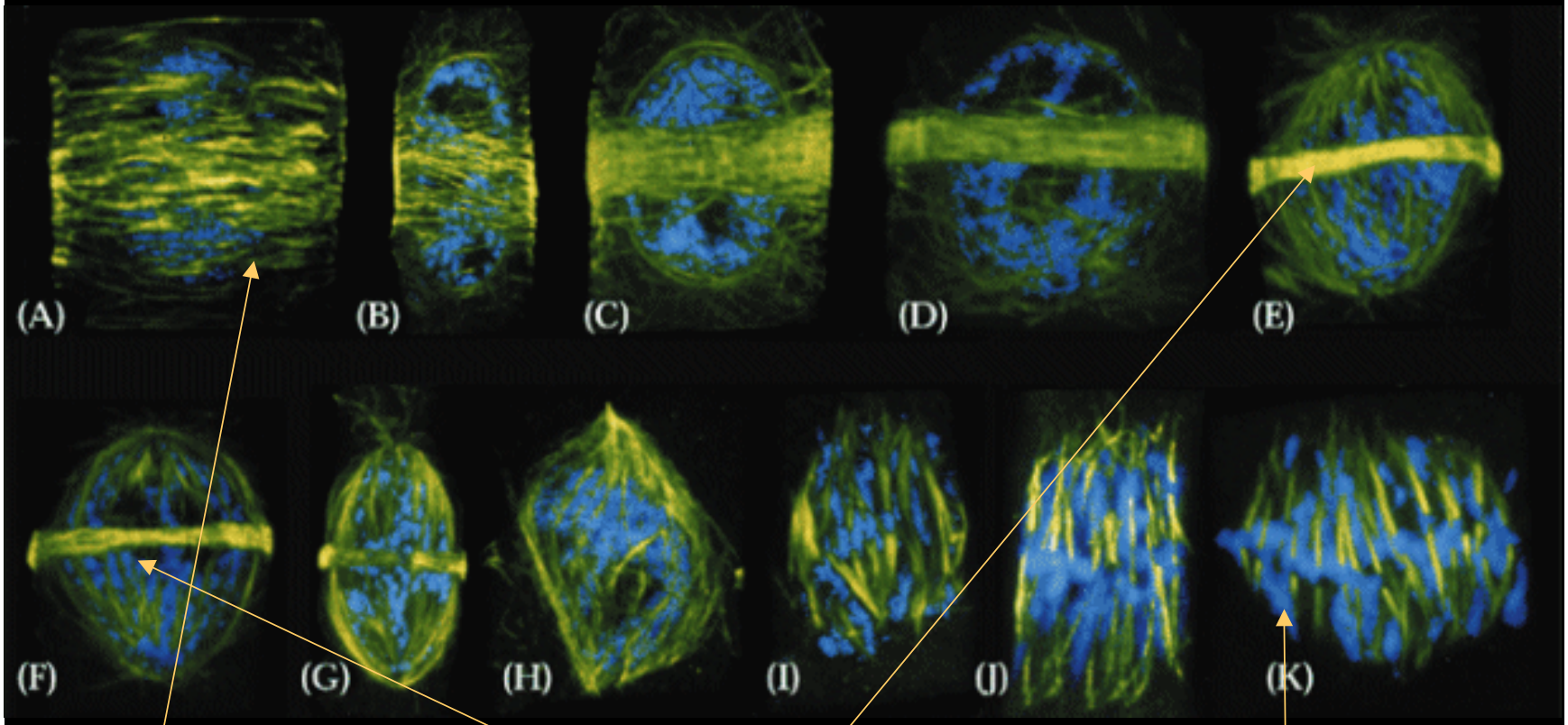
Zpět ke strukturním událostem cyklu …

1. Segregace chromozomů a karyokinese
2. Cytokinese

… aneb cytoskeletální efektory CDK (hlavně MT)


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

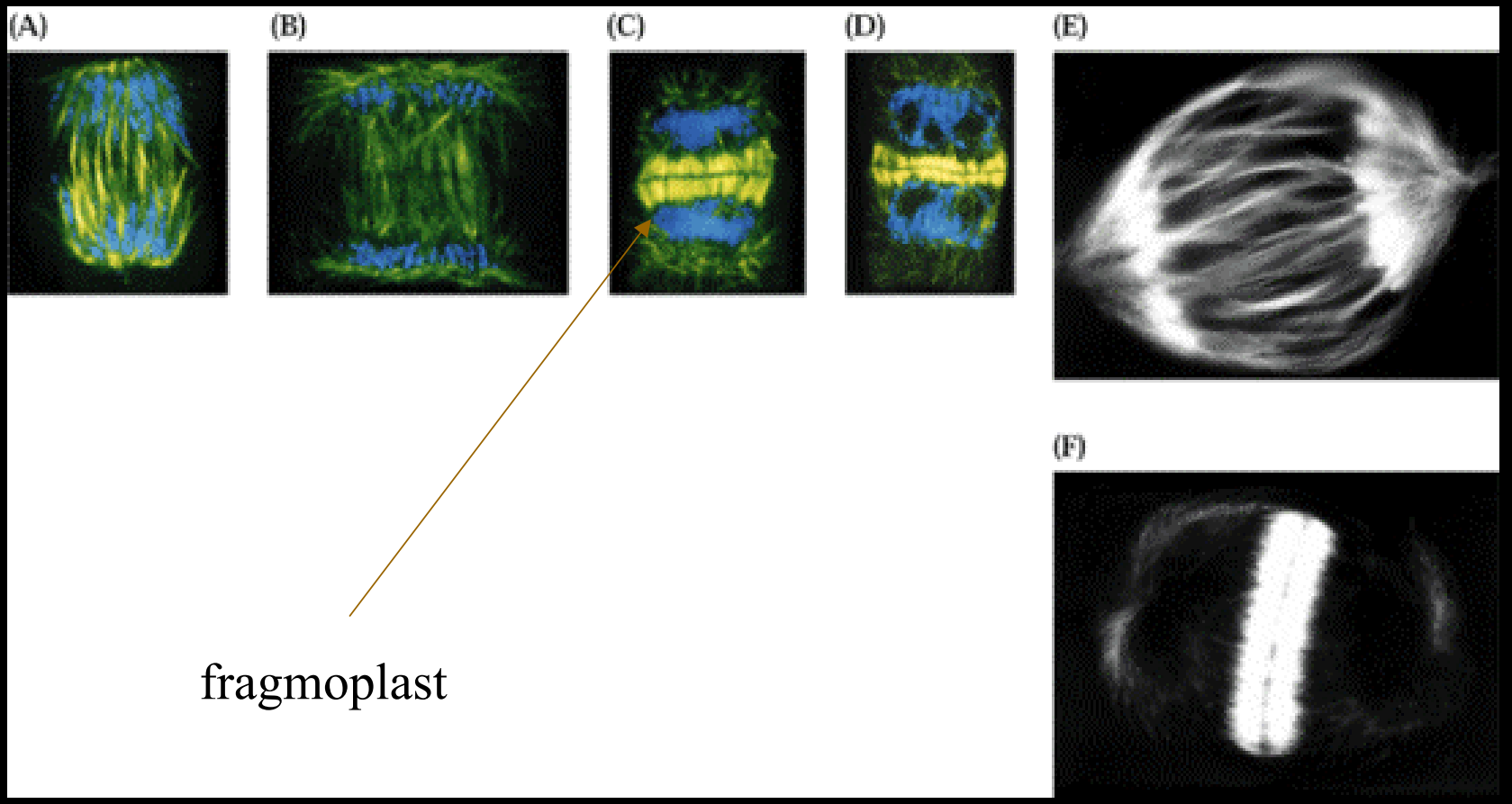
Mikrotubulární cytoskelet v cyklu somatické buňky



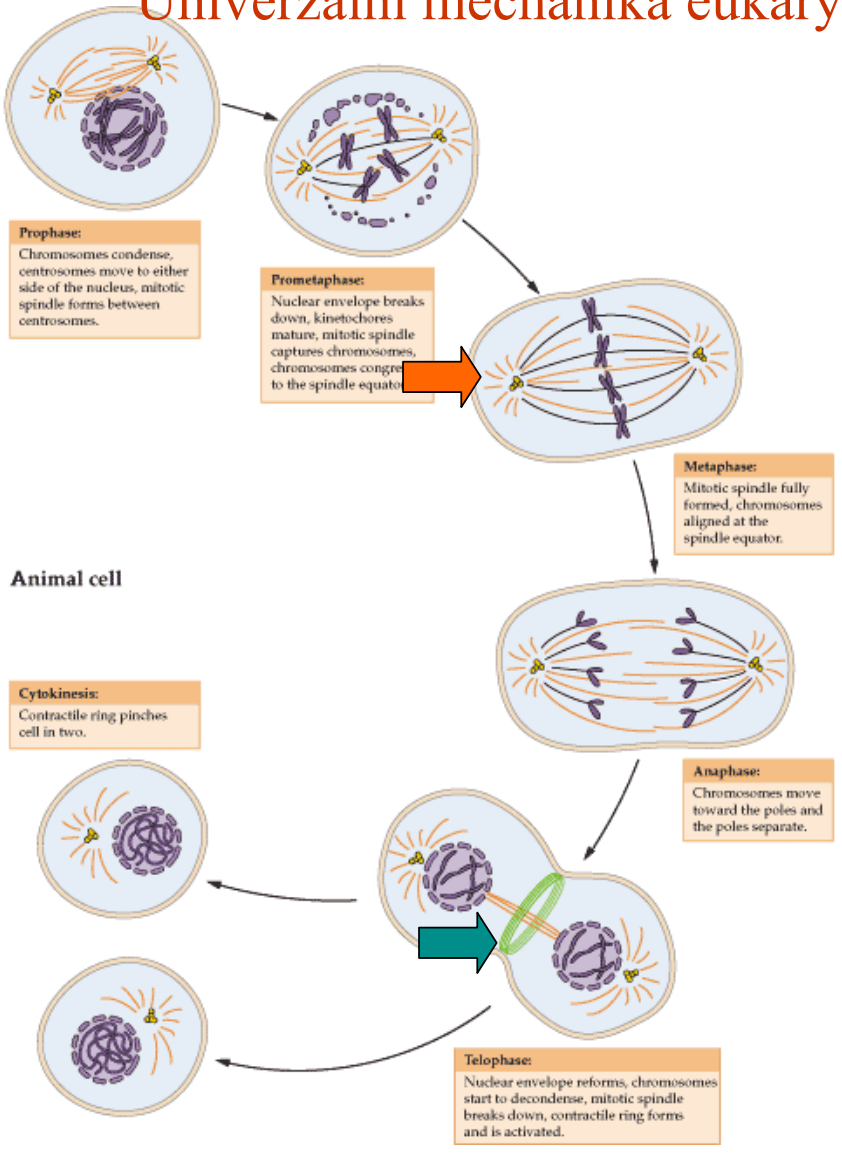
cortical array

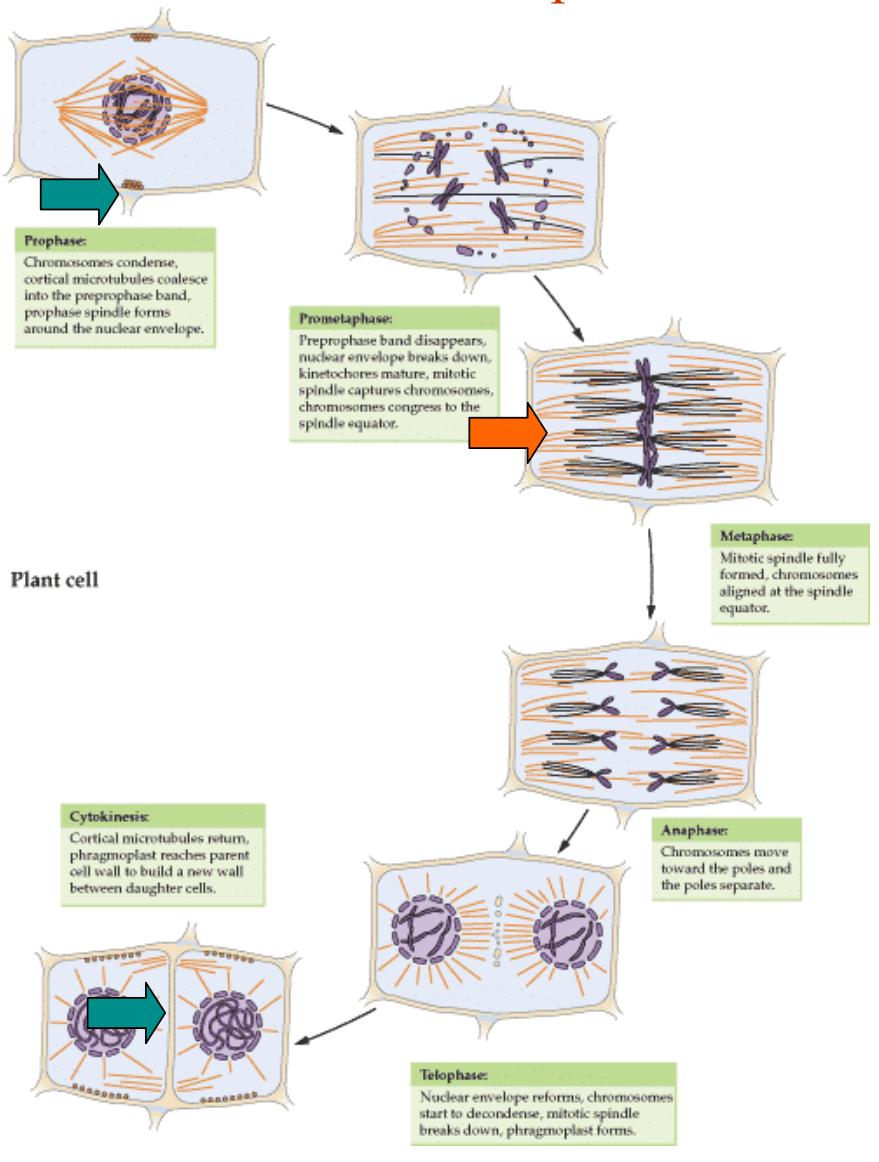
preprophase band

spindle

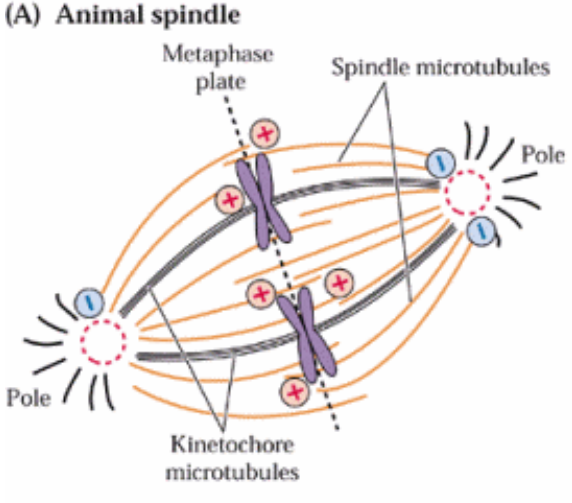

(A)
(B)
(C)
(D)
(E)
(F)
fragmoplast

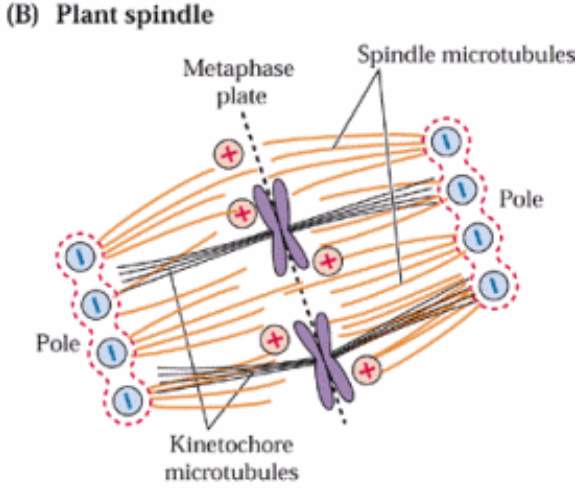
Univerzální mechanika eukaryotního BC vs. rostlinná specifika



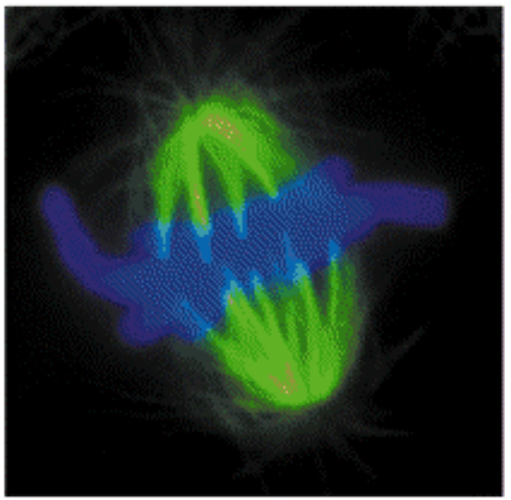


Mitotické vřeténko a segregace chromosomů

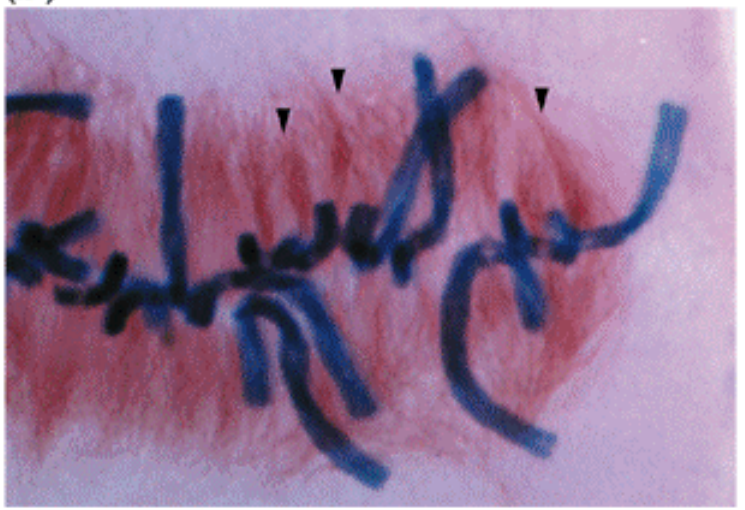




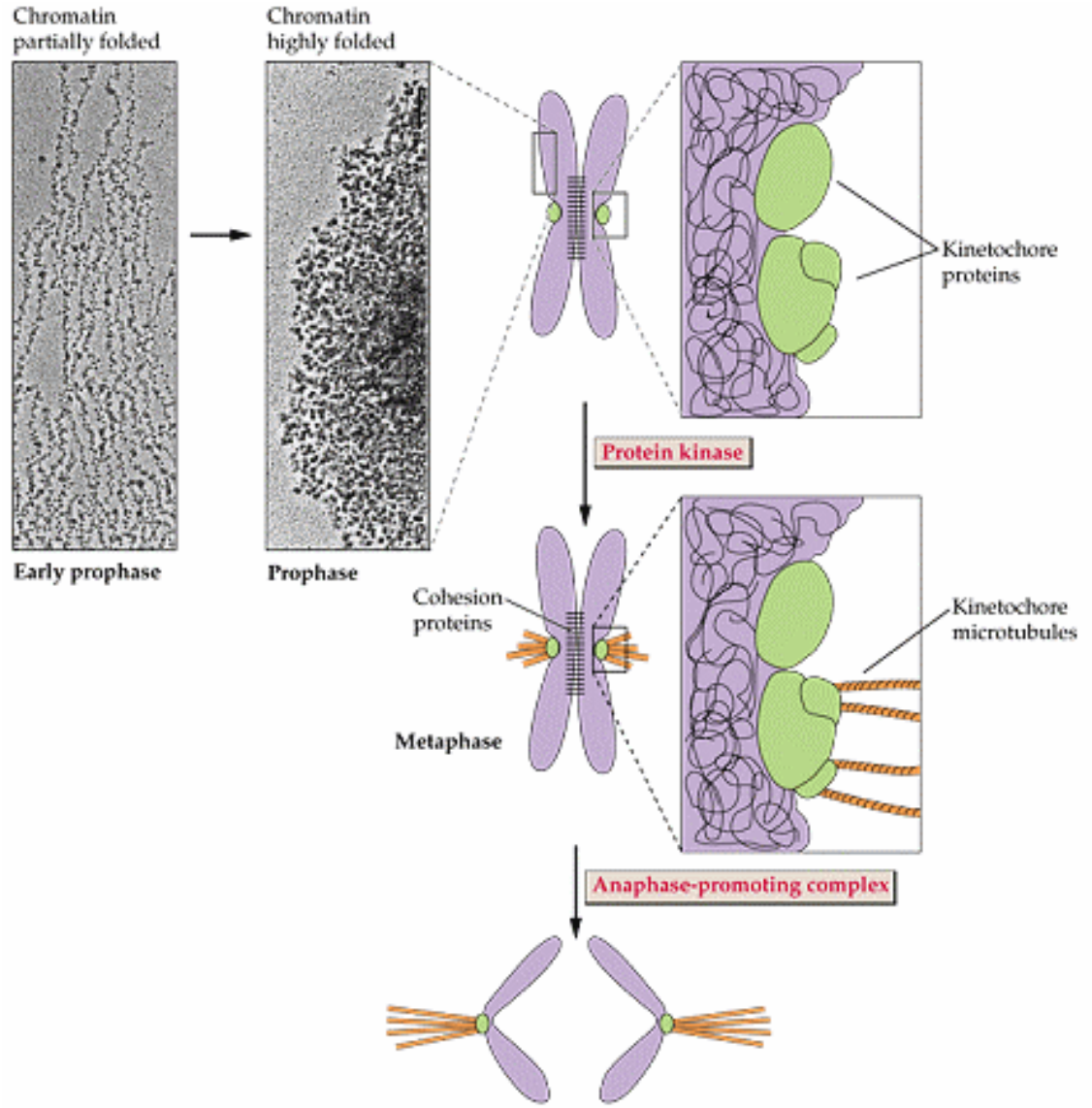
(C)



(D)



Kondensace a rozchod chromosomů



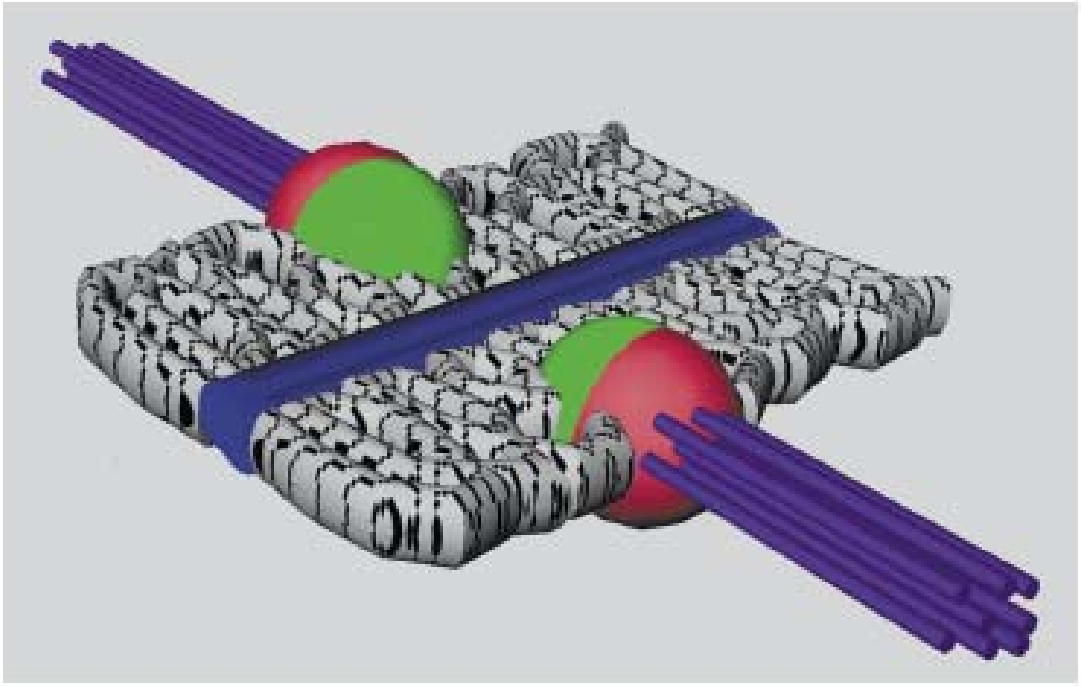


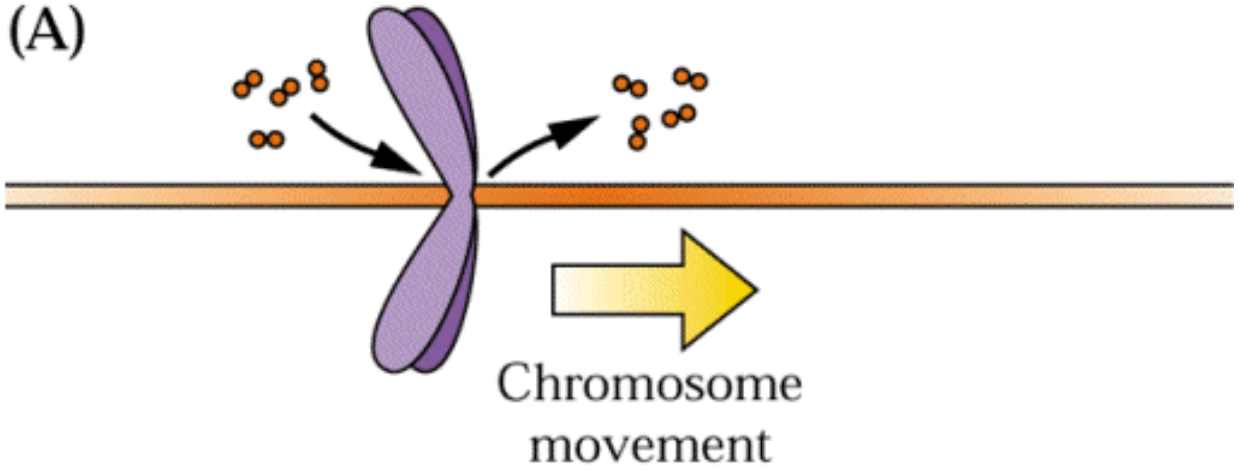
Fig. 2. A model of the maize meiotic kinetochore showing the centromeric region of a meiosis II chromosome. The kinetochore is depicted as a spherical structure with two subdomains. The inner (green) domain contains the maize protein CENP-C and the outer (red) domain contains the MAD2 protein and the 3F3/2 antigen. The chromatids, indicated by wavy lines, are attached by chromosome cores (blue). Microtubules are shown in purple.

Table 1. Plant kinetochore components

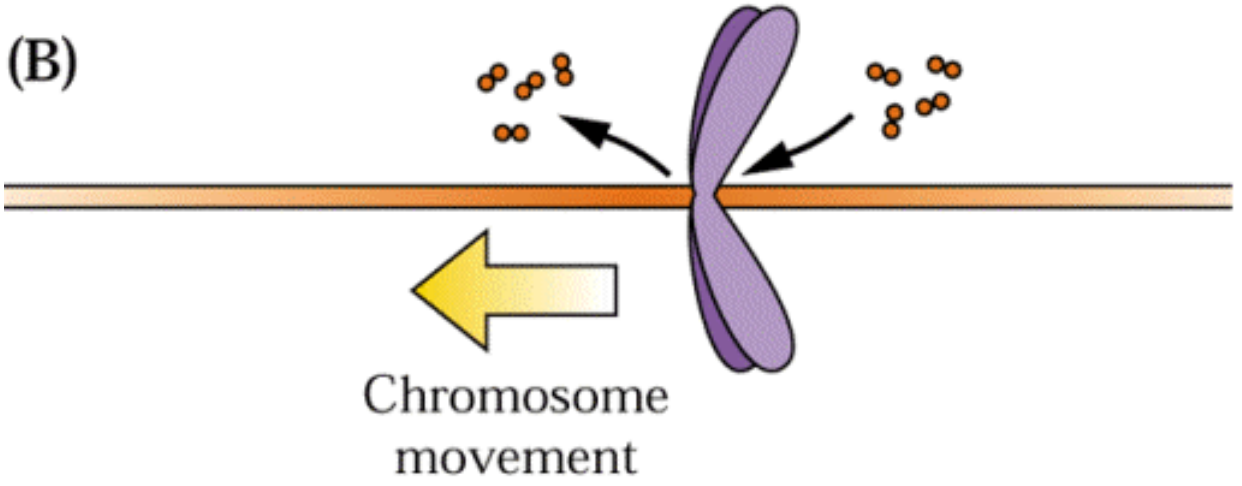
Kinetochore component	Apparent function	Plant species	Kinetochore localization[a]	Gene cloned	Refs
CBF5	Unknown	*Vicia faba*, *Hordeum vulgare*	Yes	Yes (*Hordeum vulgare*)	27
CENPC	Structural	*Zea mays*, *Vicia faba*, *Hordeum vulgare*	Yes	Yes (*Zea mays*)	26,27
CENPE	Chromosome motility	*Vicia faba*, *Hordeum vulgare*	Yes	No	27
CENPF	Unknown	*Hordeum vulgare*	Yes	No	27
MAD2	Spindle checkpoint	*Zea mays*	Yes	Yes	19
Meiotic Histone	Unknown	*Lilium longiflorum*	Yes	Yes	48,49
MPM2 antigen(s)	Unknown	*Vicia faba*	Yes	No	18
SKP1	Unknown	*Vicia faba*, *Hordeum vulgare*	Yes	Yes	27
ZW10	Spindle checkpoint	*Arabidopsis*	NA[b]	Yes	45
3F3/2 antigen	Spindle checkpoint	*Zea mays*	Yes	No	19
6C6 antigen	MTOC	*Allium sativum*, *Tulbaghia violacea*	Yes	No	17
γ-tubulin	MTOC	*Vicia faba*	Yes	No[c]	35

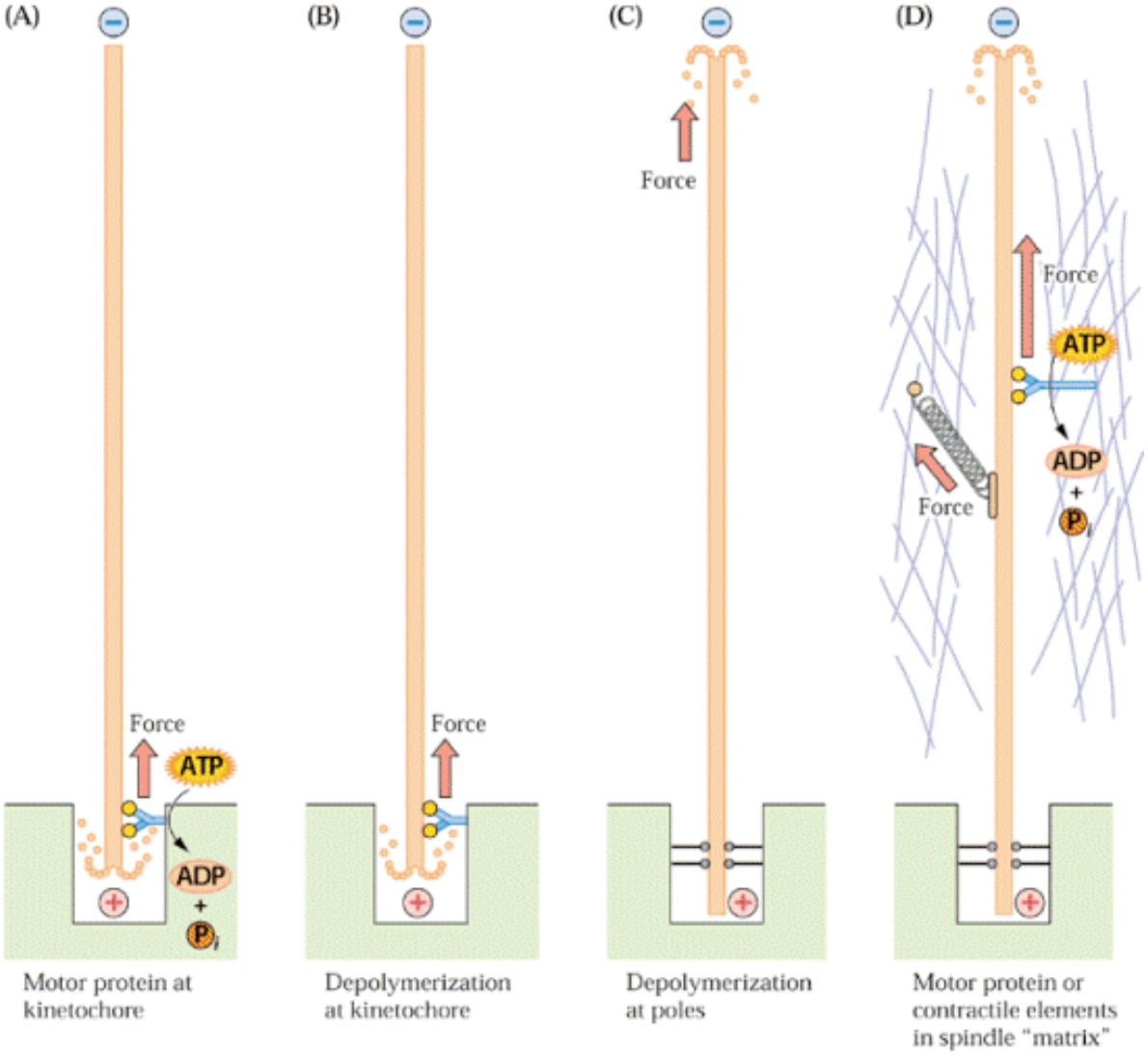
[a]The protein has been localized to the kinetochore by immunofluorescence.
[b]NA, information not available.
[c]γ-tubulin DNA sequences from other plant species are available in GenBank.

Co pohání pohyb chromosomů?



kinesiny (ATK1 a jiné?)




(A)
Force
ATP
ADP
+
P_i
Motor protein at kinetochore
(B)
Force
Depolymerization at kinetochore
(C)
Force
Depolymerization at poles
(D)
Force
ATP
ADP
+
P_i
Force
Motor protein or contractile elements in spindle "matrix"

Lokalizace chromozomů v jádře není náhodná!

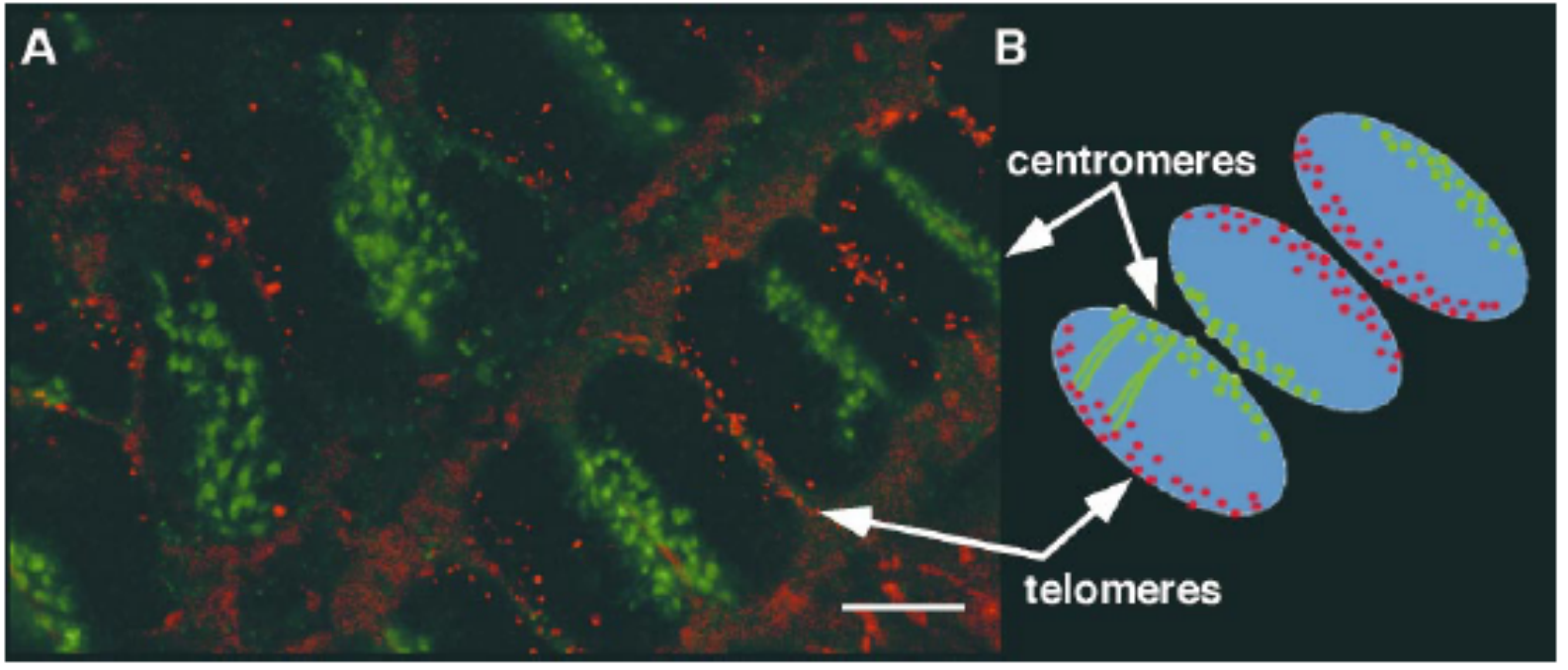


Figure 4. The Rabl Configuration in Somatic Nuclei.

(A) Projections of wheat root tissue double labeled by FISH with probes to centromeres (green) and telomeres (red).
(B) Interpretation of the labeling presented in (A). The Rabl configuration is suggested by chromosomes lying parallel to each other, with centromeres clustered on one side of the nucleus and telomeres on the other side.
Bar in (A) = 10 μm for both panels. Figure courtesy of Peter Shaw (John Innes Institute, Norwich, UK); adapted from Abranches et al. (1998).

Rabl, C. (1885). Über Zelltheilung. Morphol. Jahrb. 10, 214–330.

Mitosa vs. meiosa

Prophase I
Leptotene
Zygotene
Pachytene
Diplotene

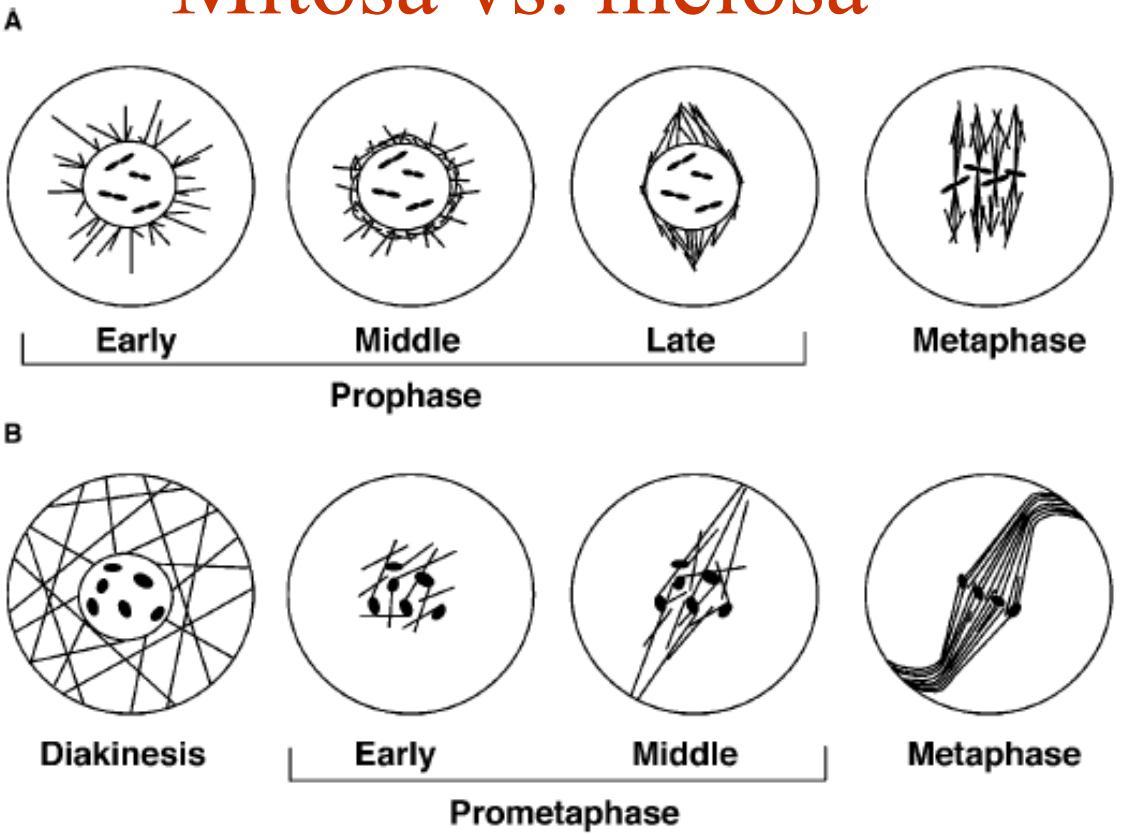


Figure 3. Diagram of Spindle Development in Meiotic versus Mitotic Cells.

(A) Mitotic spindle formation (adapted from Smirnova and Bajer, 1998). During prophase, radial microtubule arrays accumulate as a cage around the nuclear envelope. These arrays then are transformed into a multipolar and ultimately a bipolar array. After nuclear envelope breakdown, microtubules are captured by the kinetochores, and the bipolarity of the array is reinforced by the bilateral symmetry of the kinetochores attached to the sister chromatids. A key early step in the process is the formation of MTCCs near the nuclear envelope, which aggregate into two caps during prophase.

(B) Meiotic spindle formation (adapted from Chan and Cande, 1998). At diakinesis, the microtubules are organized as a cytoplasmic network. After nuclear envelope breakdown during early prometaphase, the preexisting microtubules and newly forming microtubules interact with chromatin and are stablized. With the involvement of motors, such as kinesin-related proteins and dynein, during late prometaphase, the microtubule arrays become organized into antiparallel assemblies, and pole material is recruited to the ends of the microtubules. At this stage, plus ends of microtubules also are captured by the kinetochores. By metaphase I, the spindle extends the width of the cell, and the spindle poles may interact with the plasma membrane, becoming more focused over time.

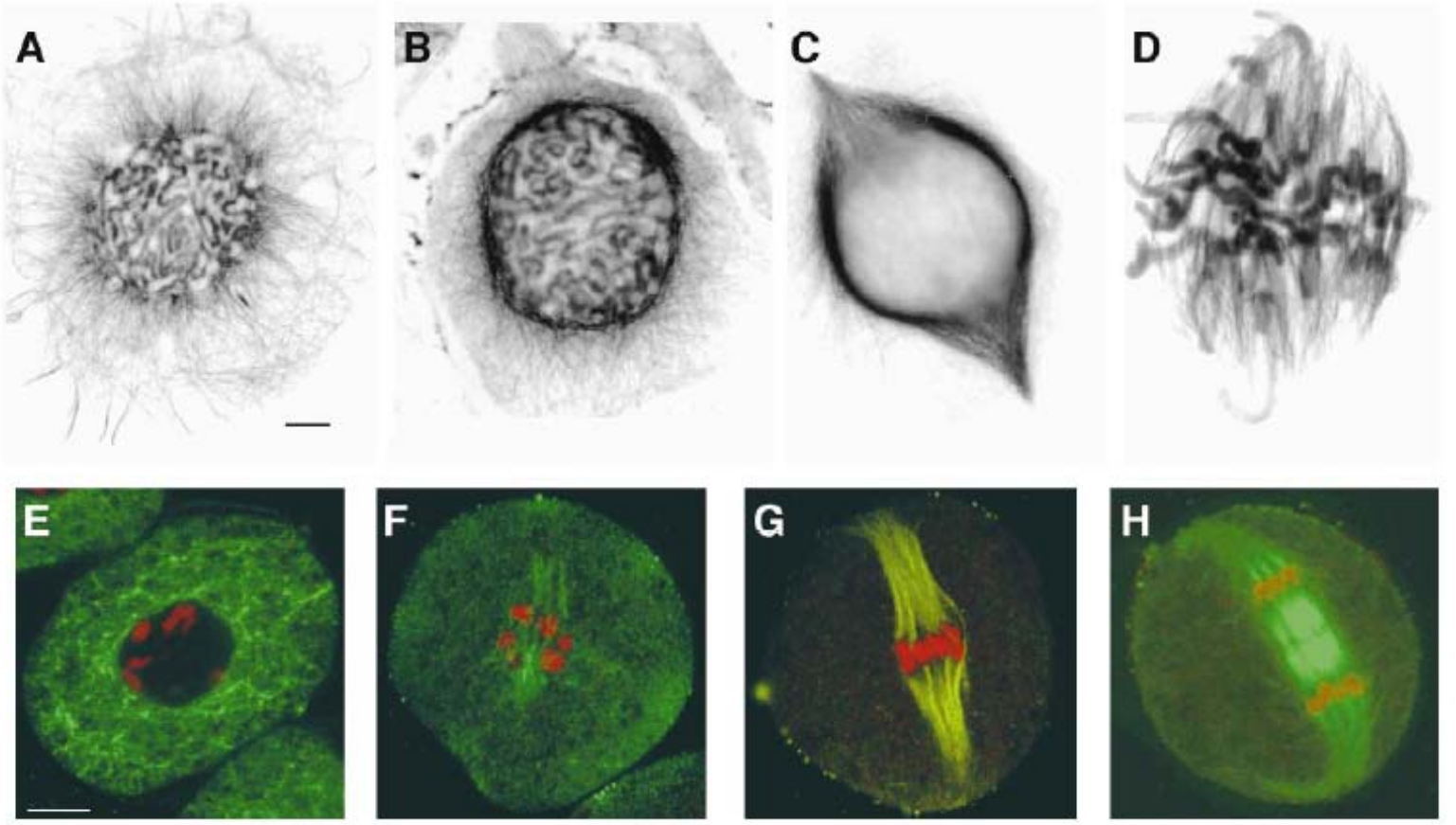


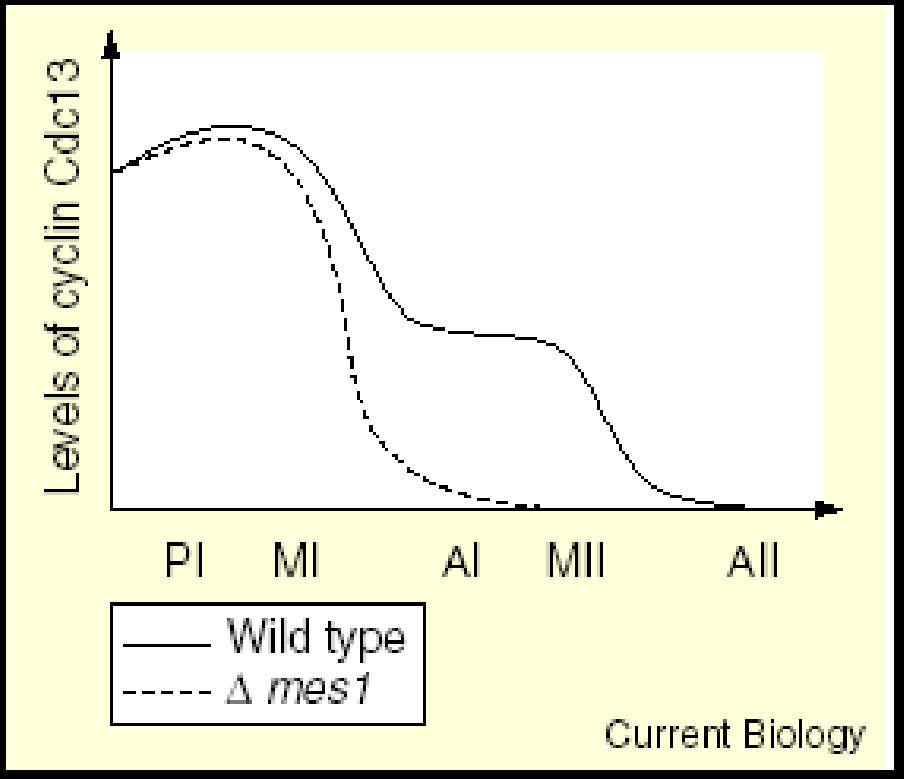
Figure 2. The Pathway of Spindle Assembly in Mitotic and Meiotic Plant Cells.

(A) to **(D)** The distribution of microtubules in somatic spindles, as viewed by using the immunogold-enhanced silver technique in *Haemanthus* endosperm cells at early prophase **(A)**, midprophase **(B)**, late prophase **(C)**, and prometaphase **(D)**. Bar in **(A)** = 10 μm. **(A)** to **(D)** courtesy of Andrew Bajer and adapted from Smirnova and Bajer (1994, 1998).

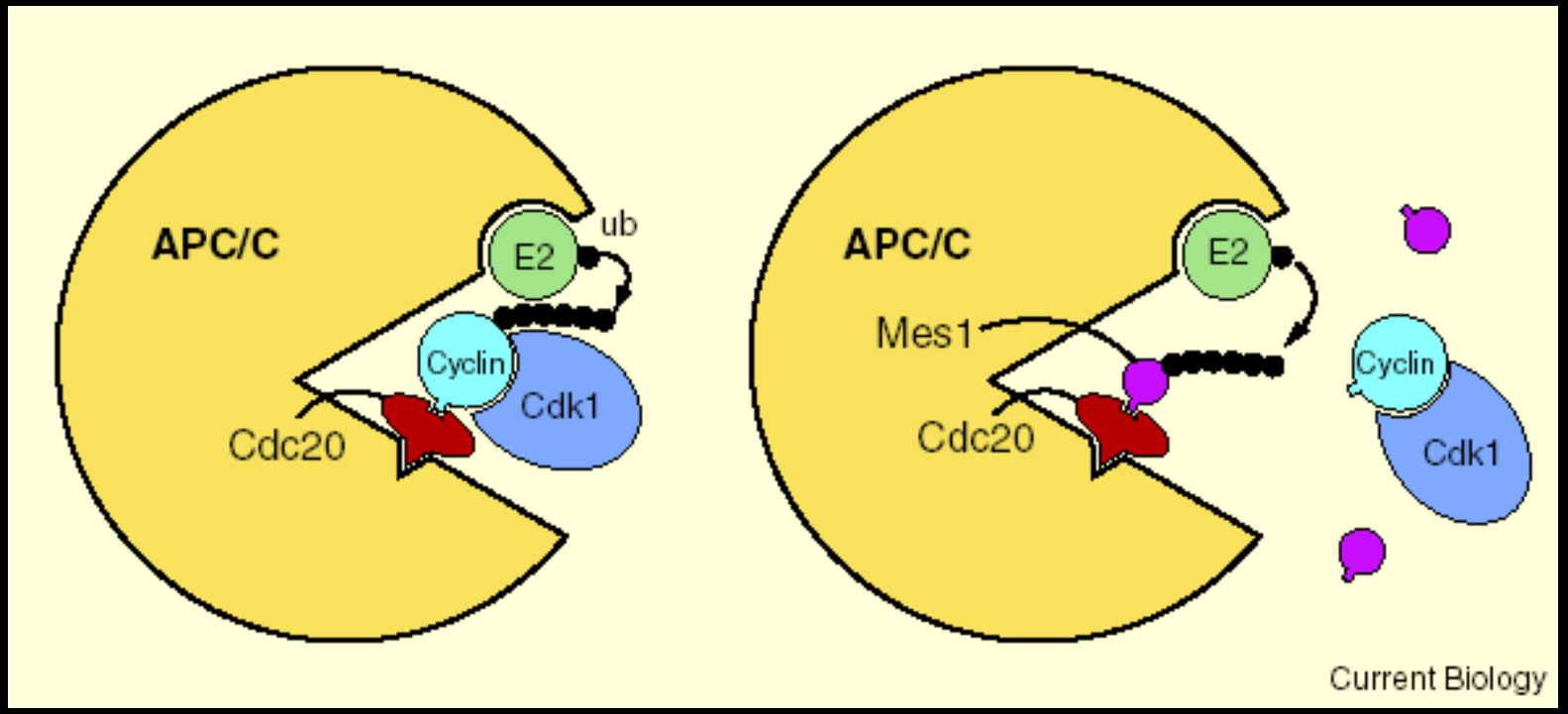
(E) to **(H)** The distribution of microtubules in maize meiocytes. Single optical sections taken by confocal laser scanning microscopy of a meiocyte in diakinesis **(E)**, early prometaphase **(F)**, metaphase I **(G)**, and anaphase I **(H)**. The chromosomes, stained with propidium iodide, are shown in red, and the microtubules, stained with a monoclonal antibody against tubulin, are shown in green. Bar in **(E)** = 10 μm. **(E)** to **(H)** adapted from Chan and Cande (1998).

Meiosu II lze chápat jako „odloženou část anafáze“

- Mutace *mes1* (meiotic segregation? – běží jako mitosa) u *S. pombe* - porucha meiose II

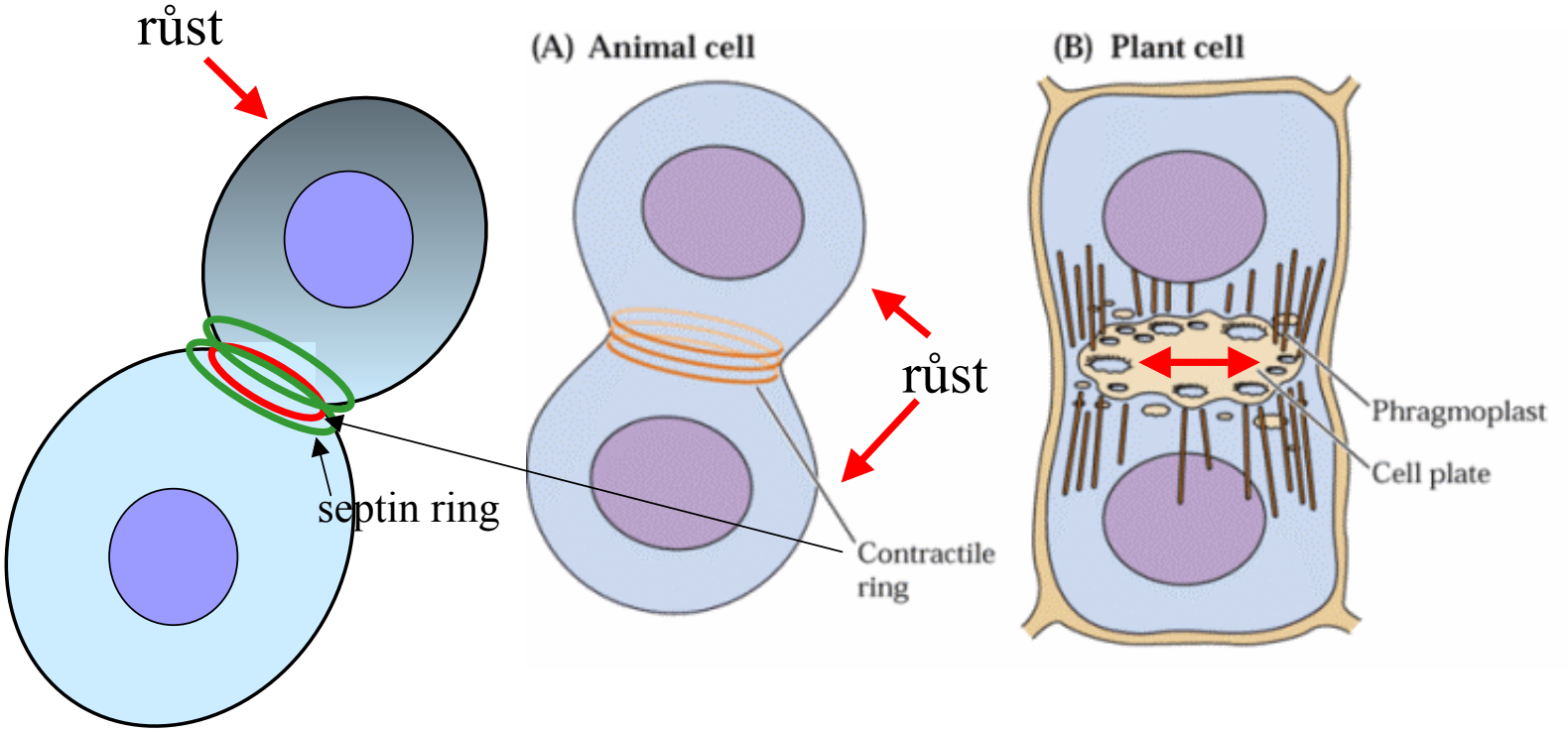


mes1+ (asi) kóduje kompetitivní inhibitor APC!



(Peters 2005)

Cytokinese



- jak rozdělit buňku ...

Je rozdíl mezi rostlinami a živočichy opravdu tak zásadní?

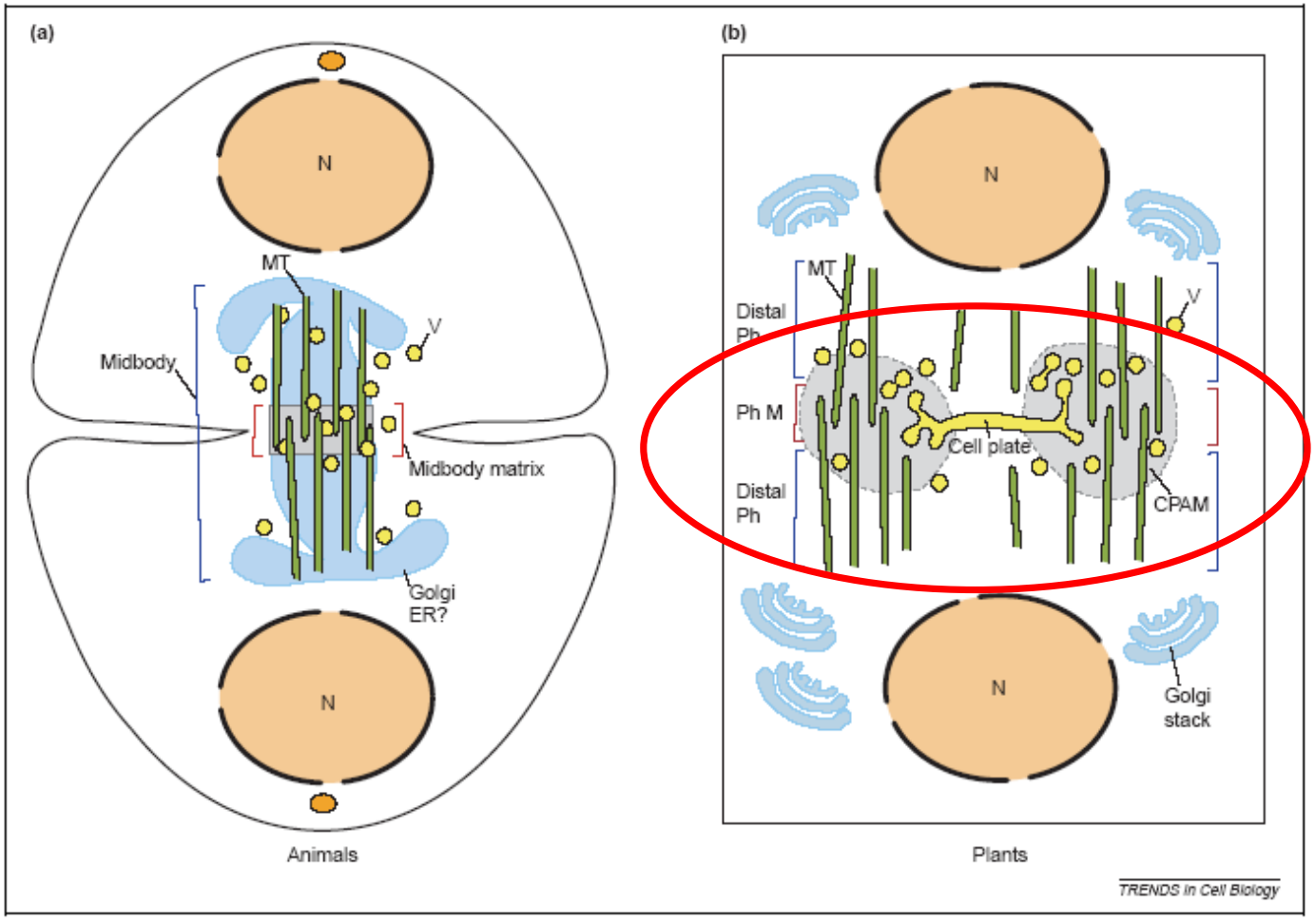
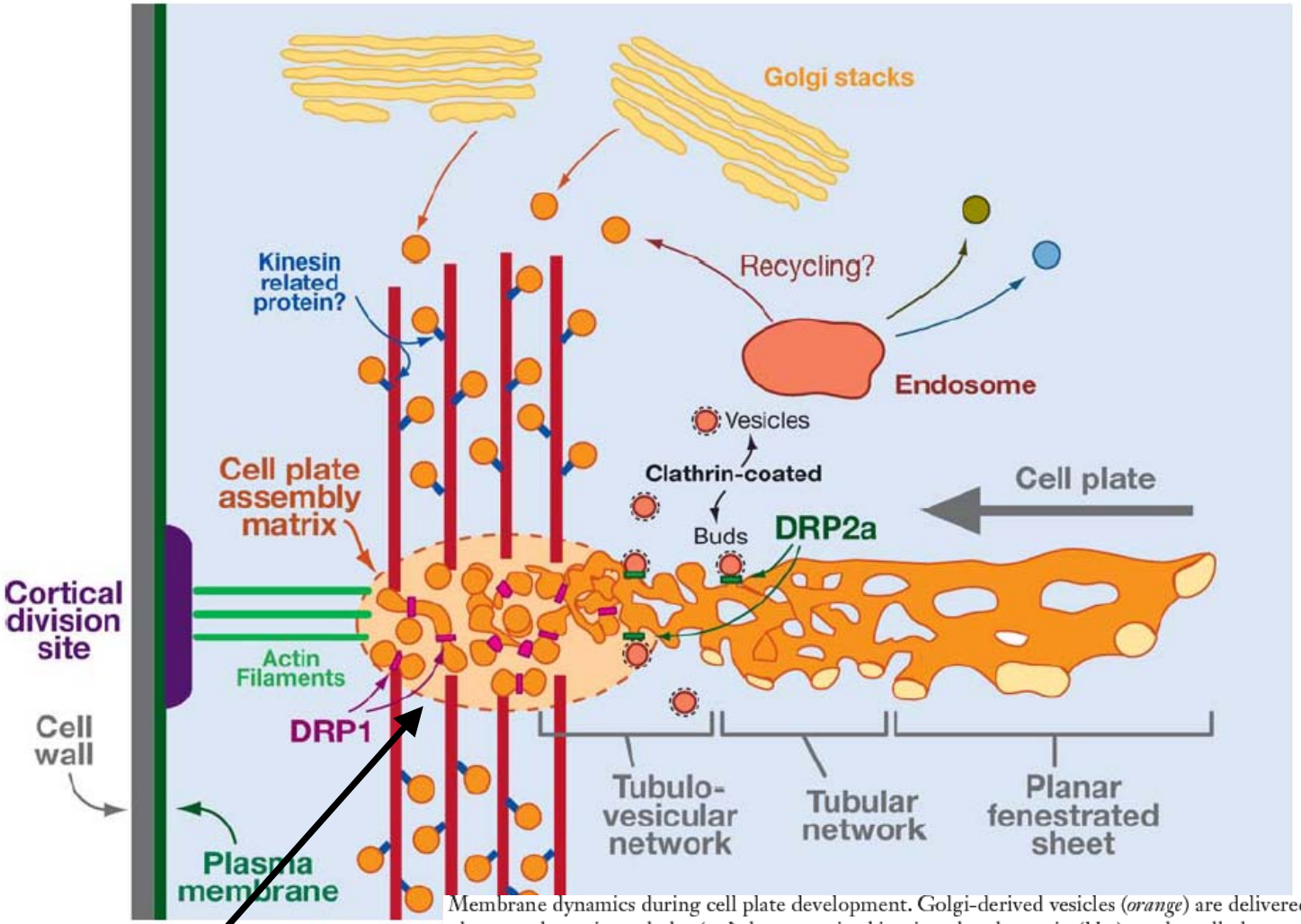


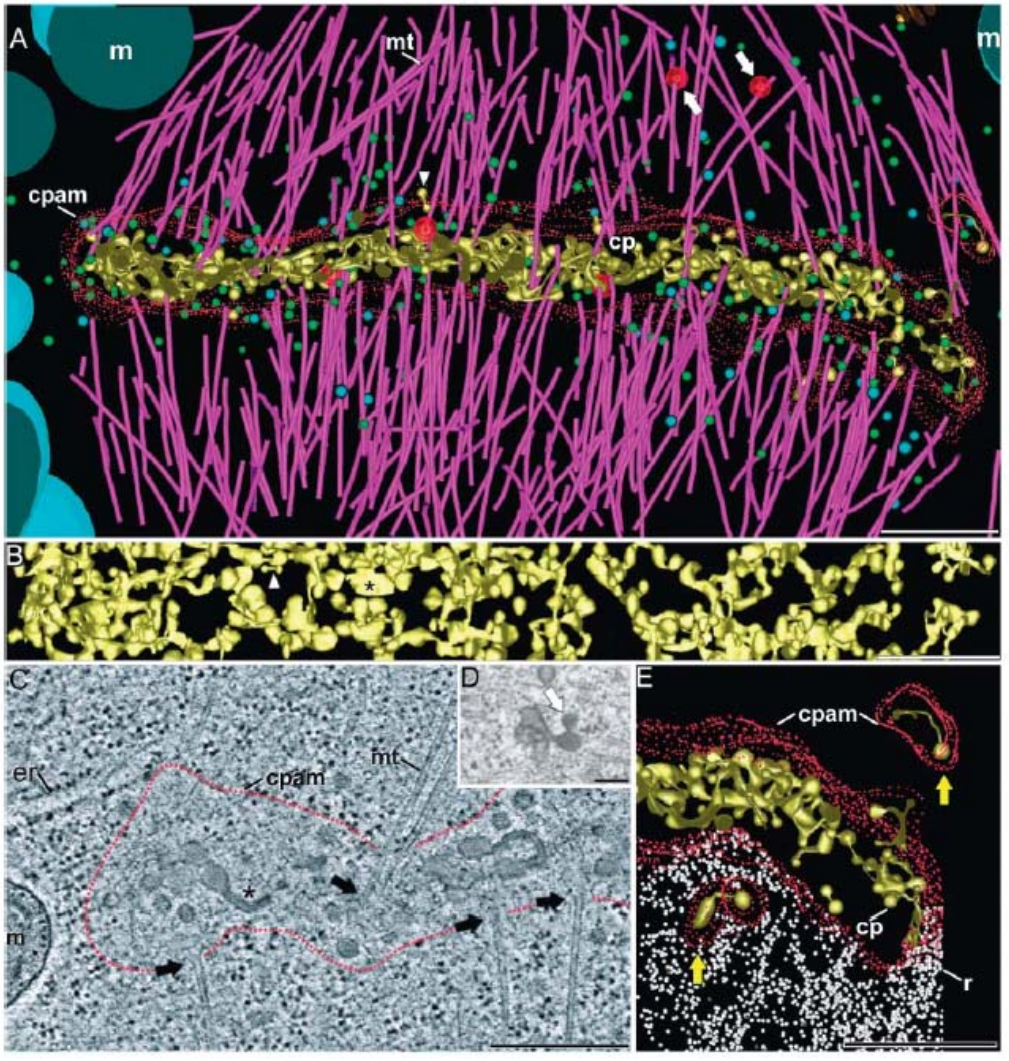
Figure 1. Overview of animal and plant cytokinesis. (a) Cytokinesis in animal cells. The spindle midzone/midbody forms when microtubules (MTs) from opposite poles overlap. It consists of the overlapping microtubules as well as associated proteins that bundle these MTs and other proteins that together form a dense protein matrix. This matrix excludes antibodies against MTs, giving a stereotypical region devoid of staining. As the furrow ingresses, the midzone is swept into one larger structure called the midbody. The Golgi and endoplasmic reticulum (ER) membranes are also found in the midbody during telophase to cytokinesis. It is proposed that vesicles (V) traffic along the midbody microtubules toward the ingressing furrow. (b) Cytokinesis in somatic plant cells. The forming cell plate is assisted by the phragmoplast at the future site of the new cell wall. Two topographic regions can be distinguished in the phragmoplast: the phragmoplast midline (Ph M), where the opposing set of microtubules interdigitate, and the distal phragmoplast (distal Ph), at both sides of the phragmoplast midline. A filamentous cell-plate assembly matrix (CPAM) accumulates at the phragmoplast midline. Key: MT, microtubule (green); N, nucleus (tan); V, vesicle (yellow); Golgi (pale blue); midbody matrix (gray box); CPAM (gray circles).



Membrane dynamics during cell plate development. Golgi-derived vesicles (*orange*) are delivered along phragmoplast microtubules (*red*), by a putative kinesin-related protein (*blue*), to the cell plate assembly matrix. Vesicle fusion generates fusion tubes and tubulo-vesicular networks as a result of the constricting activity of class I dynamin-related proteins (DRP1) (*magenta*). The tubulo-vesicular network is successively transformed into a tubular network and a planar fenestrated sheet. Lateral expansion of the cell plate (*large arrow*) toward the cortical division site is guided by actin filaments. Endocytosis from the tubulo-vesicular network and tubular network removes excess membrane, which is delivered to endosomes via clathrin-coated buds and vesicles. Dynamin-related protein 2a (DRP2a; *green*) is involved in the formation of clathrin-coated vesicles. The endosome sorts proteins for trafficking to various destinations (*blue*, *green*, *orange*), possibly including recycling to the margin of the cell plate.

fúze váčků

Vývoj fragmoplastu a CP



(electronmicroscopic tomography, Staehelin lab)

Figure 7. Solid Phragmoplast with CPAM and TVN Stage Cell Plate.

Homotypická fúze váčků: SNARE et al., Exocyst?

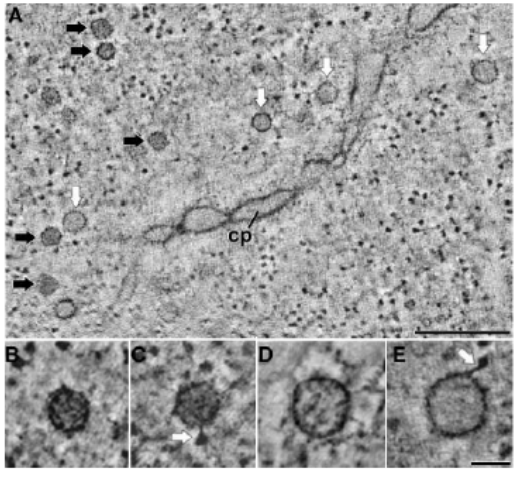
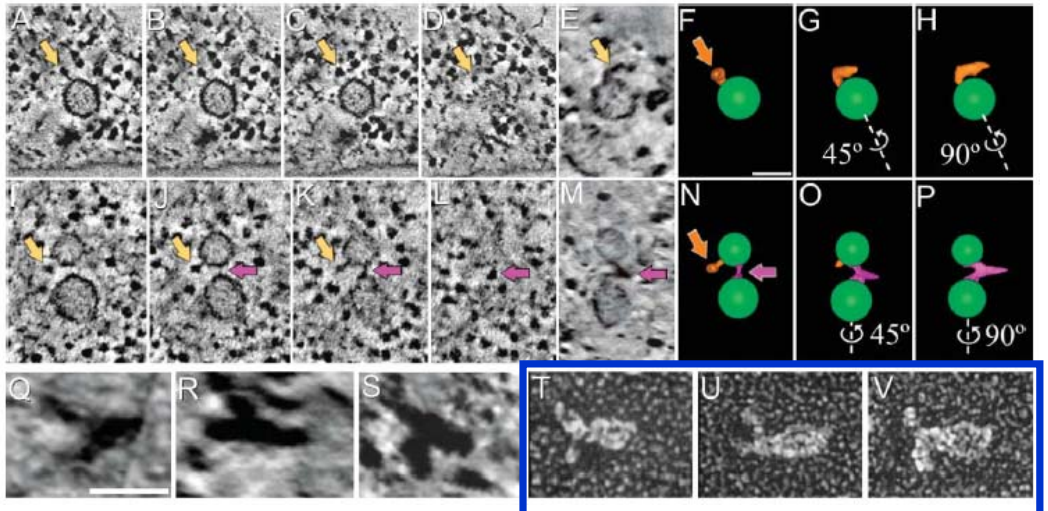


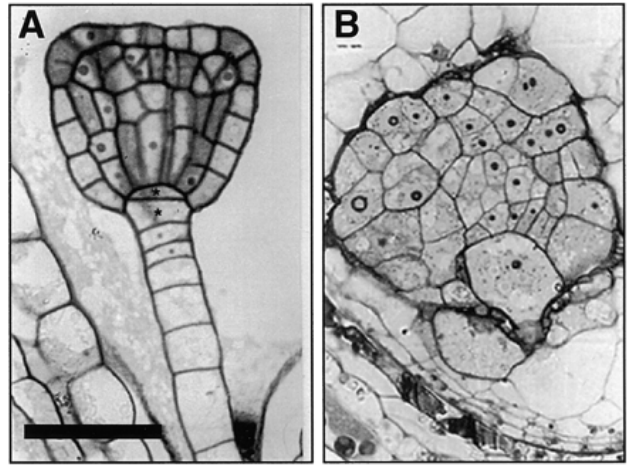
Figure 2. Two Different Types of Vesicles in the Vicinity of the Cell Plate.

(Segui-Simarro et al. 2004)



(exocyst)

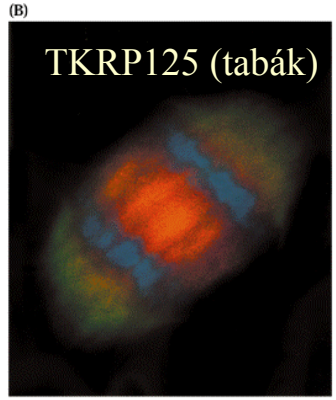
- KNOLLE : syntaxin (v-SNARE)
- přísluš. t-SNARE asi redundantní (SNAP33, SNAP29, SNAP30)
- KEULE : Sec1-related, interakce s KNOLLE
- KNOLLE a syntaxin SYP31: interakce s CDC48

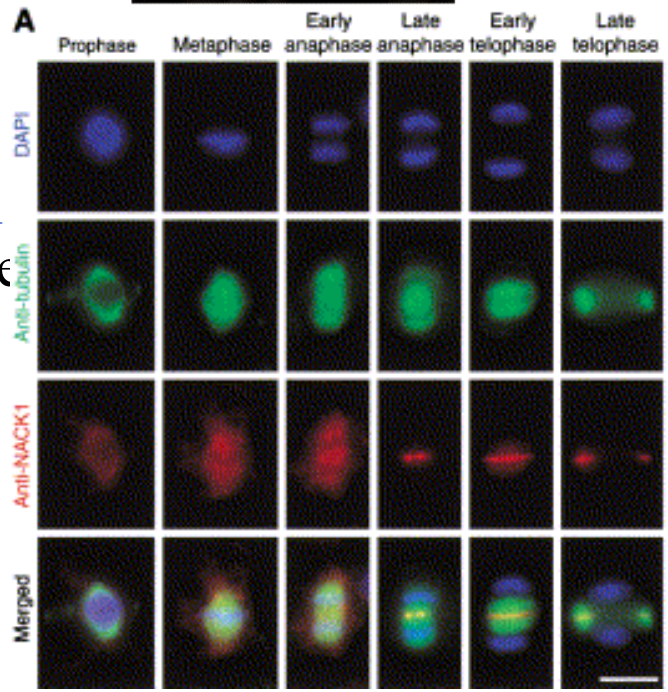


wt keule

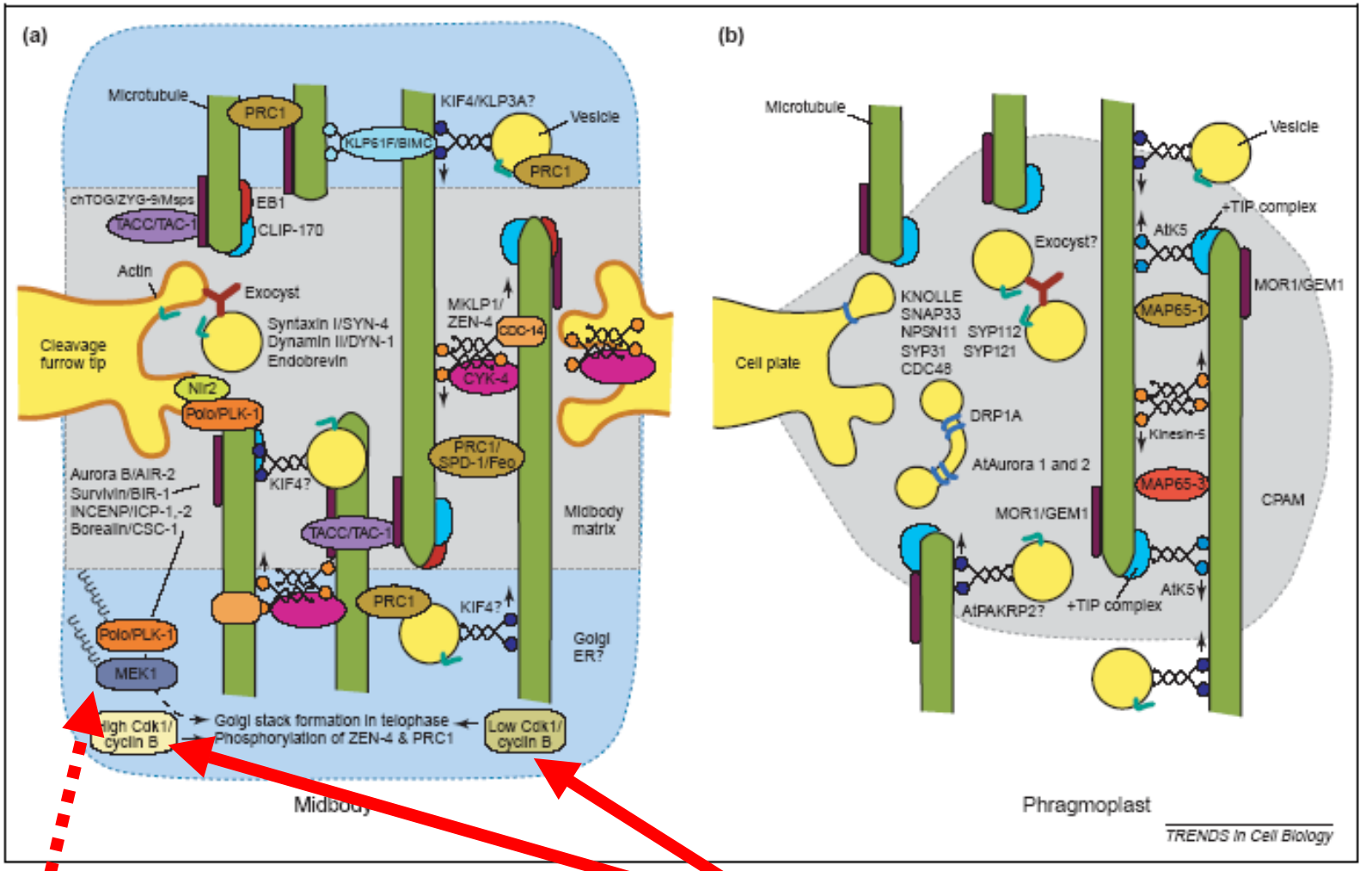
„Cytokinetické“ kinesiny

- \+ end-directed:
  - rodina TKRP125 – posun mt?
  - rodina PAKRP („phragmoplast – associated“)
- \- end-directed:
  - rodina ATK1/KatA
  - KCBP (Ca2+-calmodulin reg.)
  - HINKEL (*HIK*) a NACK1
- NACK1 nutný pro lokalizaci NPK1 (nucleus- and phragmoplast-localize protein kinase 1) MAPKKK (Arabidopsis má 3 homology)
- lokalizace závisí na fázi cyklu!





Kde by mohla působit kontrola BC?



MAPK kaskáda ... srv. NACKs!!!

CDK