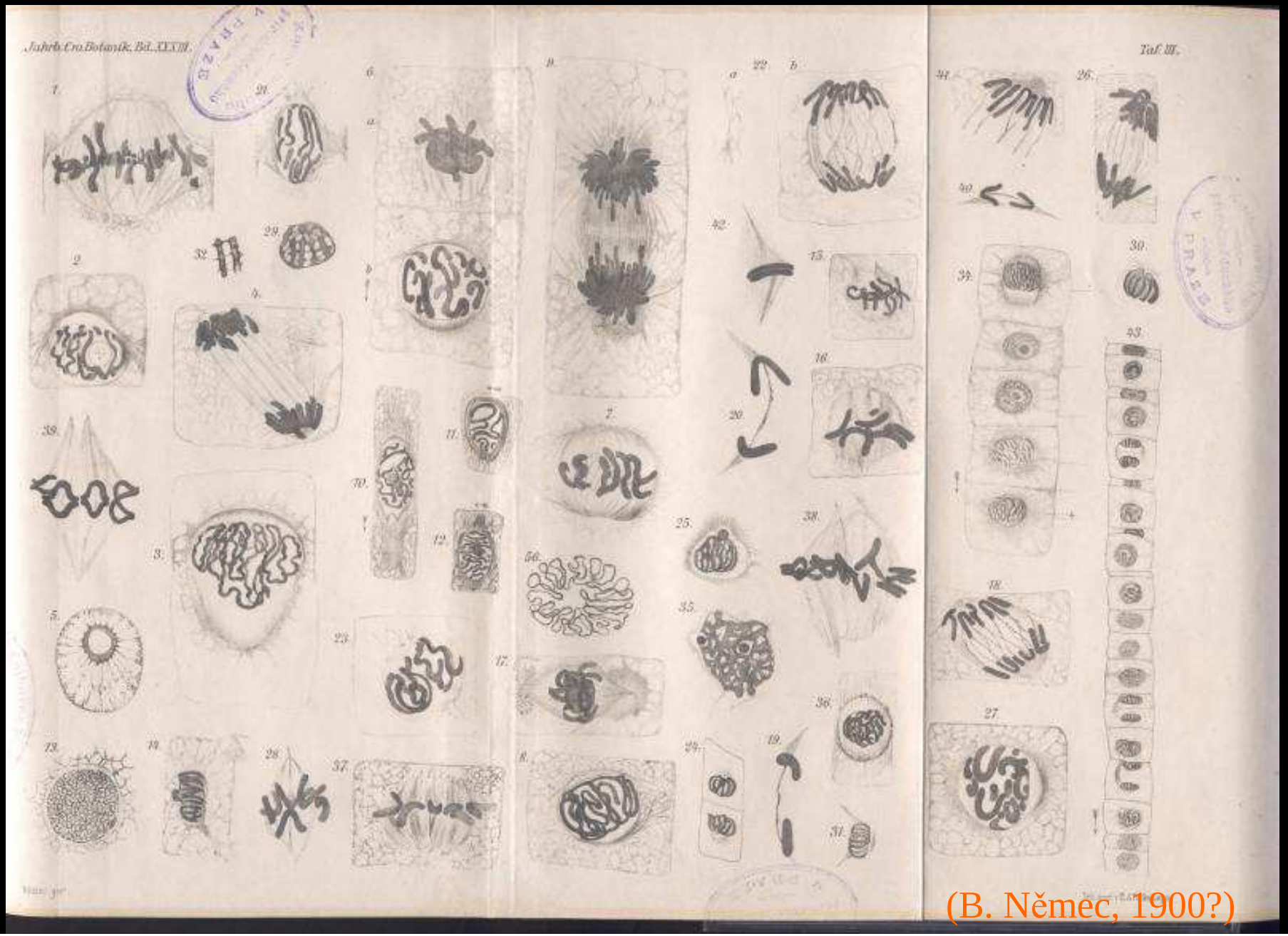
Buněčný cyklus



(B. Němec, 1900?)

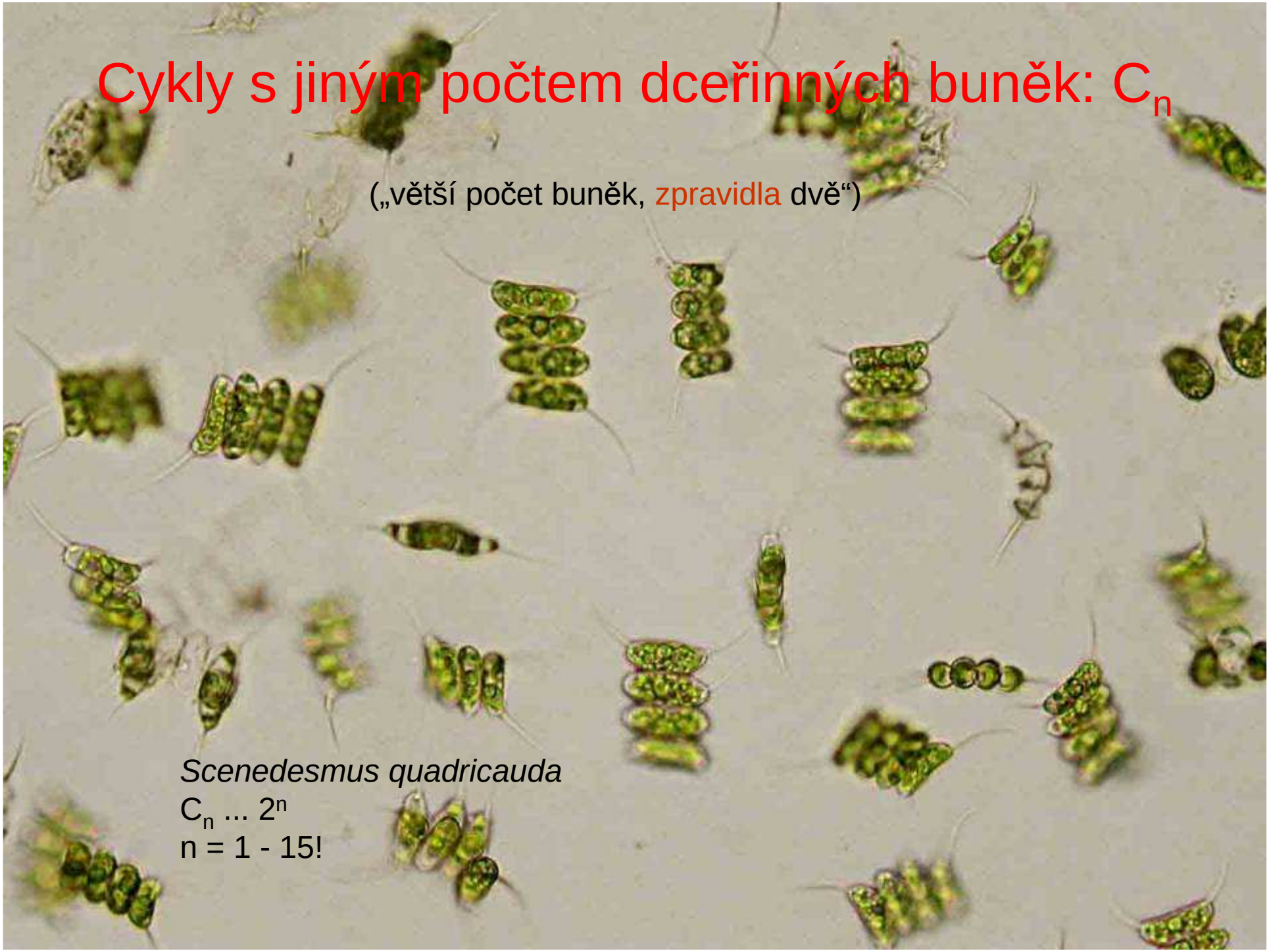
Definice BC

Buněčný cyklus je posloupnost událostí, kterými z jedné buňky vzniká větší počet buněk, zpravidla dvě.

- typická biologická definice
- BC je (také) „minimální ontogeneze“ - včetně morfogenetických aspektů!

Cykly s jiným počtem dceřinných buněk: C_n

(„větší počet buněk, zpravidla dvě“)



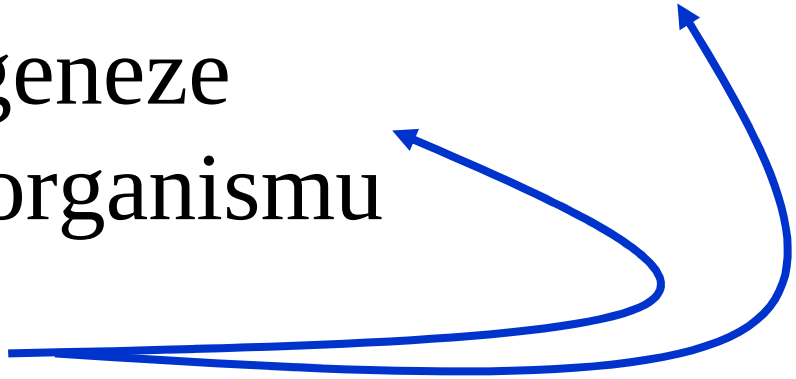
Scenedesmus quadricauda

C_n ... 2^n

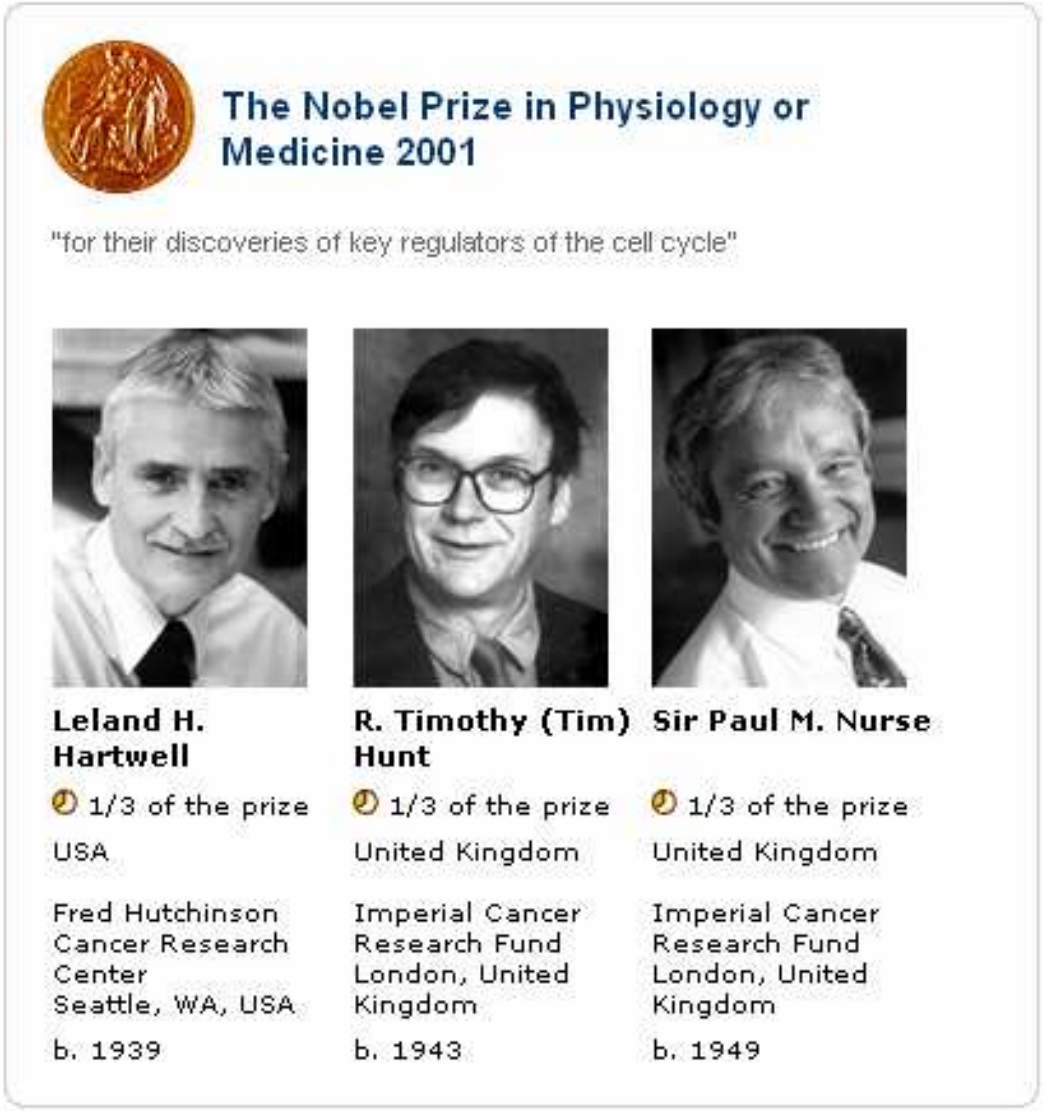
n = 1 - 15!

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

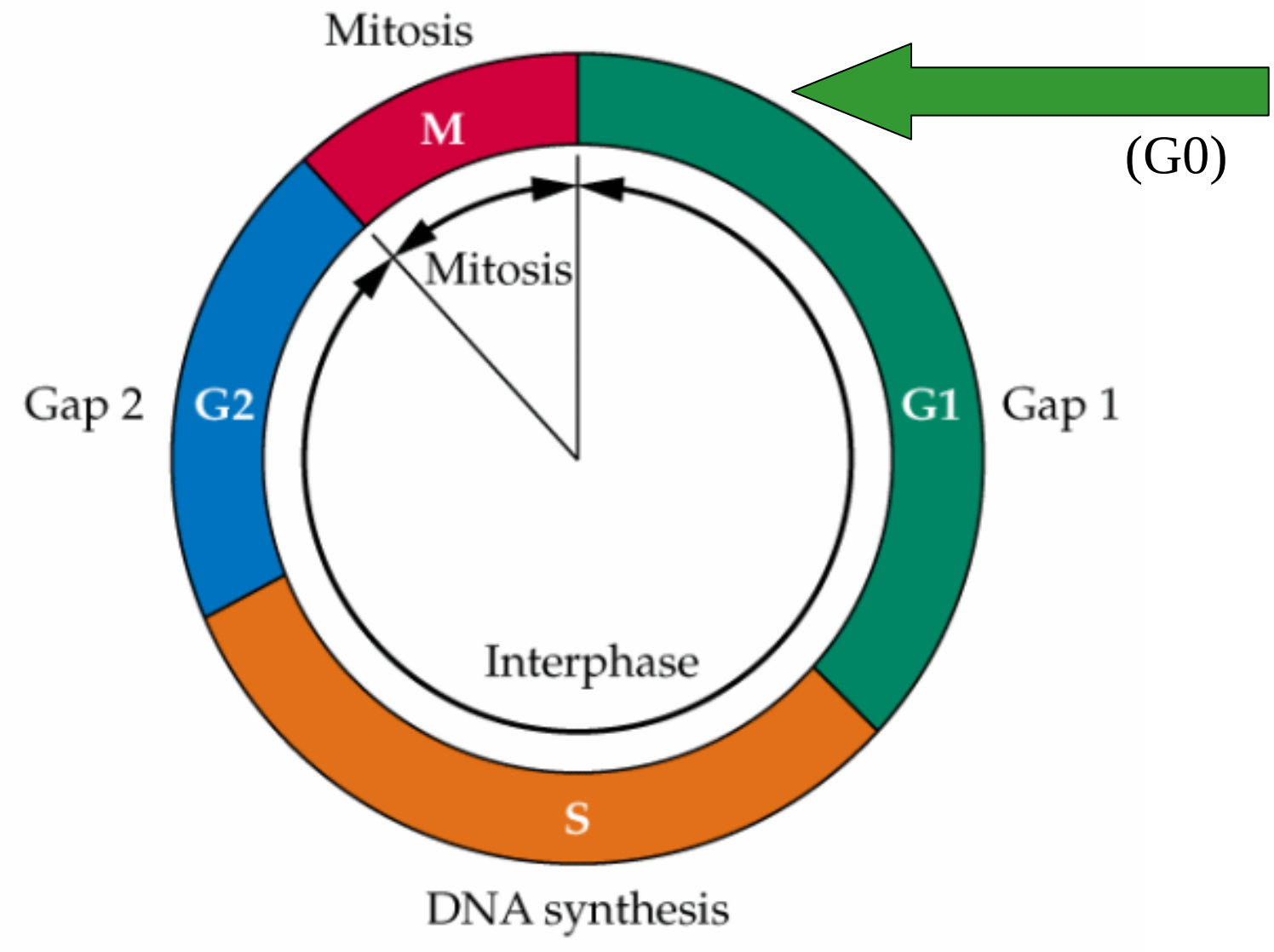
United Kingdom

Imperial Cancer Research Fund
London, United Kingdom

b. 1949

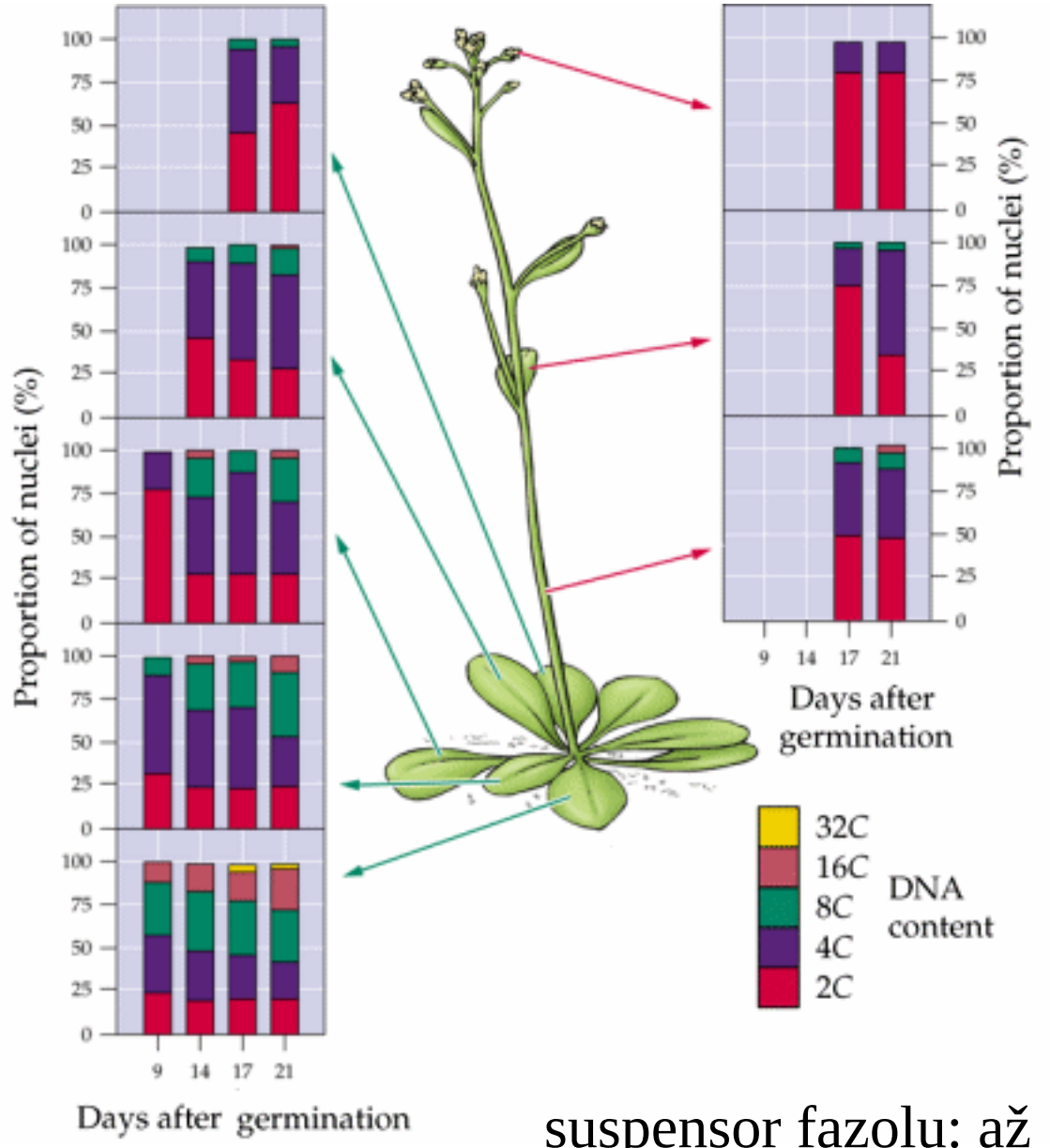
... ale začalo to mnohem dřív ...

Obecné schéma eukaryotního buněčného cyklu

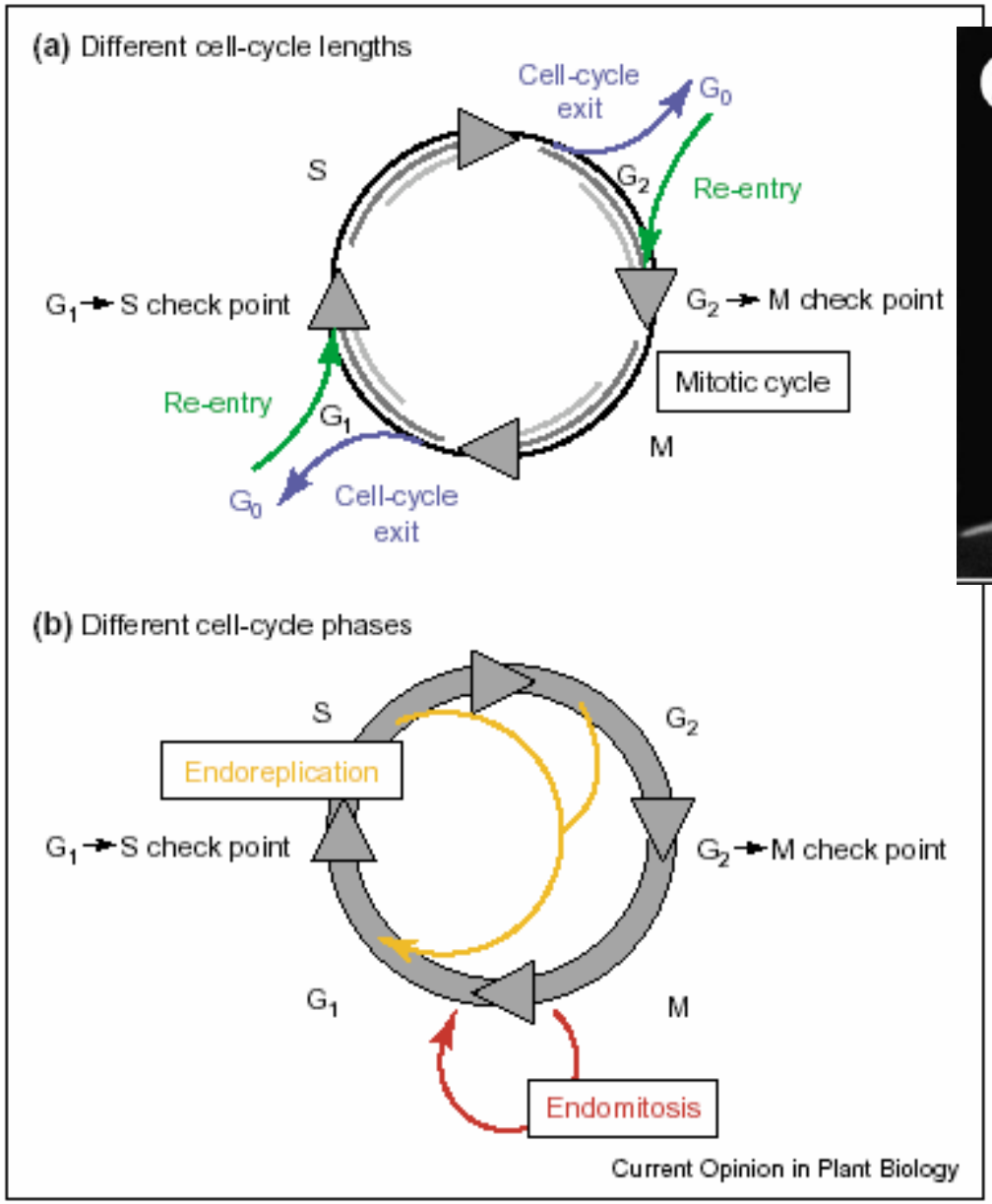


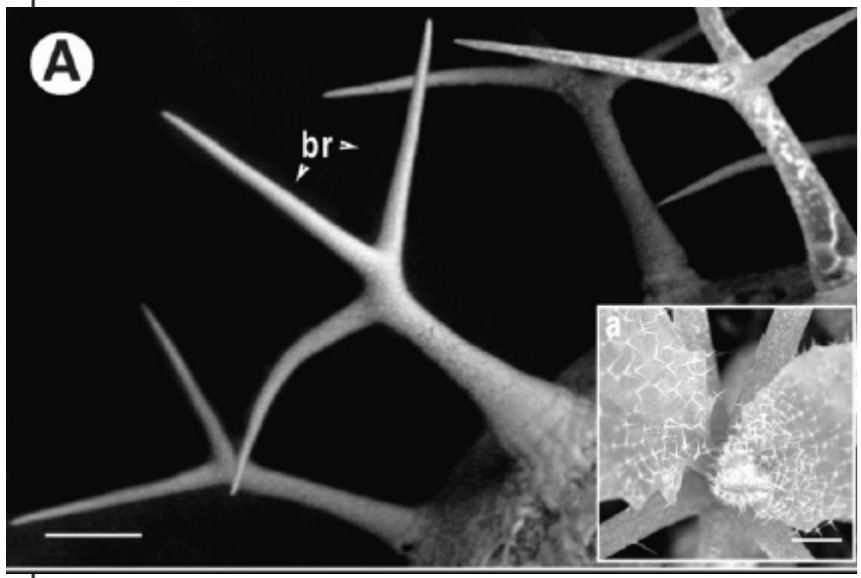
(1. pozorování - Howard and Pelc, 50. léta, rostliny!)

Výjimky z pravidel v rostlině

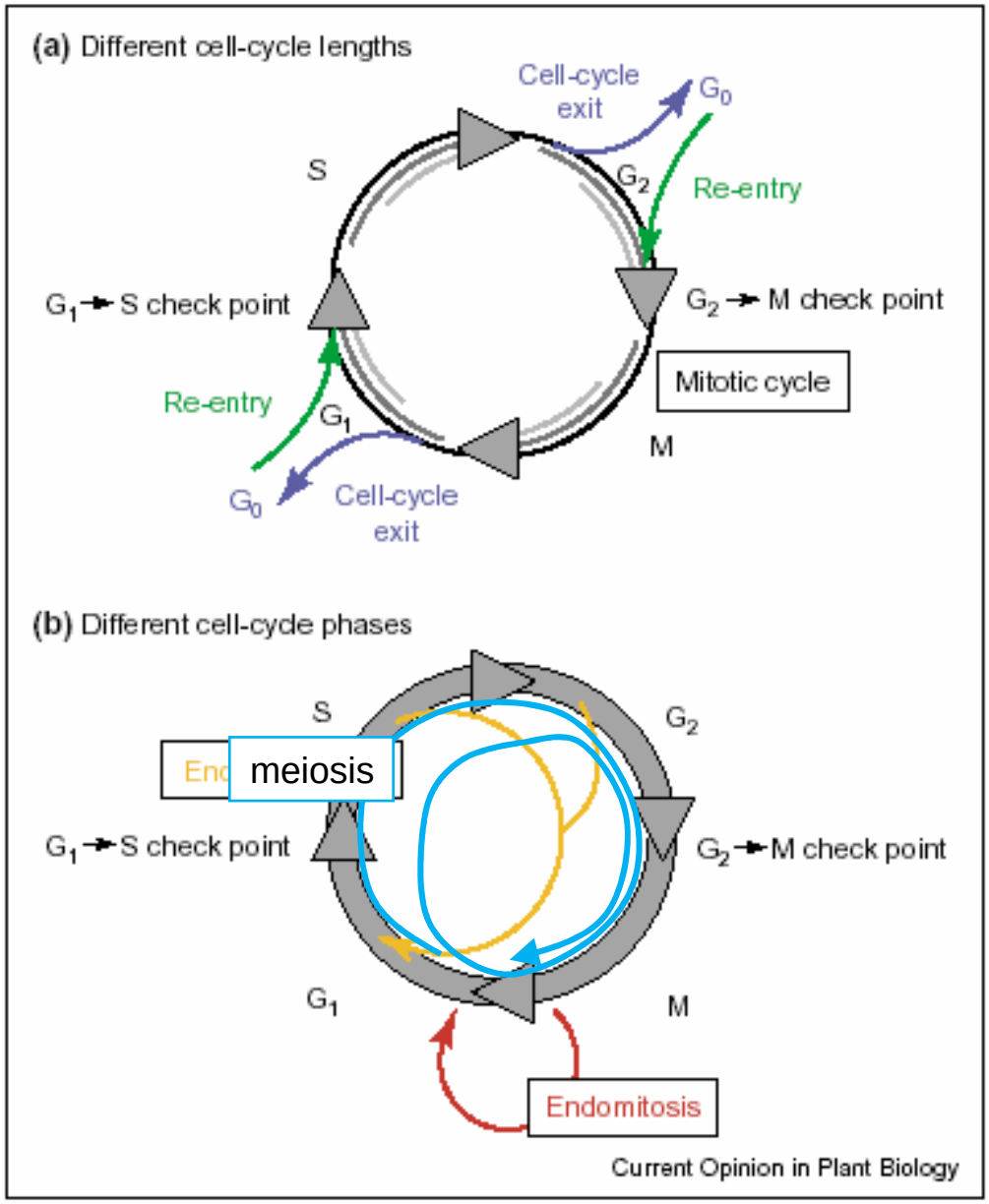


suspensor fazolu: až 10^3 C





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.

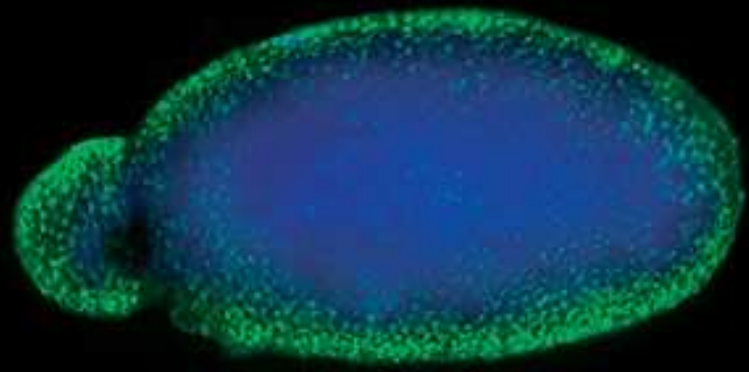


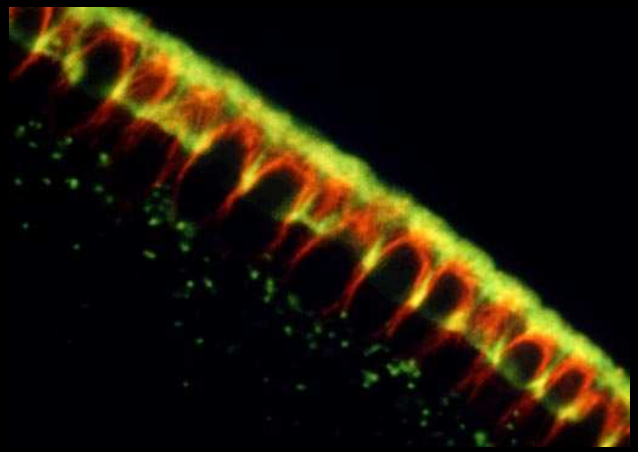
Many different cell-cycle modes are executed in plants. **(a)** The different cell-cycle modes can vary with respect to cell-cycle phase lengths, ranging from a rapid, proliferative mode to an exit from cell cycle in either G_1 or G_2. **(b)** The composition of different cell-cycle modes can also differ;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

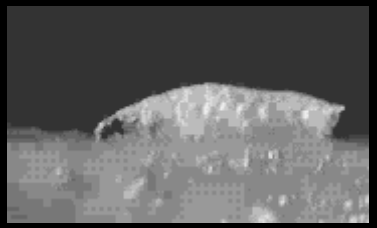
Cykly bez cytokineze: endosperm, embryo Drosophily ...

(Ohad et al. 1999)

fie (fertilisation-independent endosperm), Arabidopsis







(Sullivan et al., www)

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?

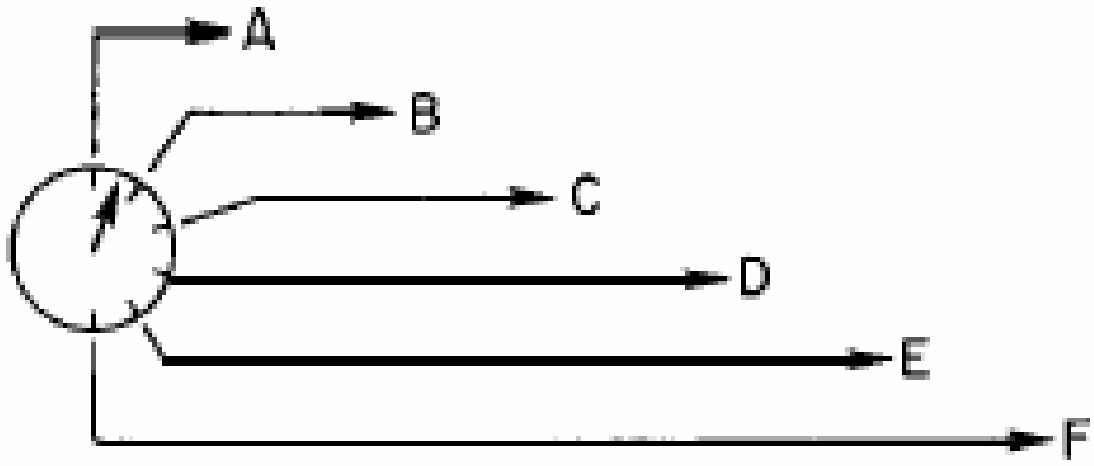
(Wheals, 1976)

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

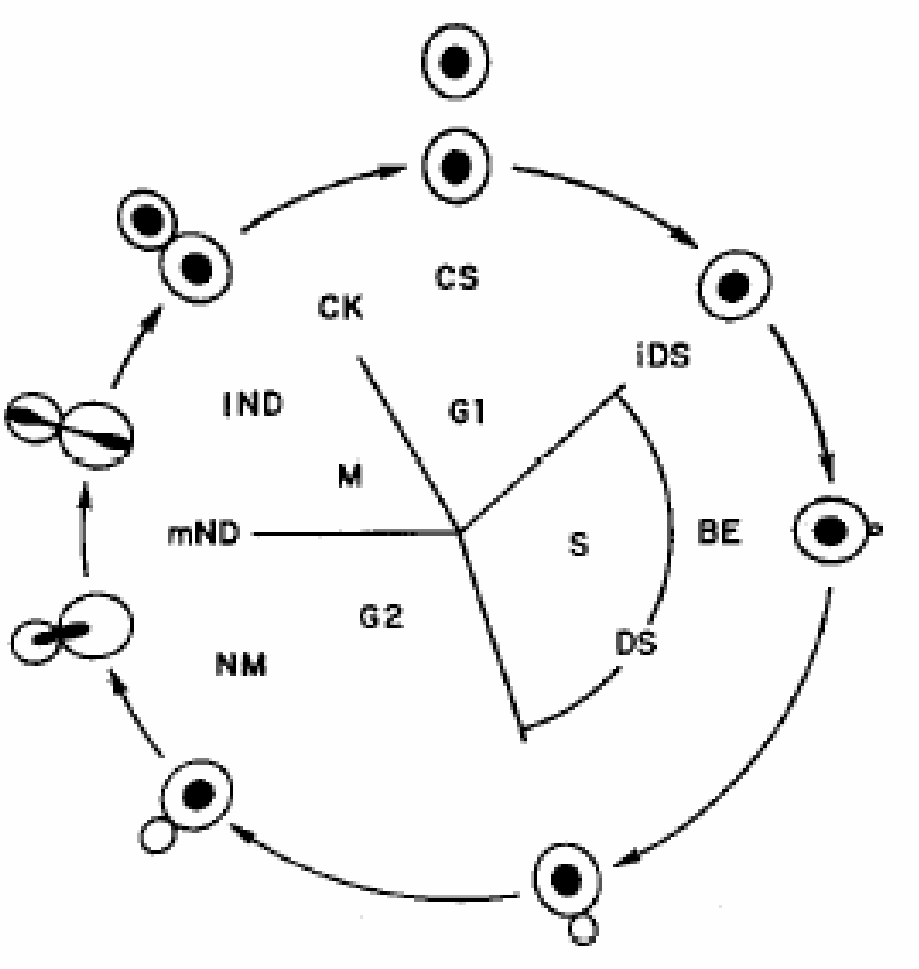
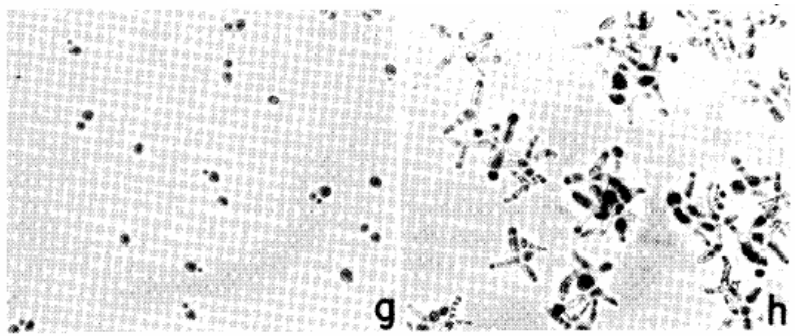


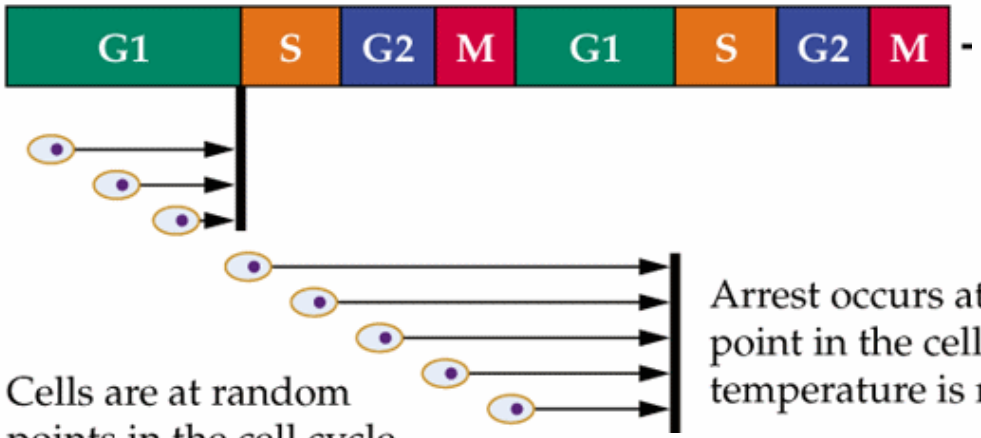
Fig. 1. The sequence of events in the cell division cycle of yeast: ***iDS,*** initiation of DNA synthesis; ***BE,*** bud emergence; ***DS,*** DNA synthesis; ***NM,*** nuclear migration; ***mND,*** medical nuclear division; ***lND,*** late nuclear division; ***CK,*** cytokinesis; CS, cell separation. 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, pm+ pm-



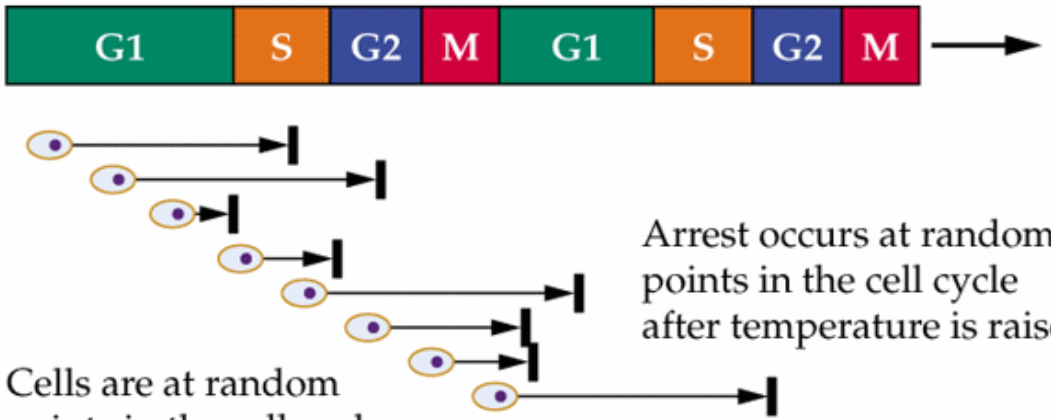
(A) Cell division cycle *(cdc)* mutants arrest uniformly after temperature is raised.



Arrest occurs at specific point in the cell cycle after temperature is raised.

Cells are at random points in the cell cycle before temperature is raised.

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

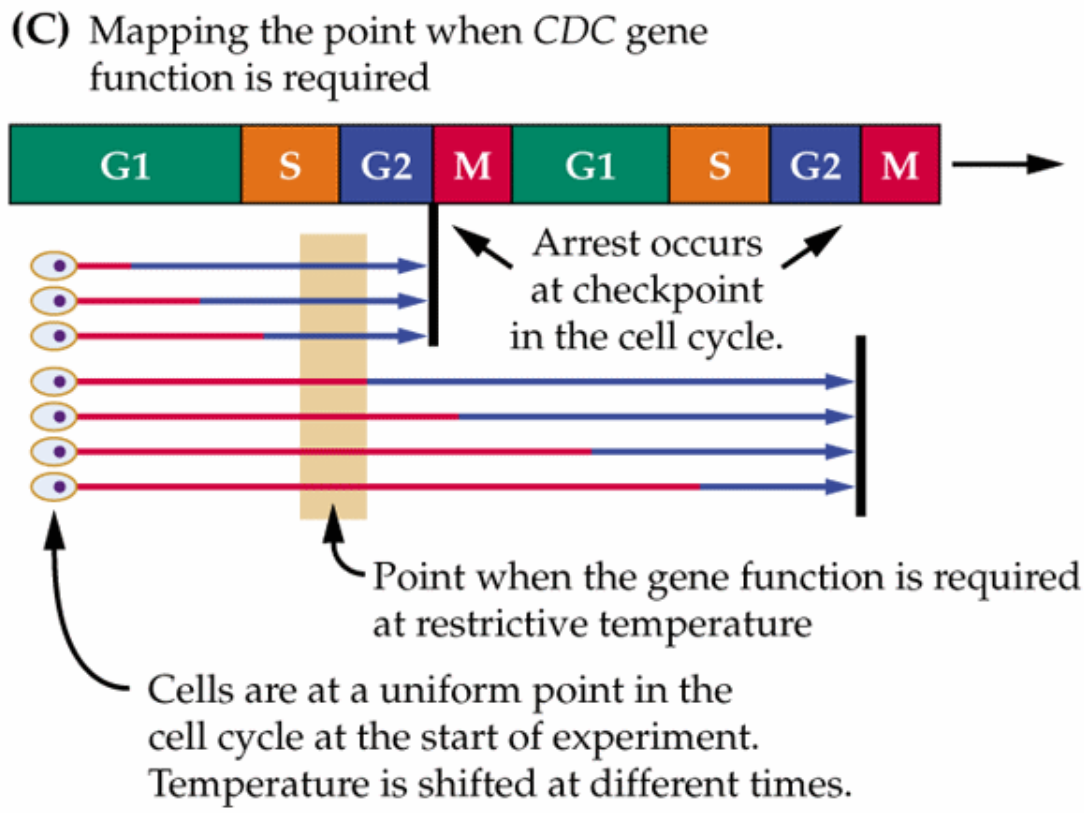


Arrest occurs at random points in the cell cycle after temperature is raised.

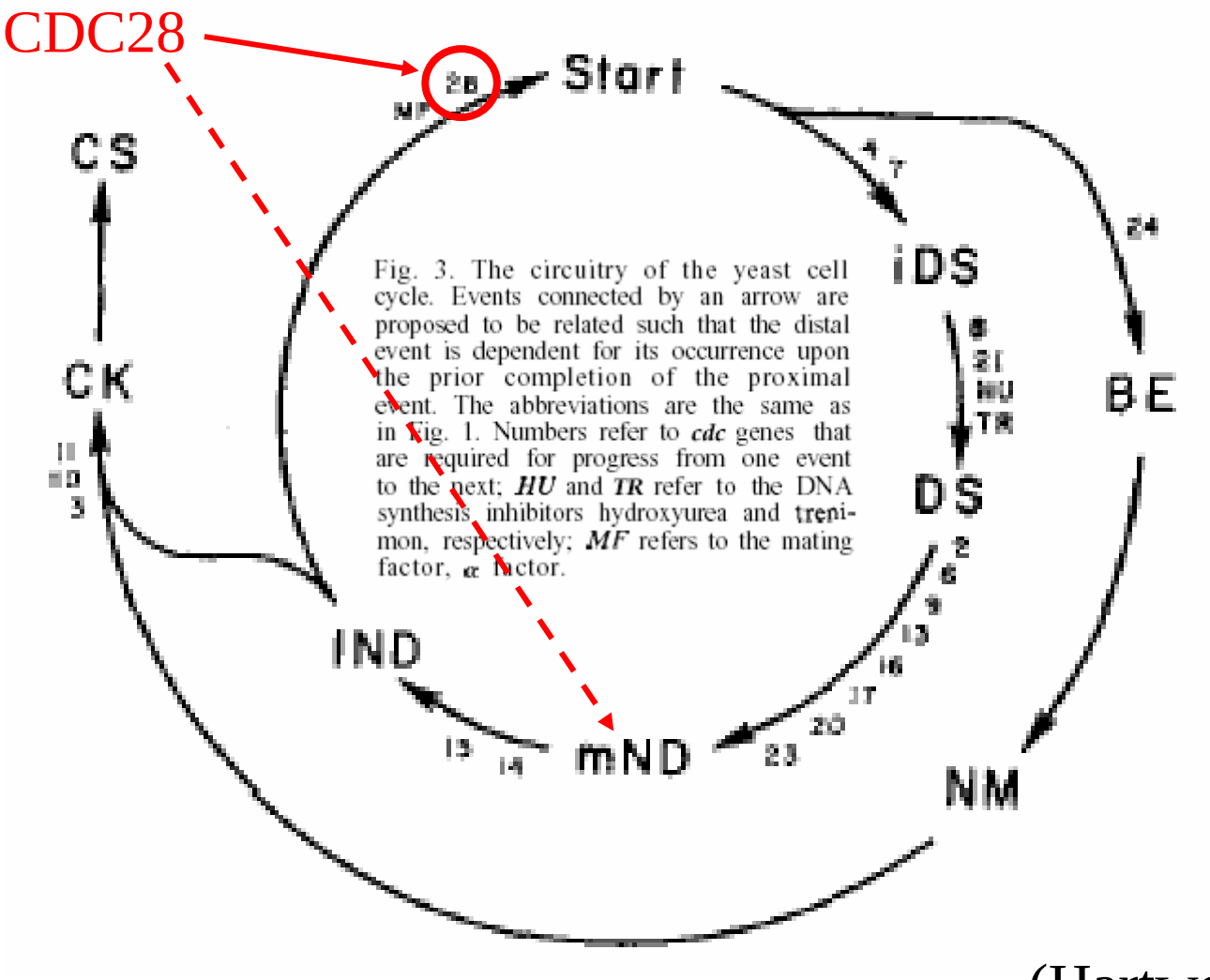
Cells are at random points in the cell cycle before temperature is raised.

Pořadí funkce CDC genů

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

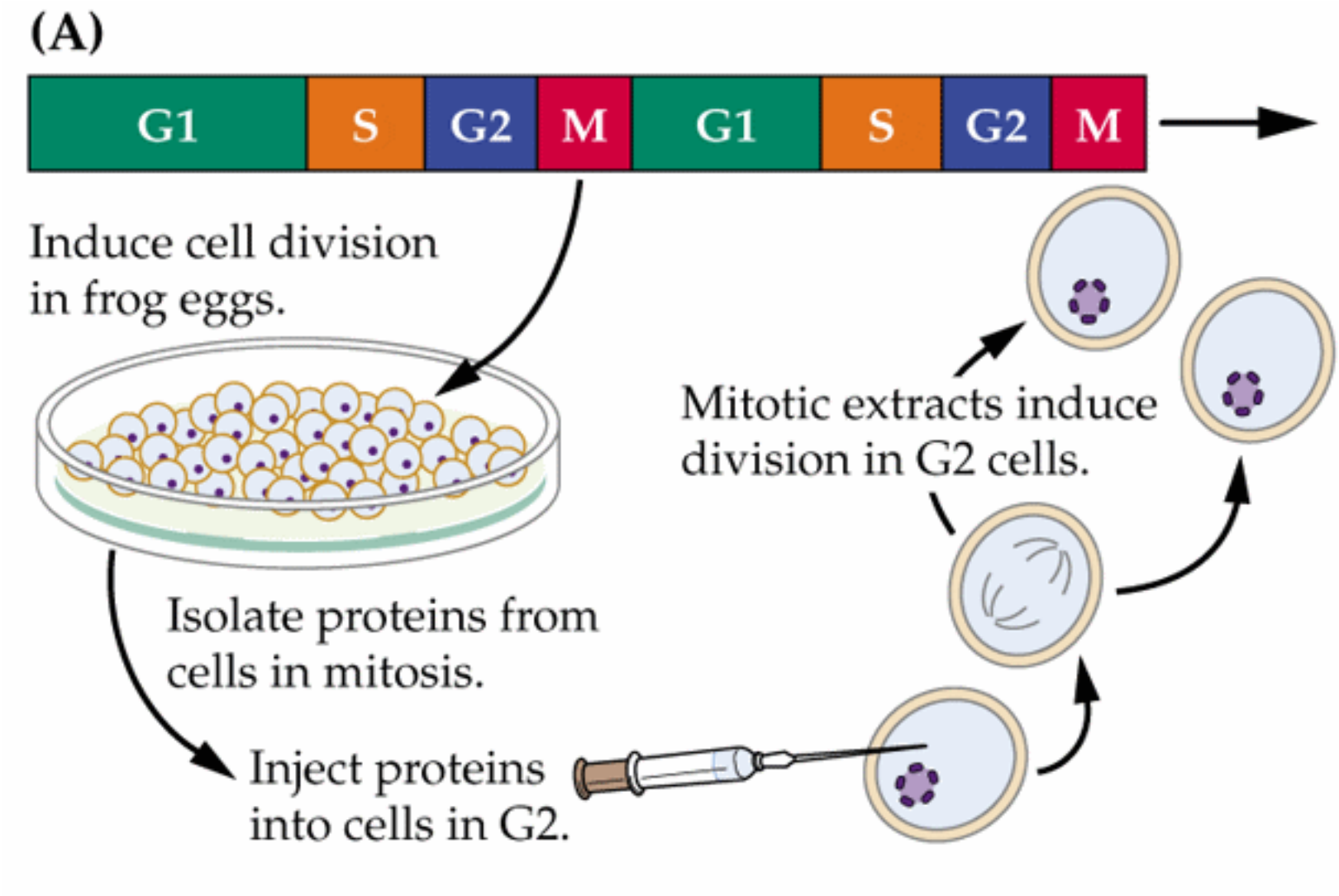


Mapa funkcí CDC genů



(Hartwell 1974)

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



MPF - maturation promoting factor

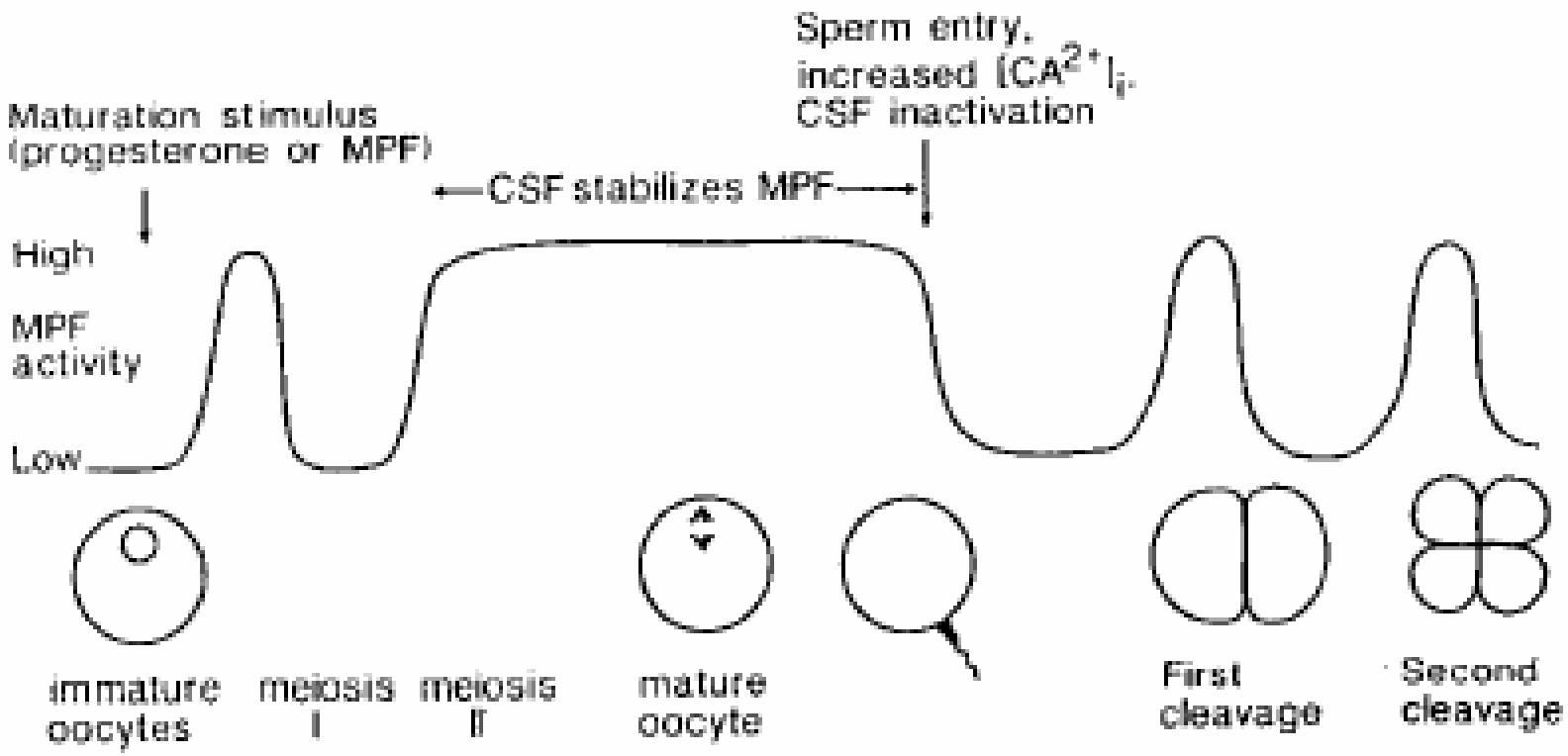
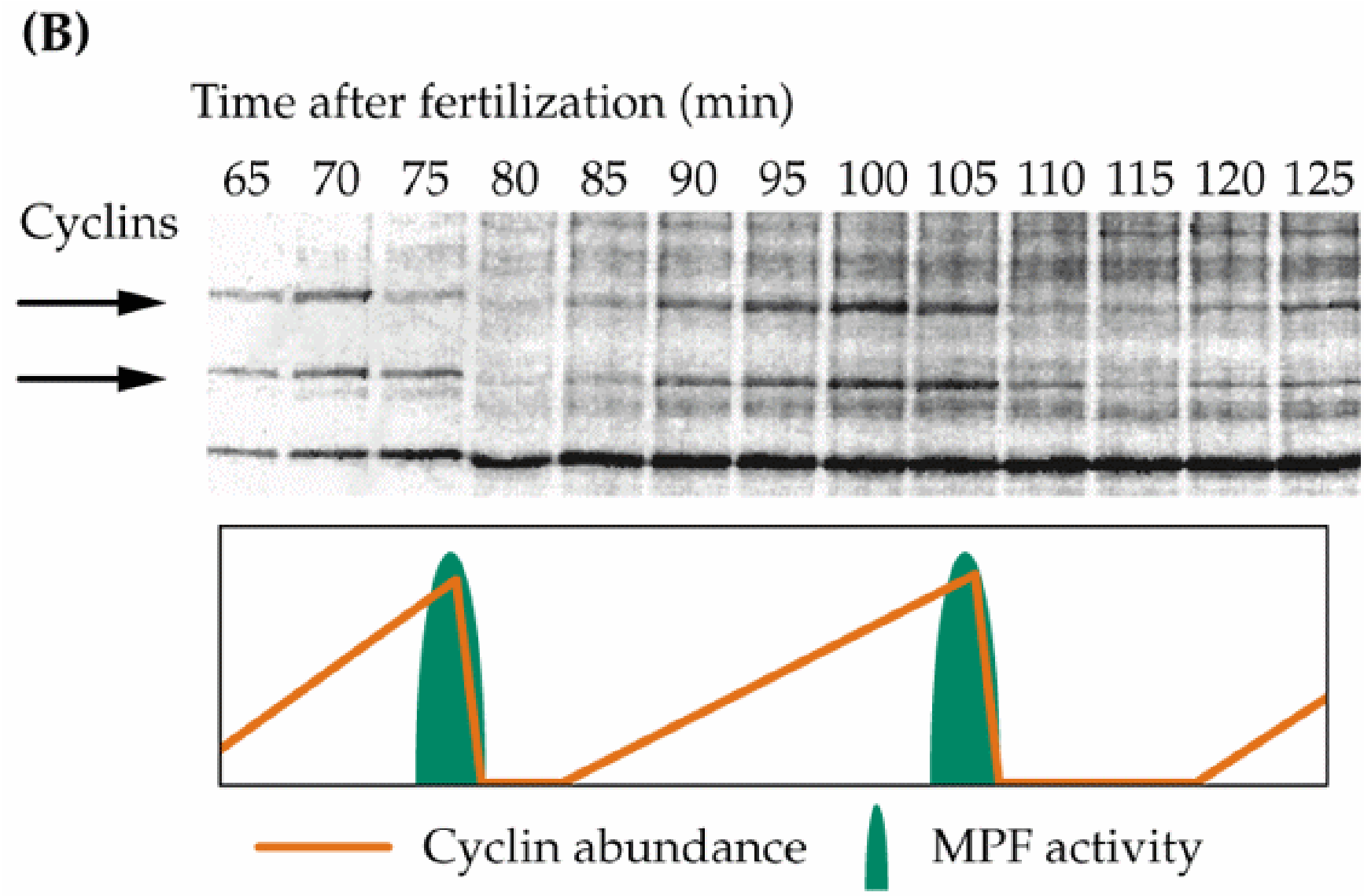
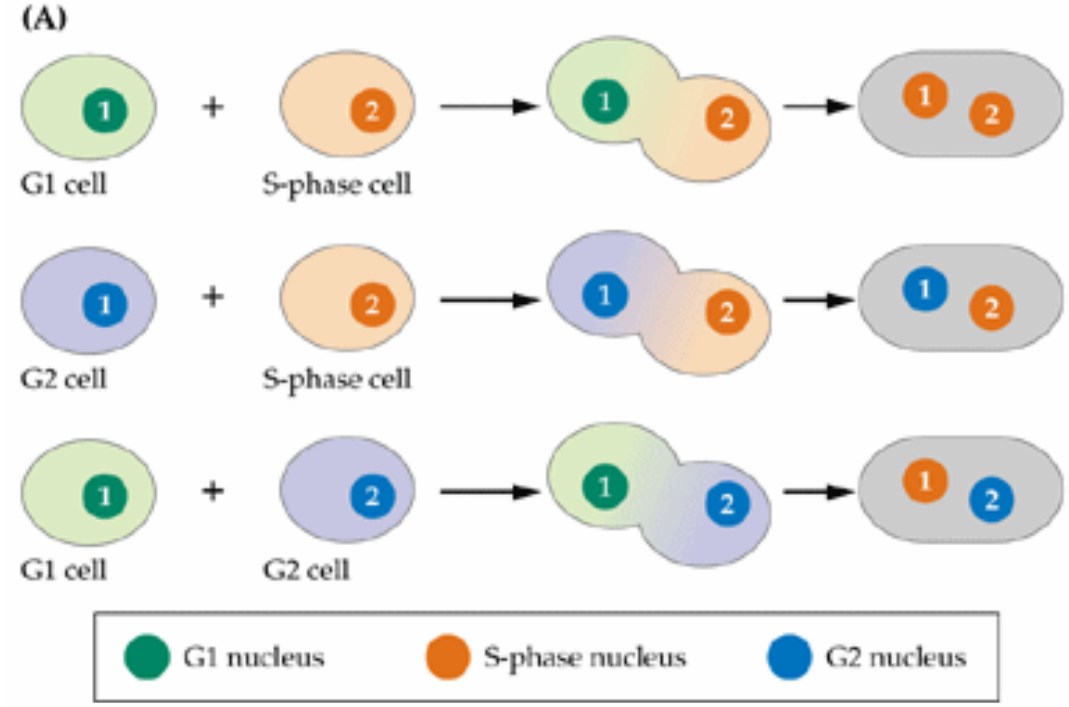


FIG. 1 MPF levels during early *Xenopus* embryonic development. The fluctuation in MPF levels as an immature oocyte passes through meiotic maturation, fertilization and the first two mitotic cell cycles is shown. For further details see the text.

MPF = p34 + „cyklin“

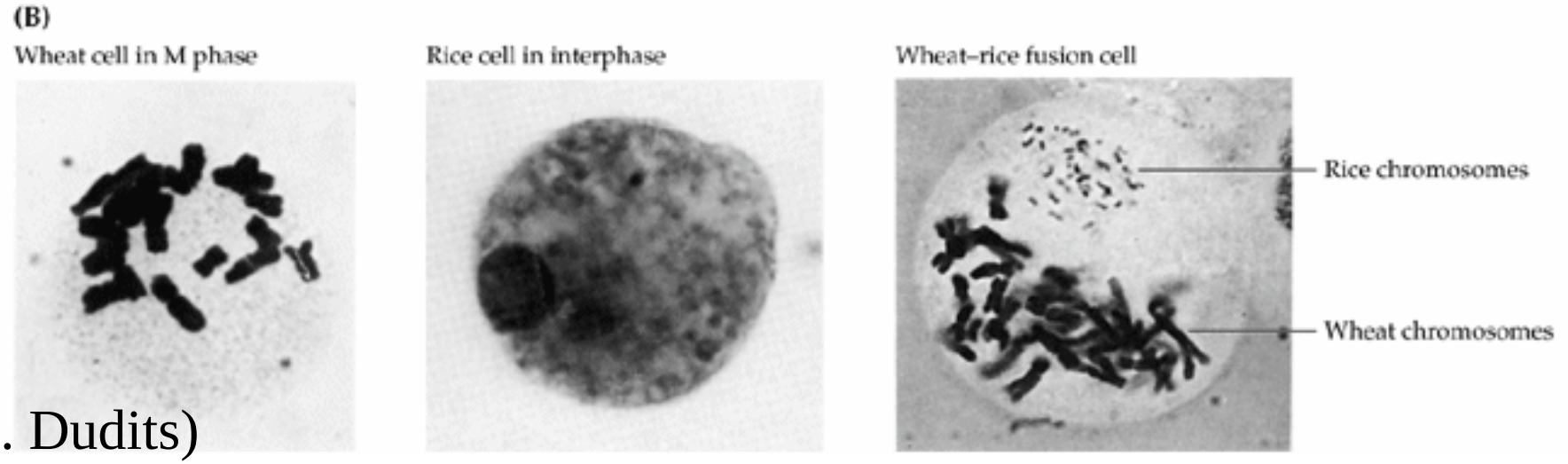


Další doklad pro „hodiny“: fúze buněk



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)

Prof. Paul M. Nurse

Scientists need to earn the trust and confidence of the public if we are to retain our “license to operate.” But to do that we have to be accurate about what science can do. It is no good exaggerating what science can deliver, as happened when the Director of the National Cancer Institute, Dr. Andrew von Eschenbach, announced the Institute’s challenge goal in 2003 as “To eliminate the suffering and death due to cancer by 2015.” This cannot be justified even as a statement of aspiration because when we fail to deliver, as we surely will with such a claim, we will lose the confidence and trust of both the politicians and the public.

generální ředitel Imperial Cancer Research Fund, Londýn; nositel Nobelovy ceny ...
nyní Rockefeller University
18. 10. 2001 – UK - doktor honoris causa přírodních věd „za zásadní příspěvky k poznání regulace buněčného dělení, které významně posouvají poznání v biologii a lékařství“ (na návrh PřF UK)

Sjednocení modelů (P. Nurse):

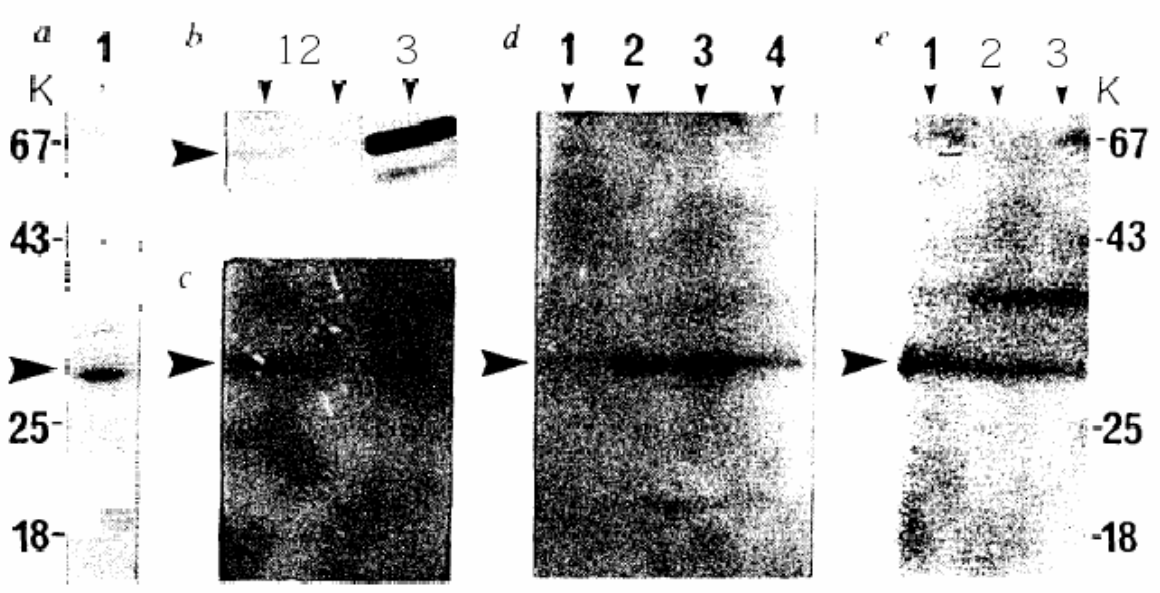


Fig. 5 Western blot analysis of S. pomhe and *H. sapiens* protein. Equivalent protein samples loaded: a, lane 1, human colonic carcinoma cell line HT29; b, lane 1, human colonic carcinoma cell line HT29; lane 2, *S. pombe* wild type; lane 3, S. pombe, *cdc2* overproducer; c, lane 1, S. *pombe* wild type; lane 2, S. *pombe* germinated spores deleted for *cdc2*Sp; lane 3, S. pombe deleted for *cdc2*Sp containing pSAB2Hs. *d, lane* 1, S. *pombe* deleted for cdc2SP containing pSAB2Hs; lane 2, human J6 T cell; lane 3, human Heb7a, HeLa-derived; lane 4, human Daudi B cell; *e*, lane 1, human J6T cell; lane 2, human Heb7a, HeLa-derived; lane 3, Human Daudi B cell. Blots a, *b, c, d* were made using affinity purified antibodies against the peptide EGVPSTAIRELLKE and *e* using serum against the carboxy-terminal 99 amino-acid residues of *cdc2*Sp. The arrowhead marks the position of the 34K protein.

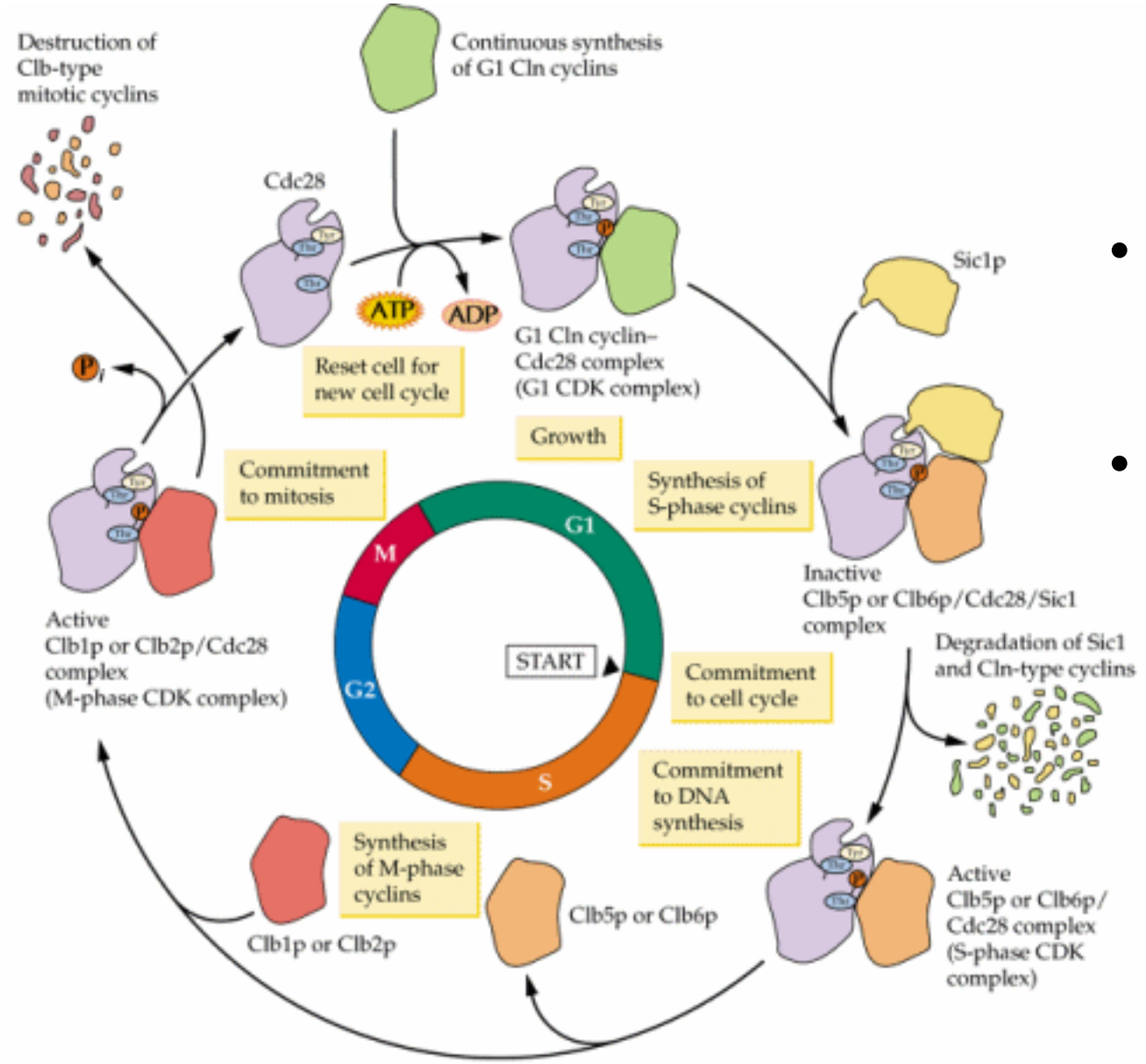
Methods. Extraction of proteins and preparation of peptides conjugates for rabbit immunization were as described in ref 9 Western blot procedures were performed using GeneScreen and GeneScreen Plus membranes according to the manufacturer's (NEN) instructions.

(Simanis a Nurse 1986)

CDC28 kóduje p34!

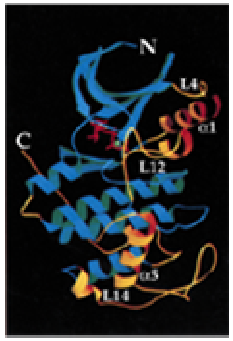
„cyclin-dependent kinase“, CDK

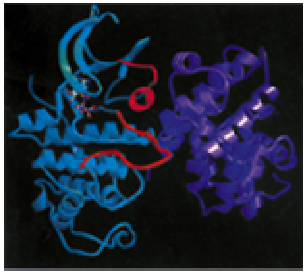
(dílčí procesy mohou běžet podle modelu „domino)

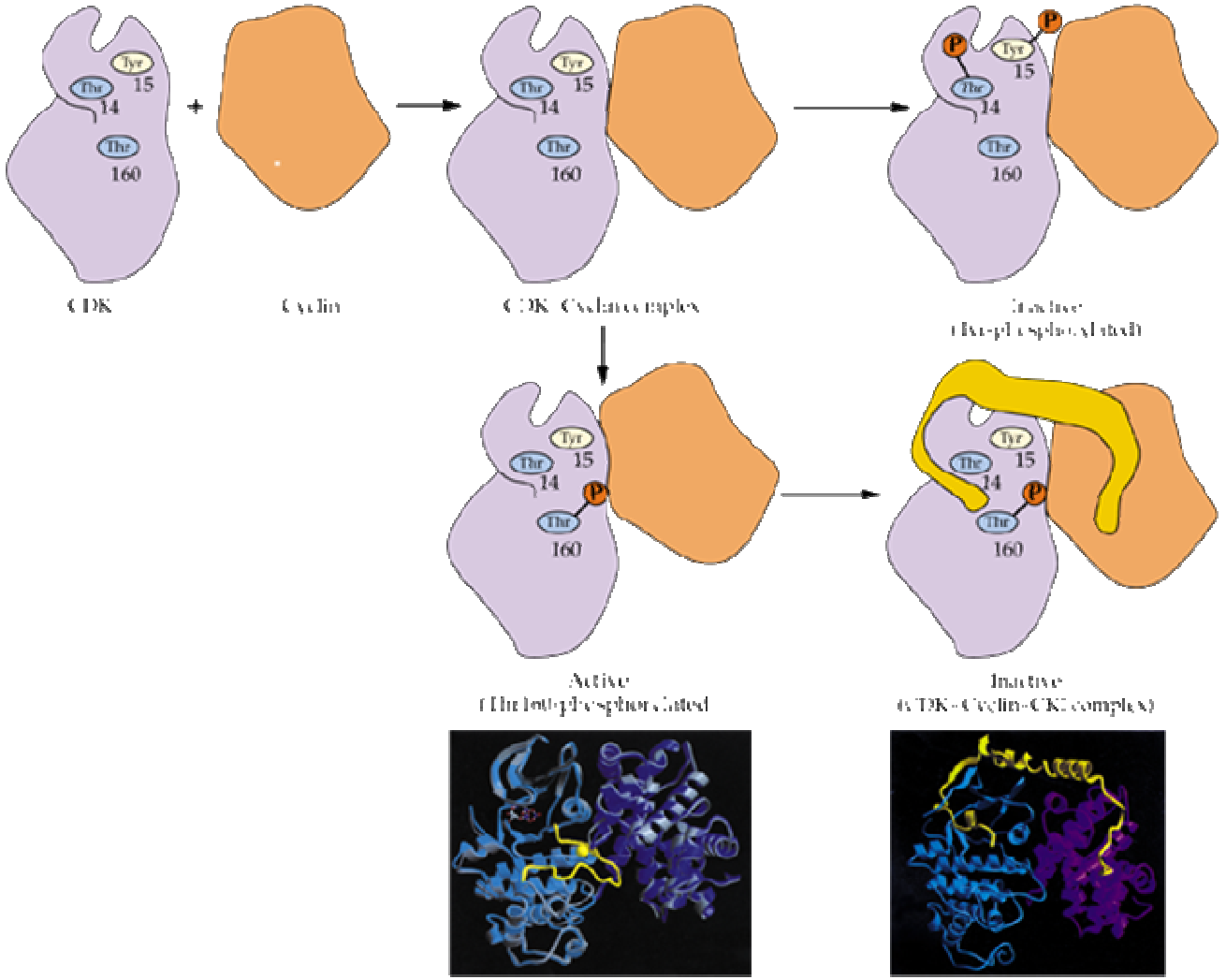


- Vlny
  - CDK
  - cyklinů
  - CDK inhibitorů
- Regulace:
  - transkripce
  - proteolýza
- Modulace aktivity CDK

Komplex CDK - cyklin - CKI







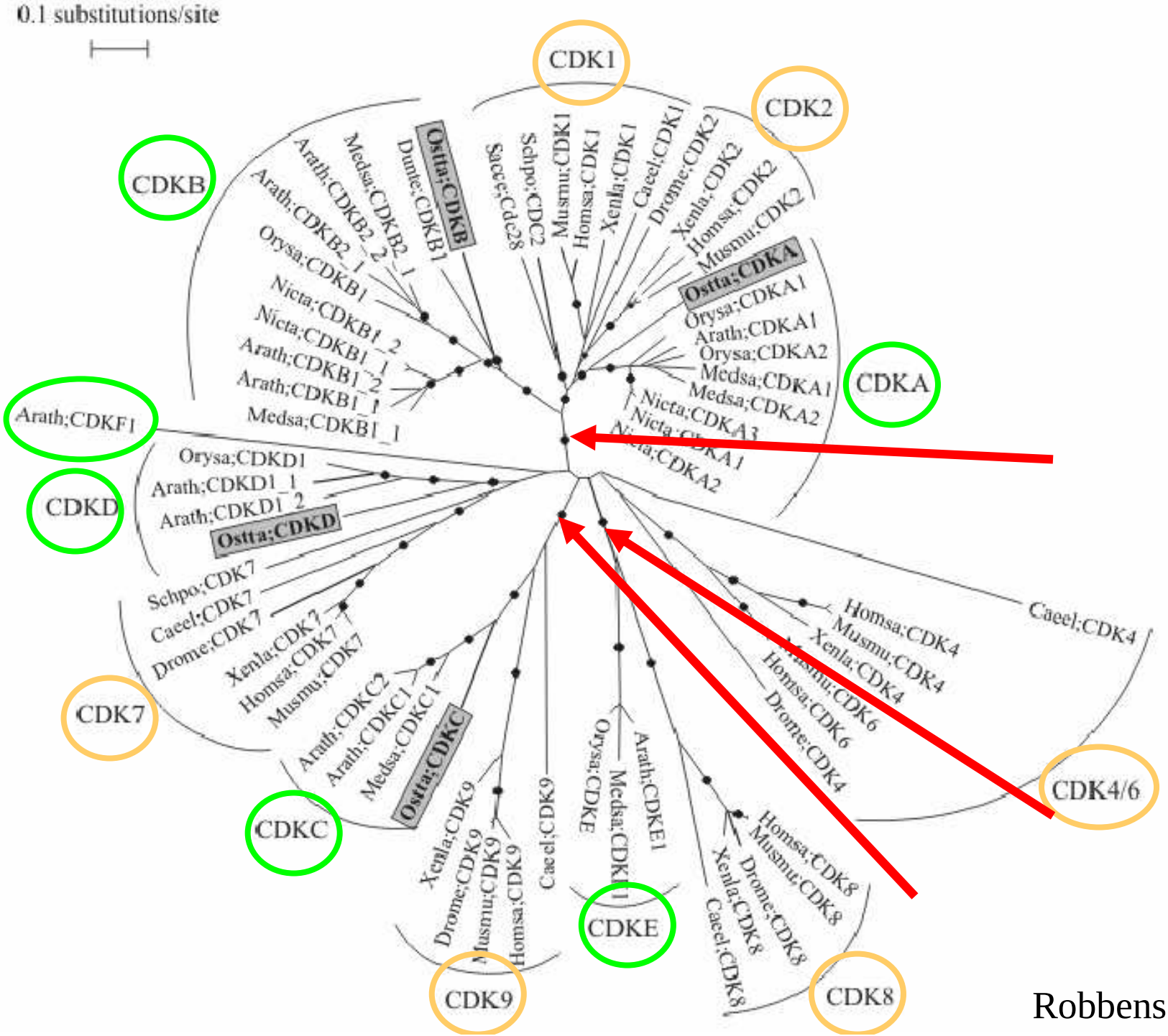
Diversita CDK a cyklinů
(živočišná terminologie)

	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)

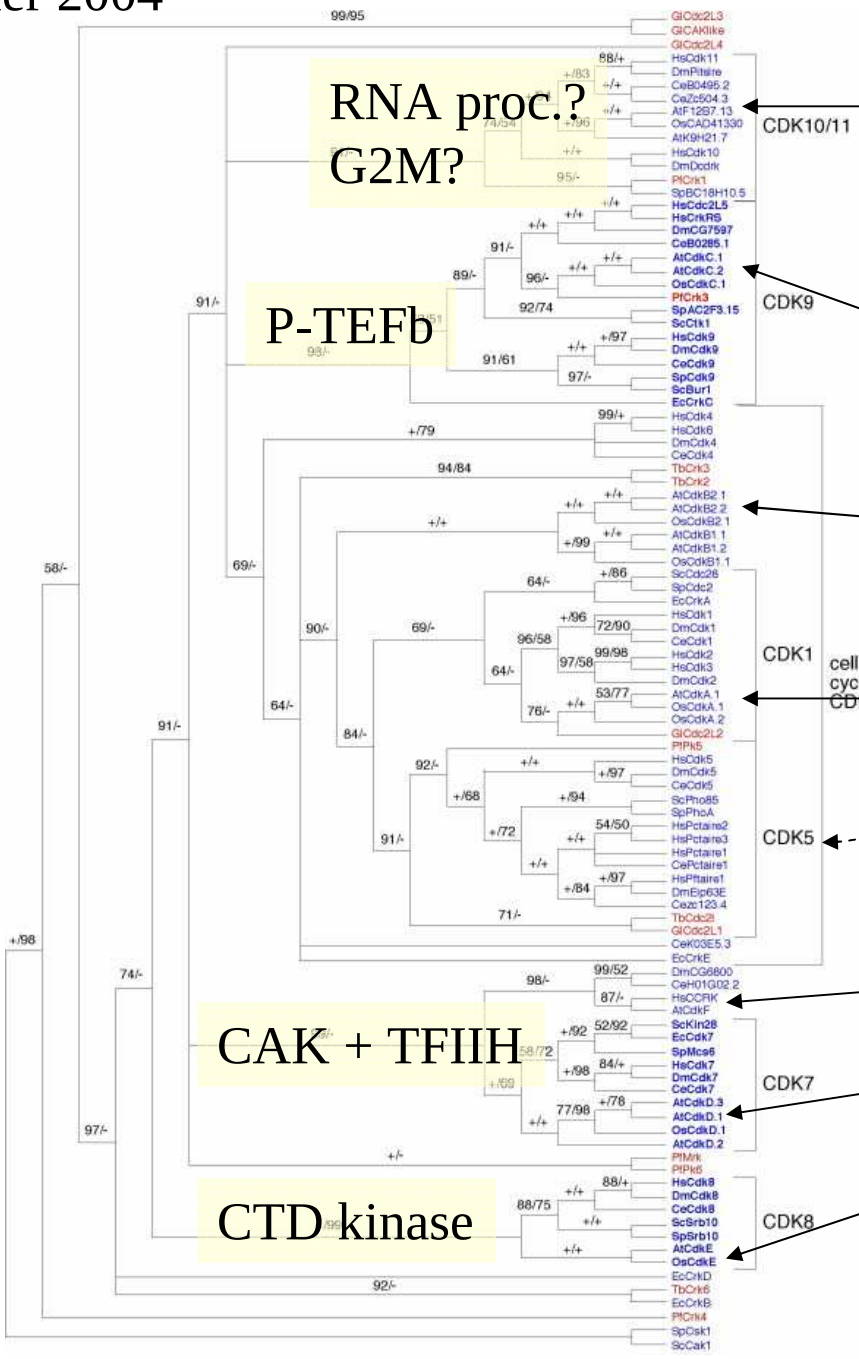
(Doerner lab 2005)

Evoluce CDK



Robbens et al. 2004

Guo and Stiller 2004



16 Arabidopsis CDK dosud zmeškáno, 2 CDK10/11 a 14 recentně duplik. CDK9

CdkC

CdkB

CdkA

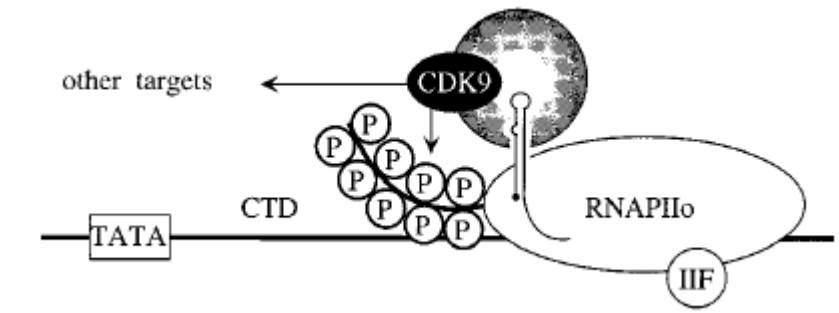
CdkF

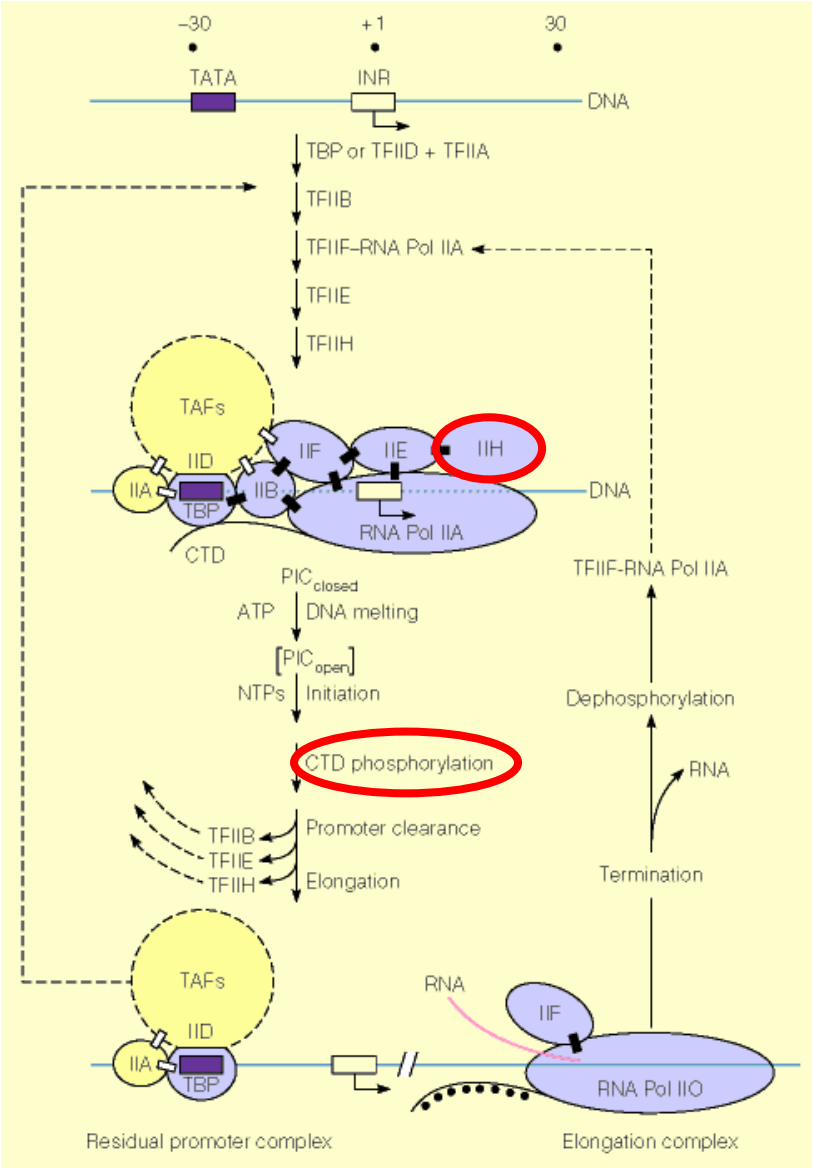
CdkD

CdkE

CDK-like kinázy a transkripce

- TFIIH
- CTD kinase (S.cer.: KIN28)
- P-TEFb (Positive-Transcription Elongation Factor)
  ... + cyclin T (referát)



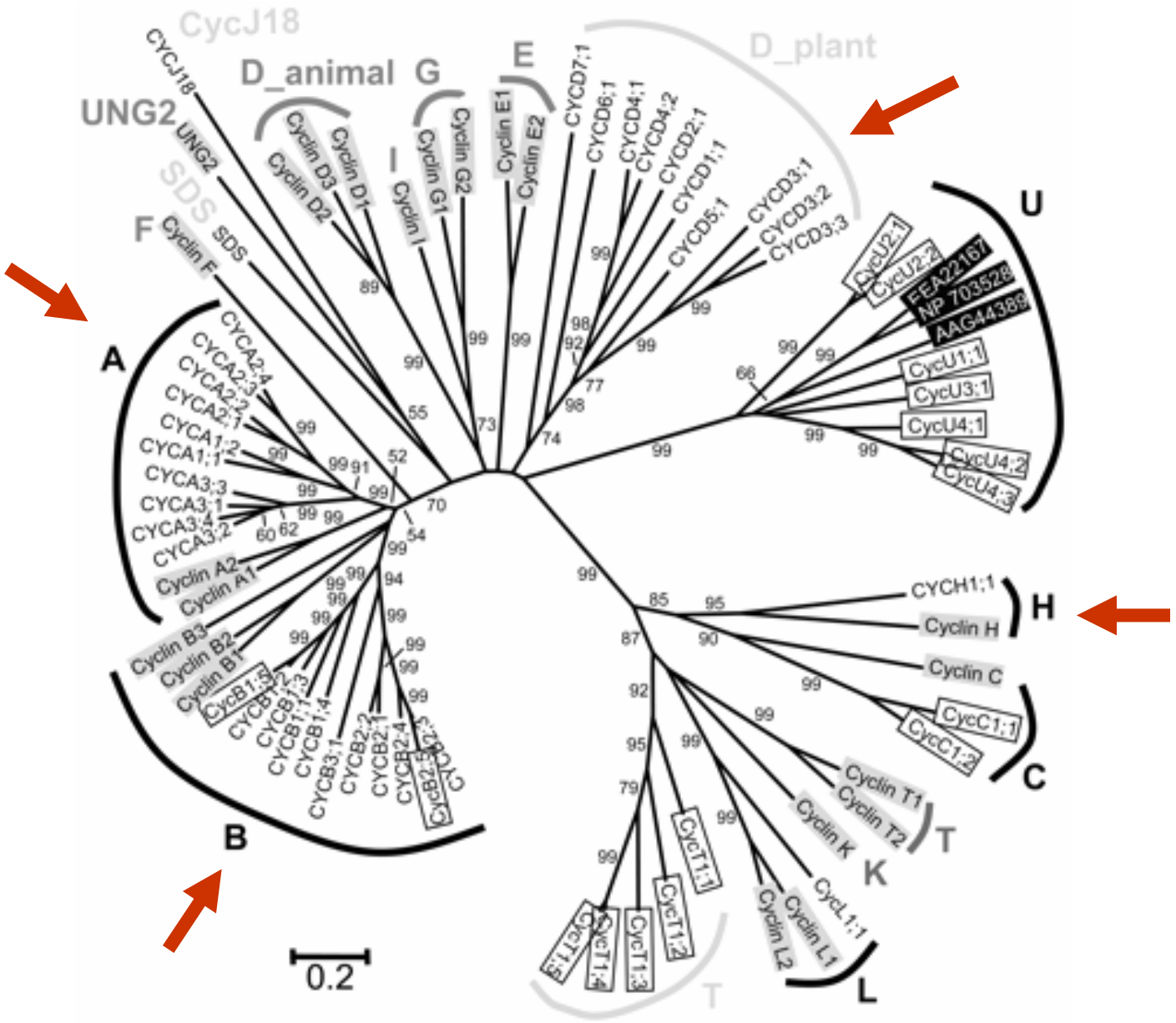


CDK: rozmanitost sekvencí i funkcí

- 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)

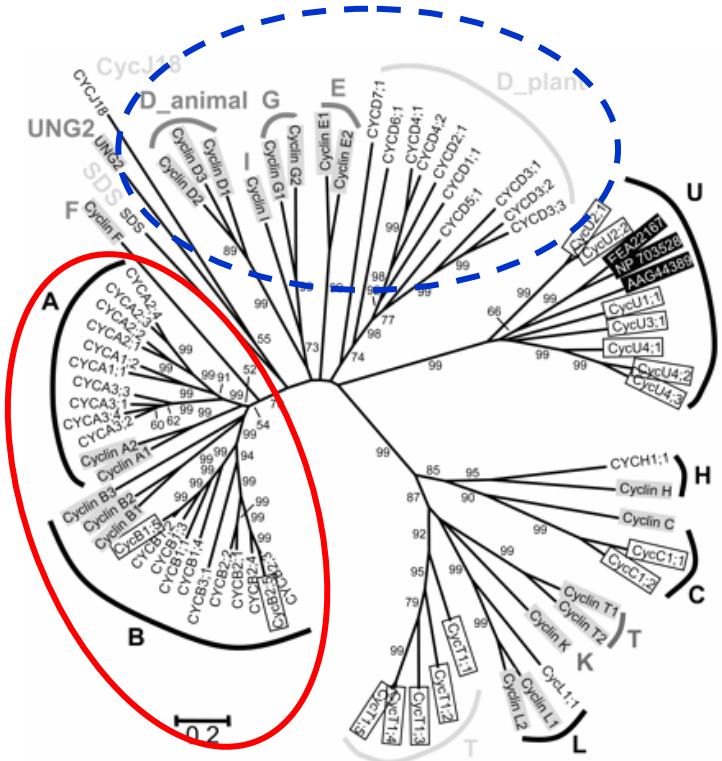
Evoluce cyklinů



Cykliny z Arabidopsis – Wang et al. 2004

Cykliny - dělba práce

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



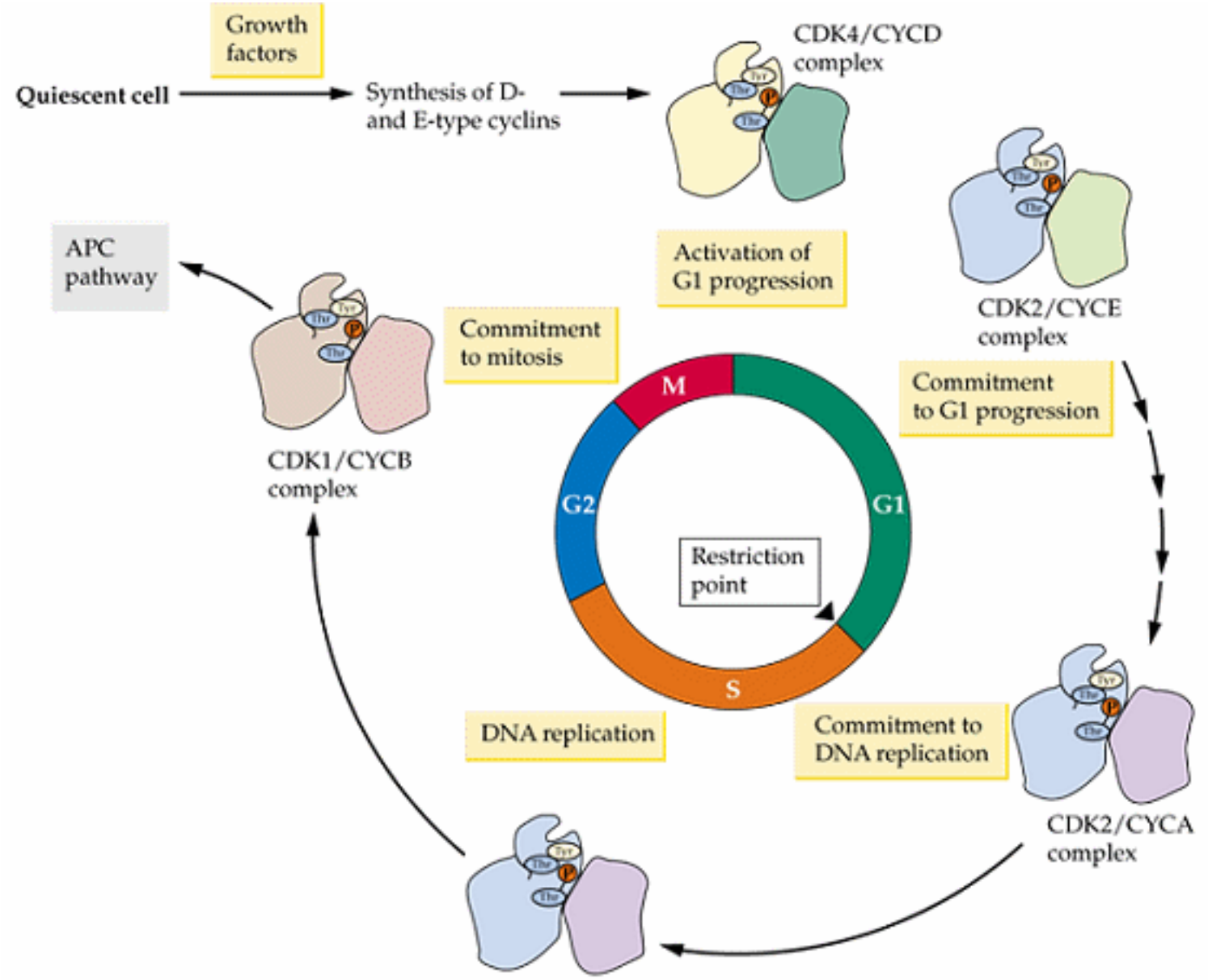
Současný stav u rostlin

(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		?

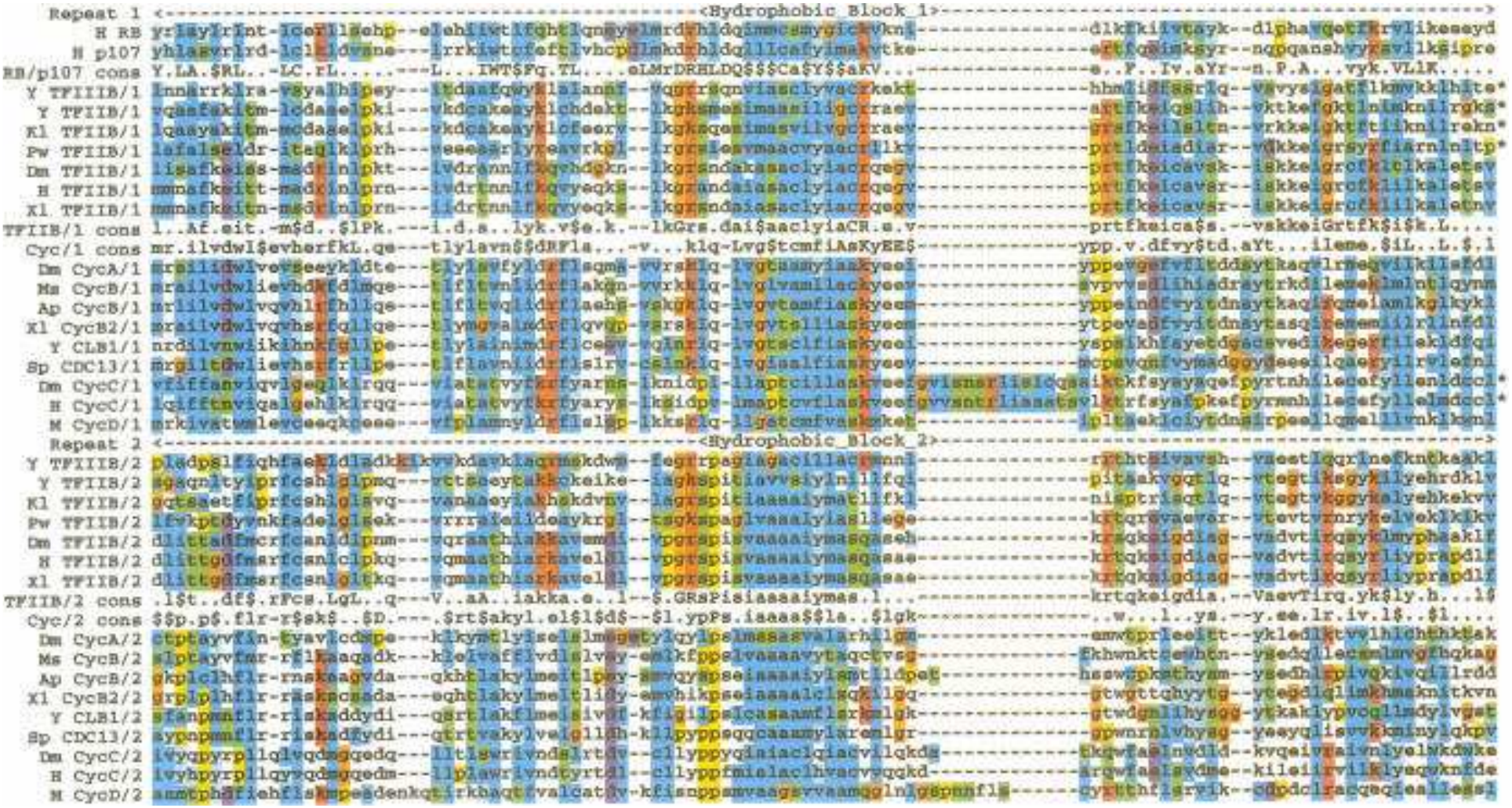
D	1;1	G0/G1/S
D	2;1	G0/G1/S
D	3;1	G0/G1/S
D	3;2	G0/G1/S
D	3.3	G0/G1/S
D	4;1	G2/M
D	4;2	G2/M
D	5;1	G0/G1/S
D	6;1	G0/G1/S
D	7;1	G0/G1/S

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 ...)

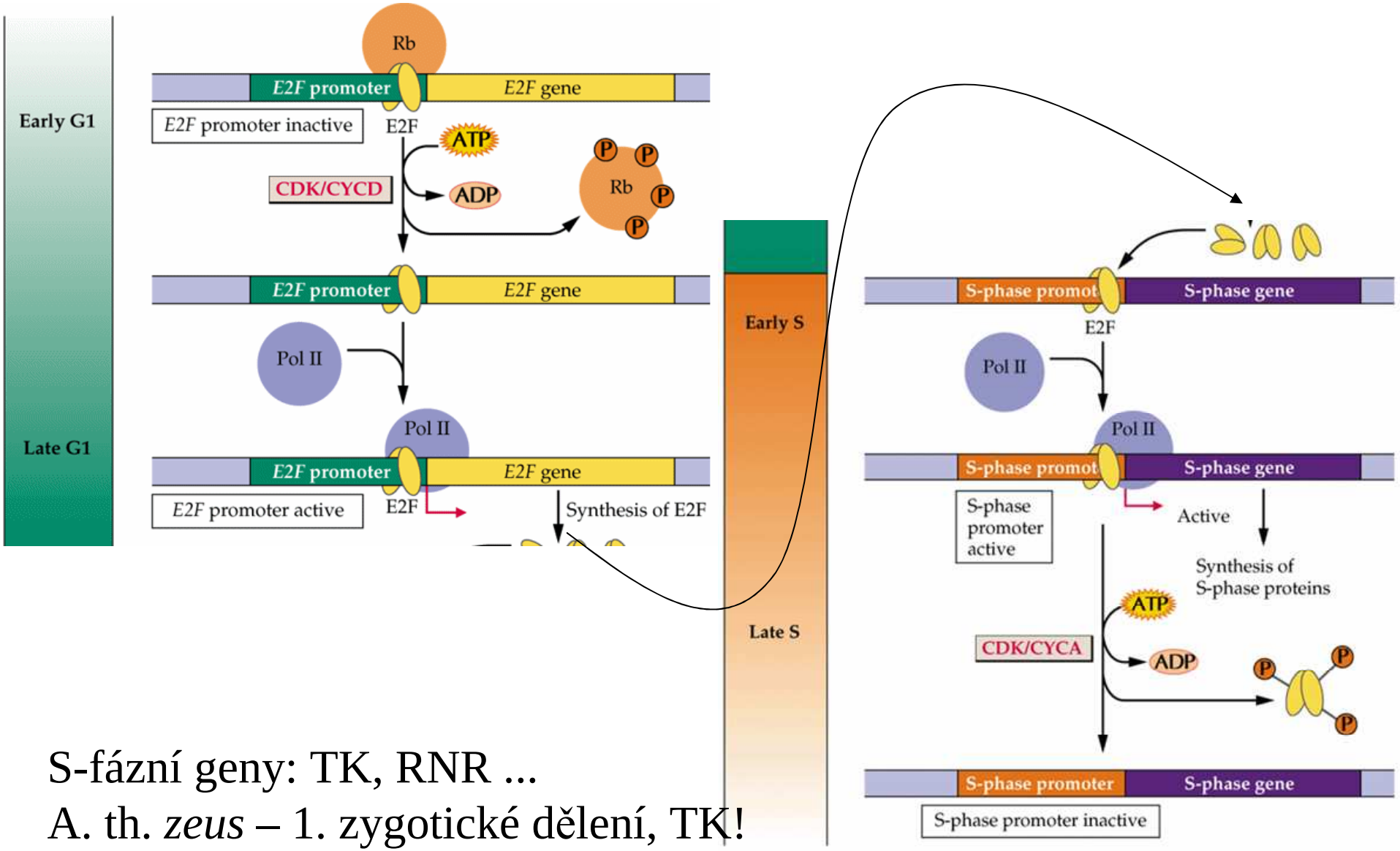
Cykliny jsou spřízněné s TFIIB a pRb!



Nucleic Acids Res. 1994 March 25; 22(6): 946–952.

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

Transkripční regulace - E2F, pRB



S-fázní geny: TK, RNR ...

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

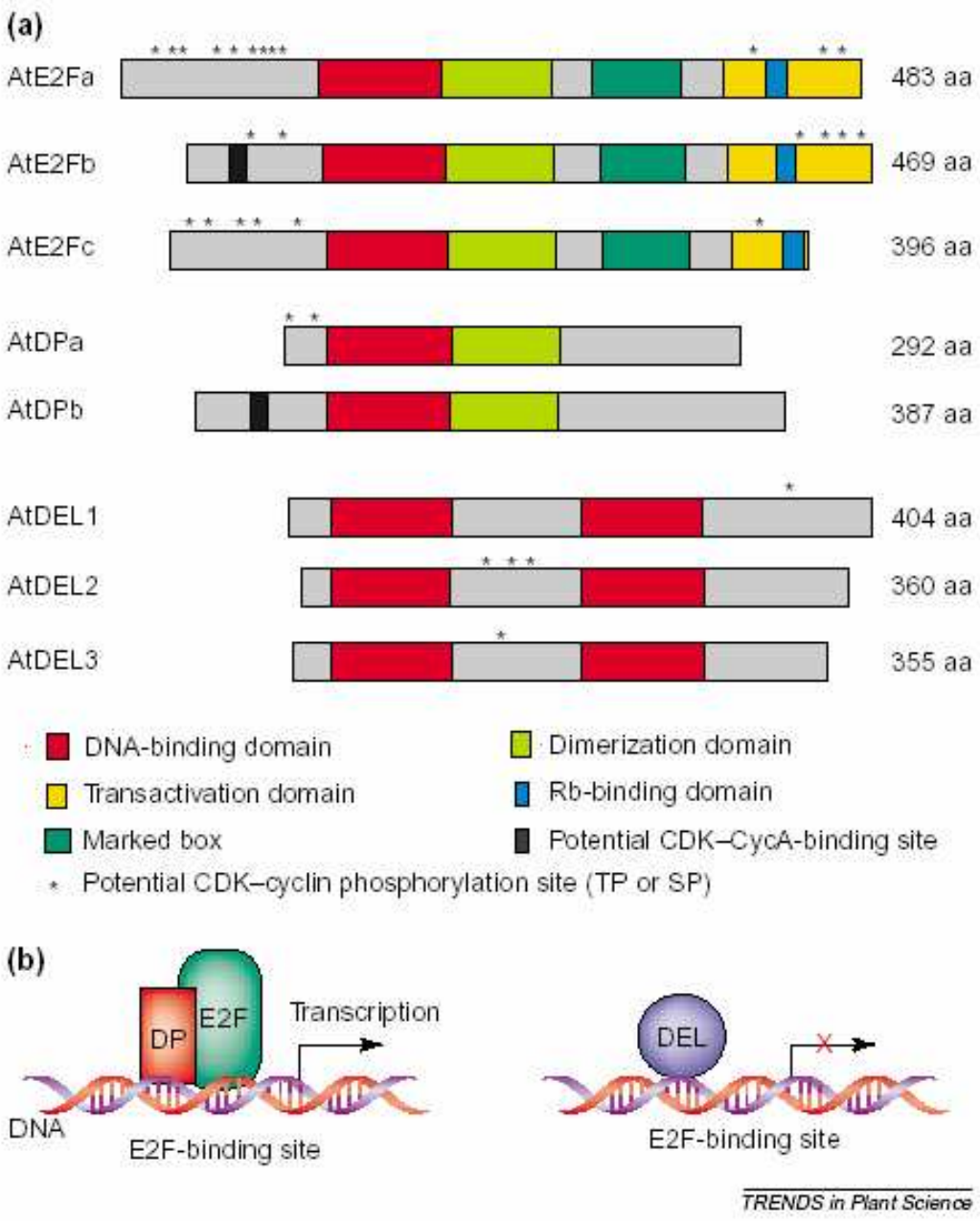


Fig. 1. Structural organization and DNA-binding properties of the *Arabidopsis* E2F-family proteins. (a) The DNA-binding, dimerization, Marked-box and transactivation domains of the E2F-family proteins. The Rb-binding domain and the potential cyclin-dependent-kinase-cyclin-A (CDK-CycA)-binding domain 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].

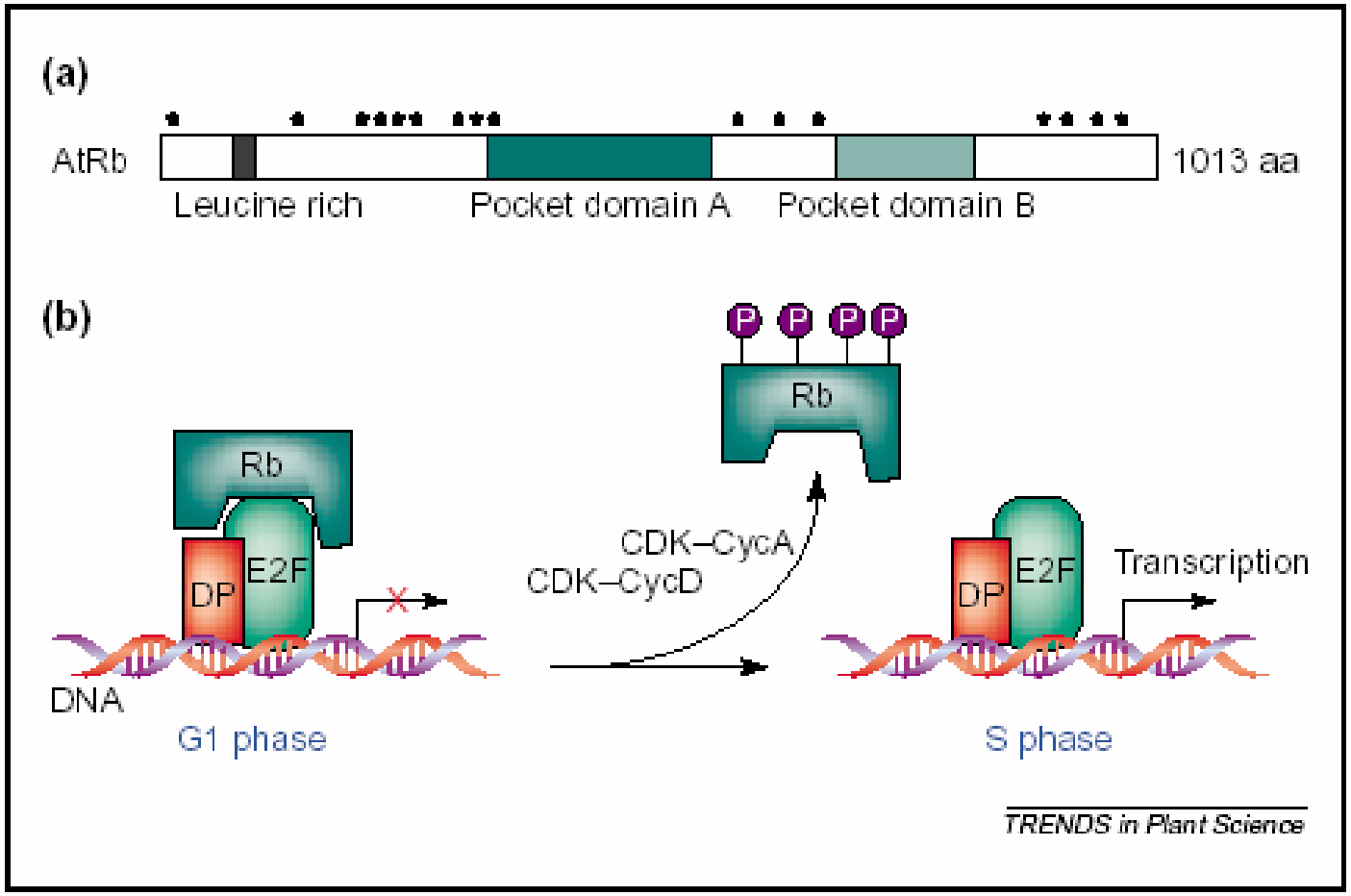
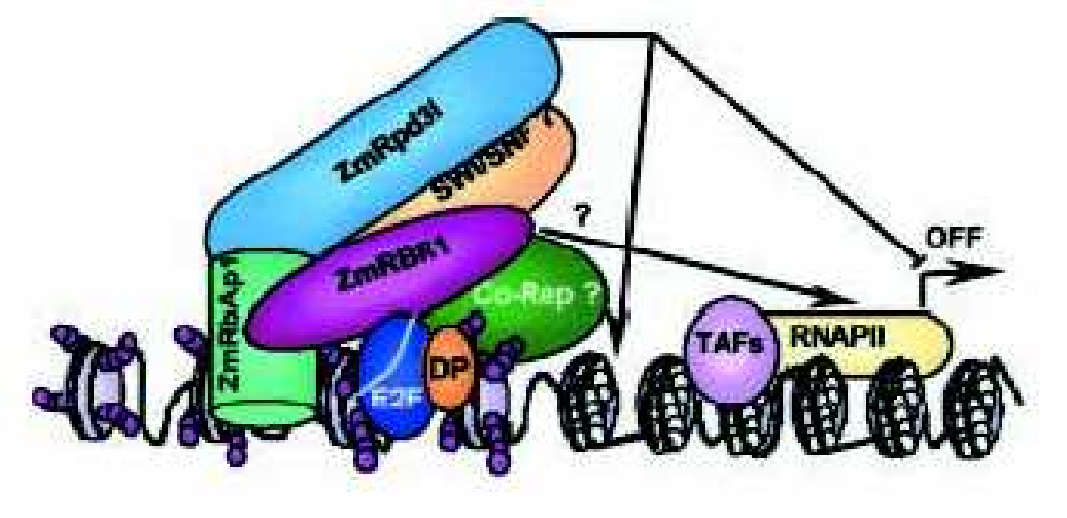


Fig. 2. (a) Structural organization of the *Arabidopsis* retinoblastoma (Rb) protein, showing the conserved pocket domains, the leucine-rich region and the potential cyclin-dependent-kinase- cyclin phosphorylation sites (asterisks). (b) Model for activation of the plant E2F- Rb pathway at the G1-to-S-phase 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.

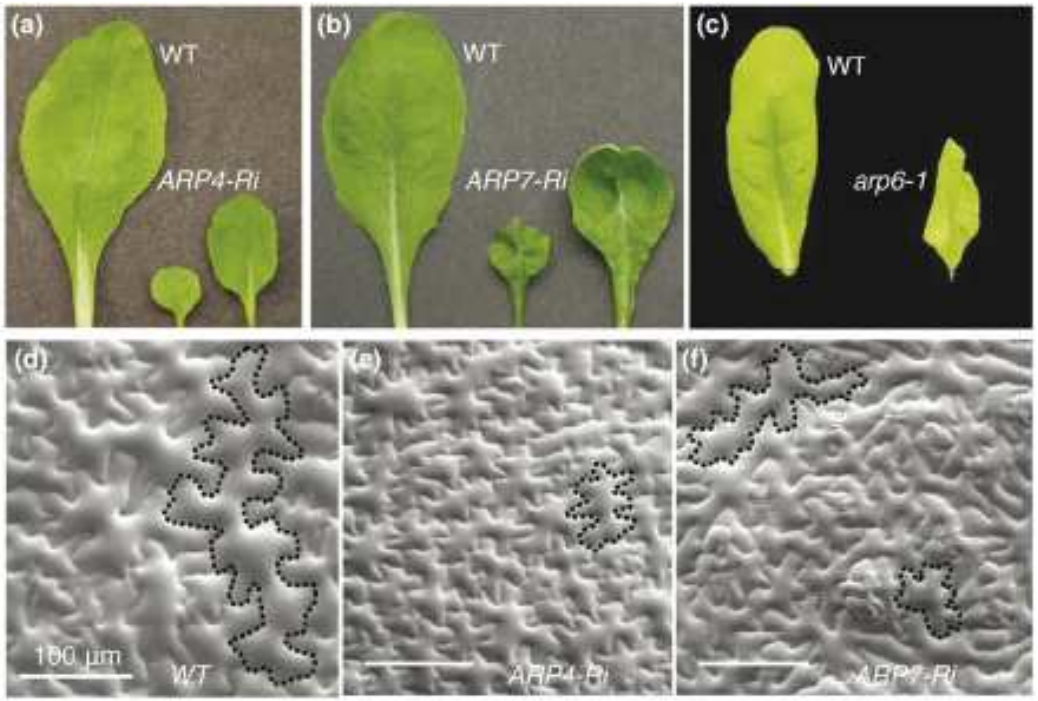
Mechanismus represe je možná trochu složitější ...



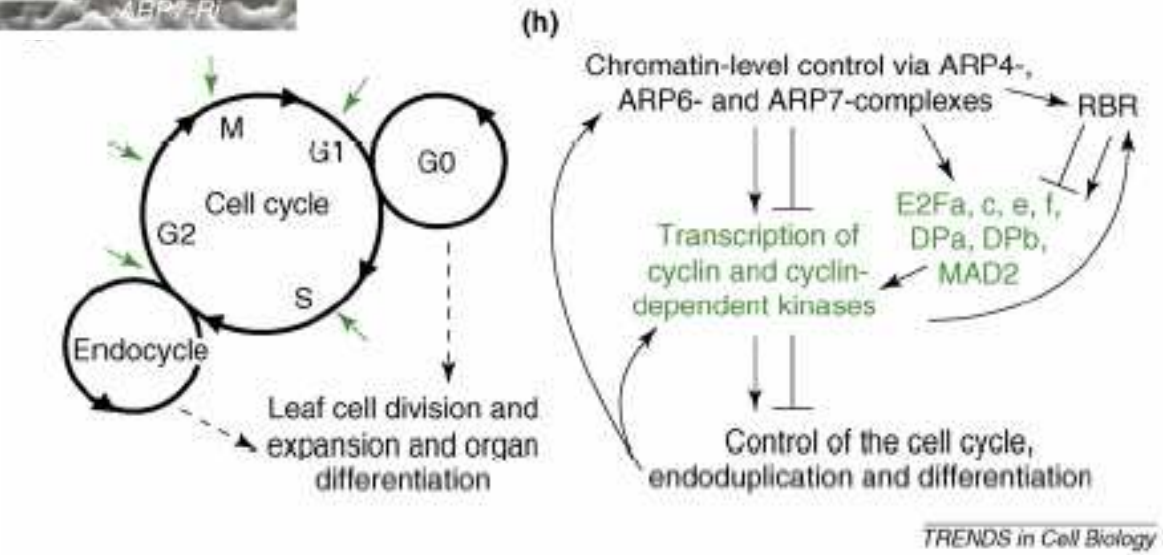
Rb indukuje deacetylaci histonů, což brání transkripc přísluš. oblasti chromatinu

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 i ARP4,6,7!

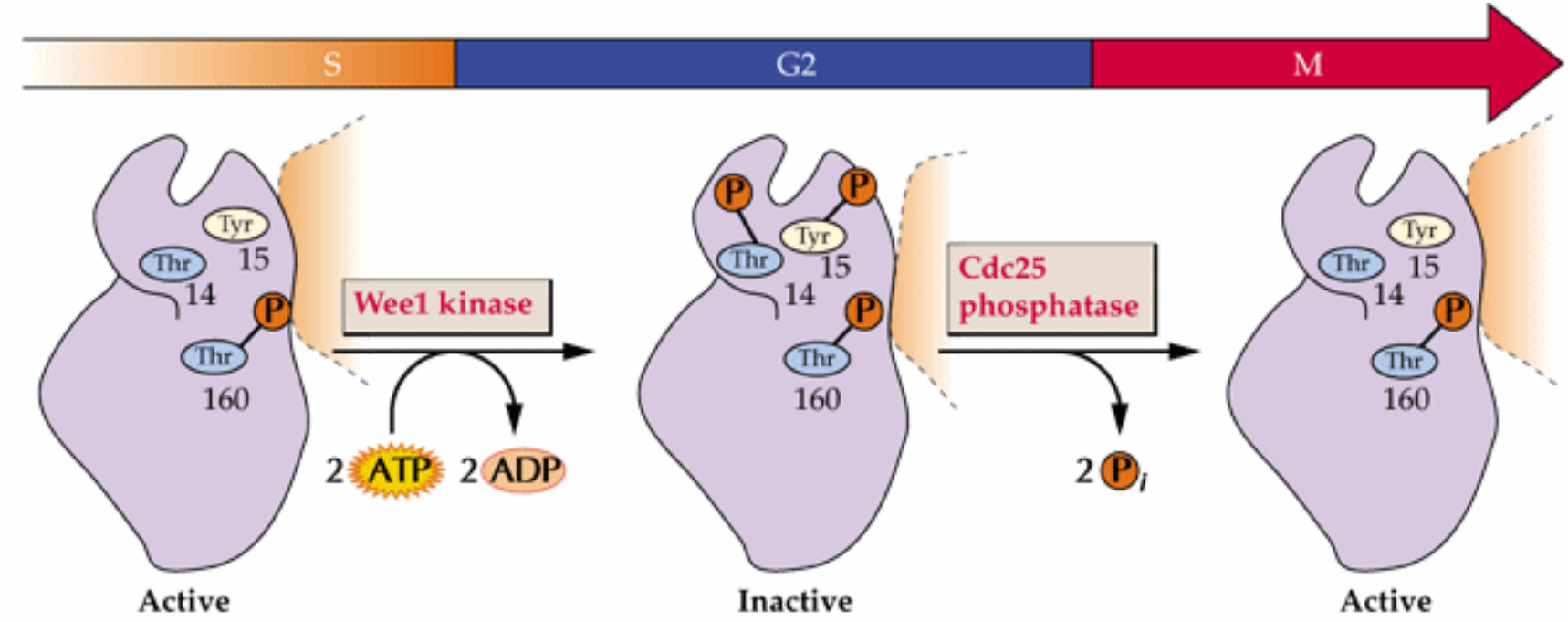


(přes kondensaci chromatinu – pleiotropní, vliv též na kvetení a senescenci květů ...)

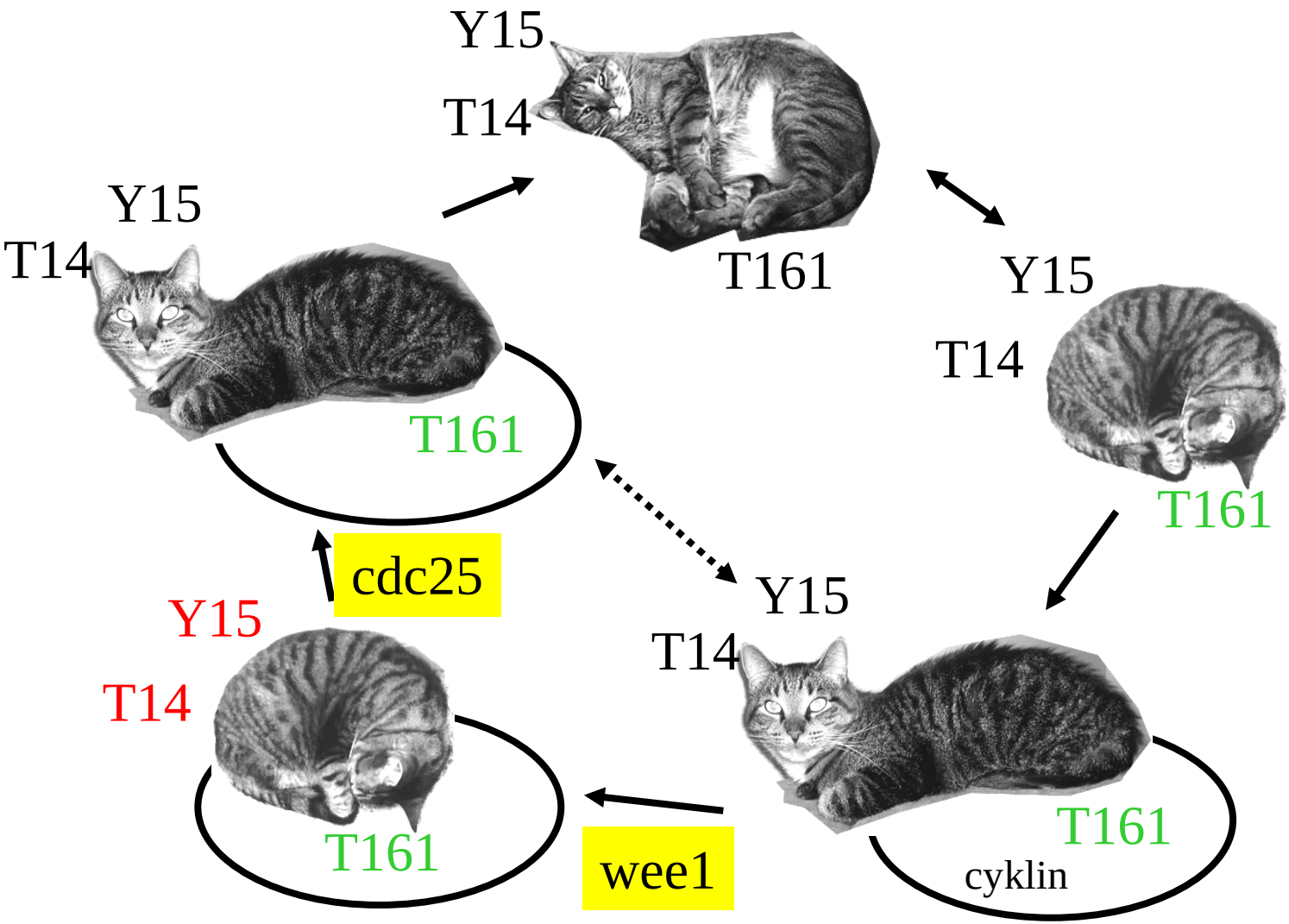


(Meagher et al. 2005, 2007)

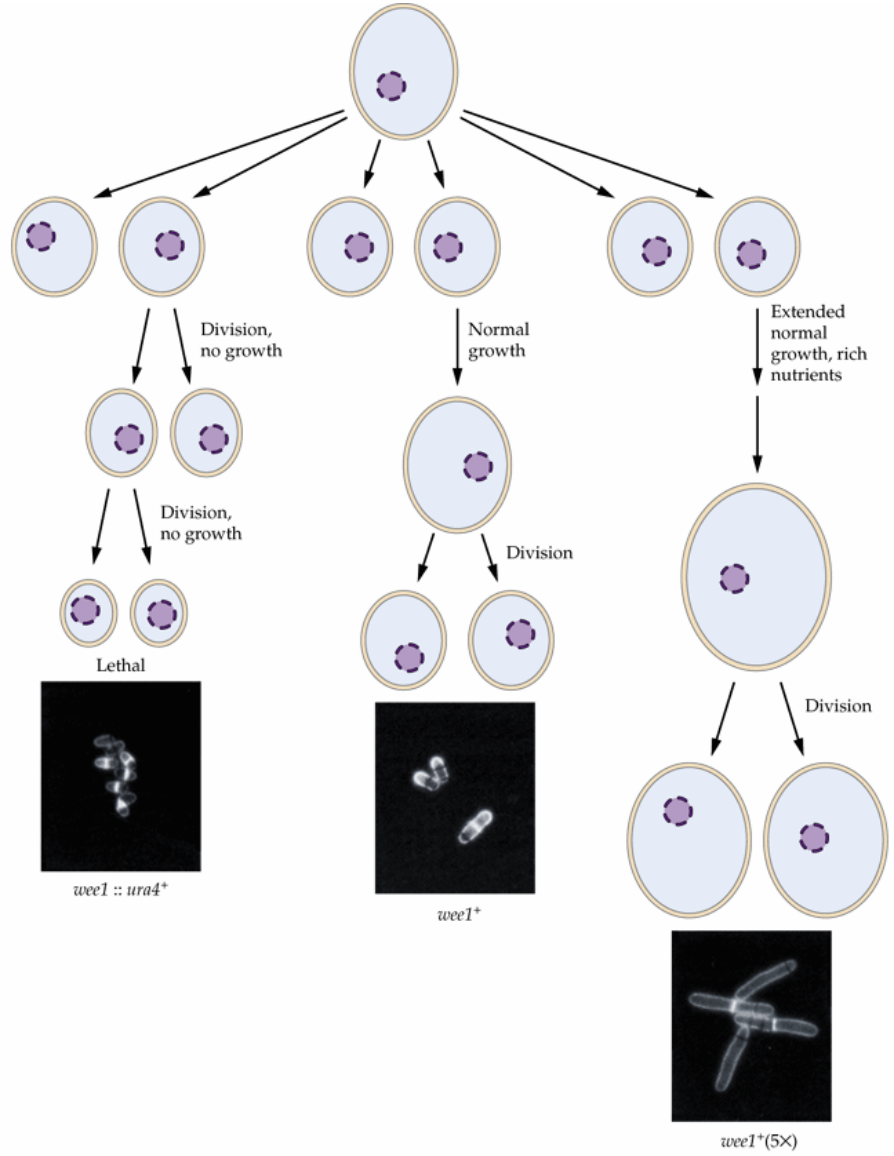
Regulace CDK: např. fosforylací



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

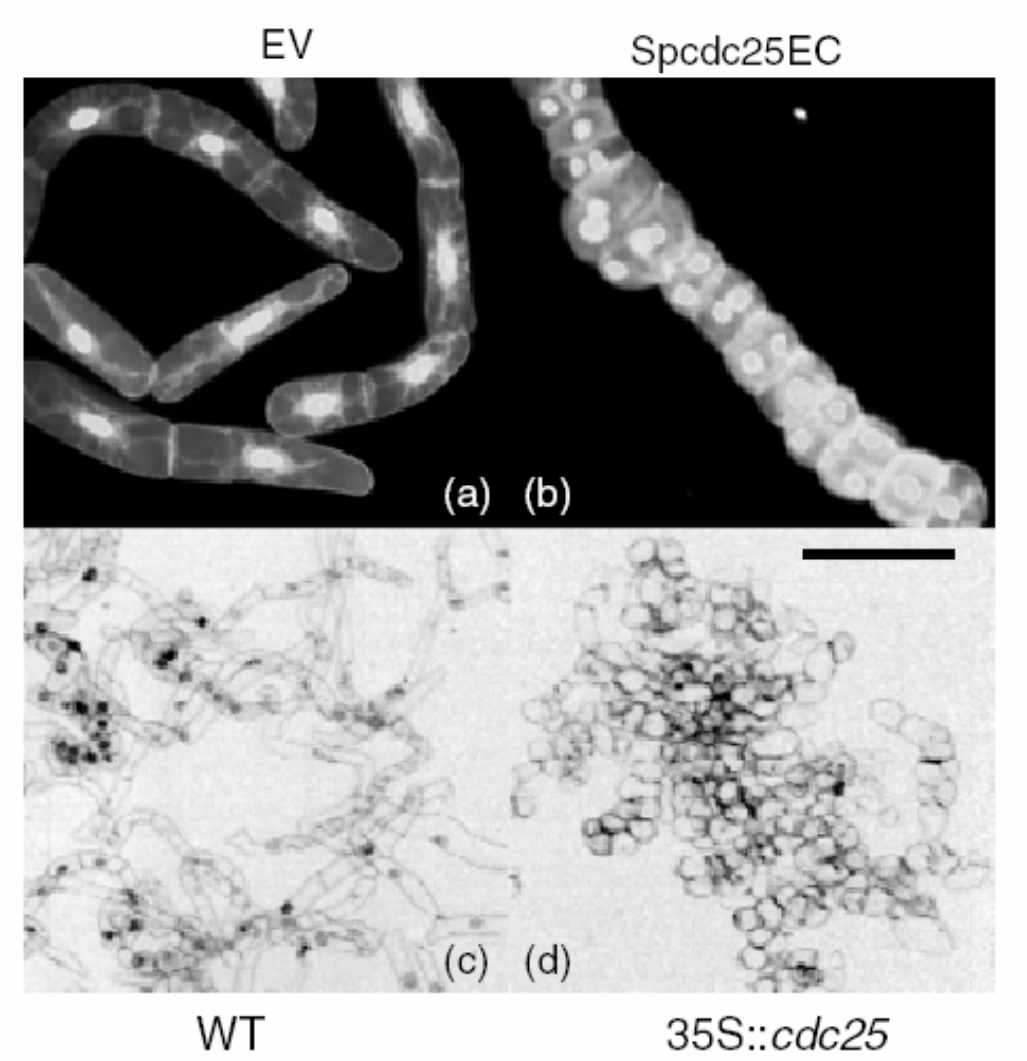


... jakožto jedna z cest ke spřažení cyklu a růstu



(S. pombe)

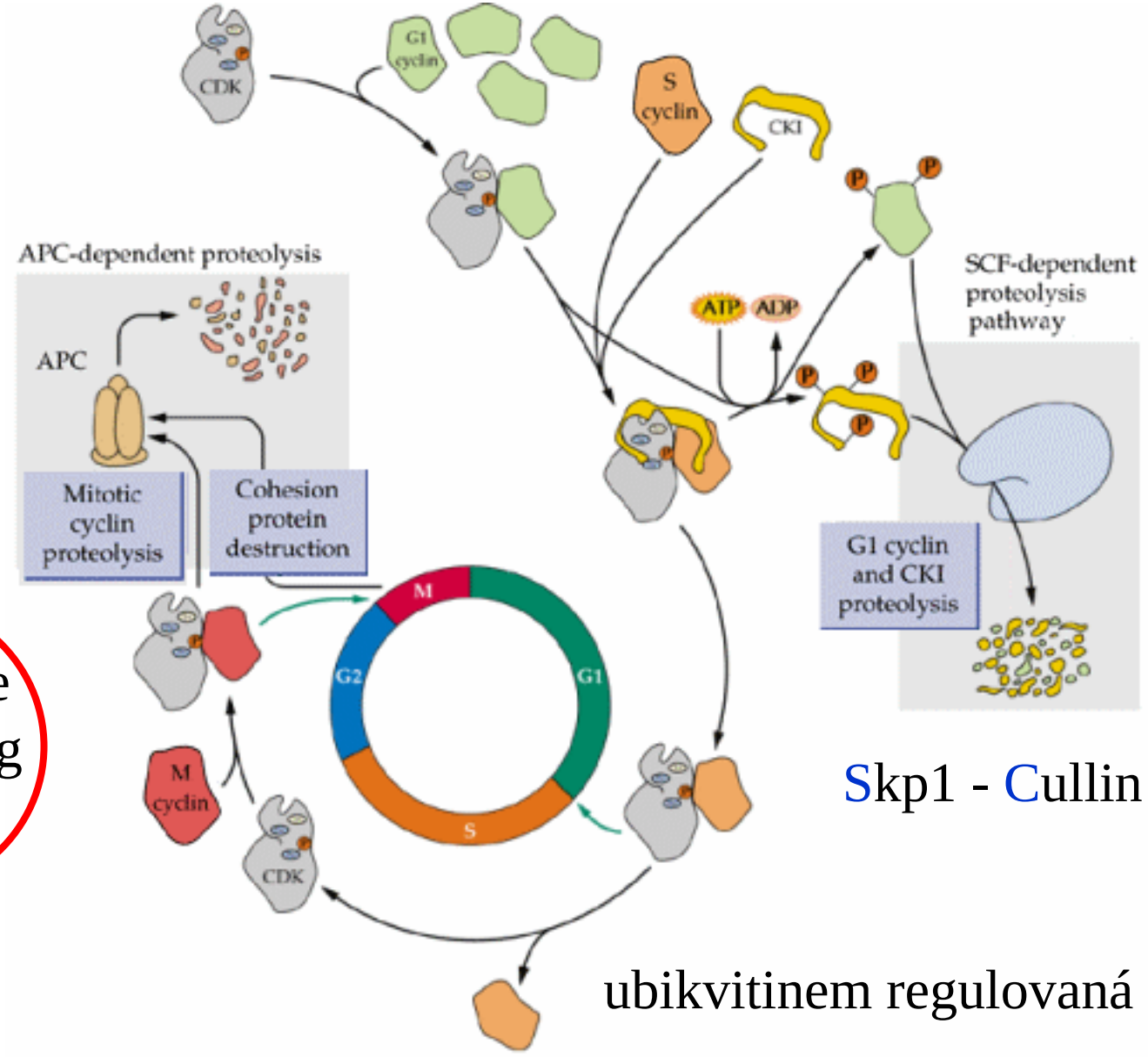
Exprese kvasinkového Cdc25 v rost. buňkách má fenotyp



Na celých rostlinách „cytokinin-like“ efekty…

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

Proteolýza v regulaci BC



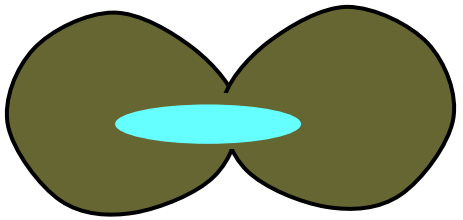
Anaphase promoting complex

Skp1 - Cullin - F-box

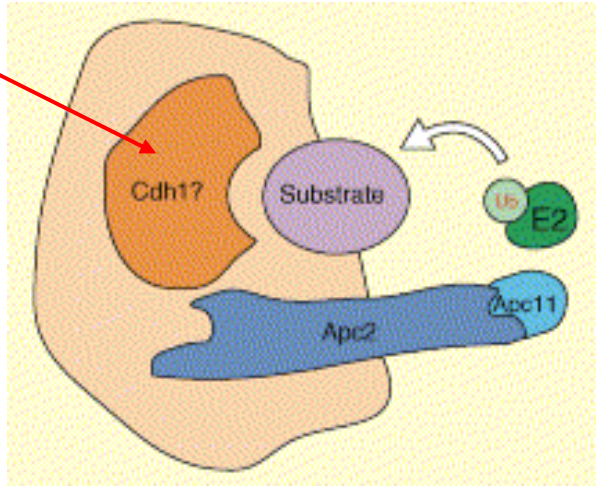
ubikvitinem regulovaná proteolýza

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 Ⓟace!)

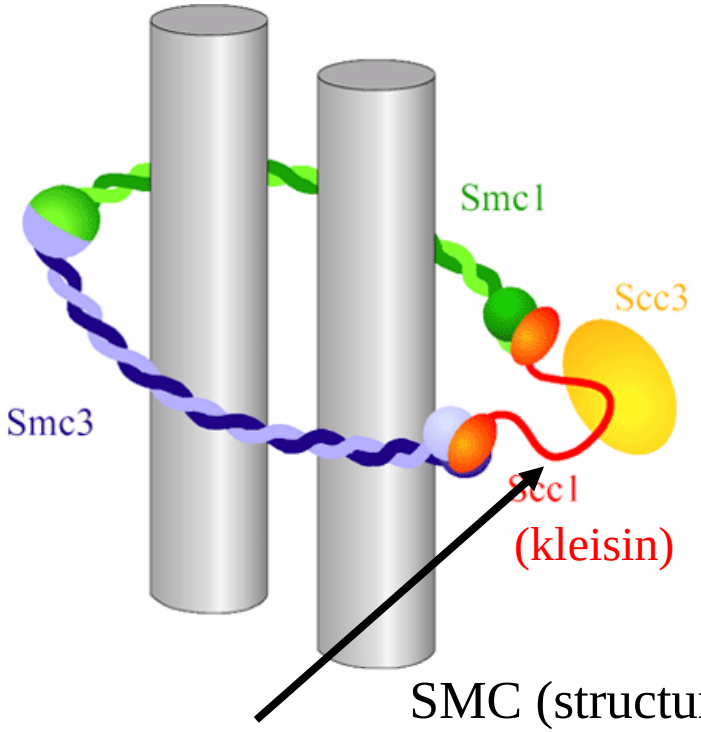


kvasinka: mND fenotyp



Další role APC: separace chromosomů

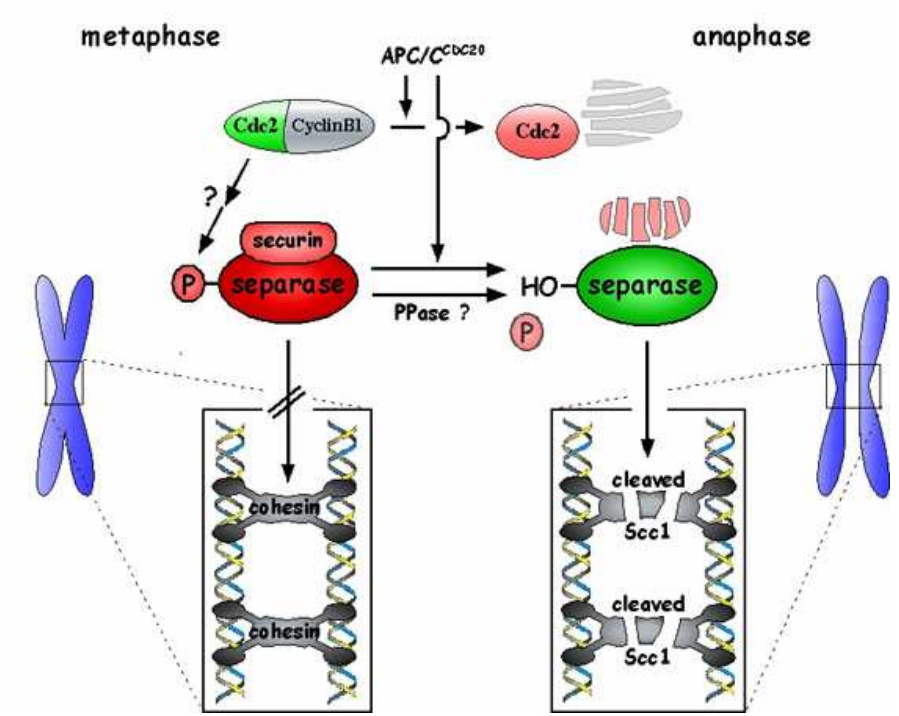
Sesterské chromatidy drží pohromadě kohesinové komplexy.



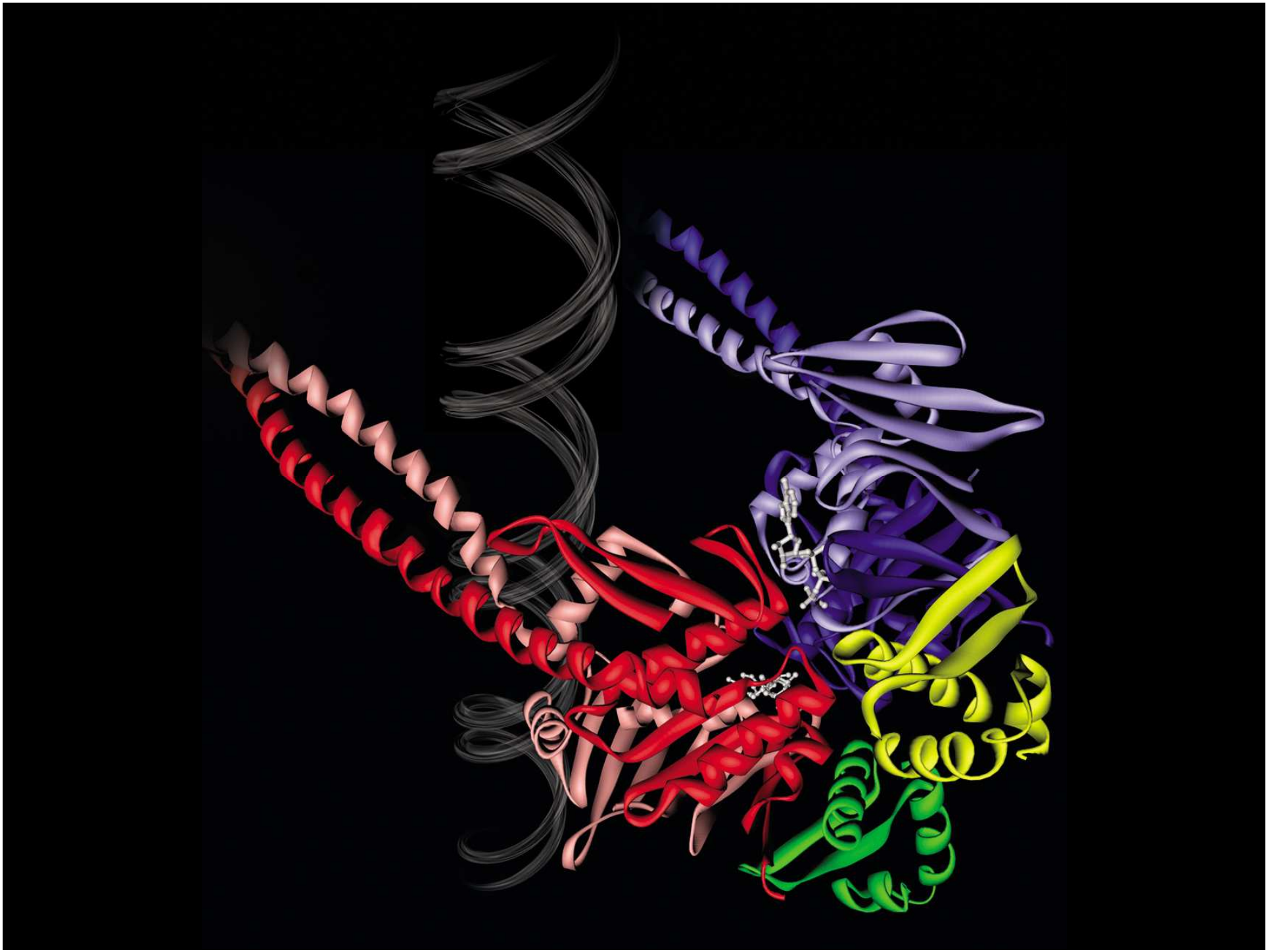
místo proteolyt. štěpení

SMC (structural maintenance of chromosomes)

SCC (sister chromatid cohesion)



(C. H. Haering)

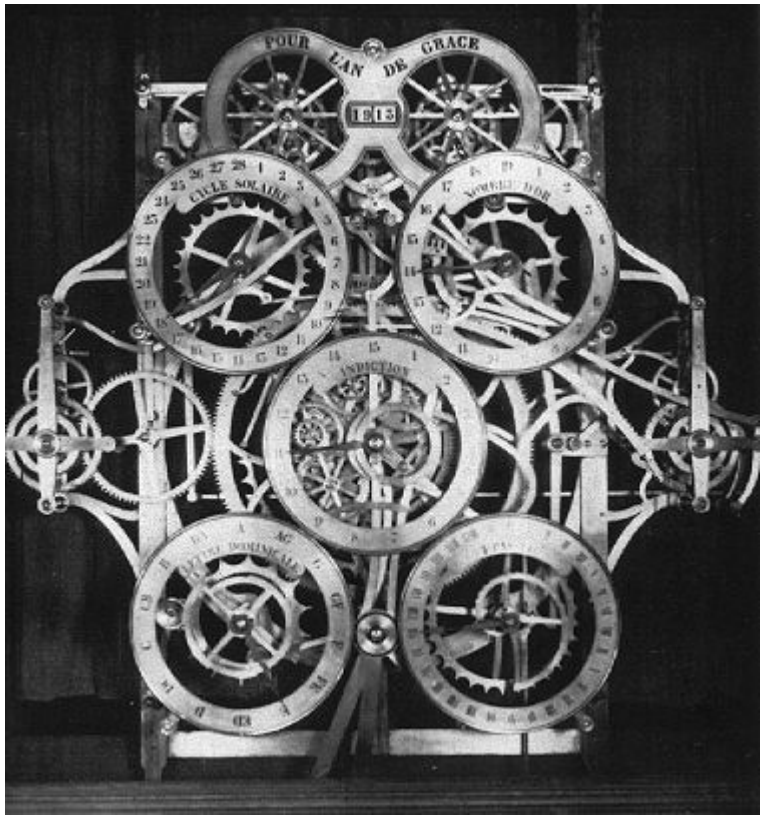


Ústřední hodiny buněčného cyklu

centrální oscilátor
(„cell cycle engine“)

vstupy

velikost
signály
poškození ...

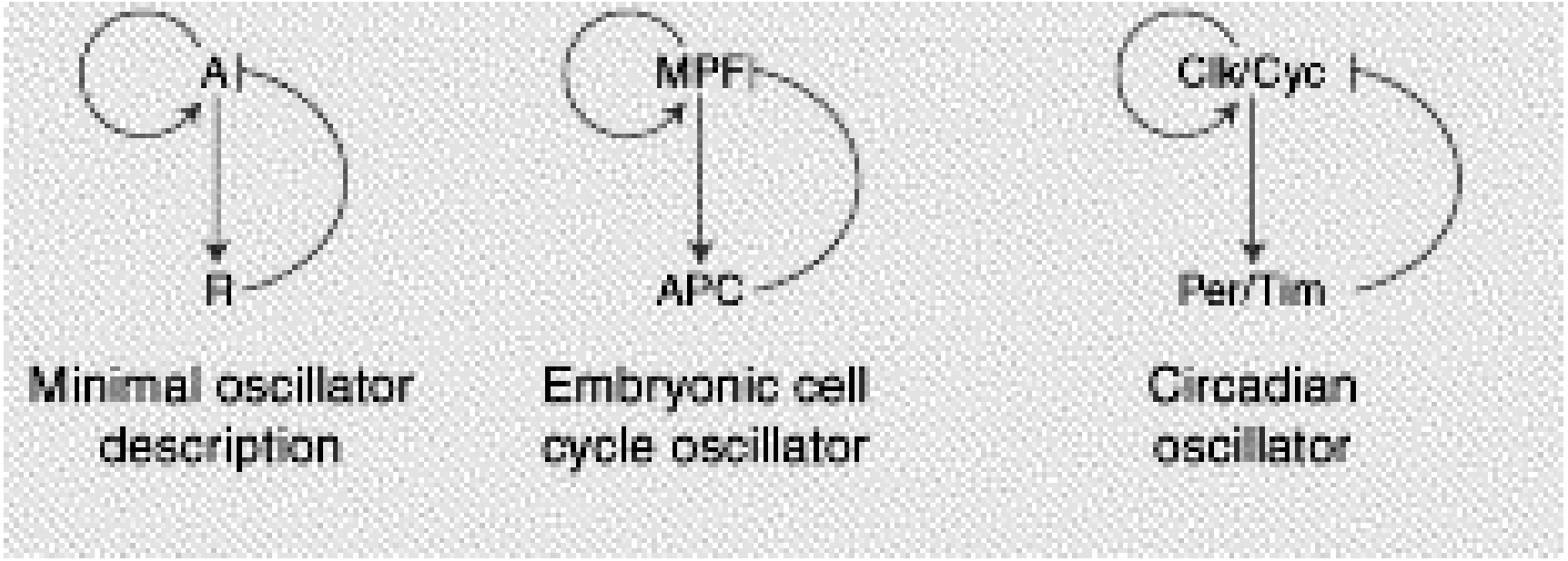


výstupy

gen. exprese
mitosa,
cytokinese ...

Jak vůbec lze zajistit pravidelný chod - oscilace?

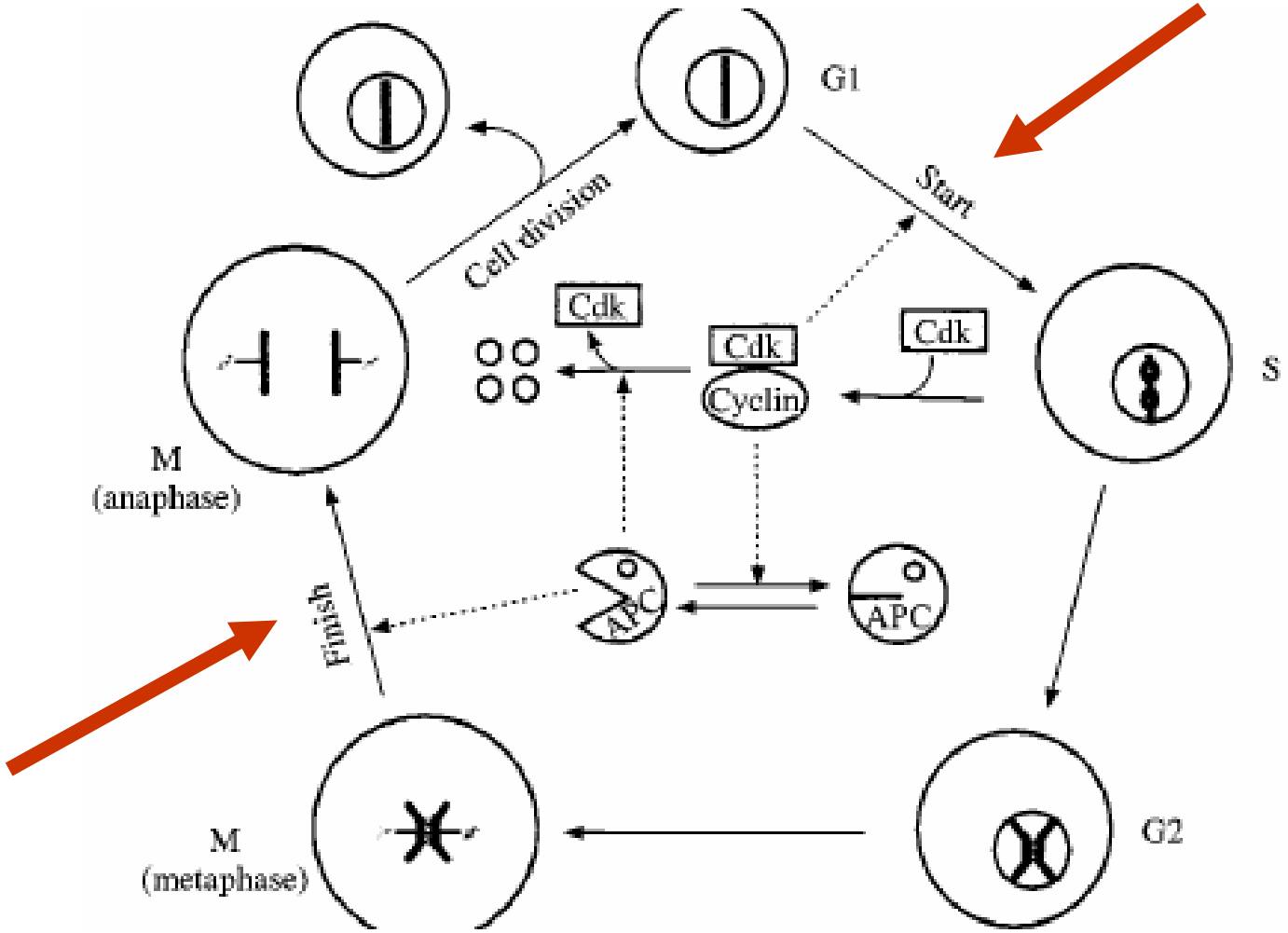
Minimální oscilátor (jeden z mnoha)



Je lepší než jiné?? Problém robustnosti!

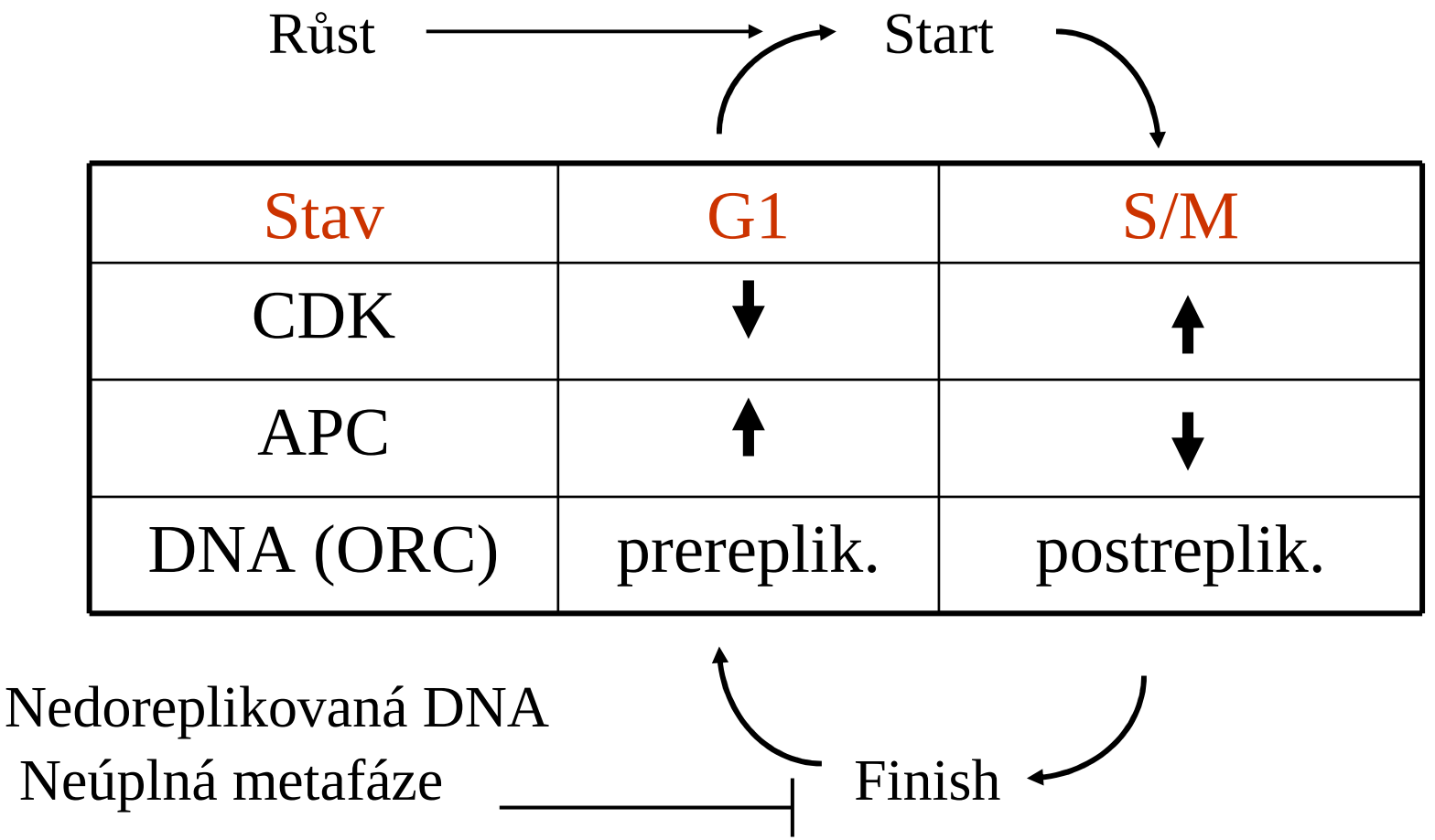
(Ingolia a Murray, Curr. Biol. 2004)

Oscilátor v kontextu tradičního pohledu



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

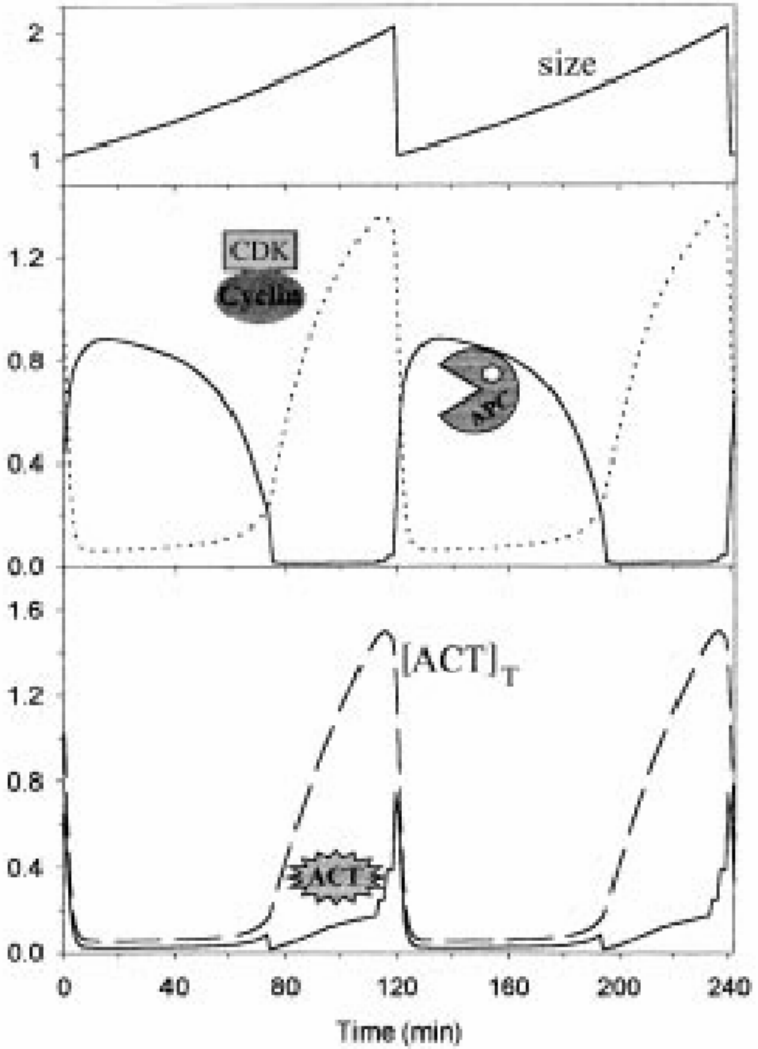
Dva stavy „cyklových hodin“



(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!



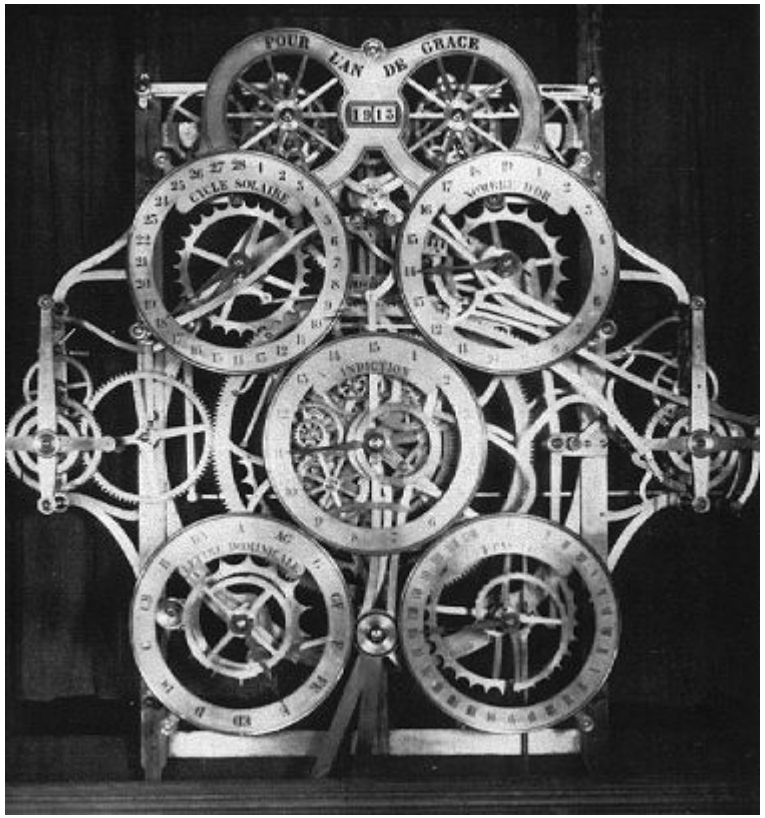
(Novak et al., Phil.Trans.R.Soc.Lond.B 353:2063-2076, 1998)

Vstupy a výstupy - (nejen) „rostlinná specifika“

centrální oscilátor
(„cell cycle engine“)

vstupy

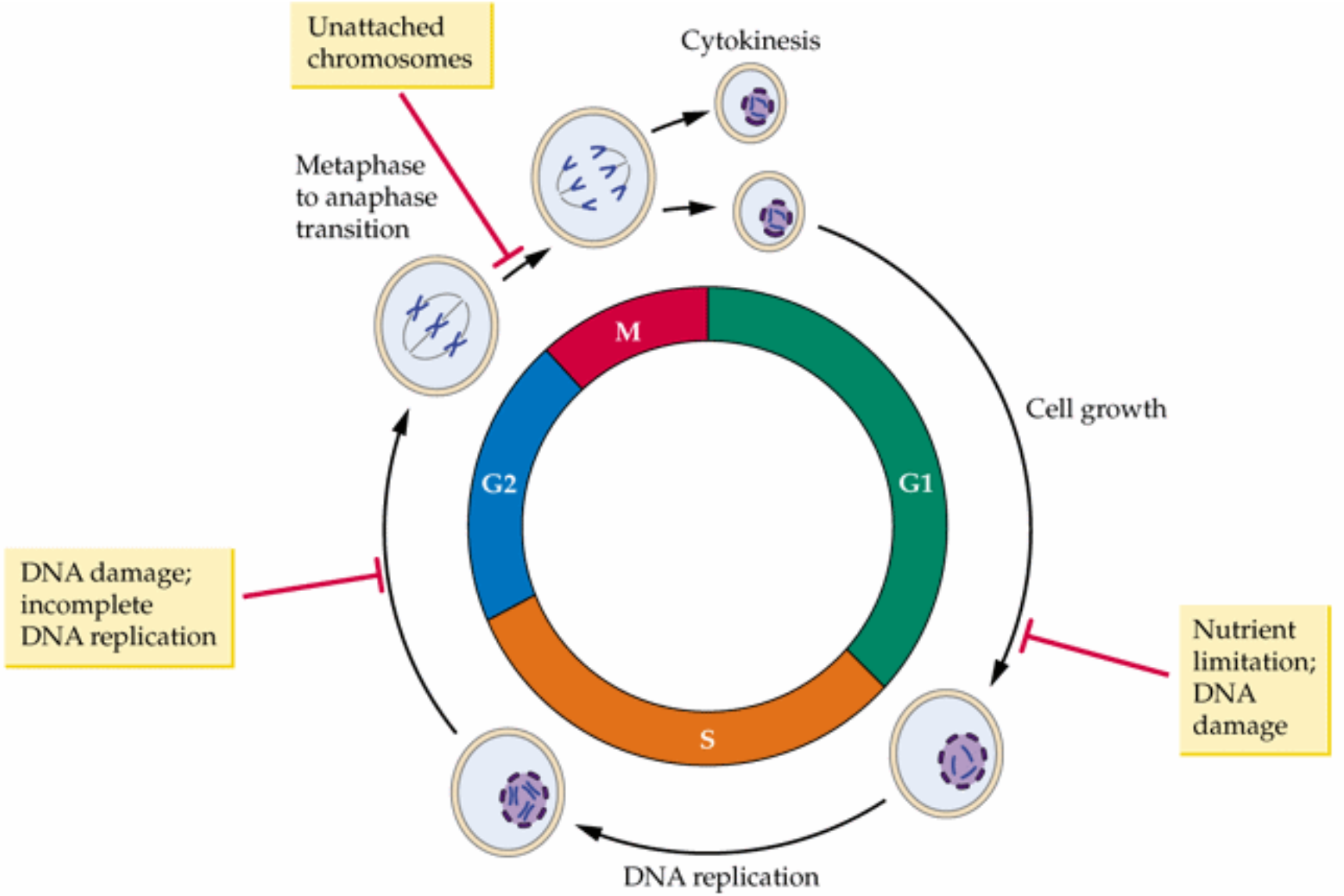
velikost
signály
poškození ...



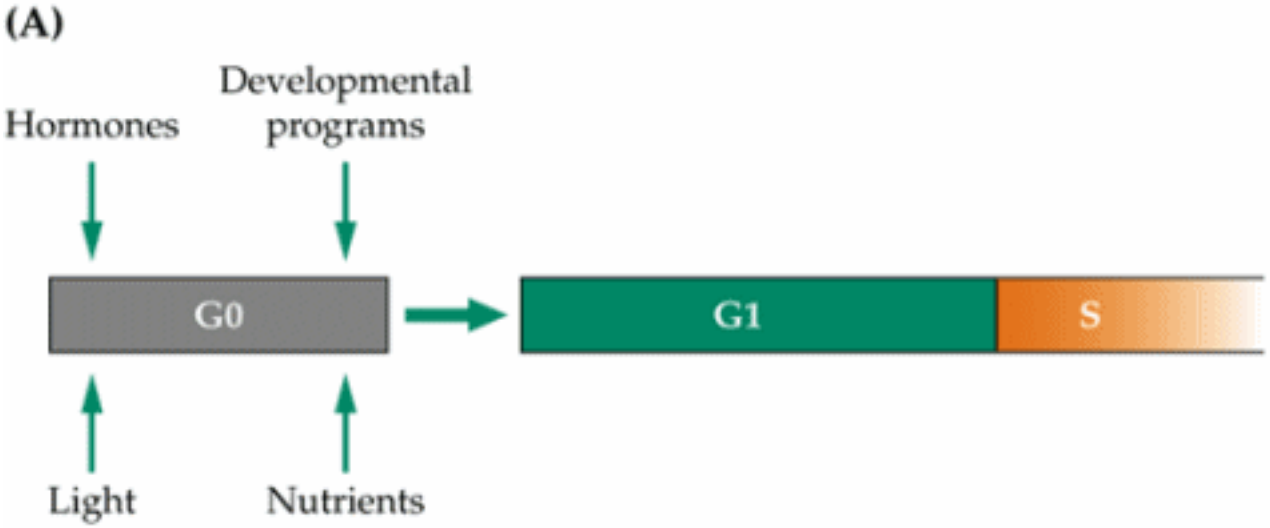
výstupy

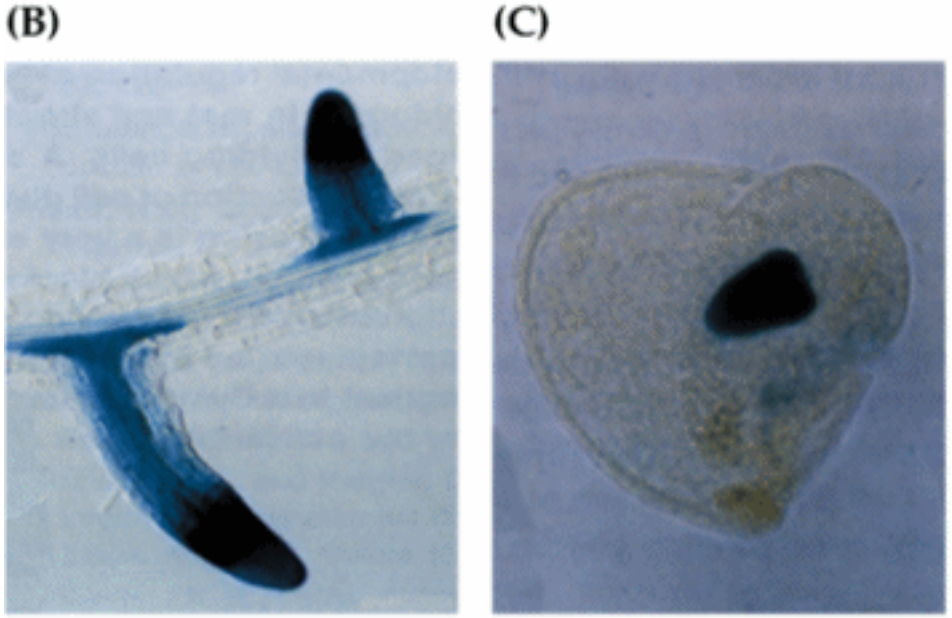
gen. exprese
replikace
mitosa,
cytokinese ...

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



Ontogenetická kontrola BC - rostlina vládne buňkám





AtCYCB1::GUS

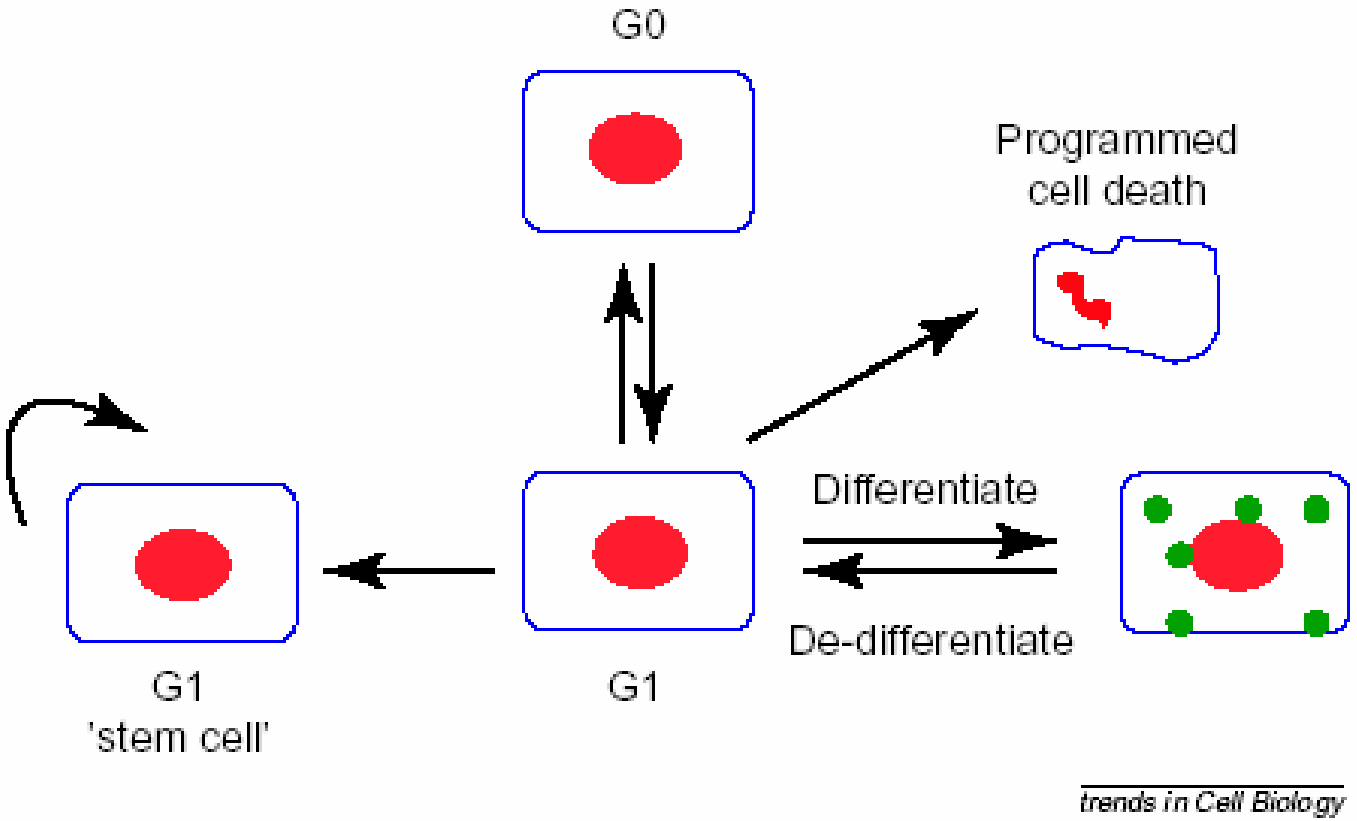
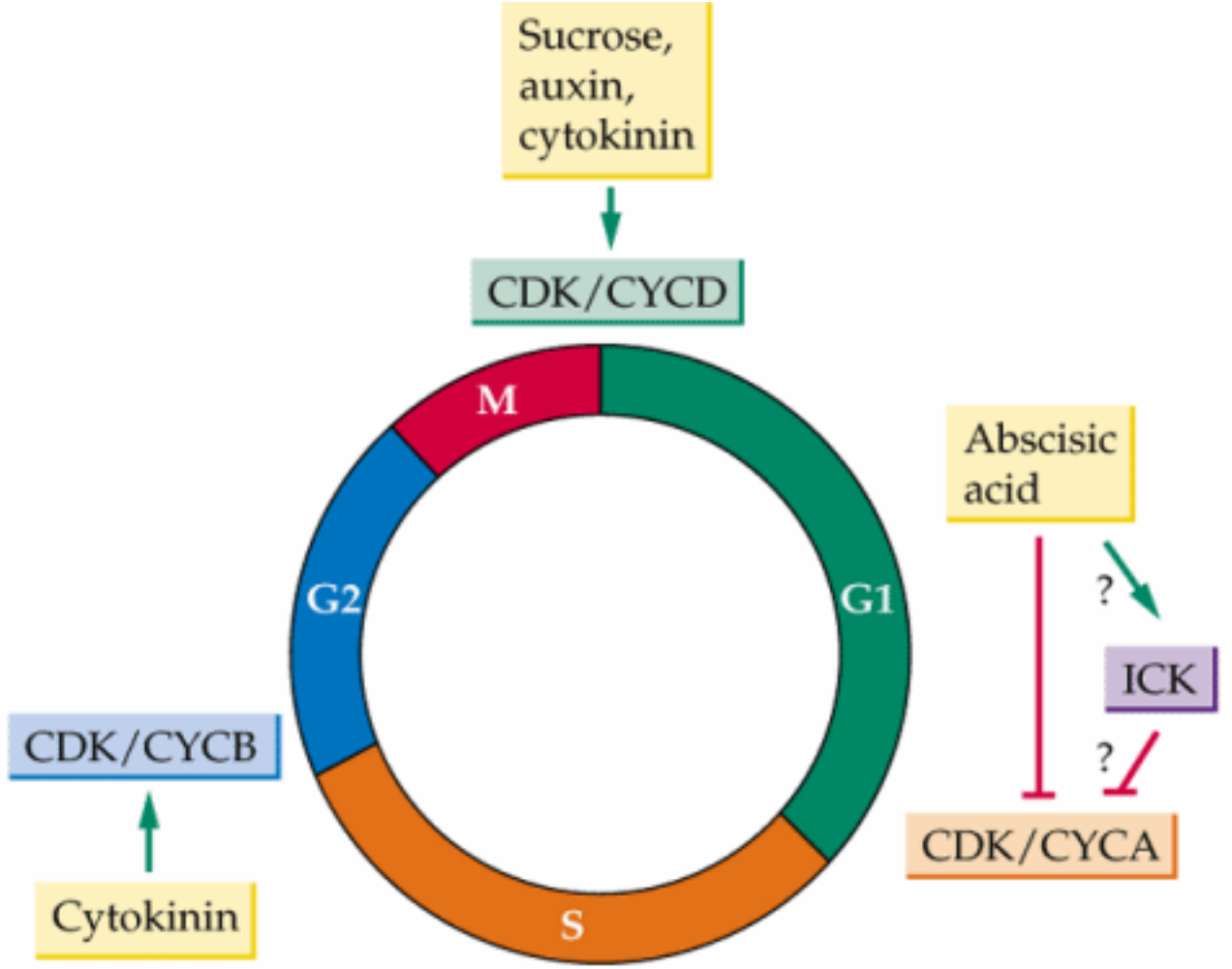


FIGURE 1

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 fytohormony



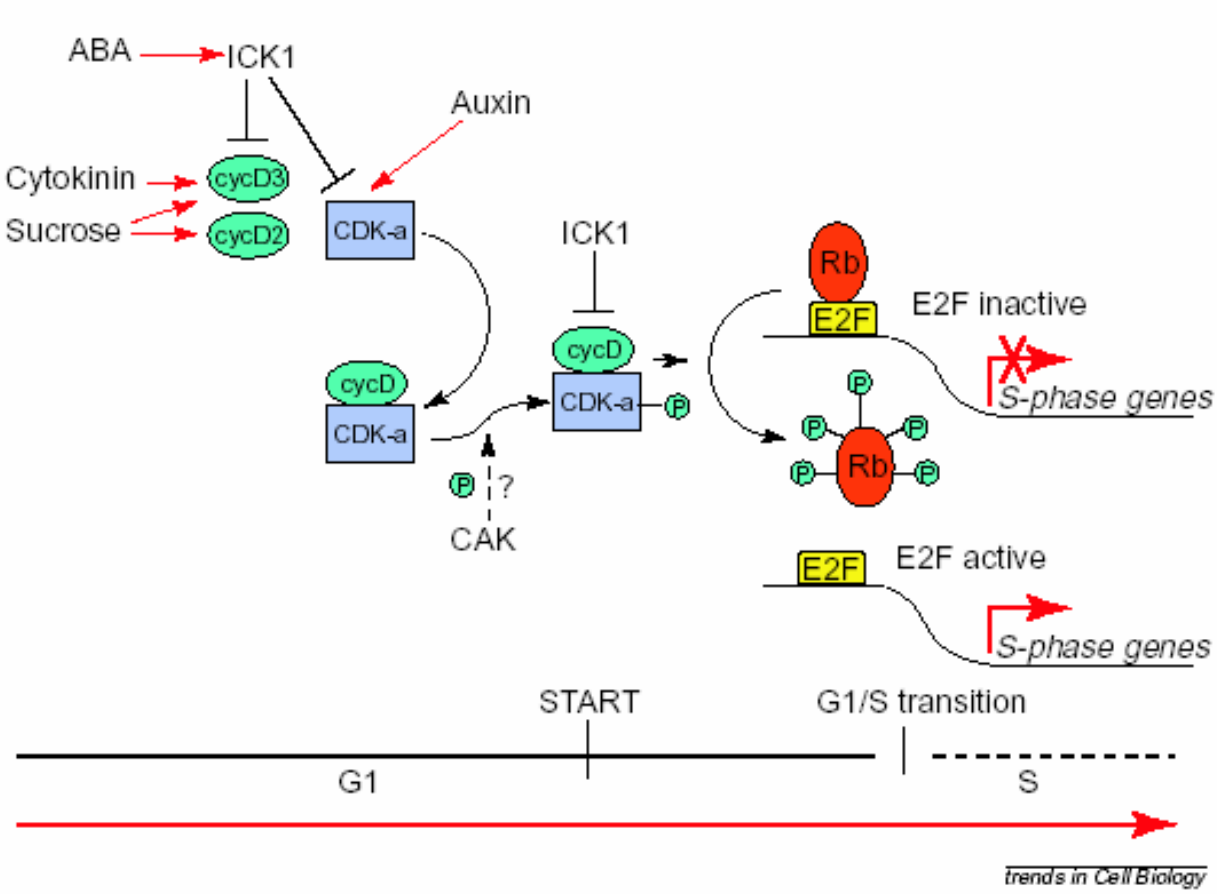
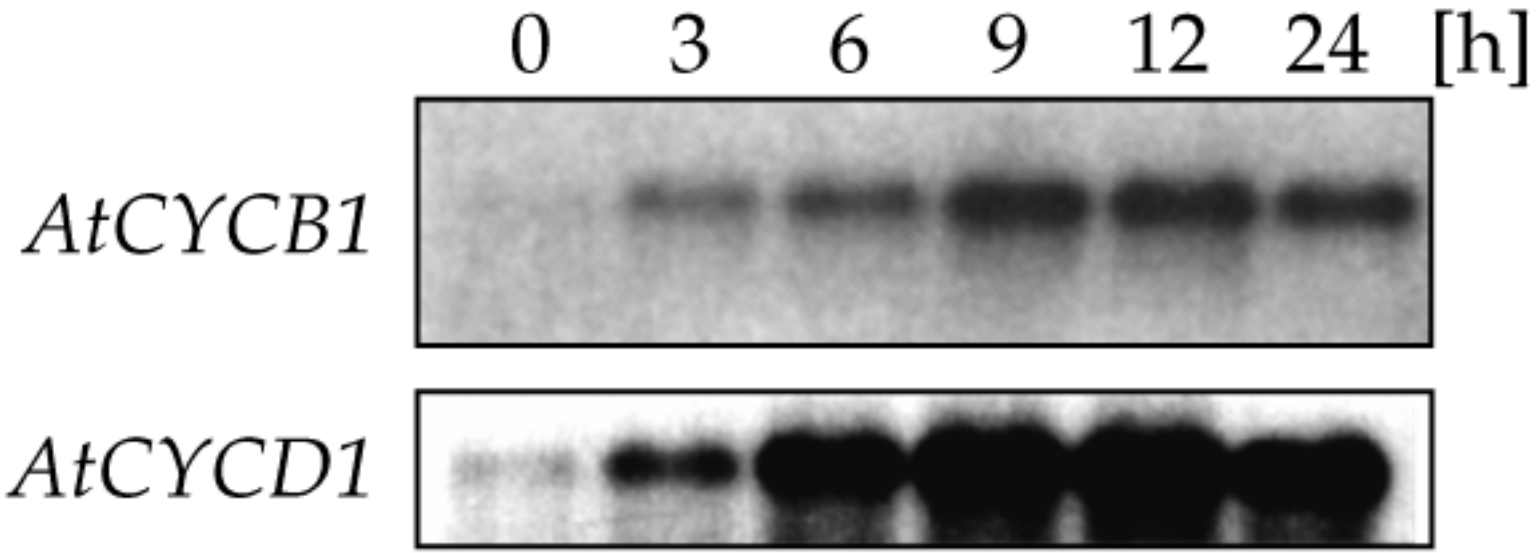


FIGURE 2

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)

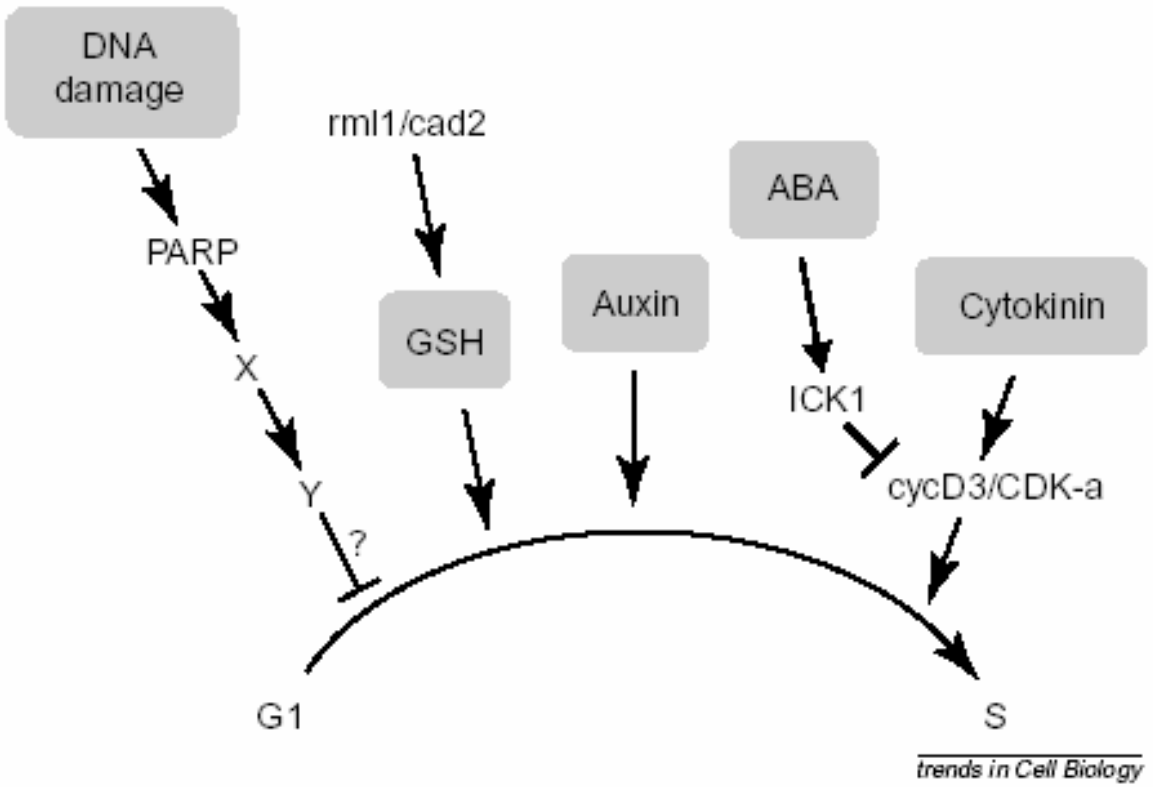
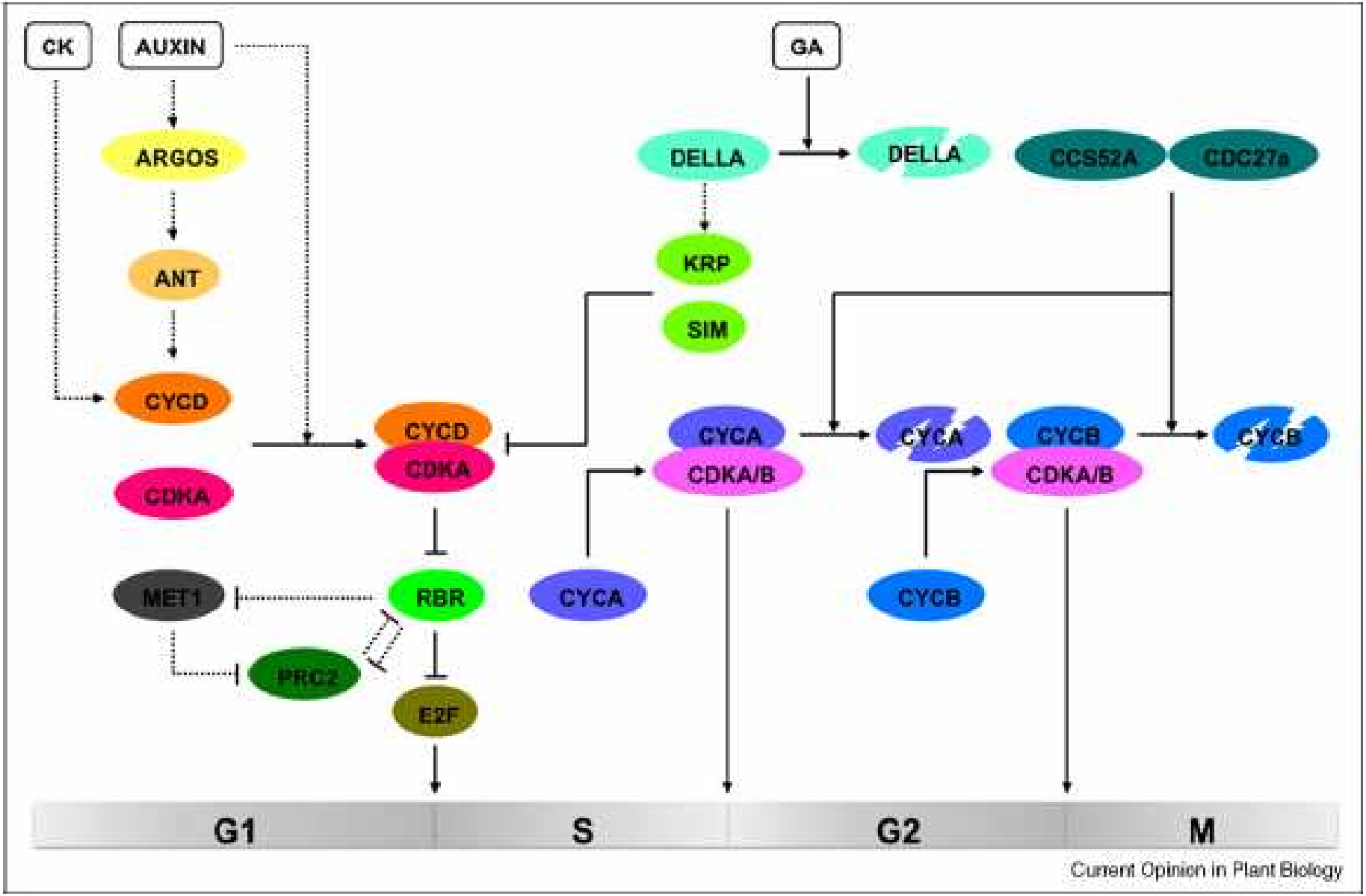
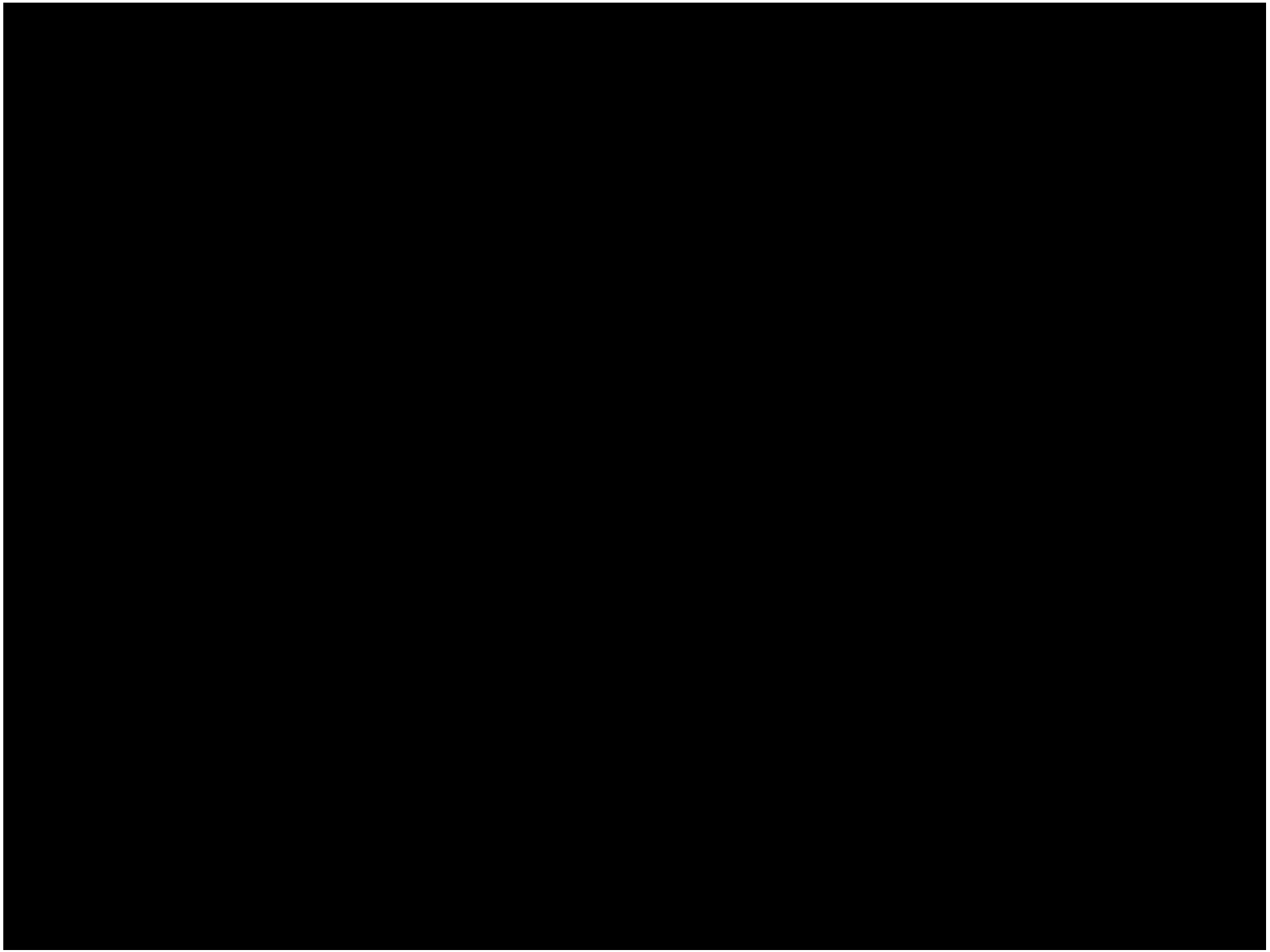


FIGURE 3

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.


CK
AUXIN
GA
ARGOS
DELLA
DELLA
CCS52A
CDC27a
ANT
KRP
SIM
CYCD
CYCD
CDKA
CYCA
CYCA
CYCB
CYCB
CDKA
CDKA/B
CDKA/B
MET1
RBR
CYCA
CYCB
PRC2
E2F
G1
S
G2
M
Current Opinion in Plant Biology

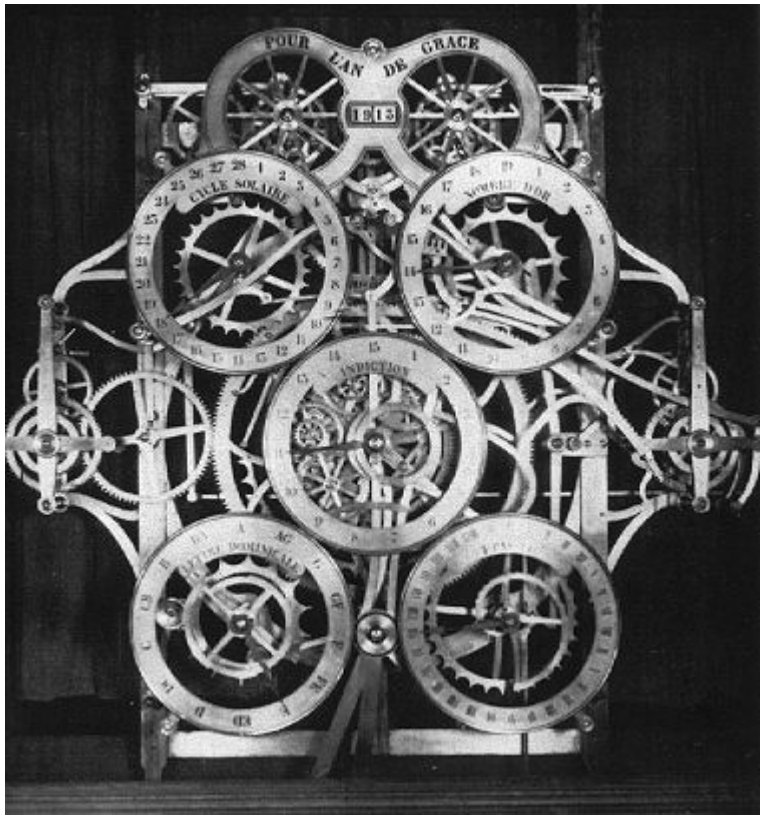


Rekapitulace:
Ústřední hodiny buněčného cyklu

centrální oscilátor
(„cell cycle engine“)

vstupy

velikost
signály
poškození ...

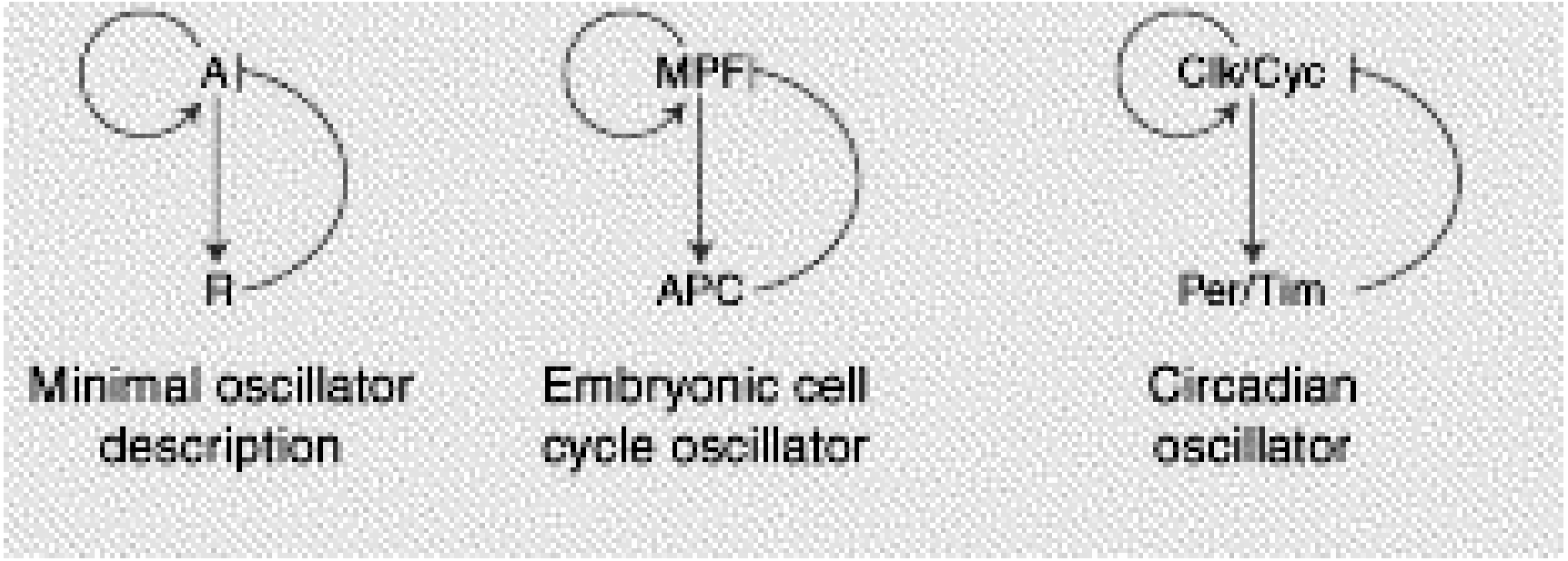


výstupy

gen. exprese
mitosa,
cytokinese ...

Jak vůbec lze zajistit pravidelný chod - oscilace?

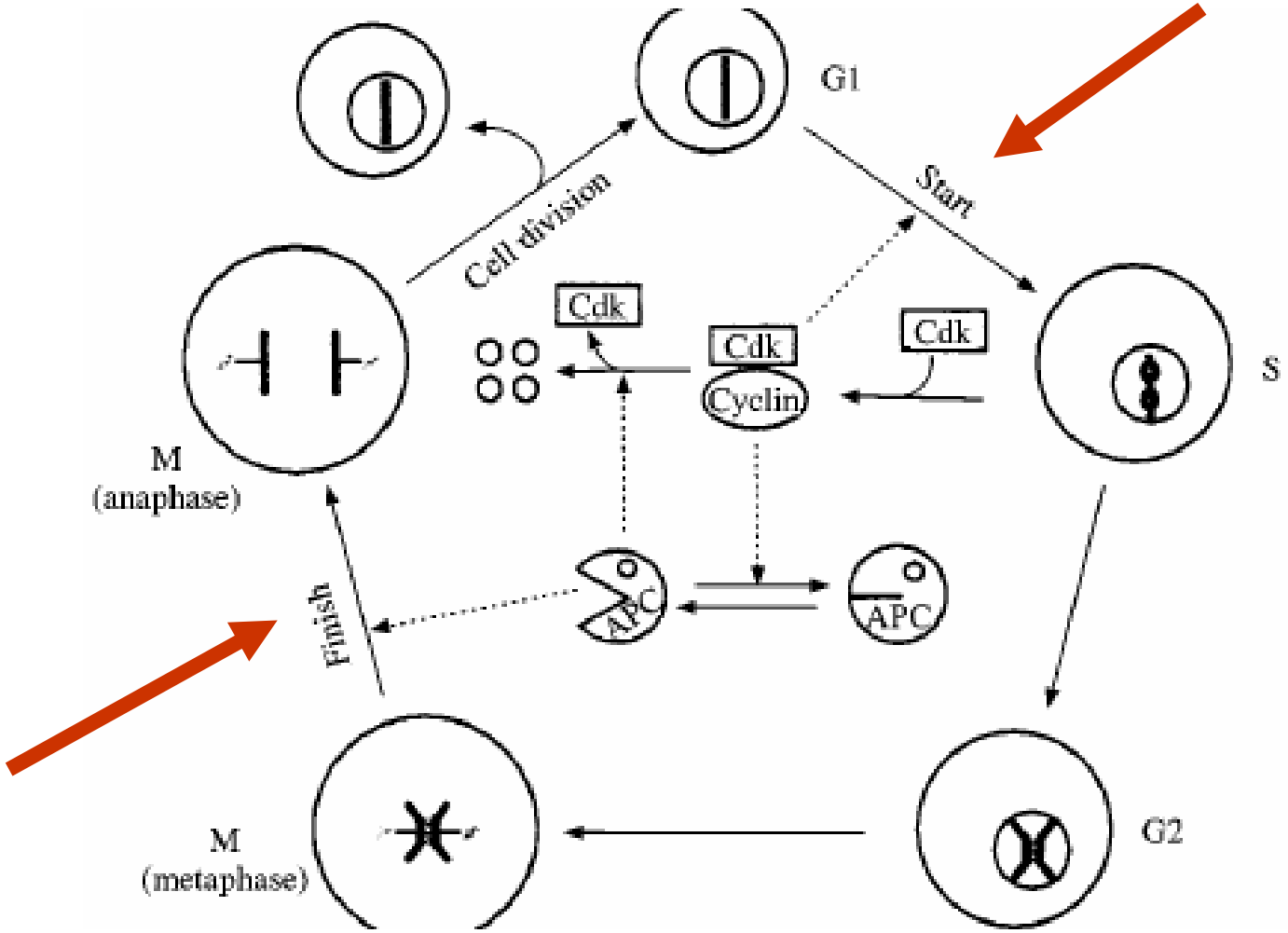
Minimální oscilátor (jeden z mnoha)



Je lepší než jiné?? Problém robustnosti!

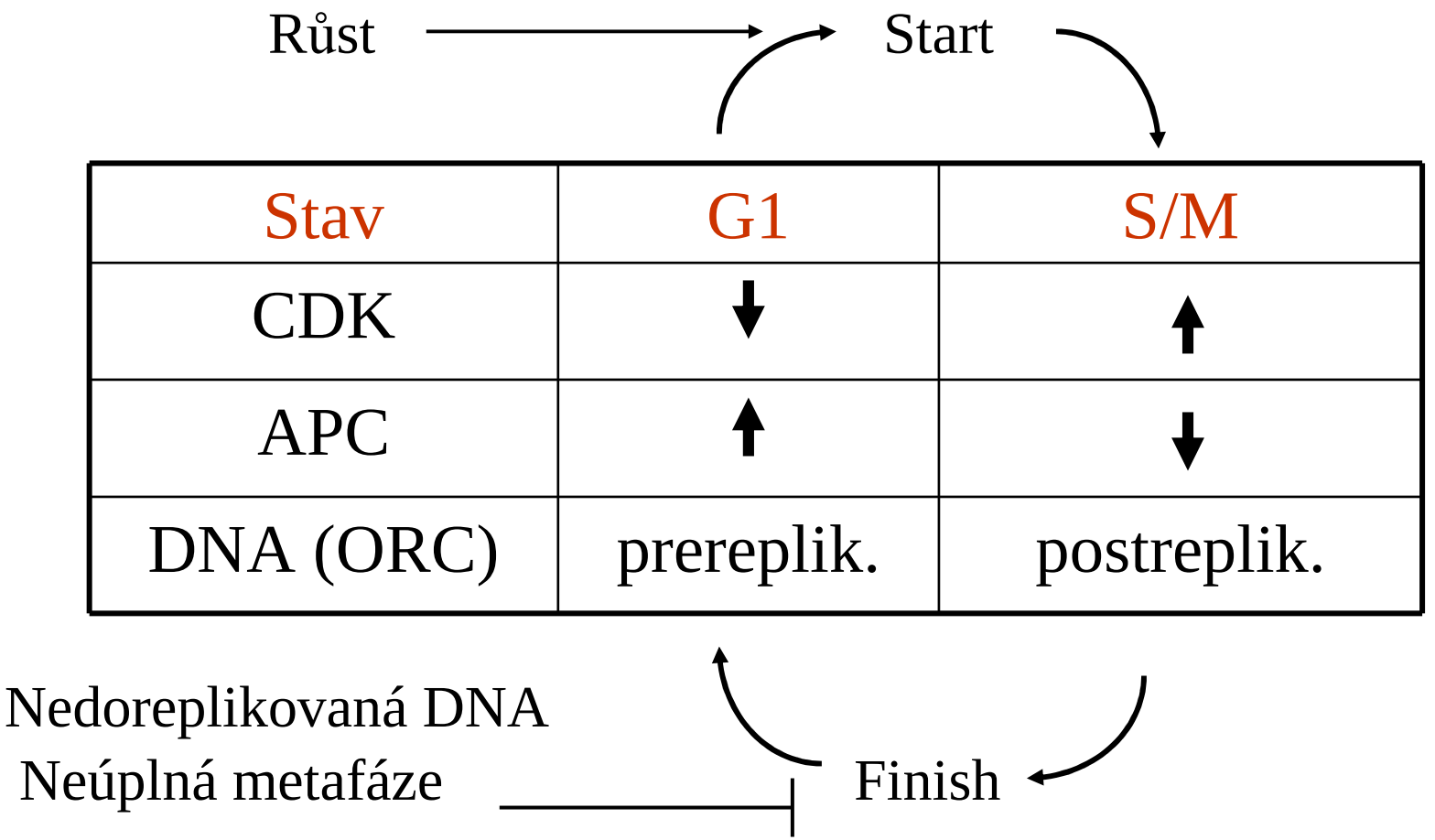
(Ingolia a Murray, Curr. Biol. 2004)

Oscilátor v kontextu tradičního pohledu

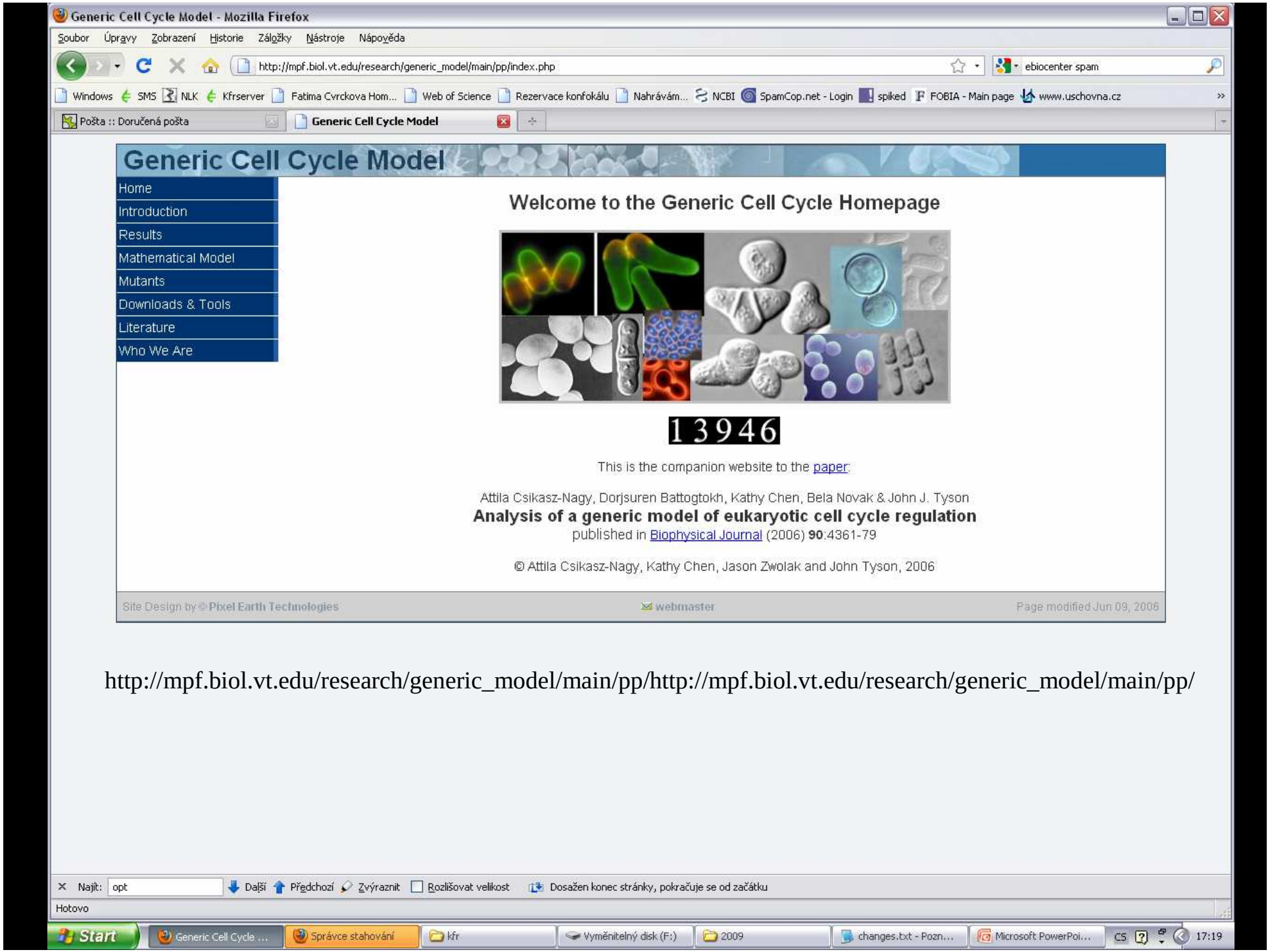


Tyson and Novak, J. Theor. Biol. 210:249-263, 2001

Dva stavy „cyklových hodin“

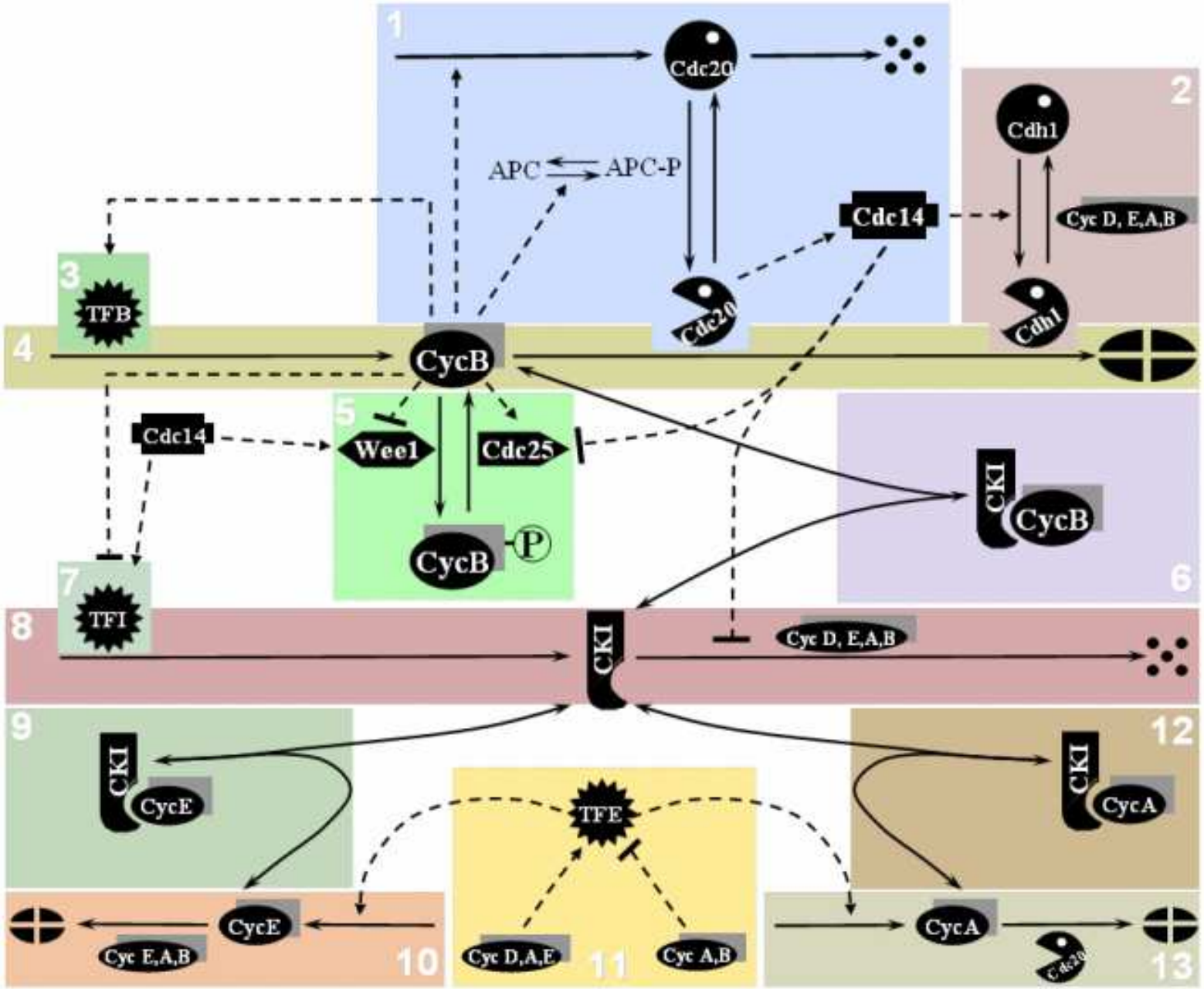


(Novak et al., Phil.Trans.R.Soc.Lond.B 353:2063-2076, 1998)

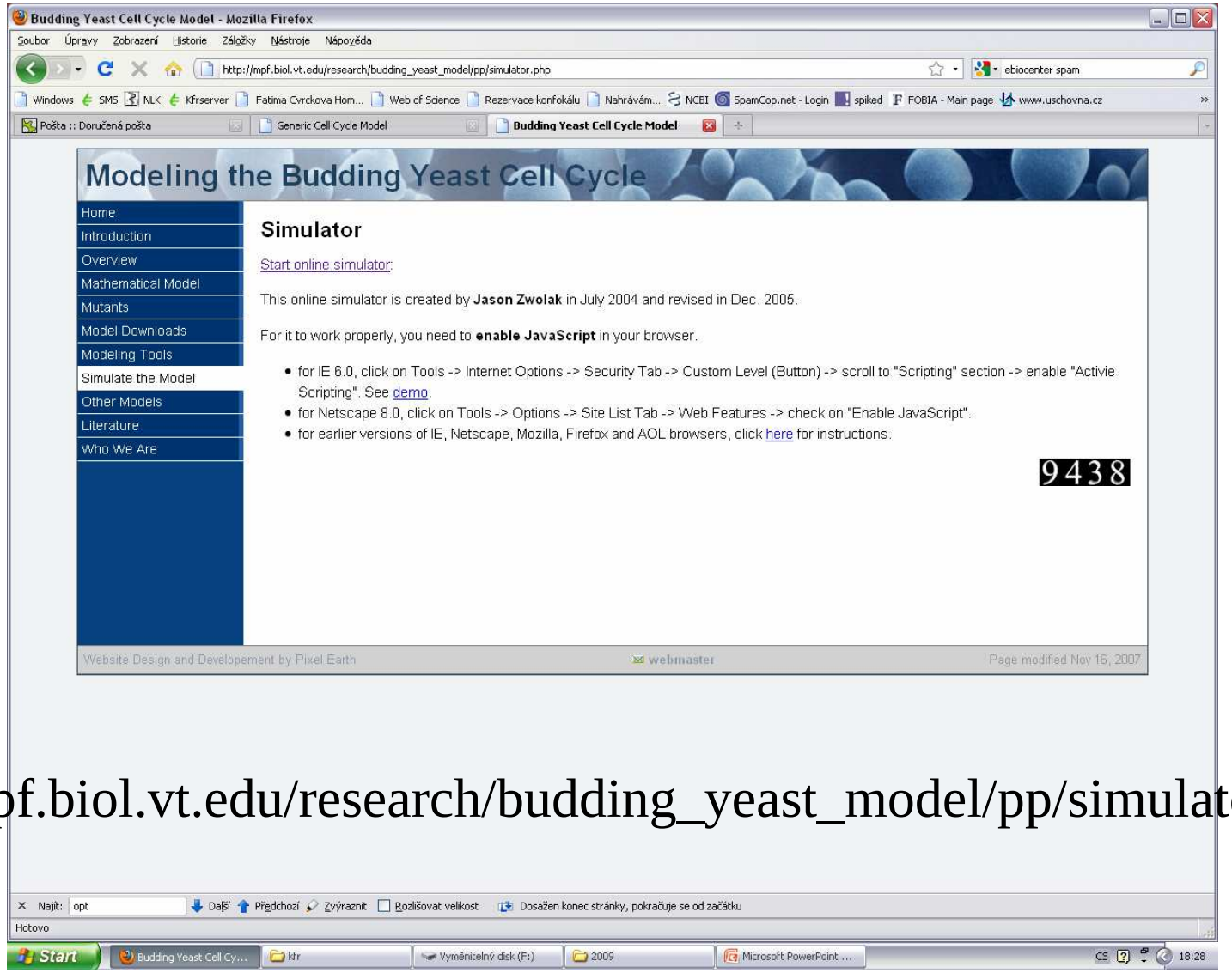


http://mpf.biol.vt.edu/research/generic_model/main/pp/http://mpf.biol.vt.edu/research/generic_model/main/pp/

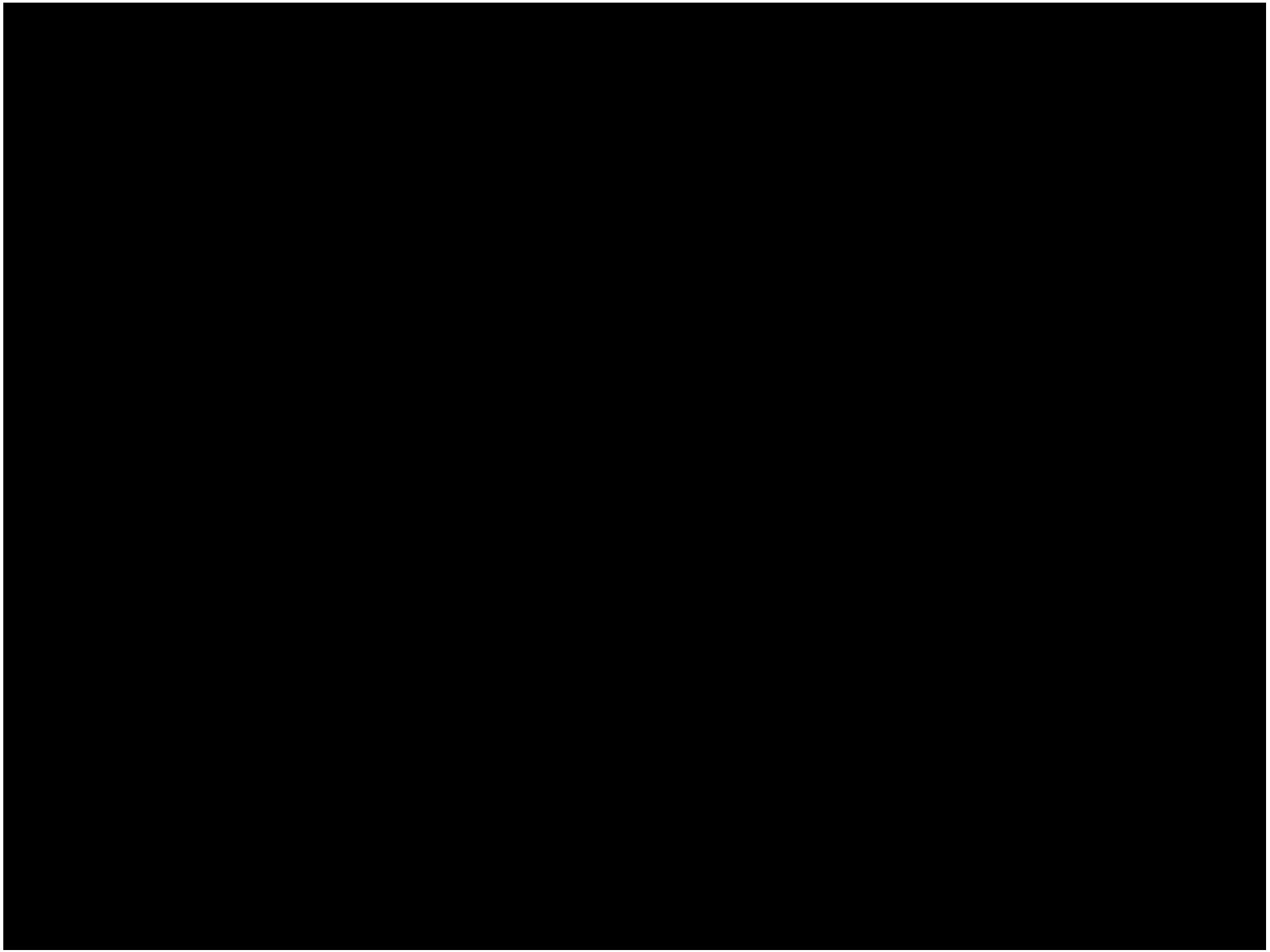
„Wiring diagram“



„Jednodušší“ případ – kvasinka:

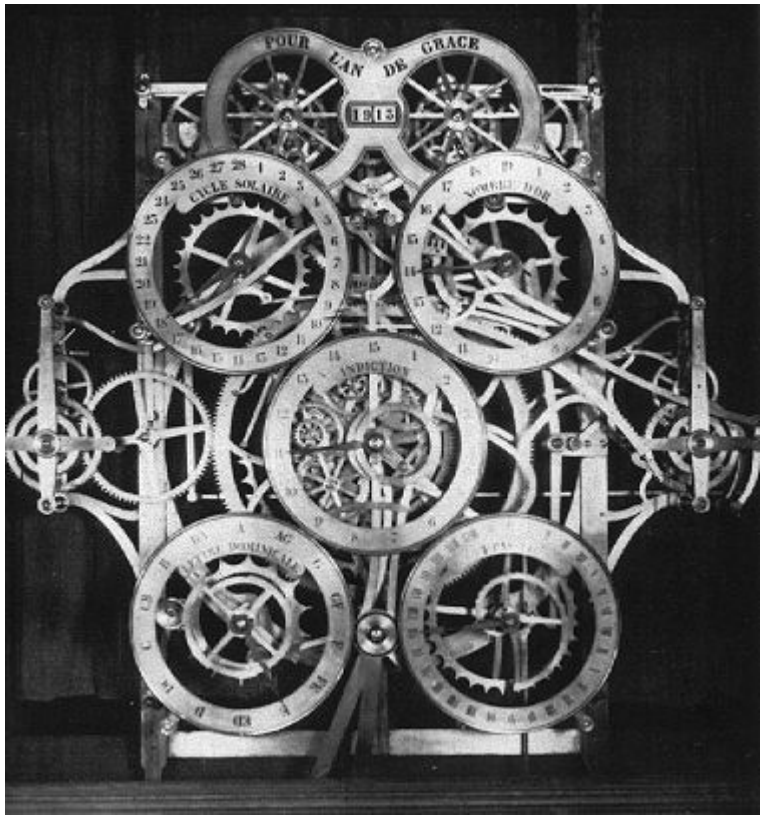


http://mpf.biol.vt.edu/research/budding_yeast_model/pp/simulator.php



Vstupy a výstupy ... zpět k cyklu

centrální oscilátor
(„cell cycle engine“)



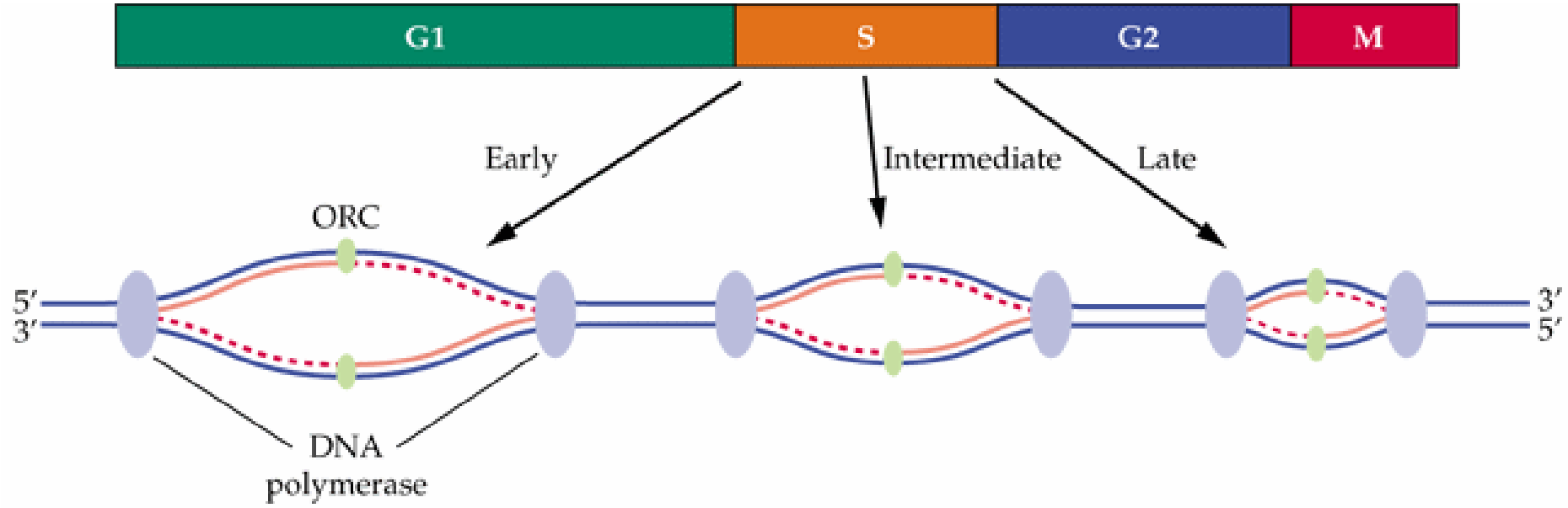
vstupy

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

ARS versus počátek replikace *in vivo*

autonomous replicating sequence

Přímá vizualizace: DNA combing (Lebofsky and Bensimon 2002)

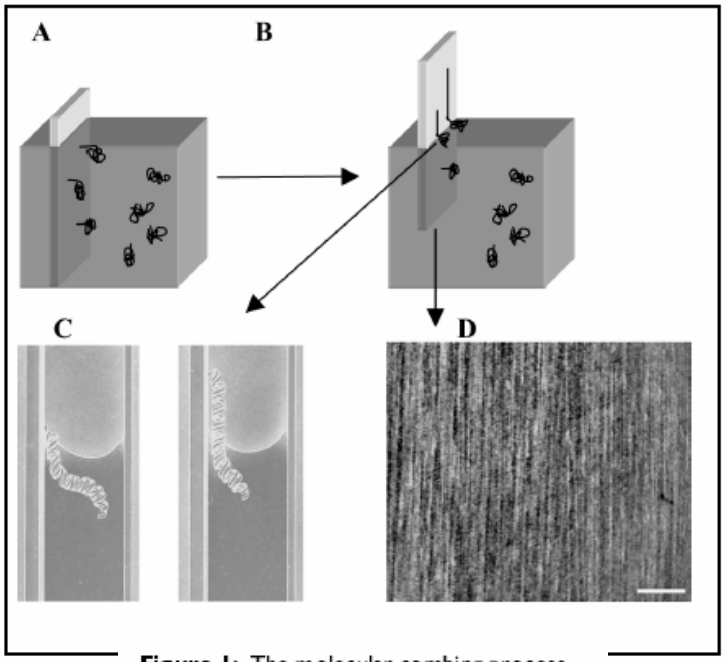


Figure 1: The molecular combing process. DNA molecules with a random-coil configuration attach to the silanised coverslip by their extremities (A). The coverslip is then removed at a constant speed (B). Molecules are uniformly stretched and aligned by the receding air–water meniscus (B, C). Molecules also attach to the coverslip along their length preventing retraction. An array of thousands of combed molecules is produced (D). Bar = 20 µm

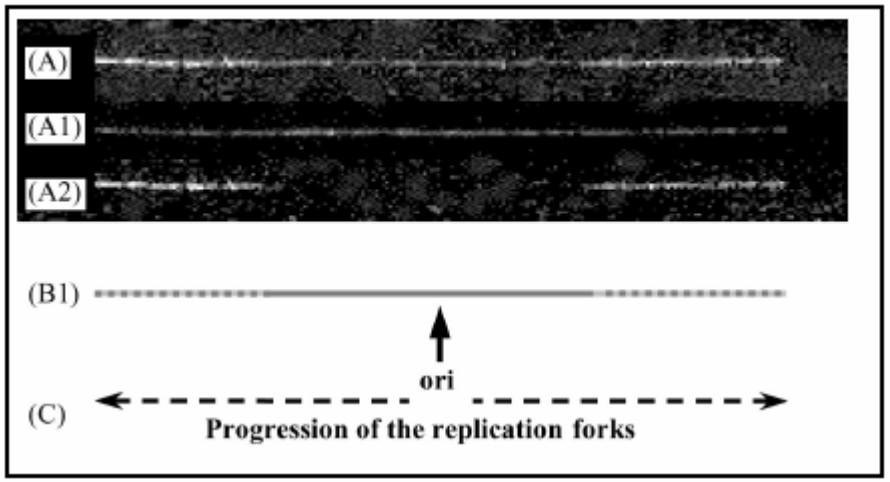
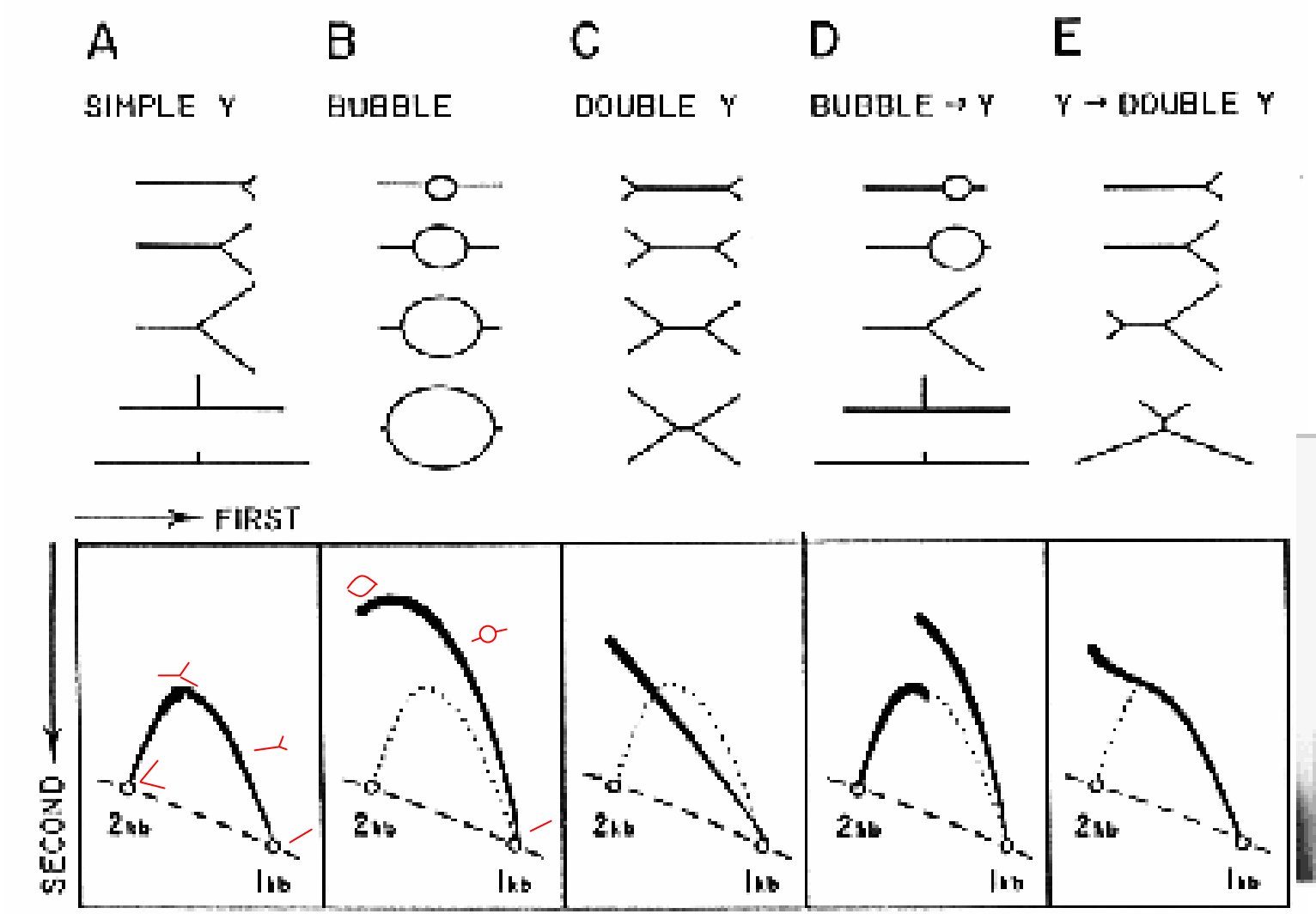


Figure 5: DNA replication studies on combed fibres. In the above experiment, CldU is added at the beginning of S phase followed by addition of IdU at time of 30 minutes for an additional 25 minutes. As replication forks proceed from an origin, the nucleotide analogues are incorporated into newly synthesised DNA. FISH on combed molecules then reveals incorporated CldU for the entire labelling period (A1) with incorporated IdU for the last 25 minutes (A2). From the fused images (A) and the relevant schematic (B1), an origin with active bidirectional replication forks can be inferred (C). Abbreviations: CldU, chloro-deoxyuridine; IdU, iodo-deoxyuridine. Reprinted with permission from ref. 77

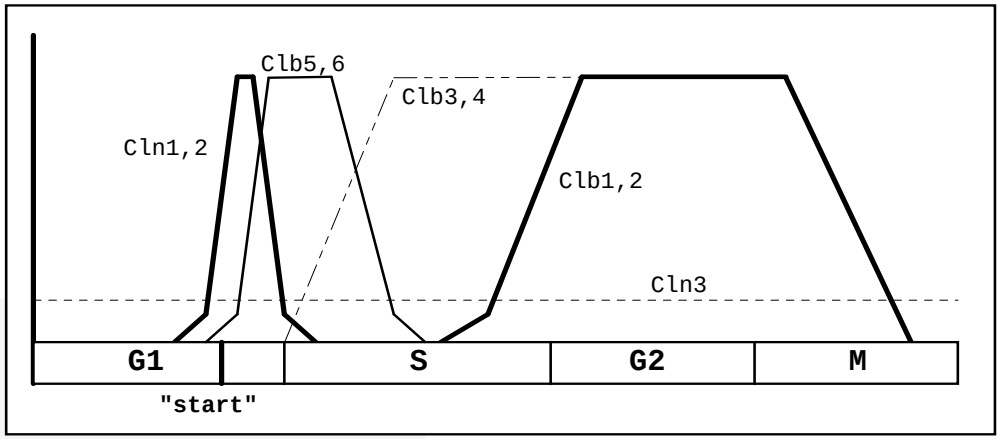
... ale jde to i méně sofistikovaně

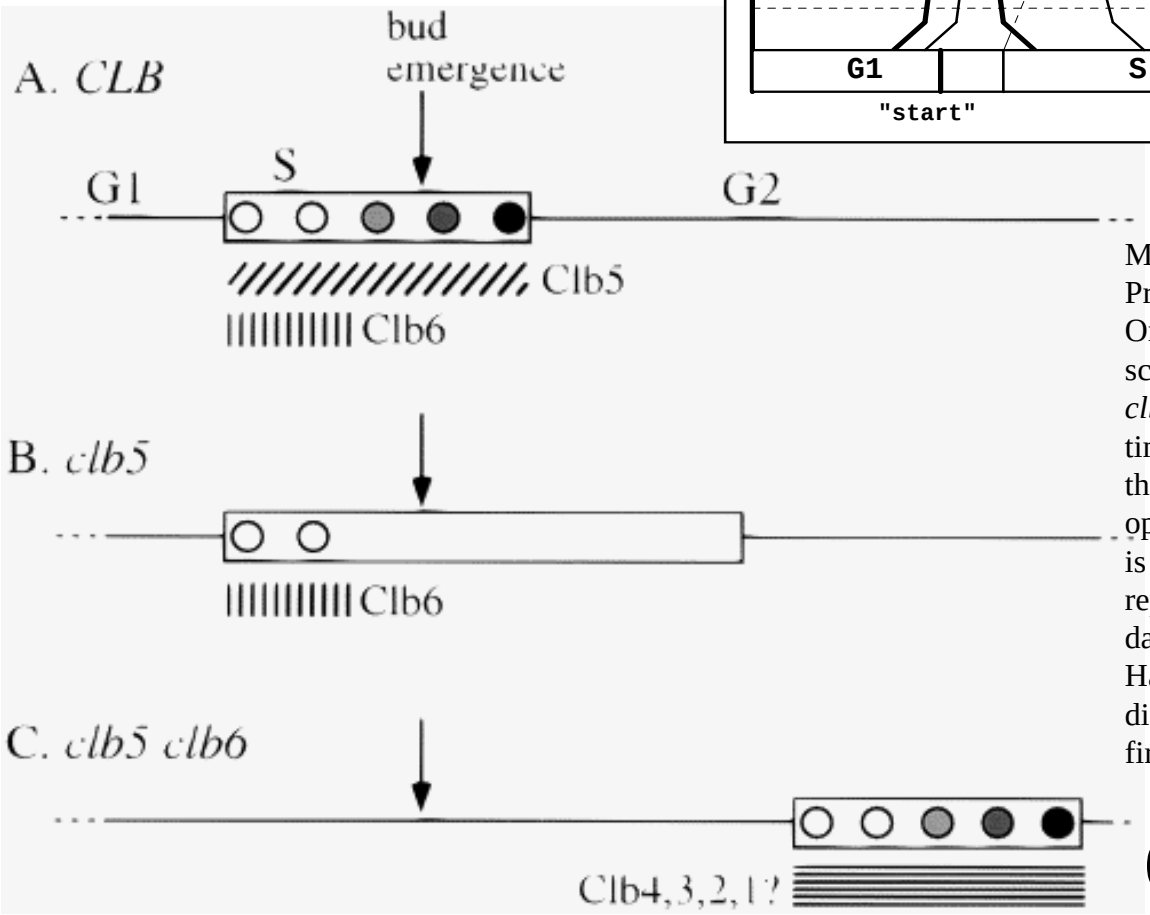


1. - EtBr
2. + EtBr

(Fangman et al.)

Každý ORI má své cykliny



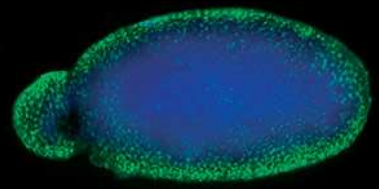


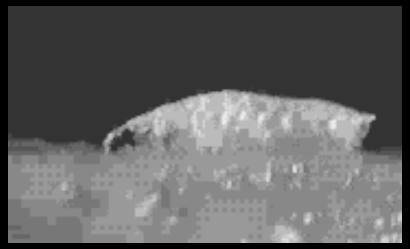
Model of the Ability of B-Type Cyclins to Promote S Phase and Activate Replication Origins(A), (B), and (C) show time lines (not to scale) representing the cell cycle in *CLB*, *clb5*, and *clb5 clb6* strains, respectively. Arrows indicate the time of bud emergence during the cell cycle, and the duration of S phase in each case is drawn as an open box. Firing of the various replication origins is shown by shaded circles. Light shading represents origins activated early in S phase, and darker shading those origins activated later. Hatched bars represent the proposed capability of different Clb-Cdk1p activities to promote timely firing of early and late replication origins.

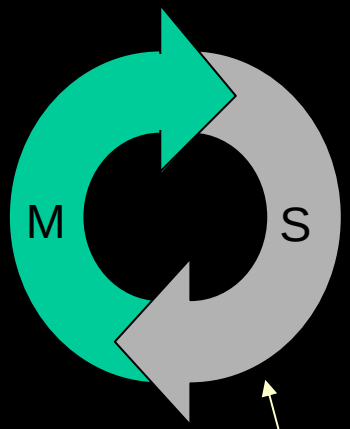
(Donaldson et al. 1998)

Trvání eukaryotního BC - příklady

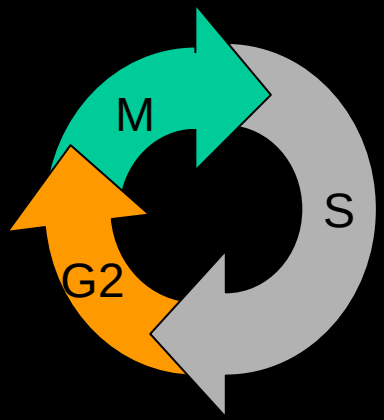
- *Saccharomyces* - v bohatém médiu 1,5-2 h
- *Drosophila*: 6 min až 10 hod

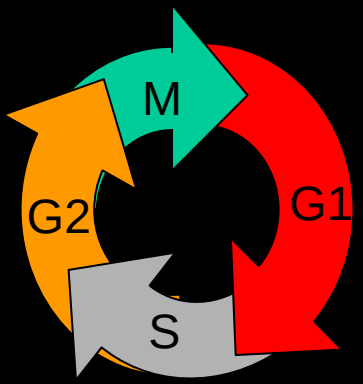






6 min

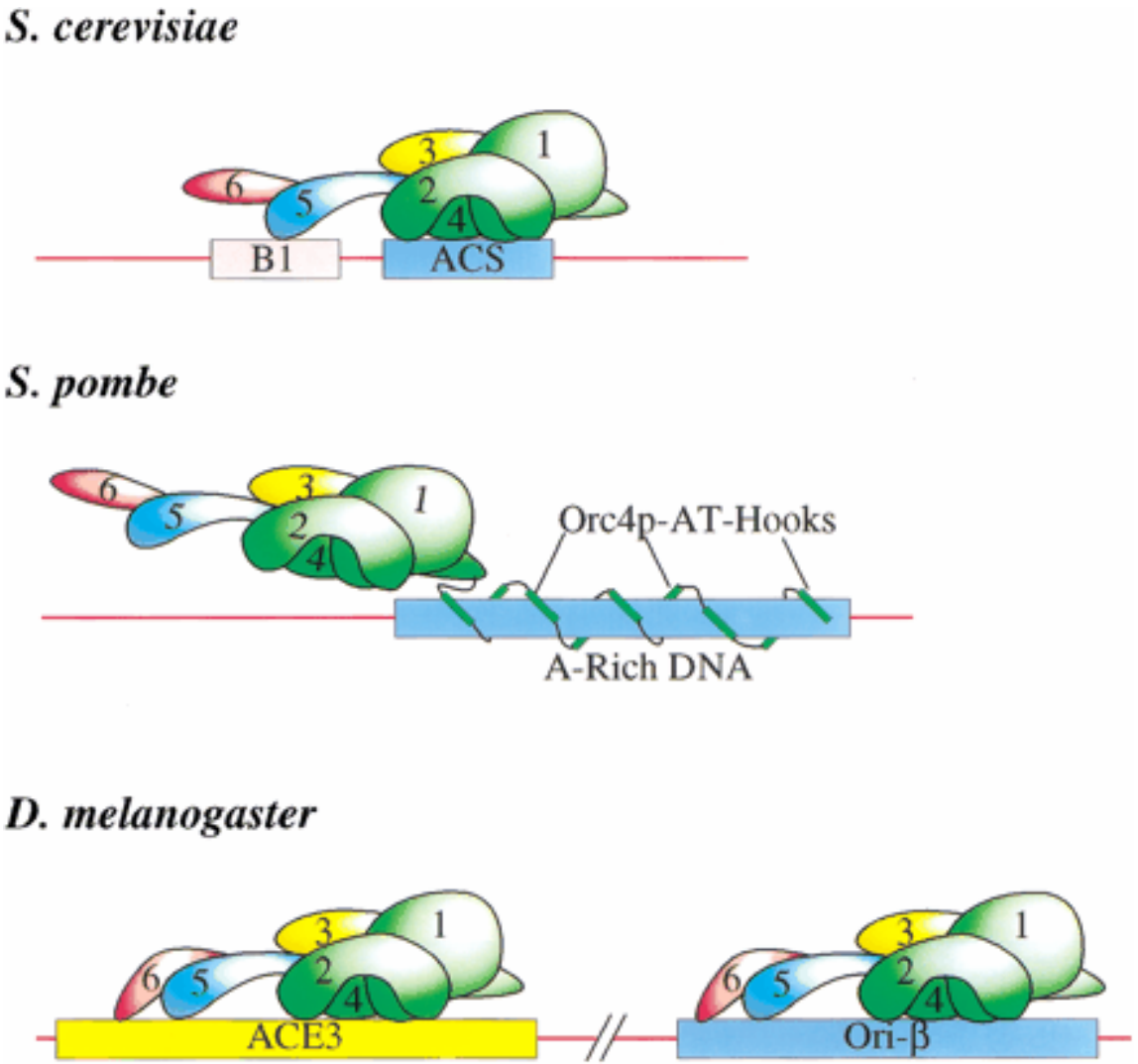




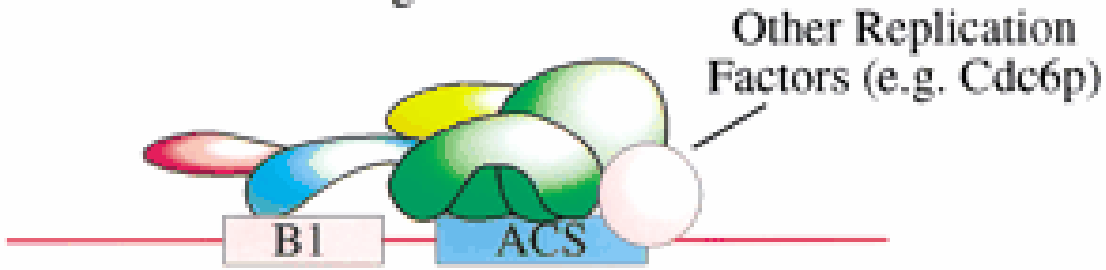
10 h

... zde používány specifické embryonální ORI!

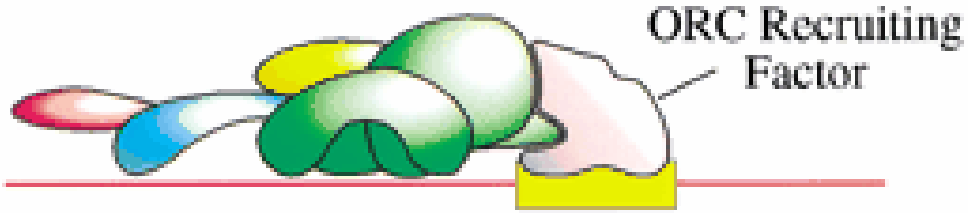
ORC – origin recognition complex



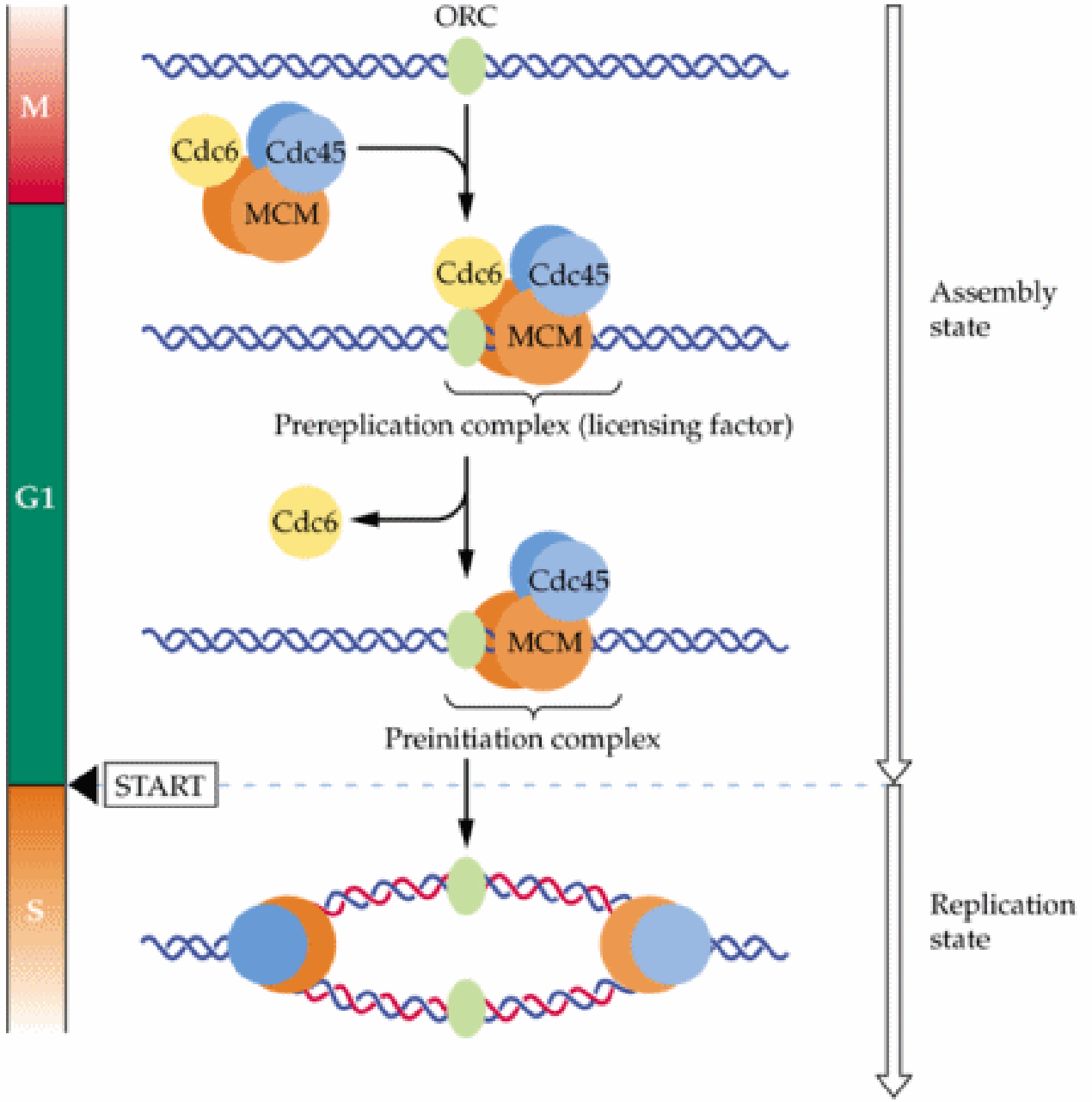
A. Stabilized DNA Binding



B. Recruitment



C. Local Chromatin Structure


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

Haploidní mitosa v buňkách bez Cdc6

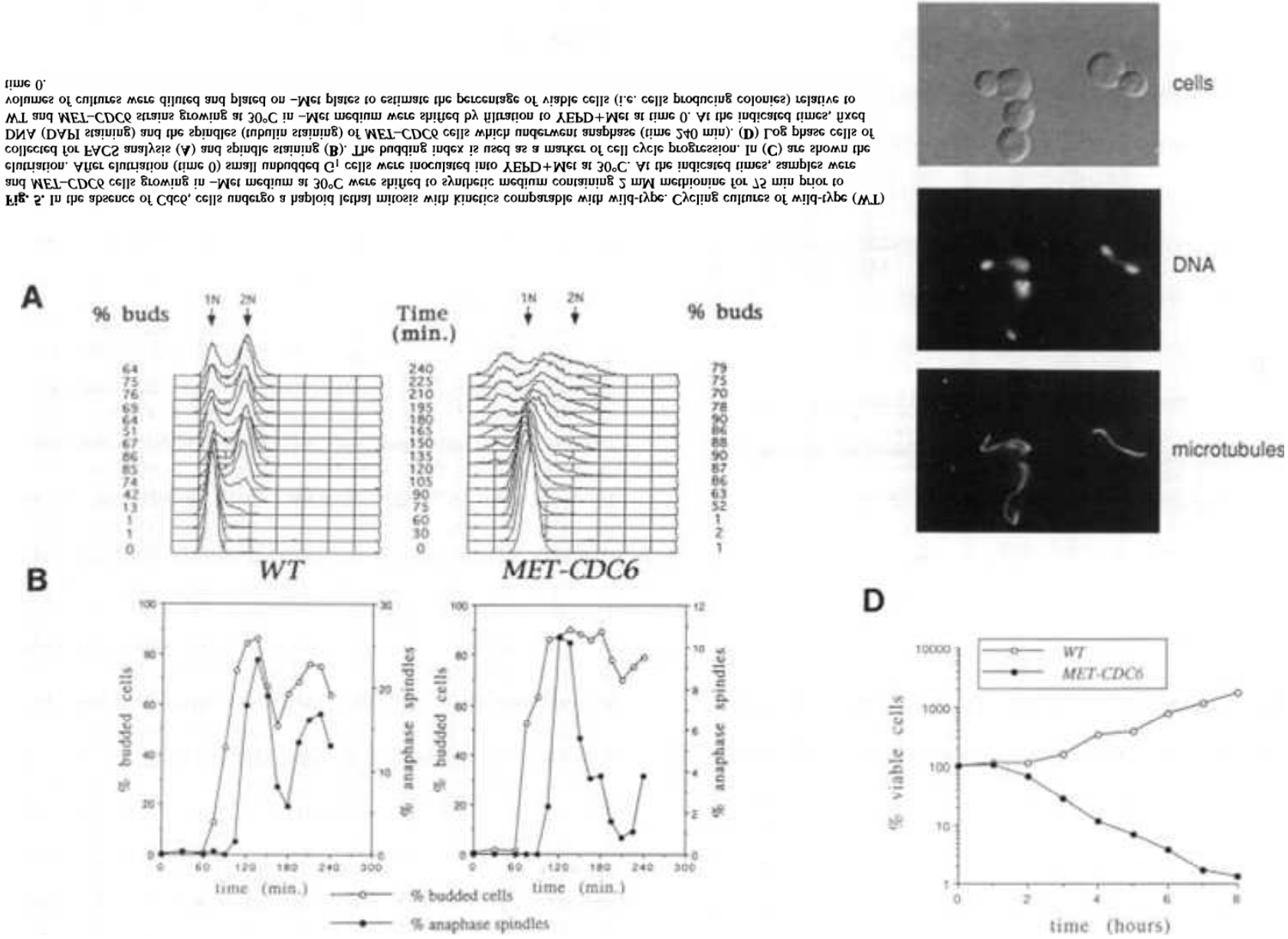
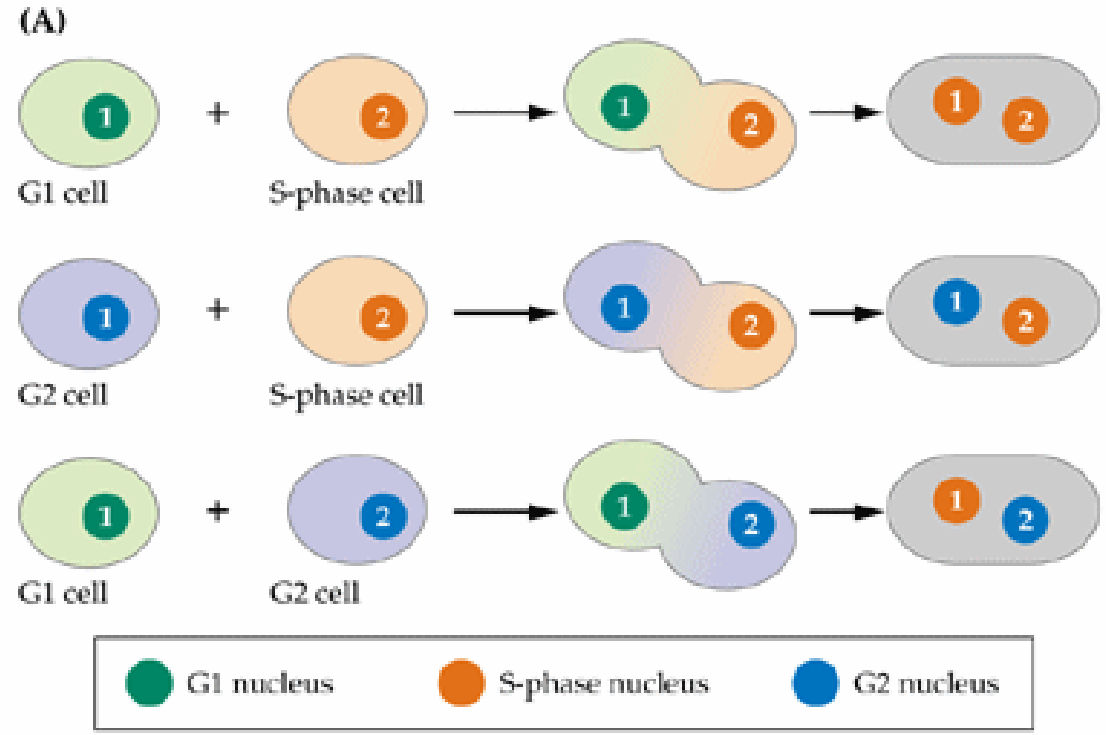


Fig. 2. In the absence of Cdc6, cells undergo a haploid-lethal mitosis with kinetics comparable with wild-type. Cycling cultures of wild-type (TW) and MET–CDC6 cells growing in −Met medium at 30°C were shifted to synthetic medium containing 5 mM methionine for 15 min prior to elutriation. After elutriation (time 0) small unbudded G_1 cells were inoculated into YEPD+Met at 30°C. At the indicated times, samples were collected for FACS analysis (**A**) and ethidic staining (**B**). The budding index is used as a marker of cell cycle progression. In (**C**) are shown the DNA (DAPI staining) and the ethidic (tubulin staining) of MET–CDC6 cells which underwent anaphase (time 150 min). (**D**) Log phase cells of TW and MET–CDC6 strains growing at 30°C in −Met medium were shifted by filtration to YEPD+Met at time 0. At the indicated times, fixed volumes of cultures were diluted and plated on −Met plates to estimate the percentage of viable cells (i.e. cells producing colonies) relative to time 0.

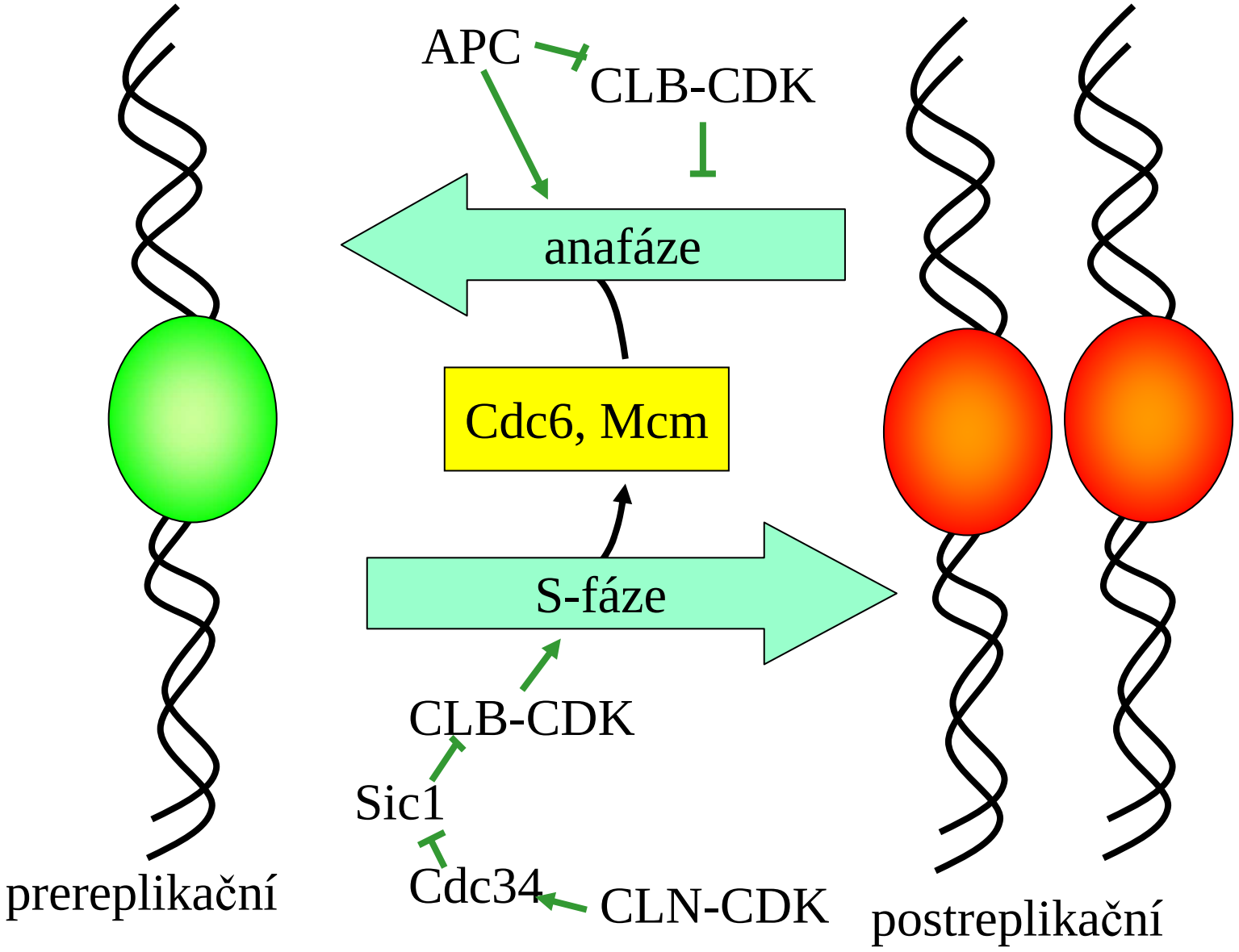
Je Cdc6 „licensing factor“?



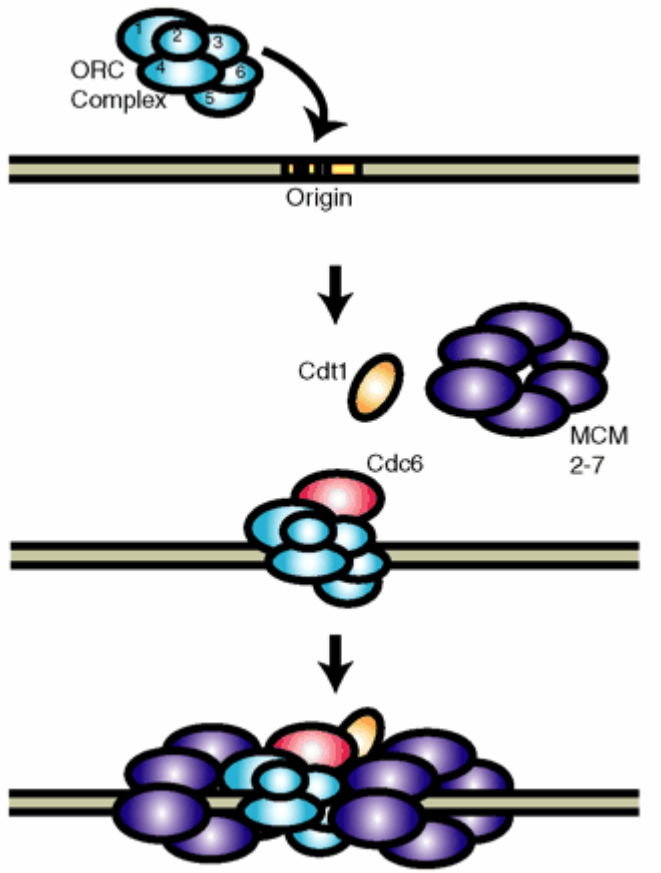
Observations	Conclusions
G1 nucleus replicates. Early S-phase nucleus continues replication.	G1 nucleus is competent to replicate. S-phase cells contain activator.
G2 nucleus does not replicate. S-phase nucleus continues replication. G2 nucleus does not progress into M phase.	G2 nuclei are not competent 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.

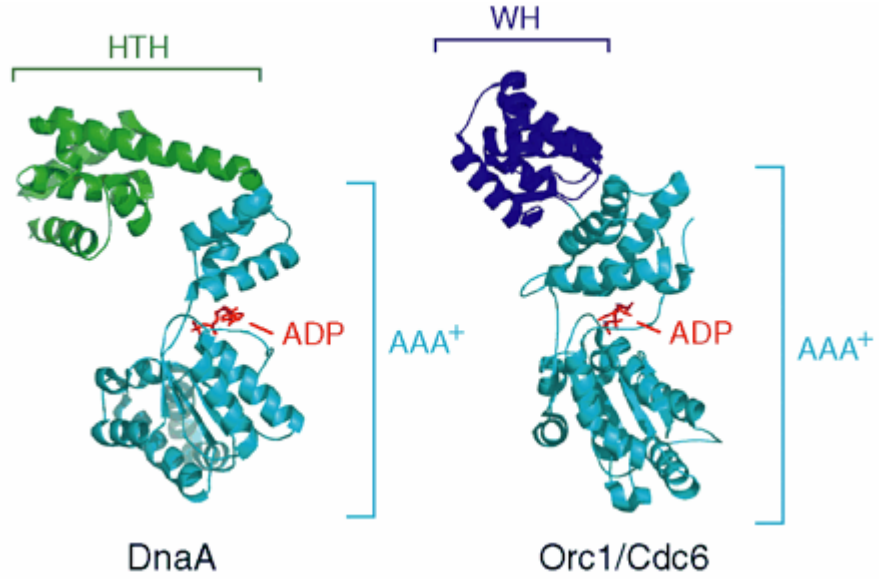
(Rao and Johnson)

Kontrola replikace: 2 stavy ORC



ORC assembly

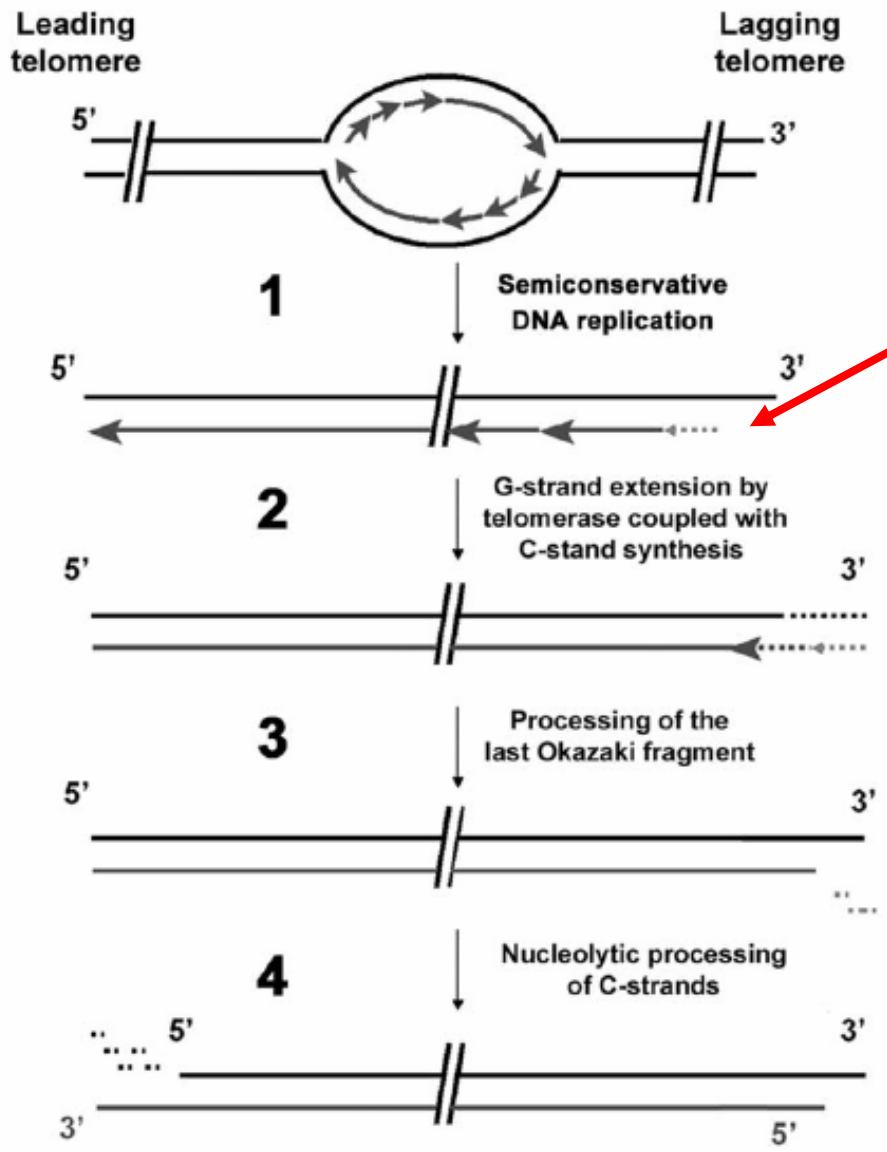




bacteria

Archaea/eukaryota

Konce - telomery



telomeráza

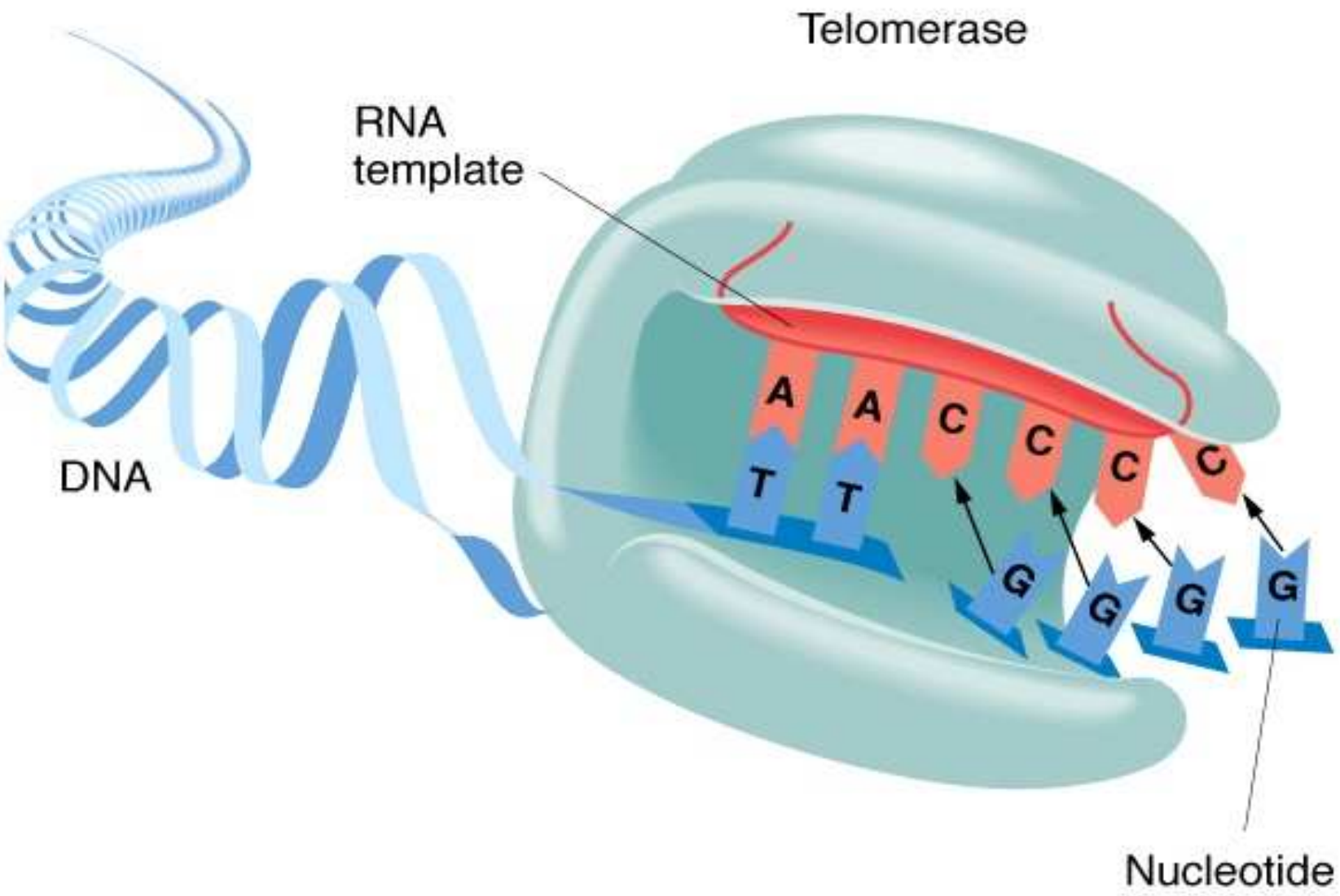
$(TTTAGGG)_n$ 2.7 - 3.5 kb

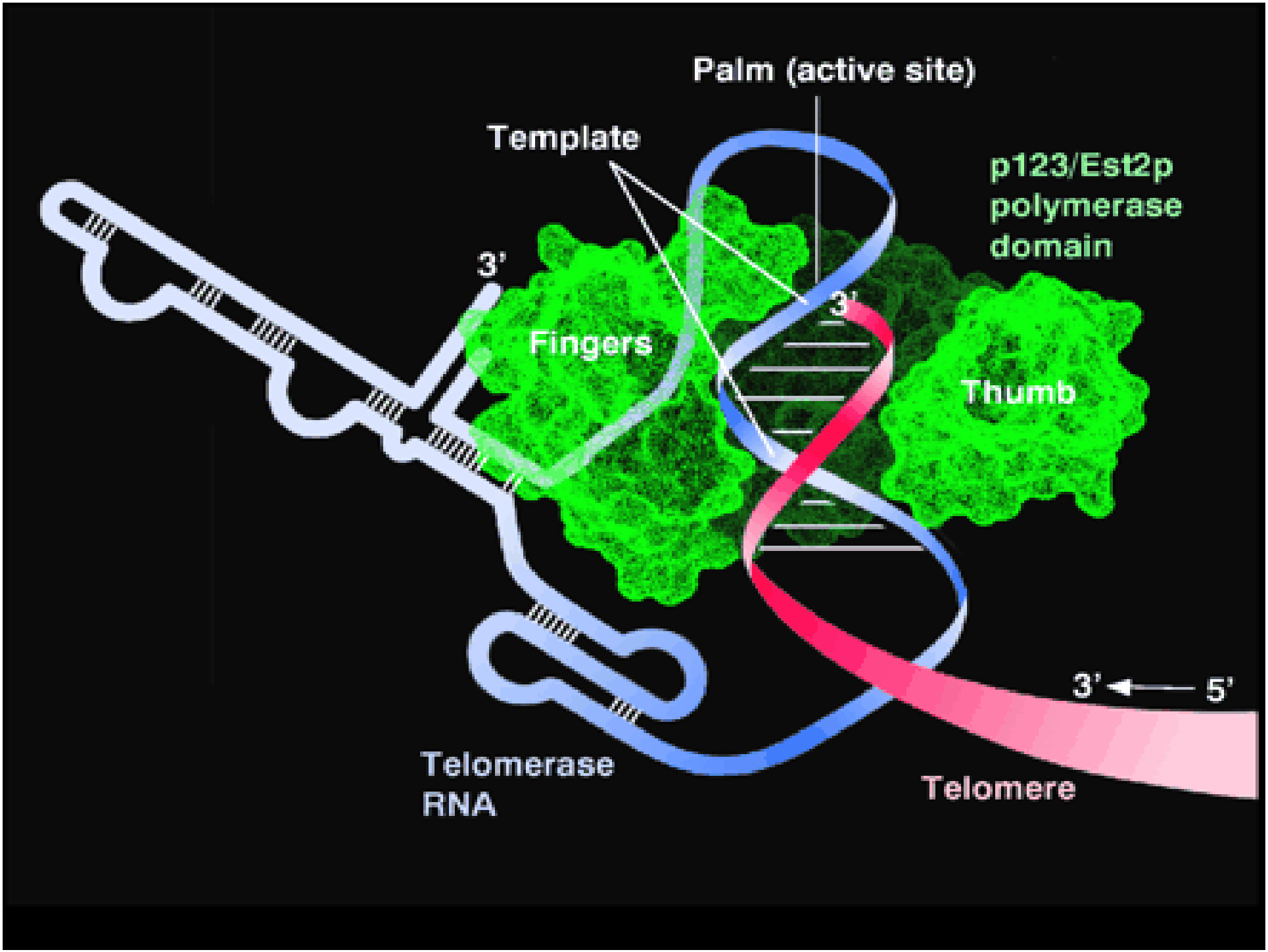
Telomeráza

biochemický důkaz:

- přidání $(TTGGGG)_4$ ve vysoké koncentraci do buněčného extraktu z *Tetrahymena* + *dGTP
- produkty na gelu zvětšující se o 6 bází

- vložení telomerové sekvence kvasinek (TTGGG) do *Tetrahymena* (TTGGGG) – rozdíl 1 báze a tedy posun v každém opakování


Telomerase
RNA
template
DNA
A
A
C
C
C
C
T
T
G
G
G
G
Nucleotide


Palm (active site)
Template
p123/Est2p
polymerase
domain
3'
3'
Fingers
Thumb
3'
5'
Telomerase
RNA
Telomere

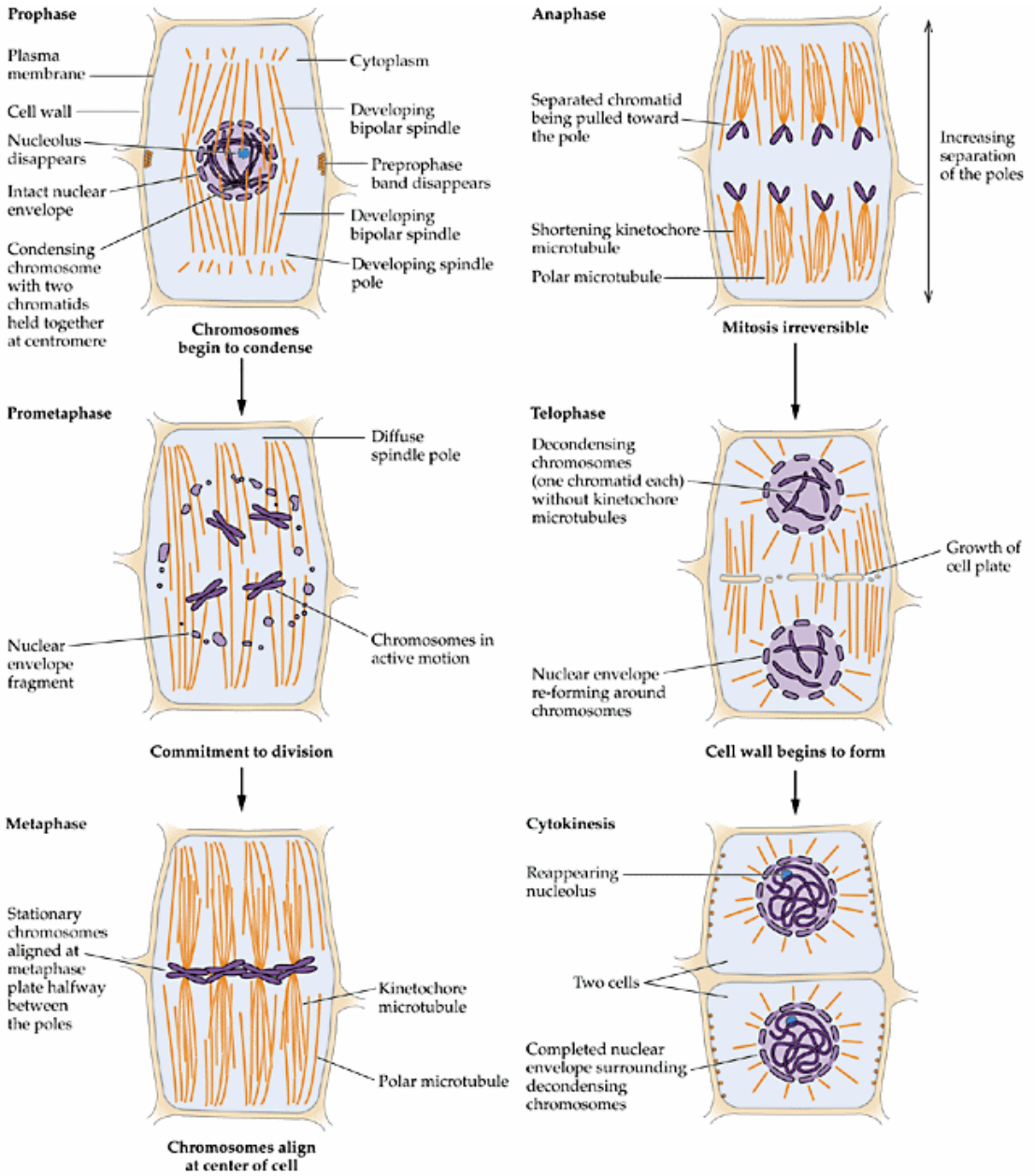
Telomery: další Nobelova cena za BC!

(Lasker prize 2006)

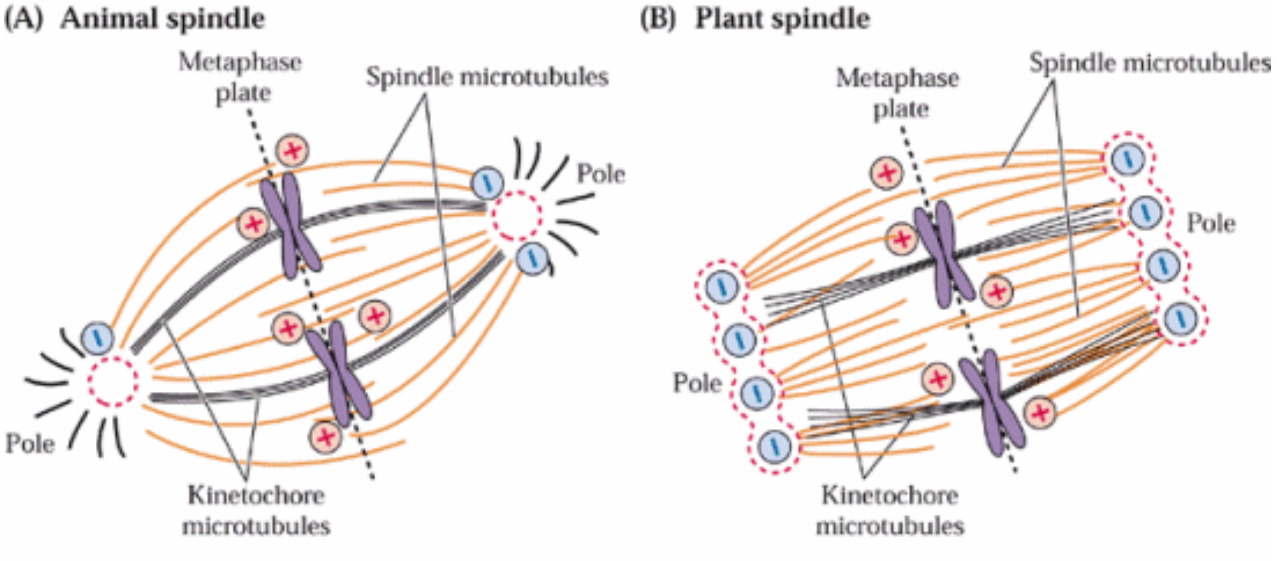
Zpět ke strukturním událostem cyklu …

1. Segregace chromozomů a karyokinese
2. Cytokinese

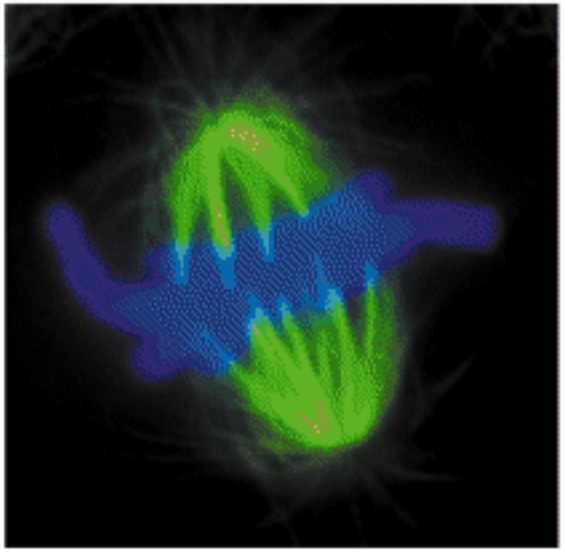
… aneb cytoskeletální efektory CDK (hlavně MT)


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

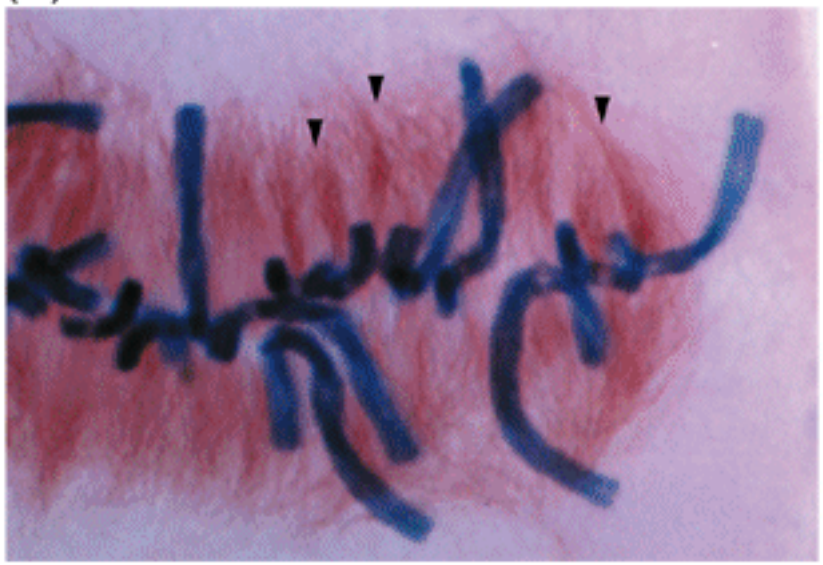
Mitotické vřeténko a segregace chromosomů



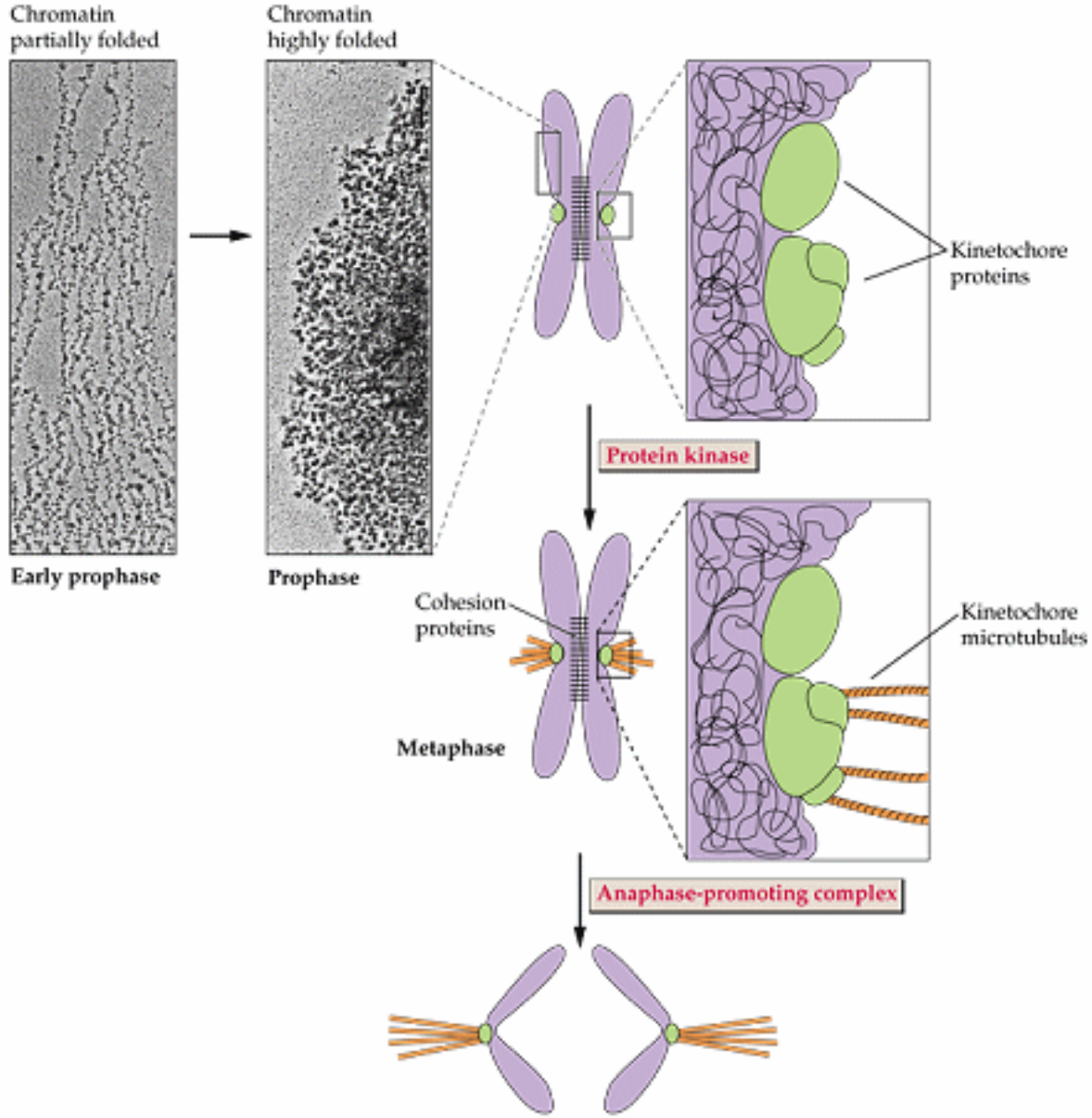
(C)



(D)



Kondensace a rozchod chromosomů



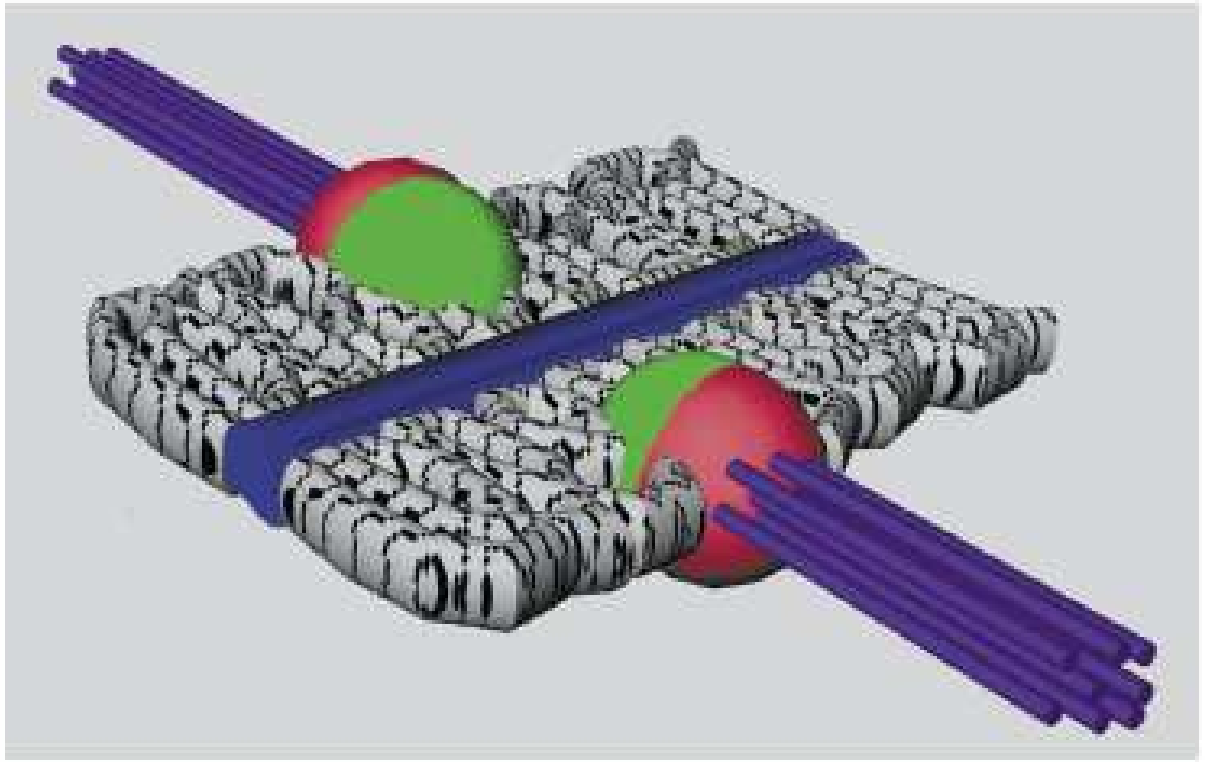


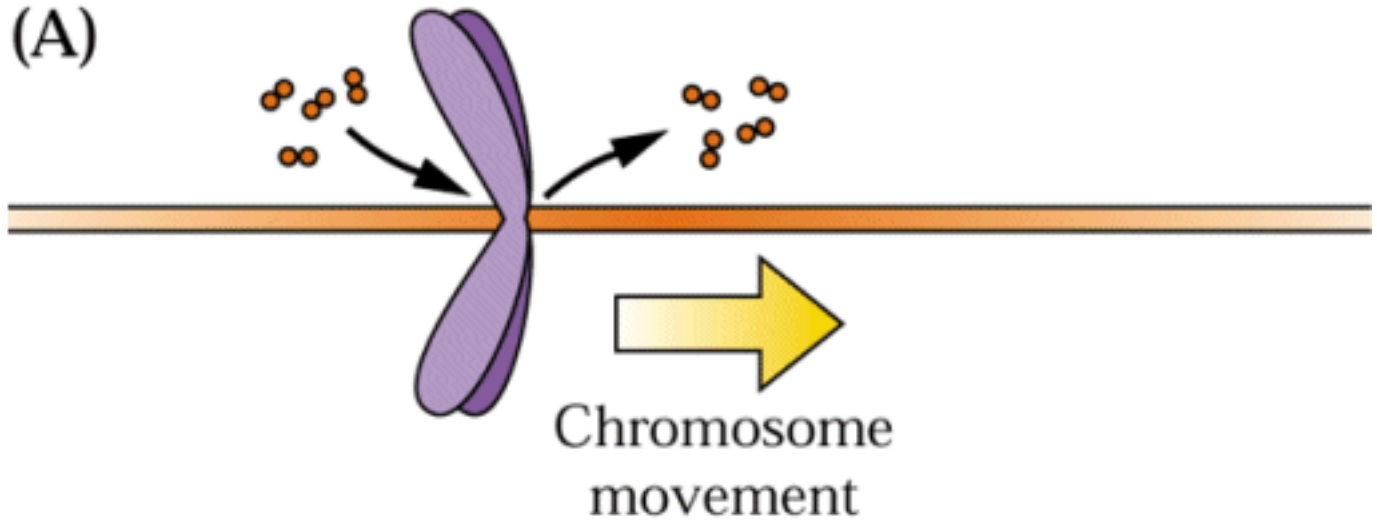
Fig. 2. A model of the maize meiotic kinetochore showing the centromeric region of a meiosis II chromosome. The kinetochore is depicted as a spherical structure with two subdomains. The inner (green) domain contains the maize protein CENP-C and the outer (red) domain contains the MAD2 protein and the 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

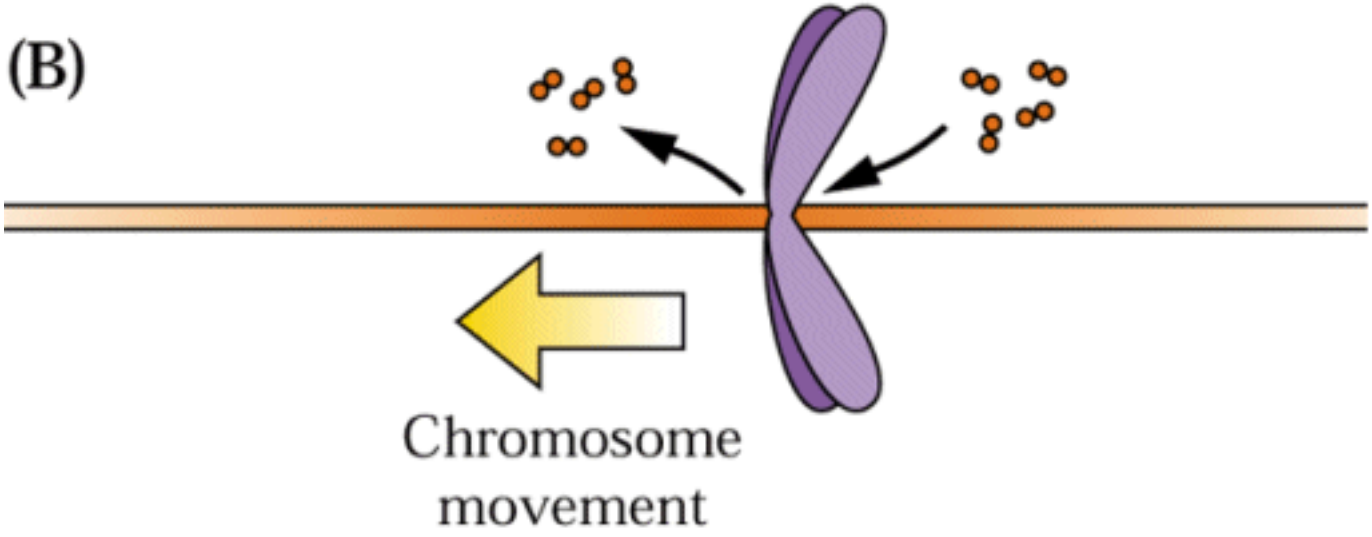
Kinetochore component	Apparent function	Plant species	Kinetochore localization[a]	Gene cloned	Refs
CBF5	Unknown	*Vicia faba*, *Hordeum vulgare*	Yes	Yes (*Hordeum vulgare*)	27
CENPC	Structural	*Zea mays*, *Vicia faba*, *Hordeum vulgare*	Yes	Yes (*Zea mays*)	26,27
CENPE	Chromosome motility	*Vicia faba*, *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

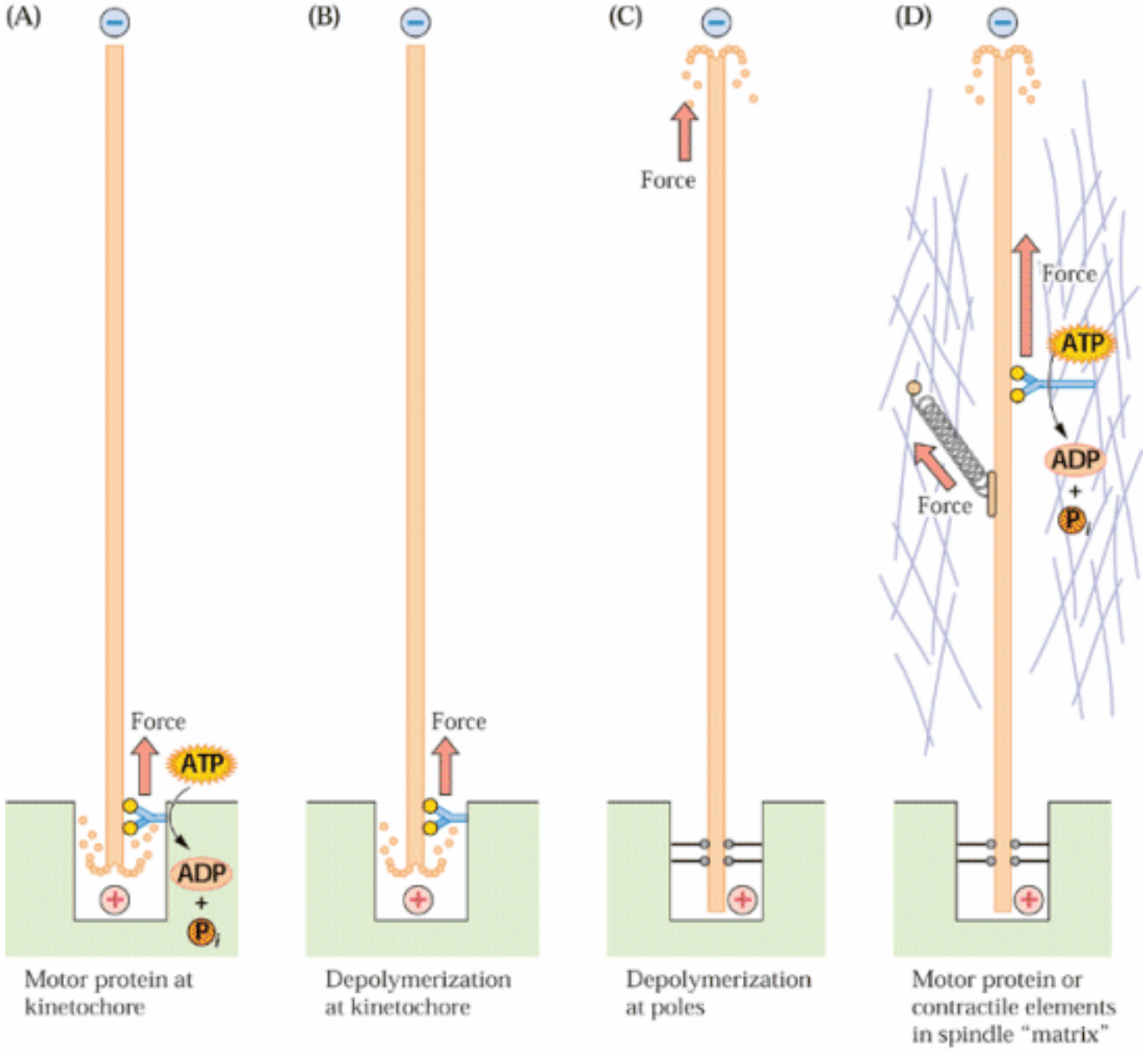
[a]The protein has been localized to the kinetochore by immunofluorescence.
[b]NA, information not available.
[c]γ-tubulin DNA sequences from other plant species are available in GenBank.

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!

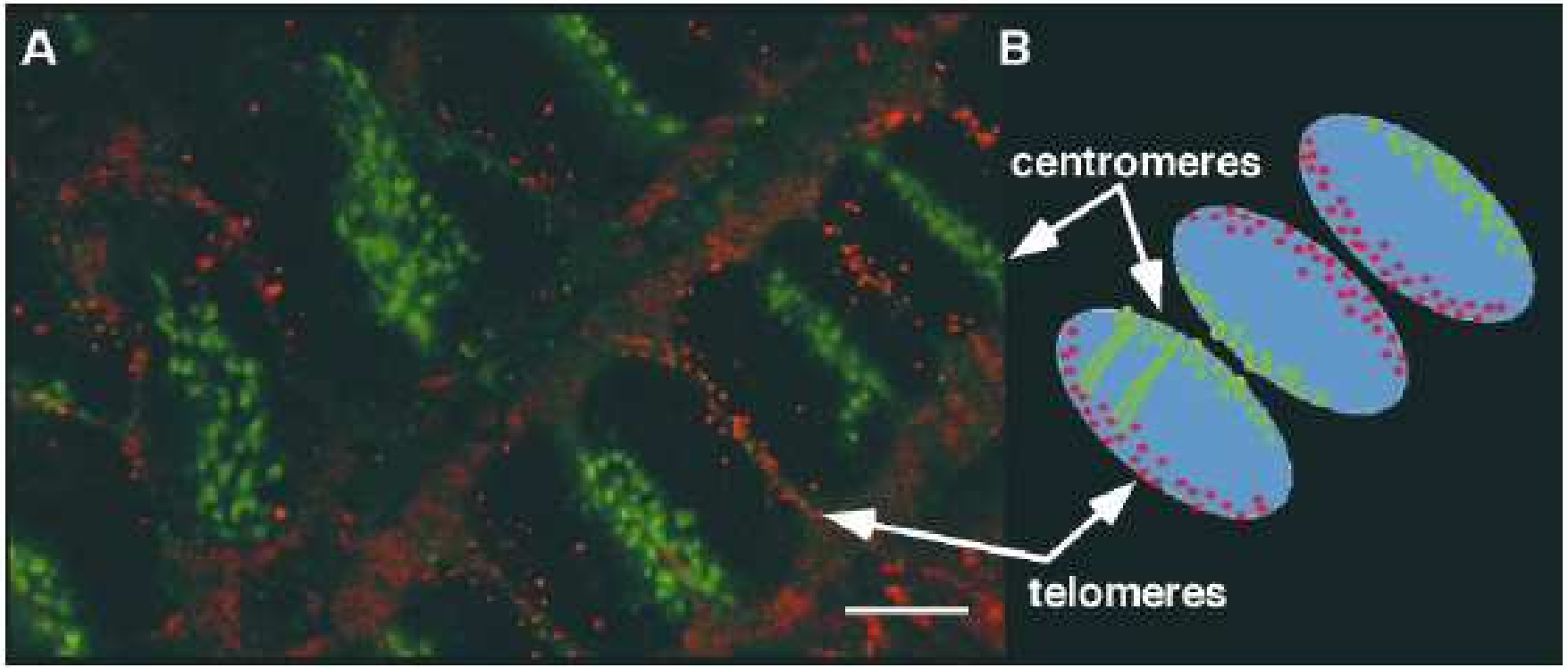


Figure 4. The Rabl Configuration in Somatic Nuclei.

(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

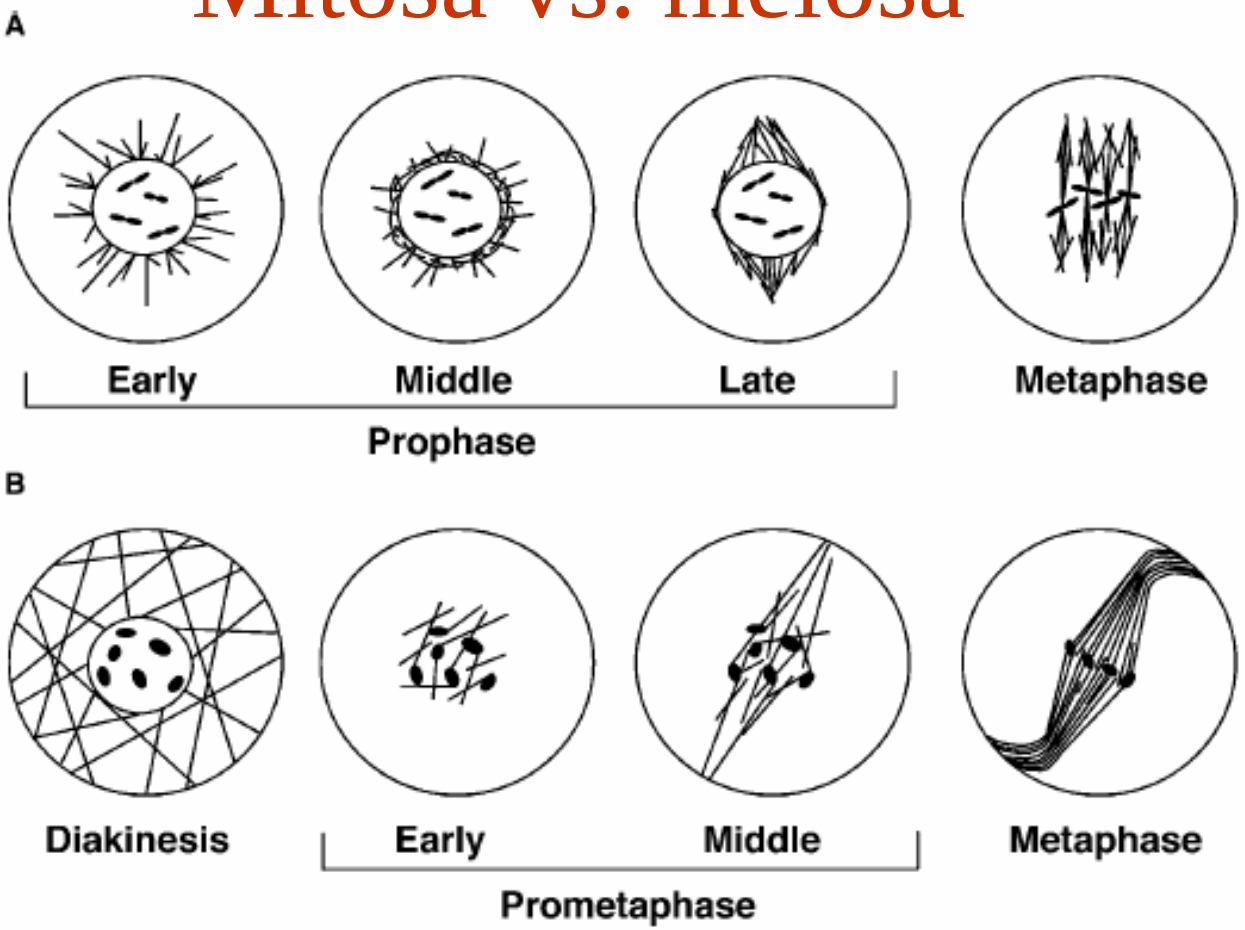


Figure 3. Diagram of Spindle Development in Meiotic versus Mitotic Cells.

(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.

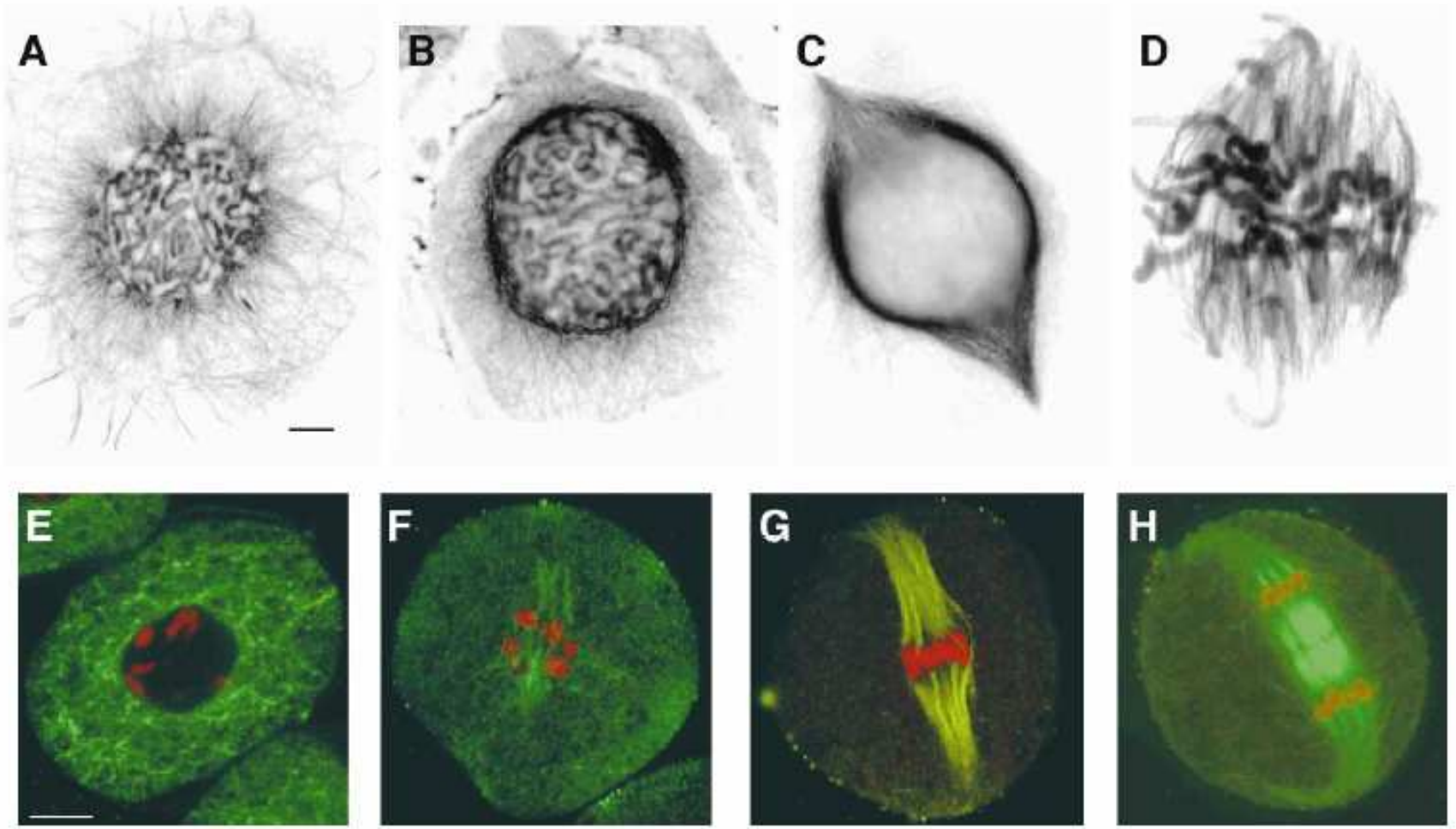


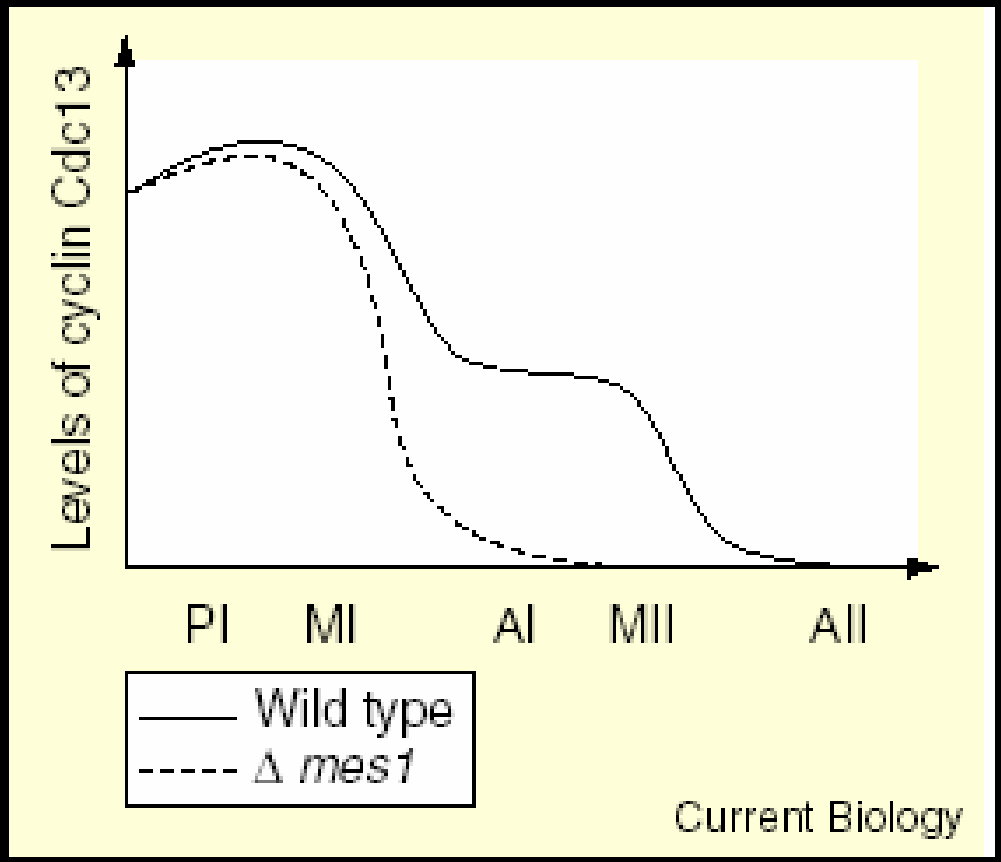
Figure 2. The Pathway of Spindle Assembly in Mitotic and Meiotic Plant Cells.

(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).

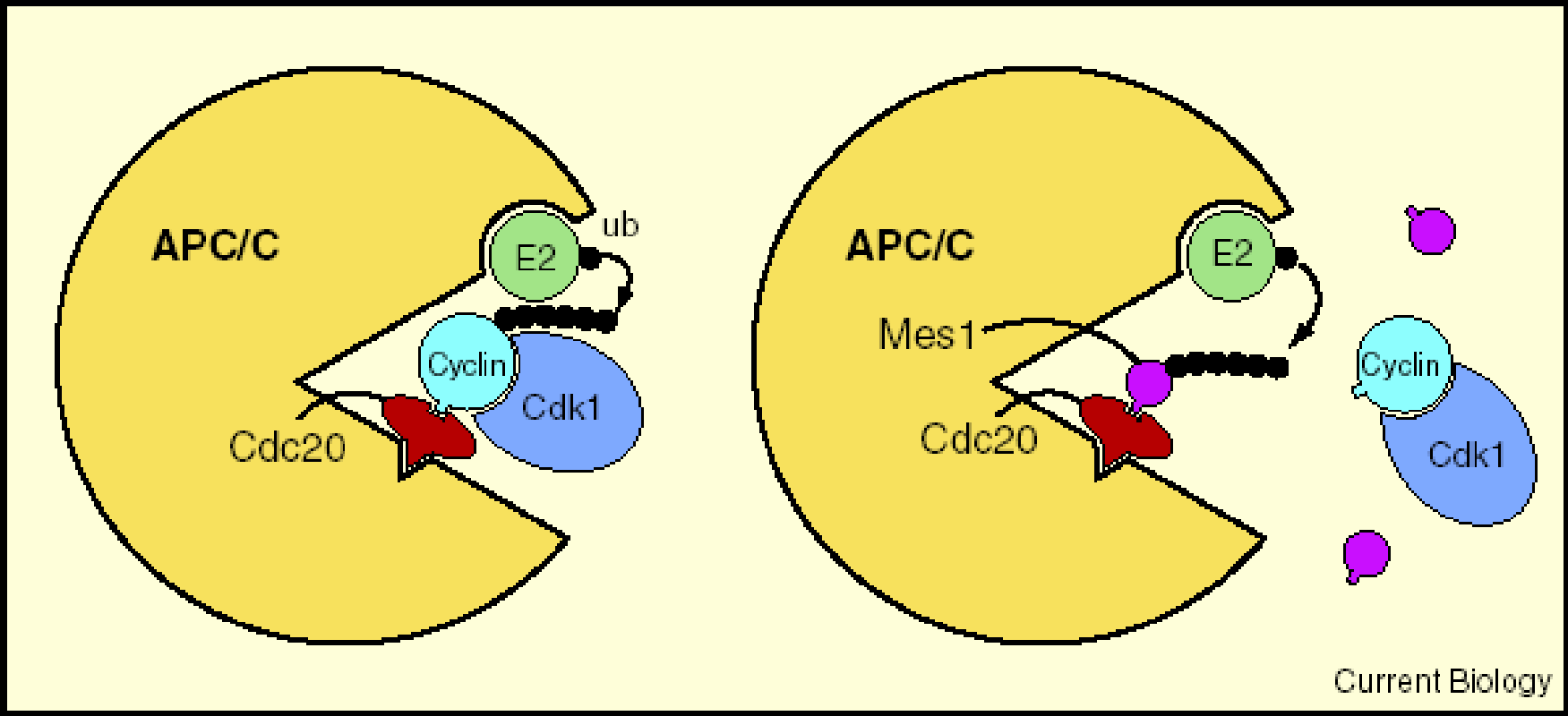
(E) to **(H)** The distribution of microtubules in maize meiocytes. Single optical sections taken by confocal laser scanning microscopy of a meiocyte in diakinesis **(E)**, early prometaphase **(F)**, metaphase I **(G)**, and anaphase I **(H)**. The chromosomes, stained with propidium iodide, are shown 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?) u *S. pombe* - porucha meiose II

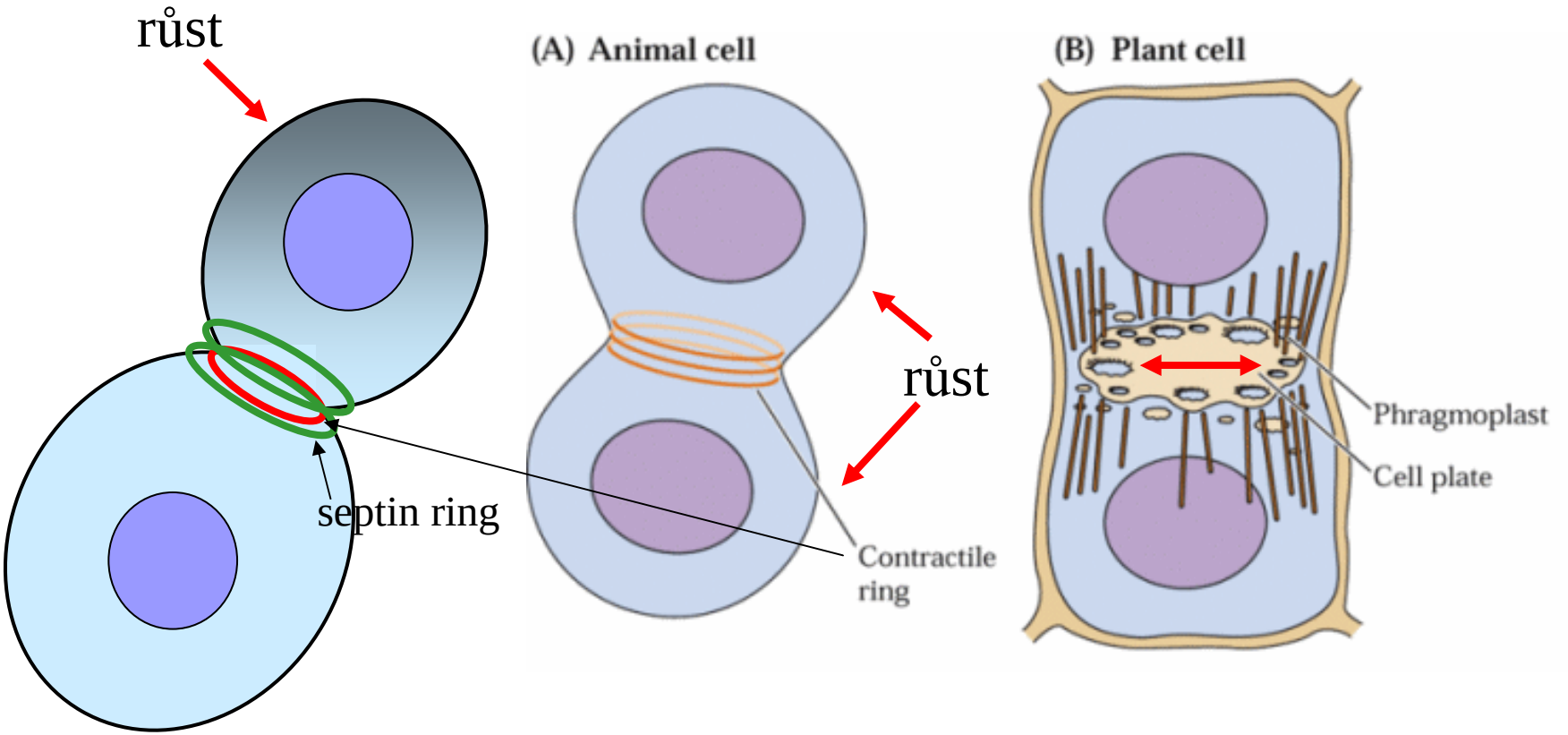


mes1+ (asi) kóduje kompetitivní inhibitor APC!



(Peters 2005)

Cytokinese



- jak rozdělit buňku ...

Je rozdíl mezi rostlinami a živočichy opravdu tak zásadní?

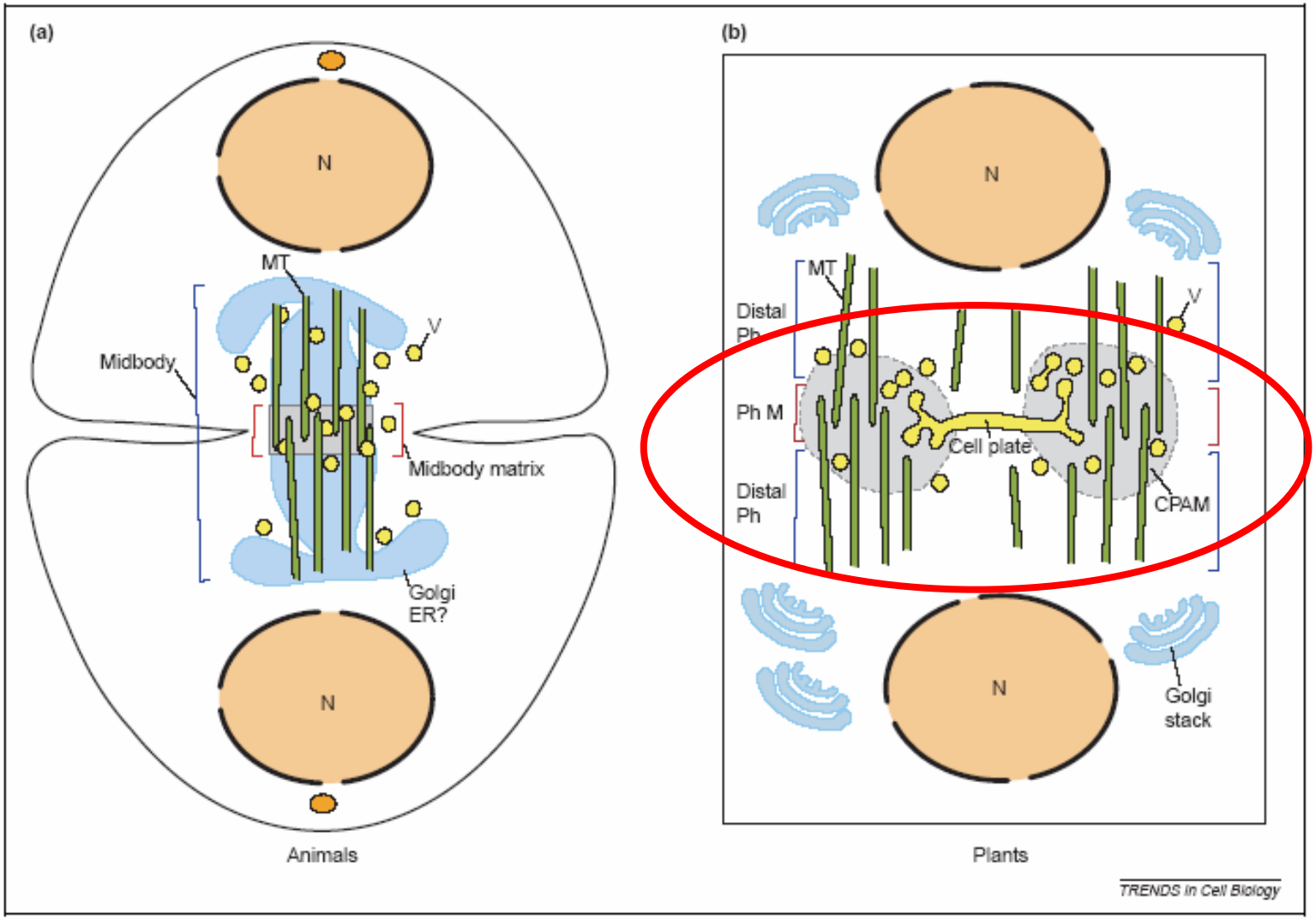
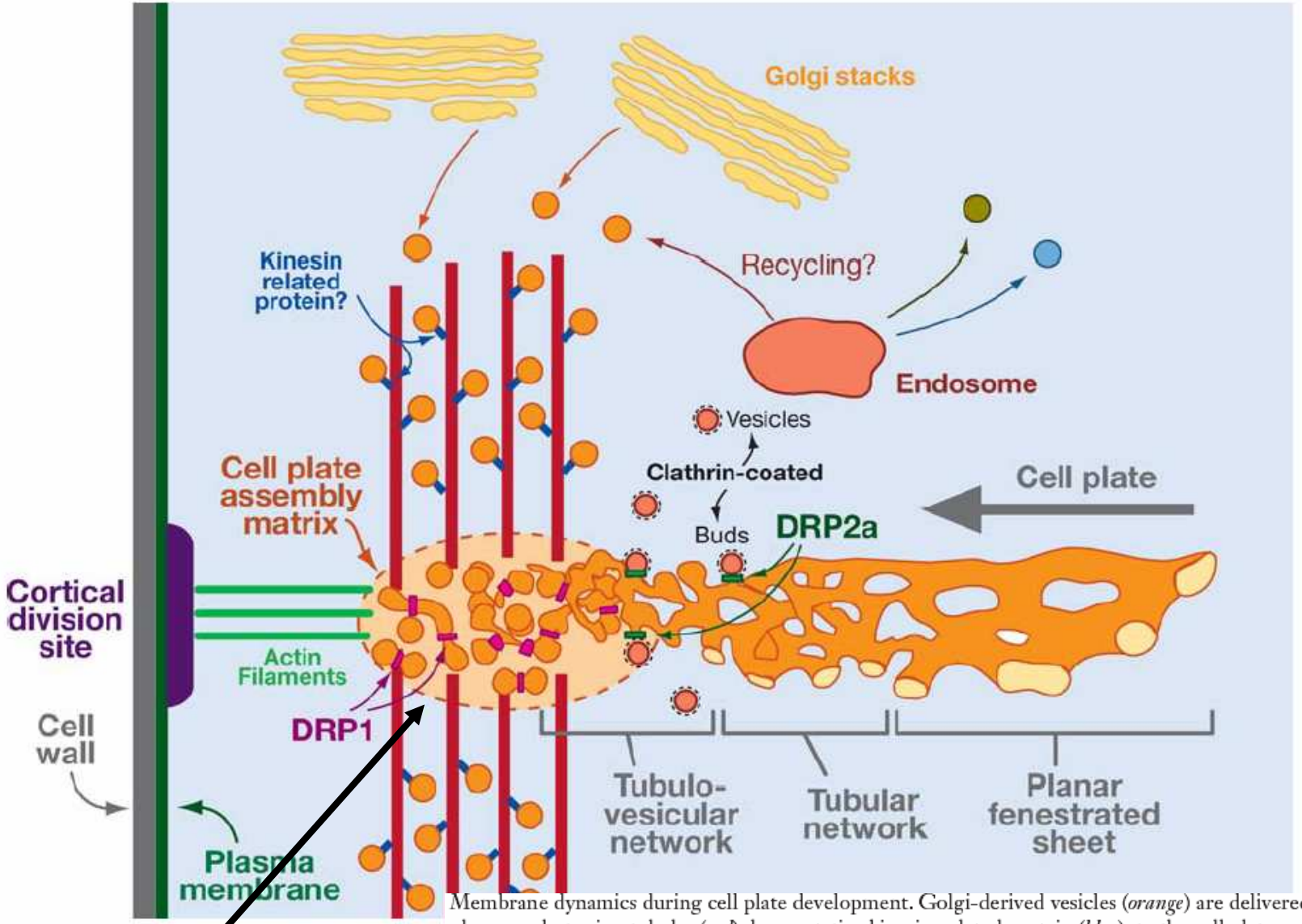


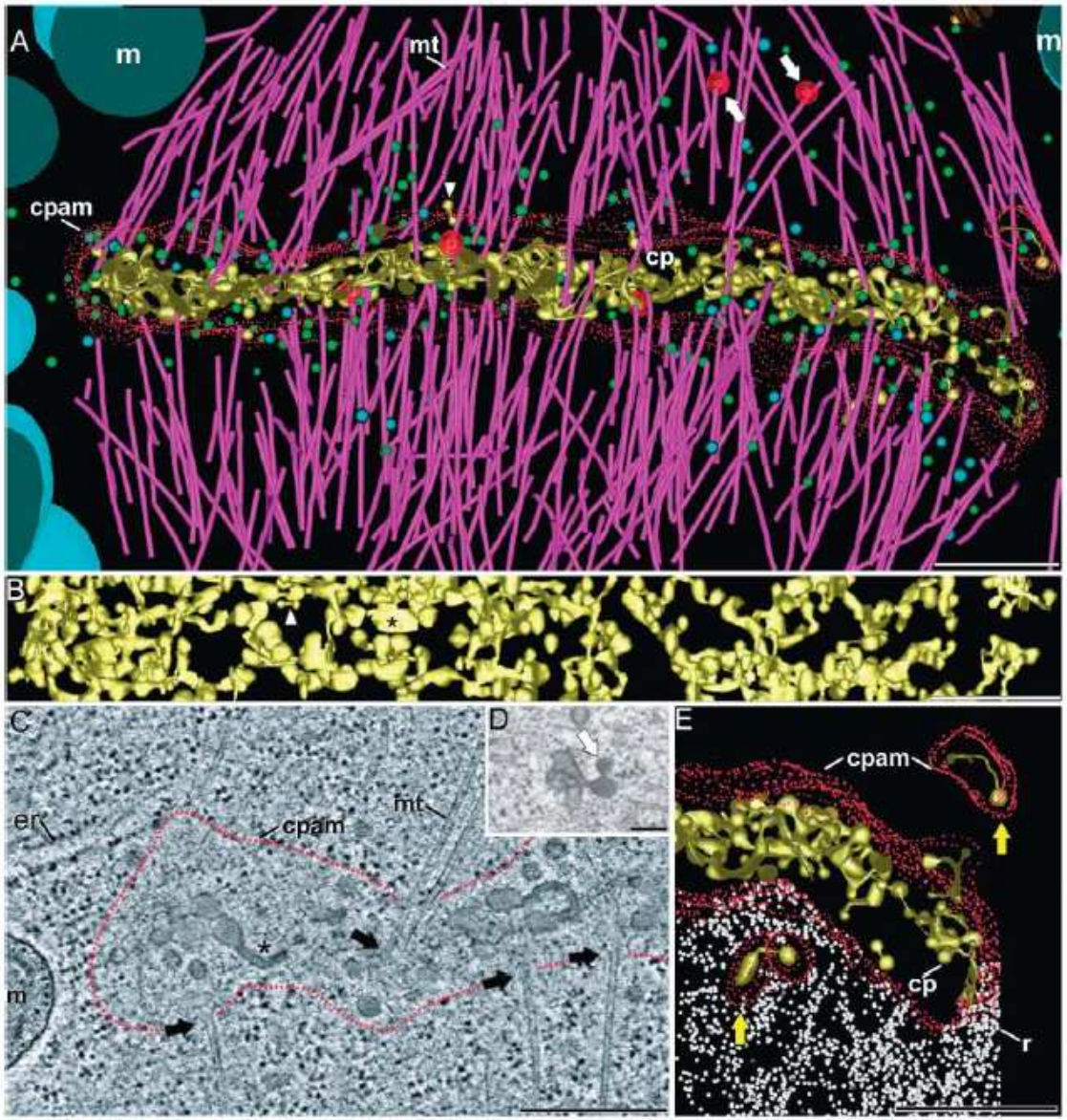
Figure 1. Overview of animal and plant cytokinesis. (a) Cytokinesis in animal cells. The spindle midzone/midbody forms when microtubules (MTs) from opposite poles overlap. It consists of the overlapping microtubules as well as associated proteins that bundle these MTs and other proteins that together 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, Hepler lab)

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

Homotypická fúze váčků: SNARE et al., Exocyst?

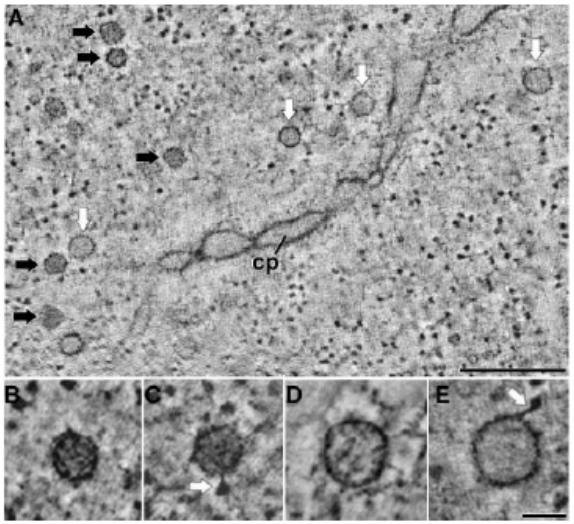
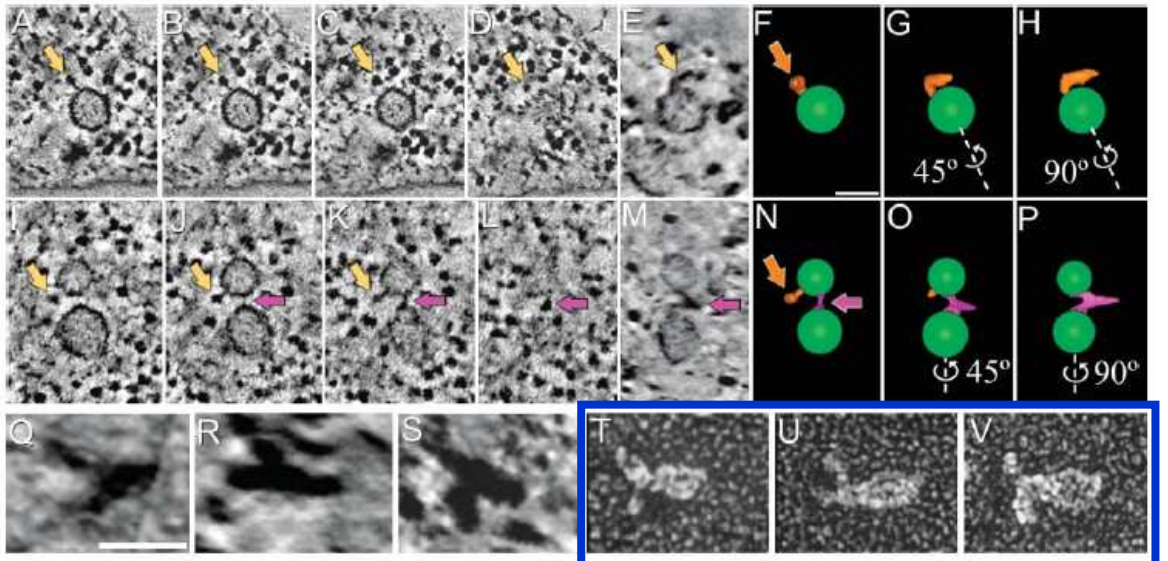


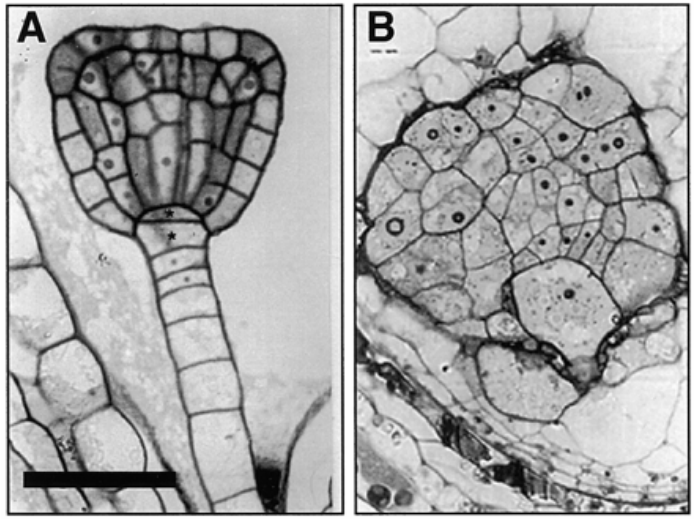
Figure 2. Two Different Types of Vesicles in the Vicinity of the Cell Plate.

(Segui-Simarro et al. 2004)



(exocyst)

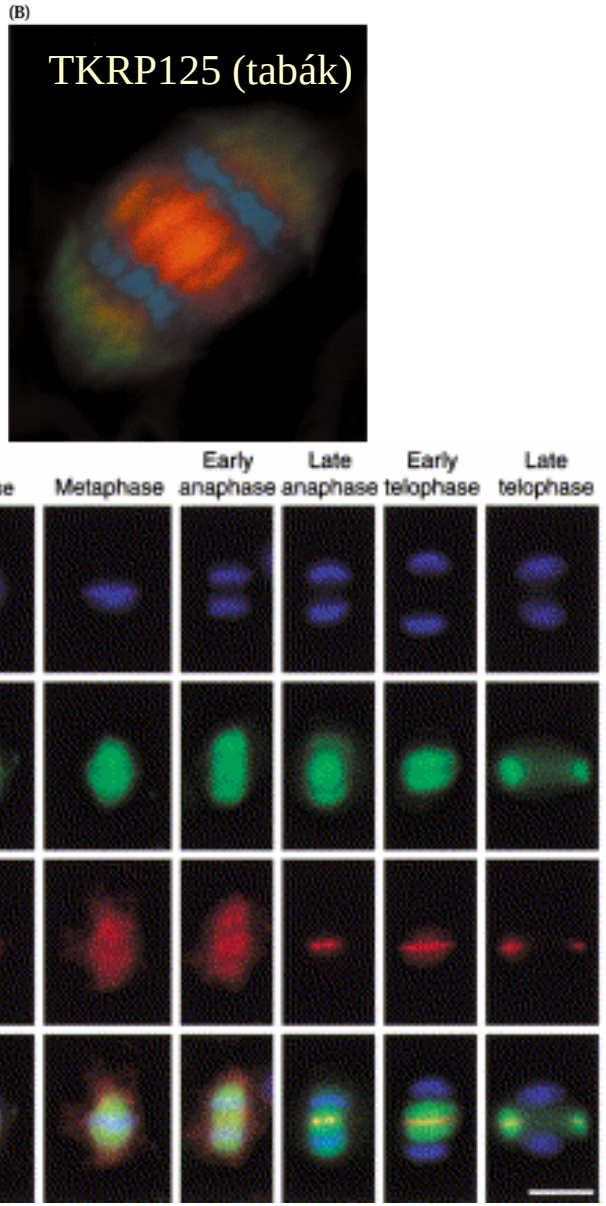
- KNOLLE : syntaxin (v-SNARE)
- přísluš. t-SNARE asi redundantní (SNAP33, SNAP29, SNAP30)
- KEULE : Sec1-related, interakce s KNOLLE
- KNOLLE a syntaxin SYP31: interakce s CDC48



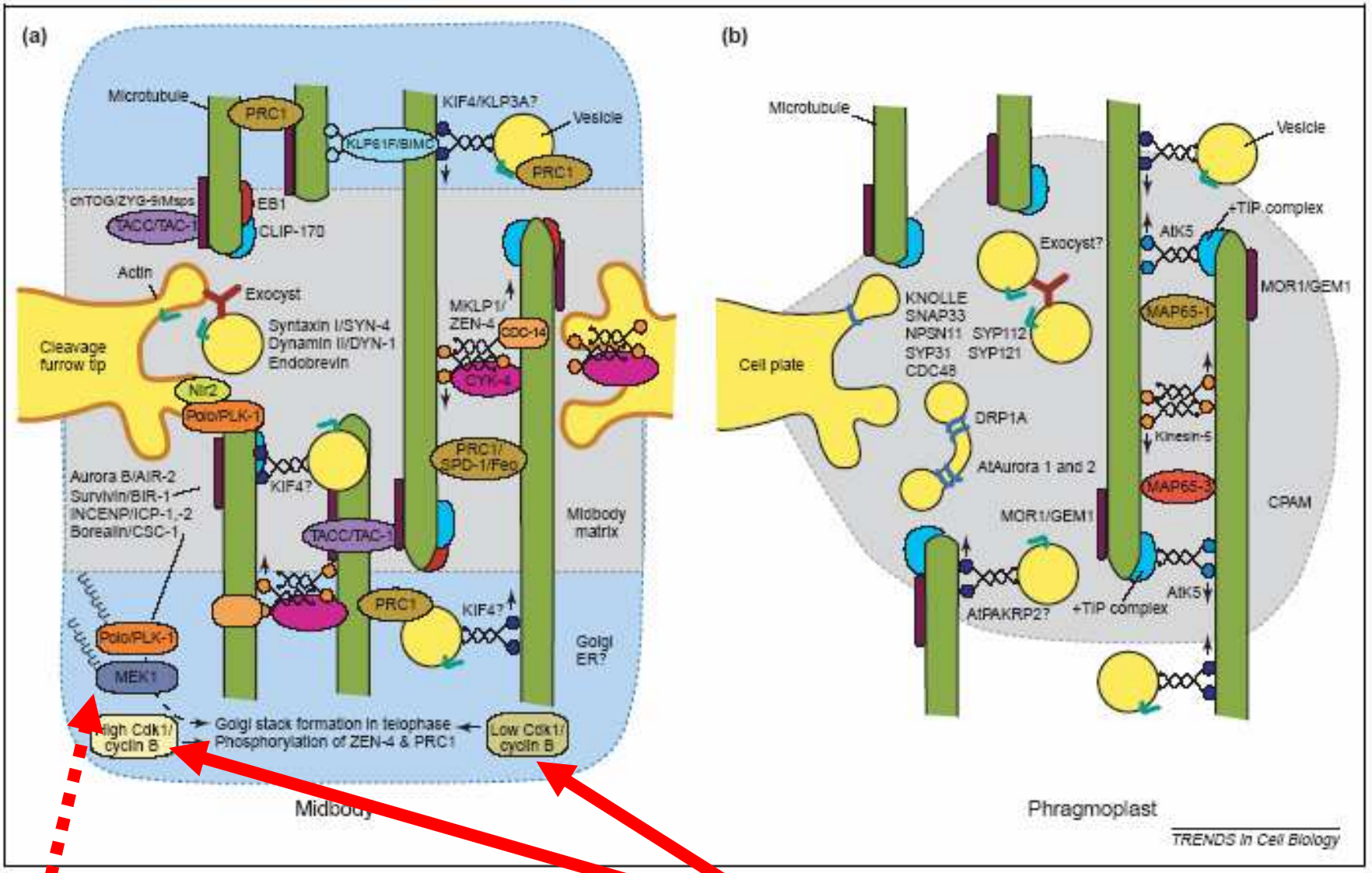
wt keule

„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!!!

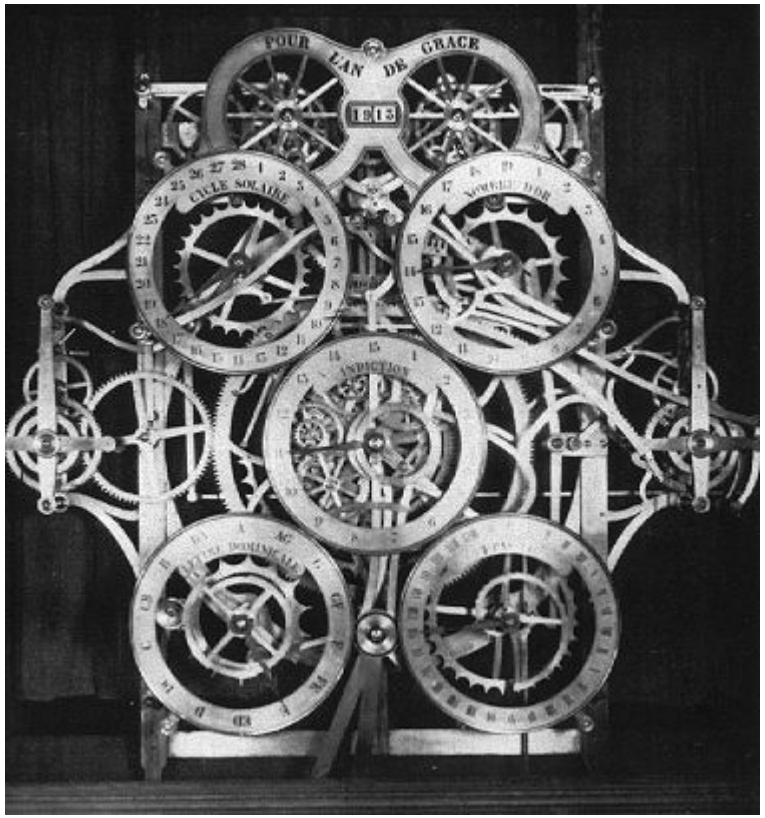
CDK

Vstupy - (nejen) „rostlinná specifika“

centrální oscilátor
(„cell cycle engine“)

vstupy

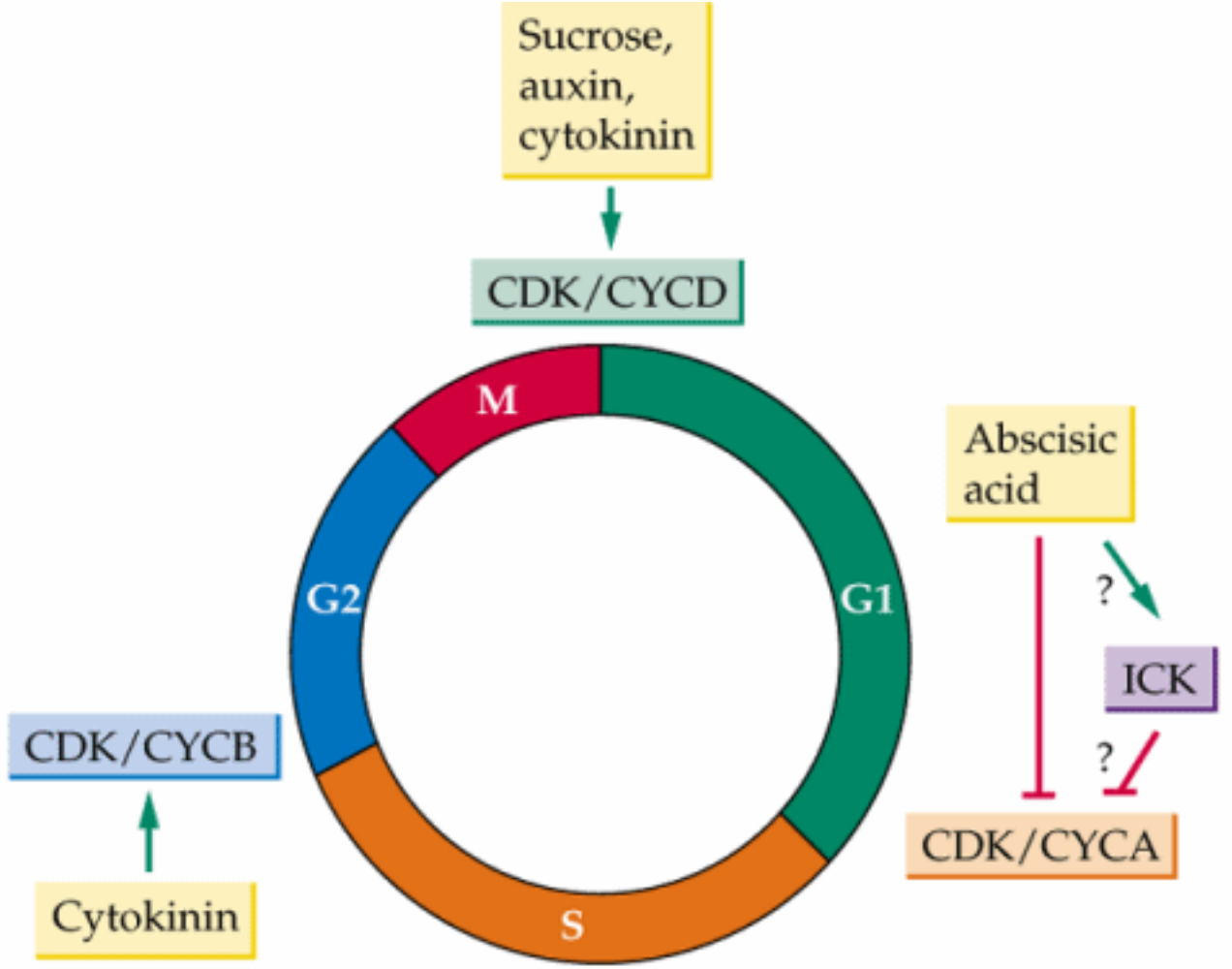
velikost
signály
poškození ...



výstupy

gen. exprese
replikace
mitosa,
cytokinese ...

Kontrola cyklu fytohormony



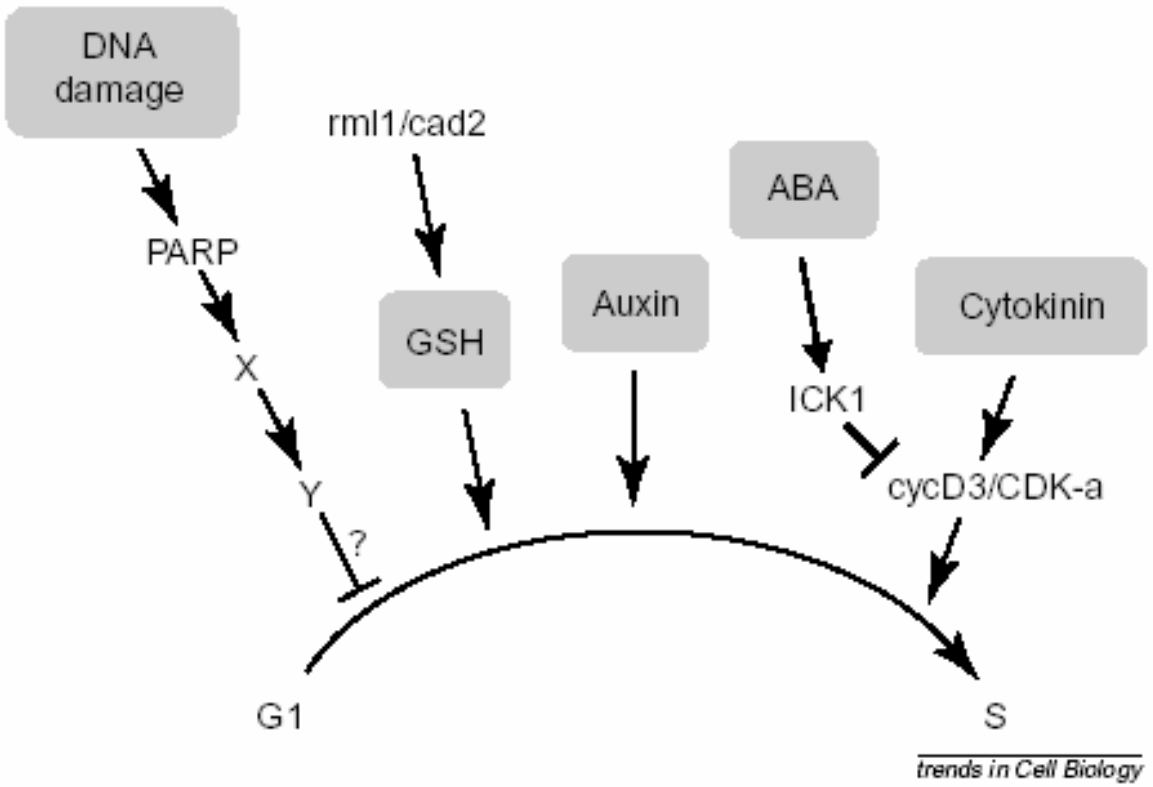


FIGURE 3

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.

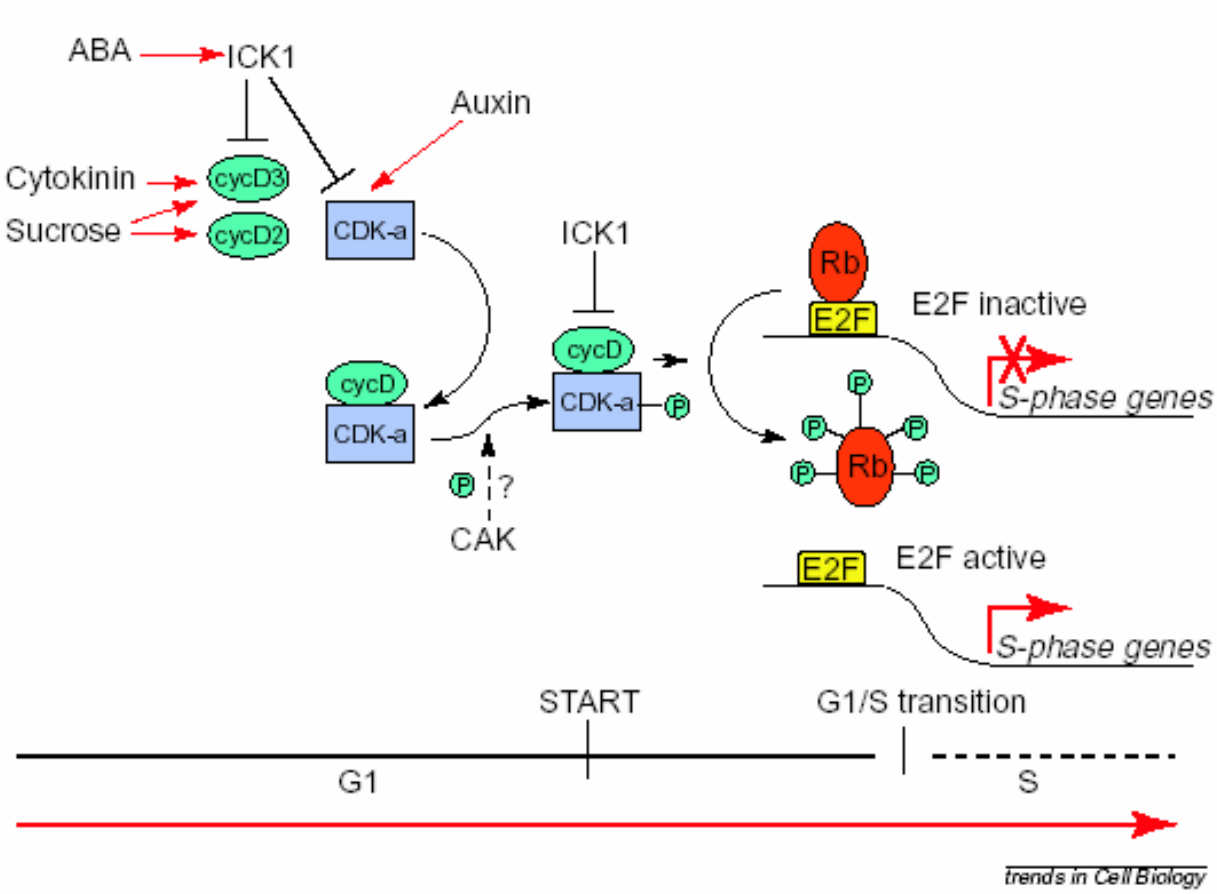
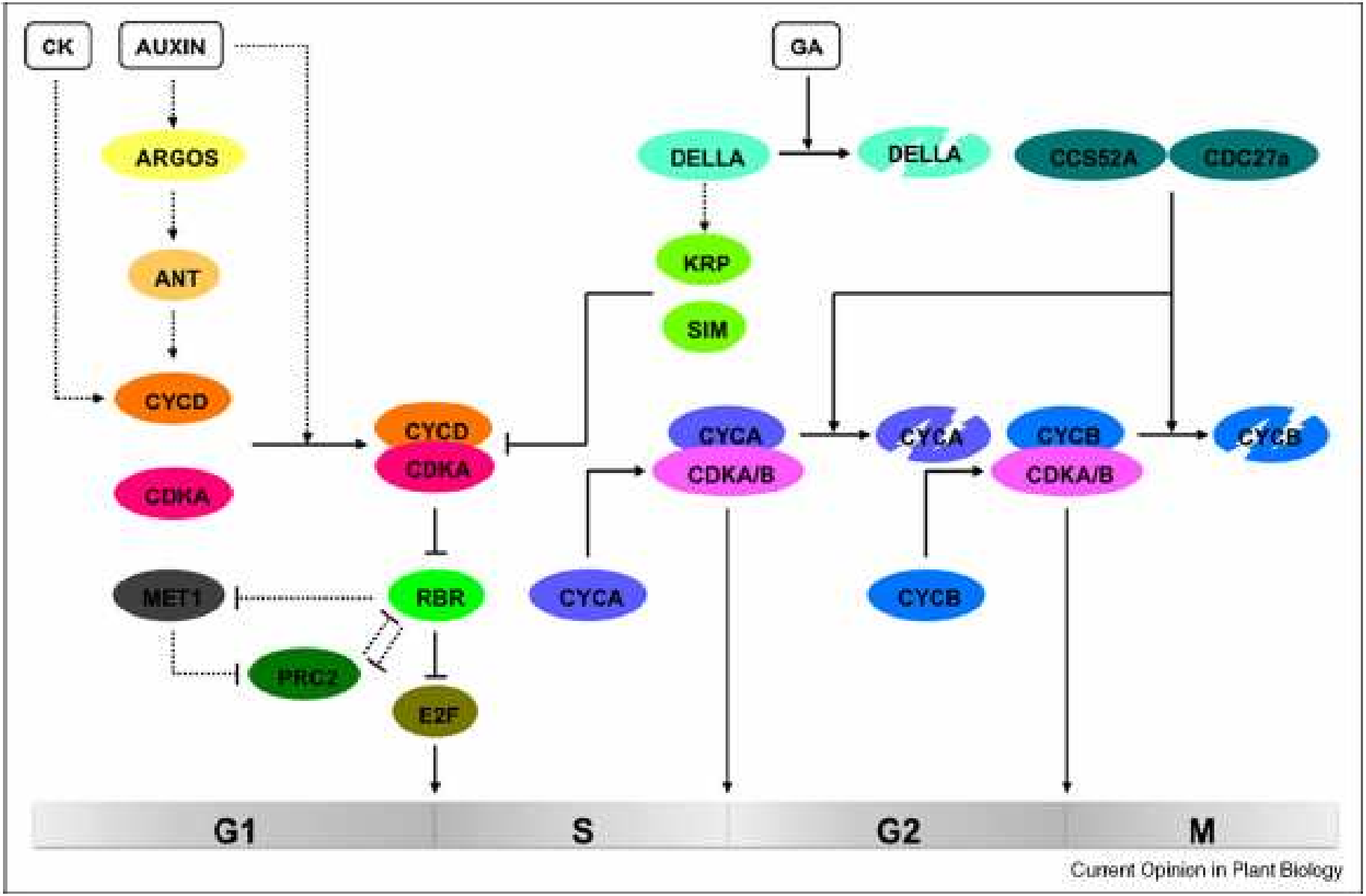


FIGURE 2

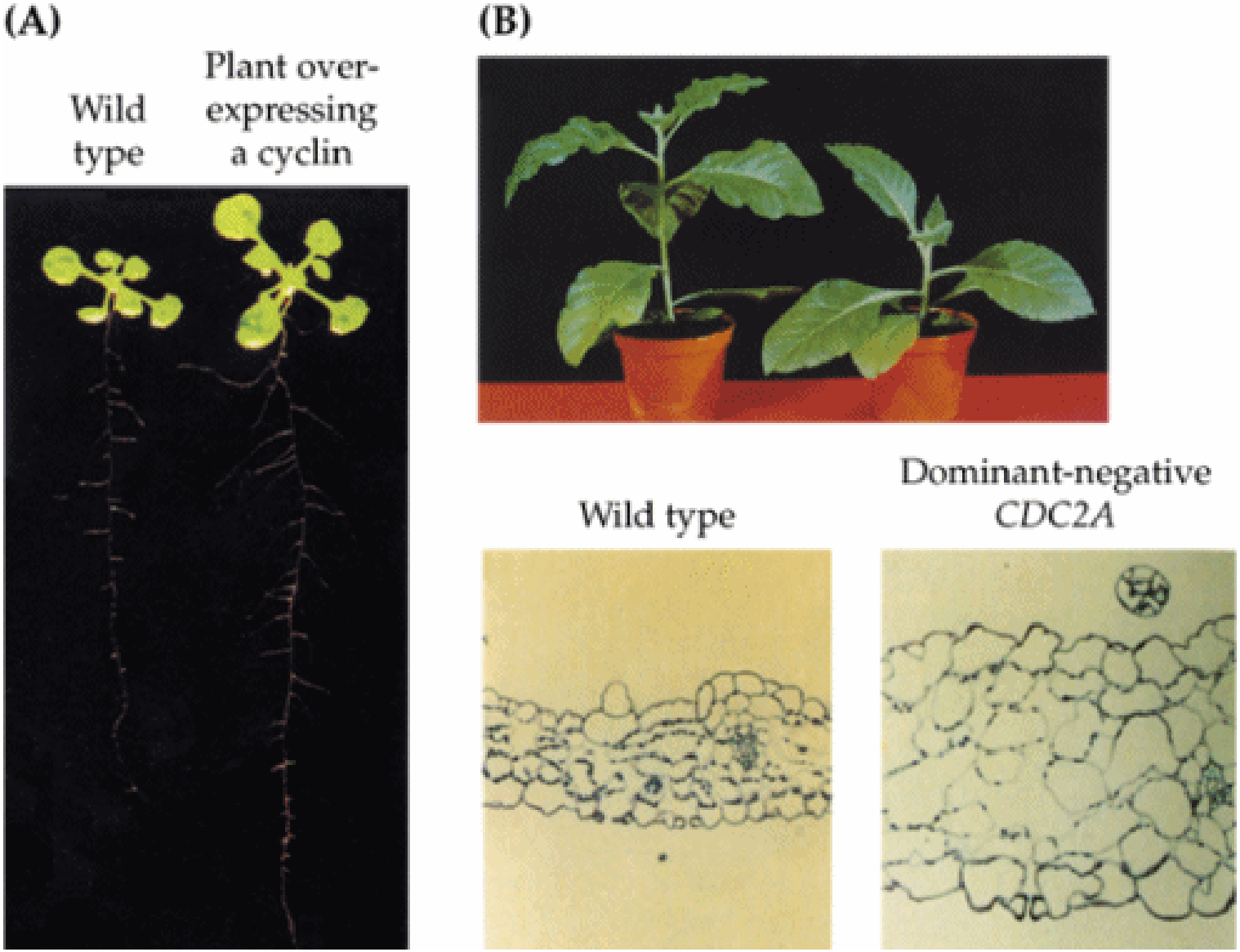
Model for G1–S transition in plants. Cytokinin- and sucrose-induced D-type cyclins bind to cyclin-dependent kinase-a (CDK-a) to form inactive heterodimers. Regulation of kinase activity after binding the cyclin might occur either by an inhibitor (ICK1) or by phosphorylation by an activating kinase (CAK). 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.


CK
AUXIN
GA
ARGOS
DELLA
DELLA
CCS52A
CDC27a
ANT
KRP
SIM
CYCD
CYCD
CDKA
CYCA
CYCA
CYCB
CYCB
CDKA
CDKA/B
CDKA/B
MET1
RBR
CYCA
CYCB
PRC2
E2F
G1
S
G2
M
Current Opinion in Plant Biology

Na úrovni buňky jasné priority???

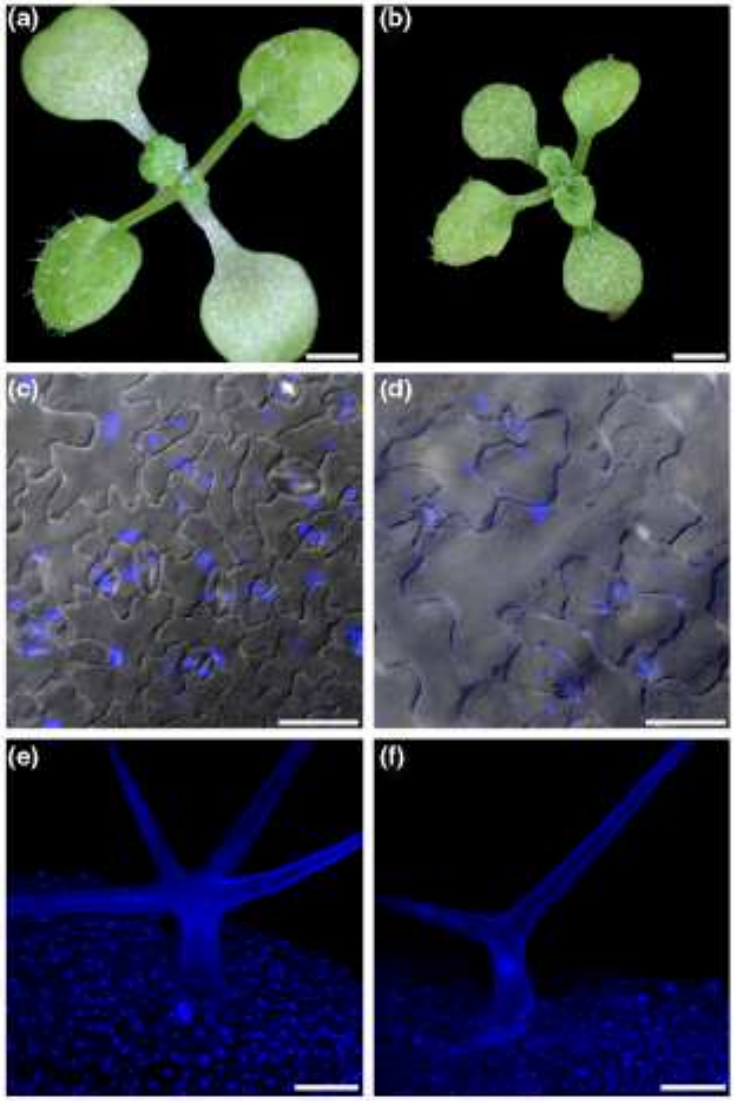
- Základem je „cell cycle engine“
- Regulace vstupů a výstupů jsou „přívěsky“
- Víme, že rostliny rakovinu nemívají
- ALE...

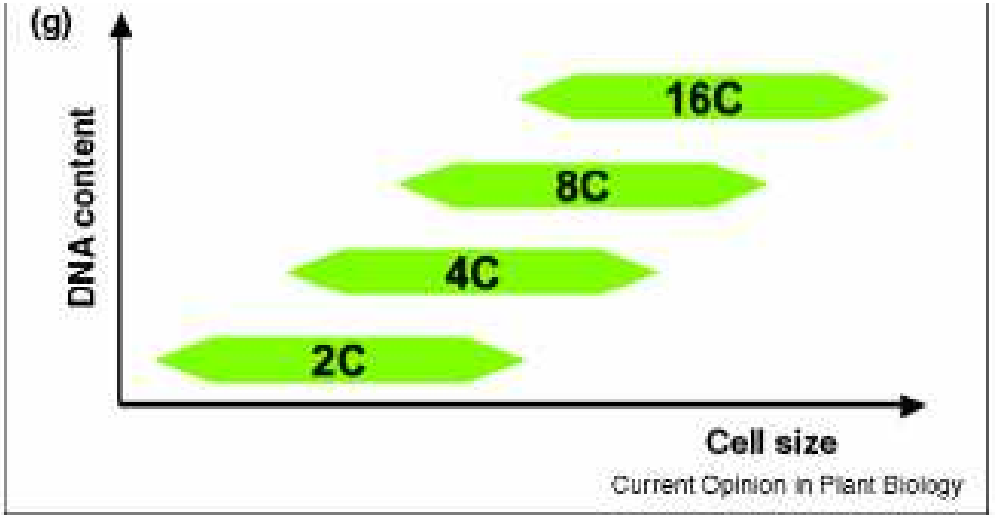
Mutace a změny exprese centrálních regulátorů cyklu



Rostlina vládne buňkám, ne naopak!

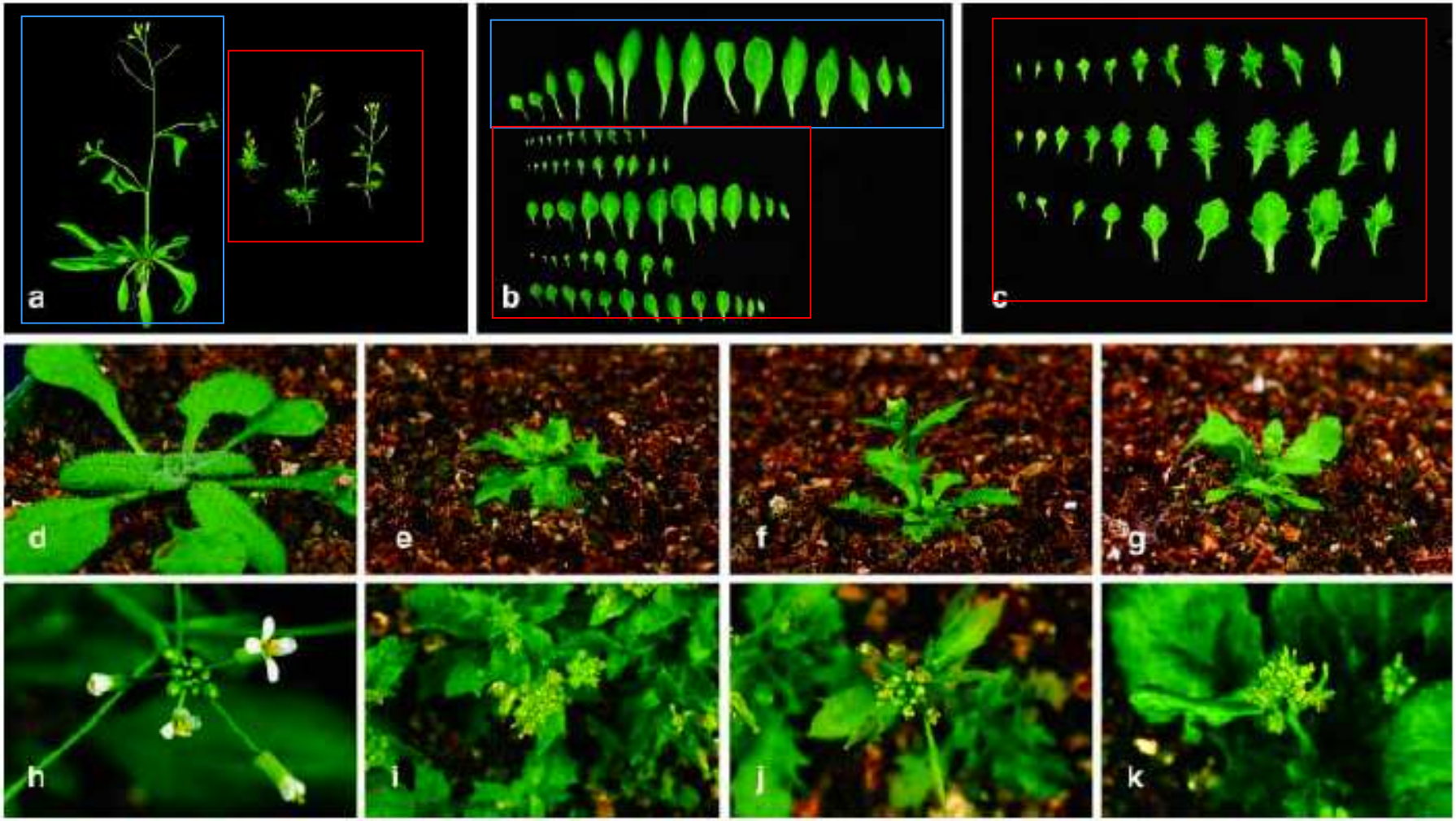
Typický fenotyp cyklových mutací?





Weak loss-of-function mutants in the central cell-cycle kinase CDKA;1 (b) are much smaller than wild-type Arabidopsis plants with much fewer cells (a). However, cell size of epidermal pavement cells is strongly increased in weak loss-of-function mutants of CDKA;1 (d) in comparison with wild type (c) as seen in DIC light micrographs overlaying an DAPI epifluorescence image of the same leaf. DAPI stained leaves showing a trichome (leaf hair) that is a typical example for an endoreplicating cell, note the large nucleus (e). A reduction of endoreplication (please note the smaller nucleus) results in smaller trichomes with fewer branches (f). (g) The relationship between cell size and nuclear size is flexible and at a given DNA content cells can reach very different sizes, as seen in (c) and (d). However, the DNA content of a cell apparently sets an upper limit for cell expansion as in trichomes on weak loss-of-function mutants of CDKA;1 (f).

(Harashima09)



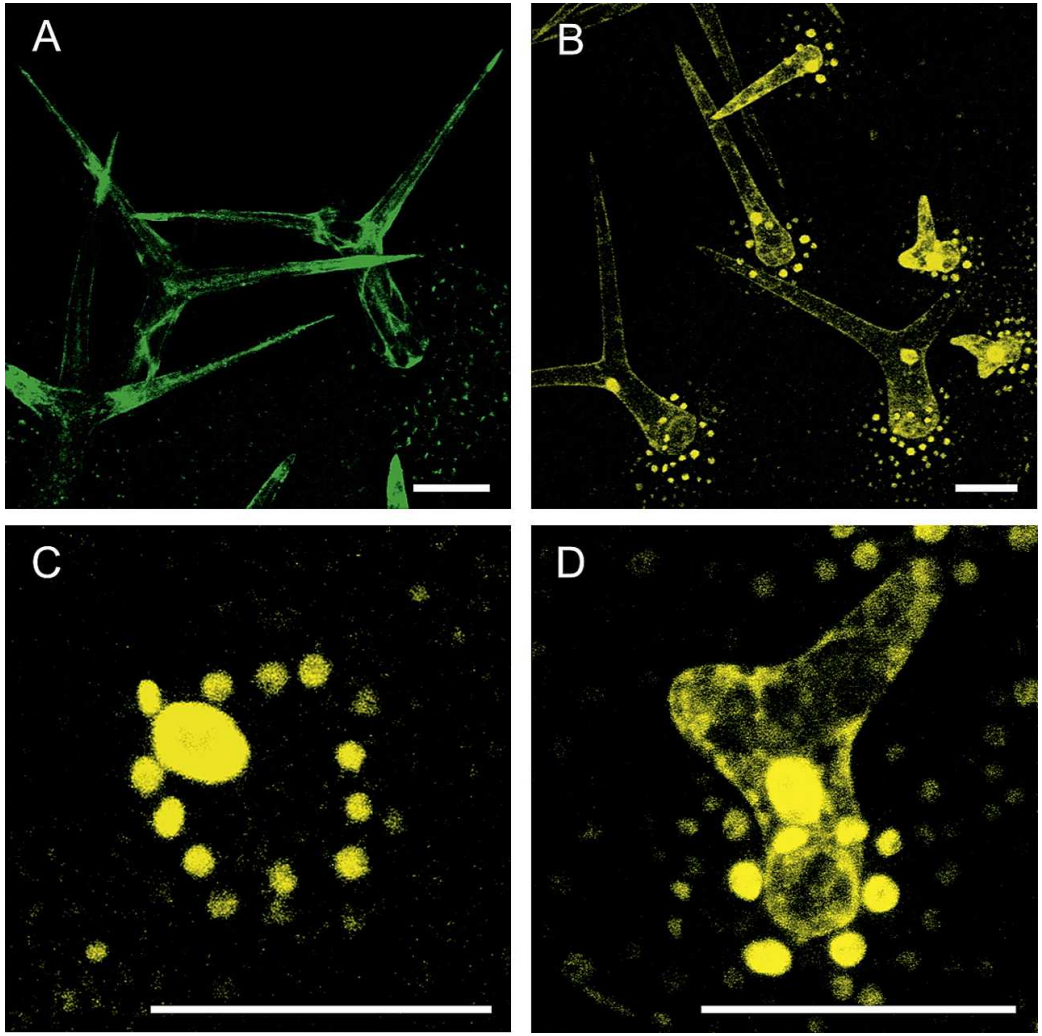
masivní overexprese KRP (Kip-related protein, inhibitor CDK):
malé rostliny, méně buněk

wt

Zhou et al. 2002

Intercellular and subcellular localization of Arath;KRP1

pGL2:GFP



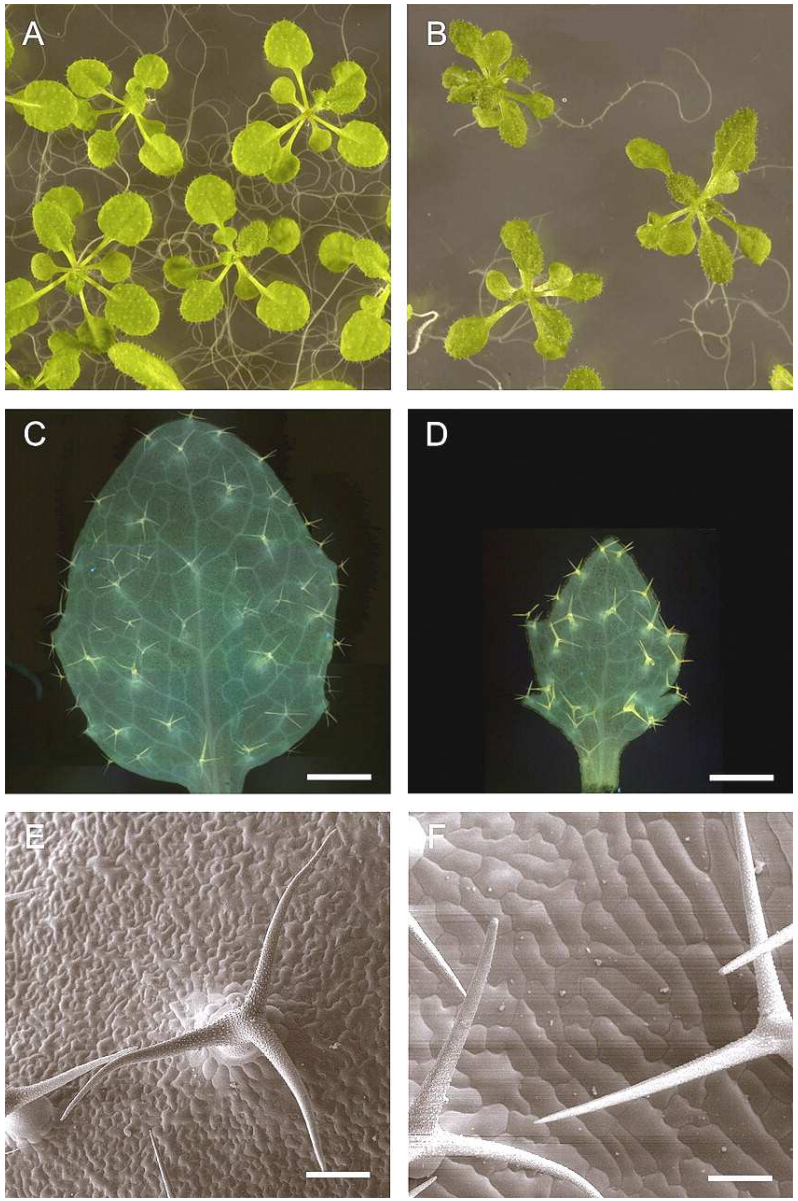
pGL2:KRP1:GFP

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!

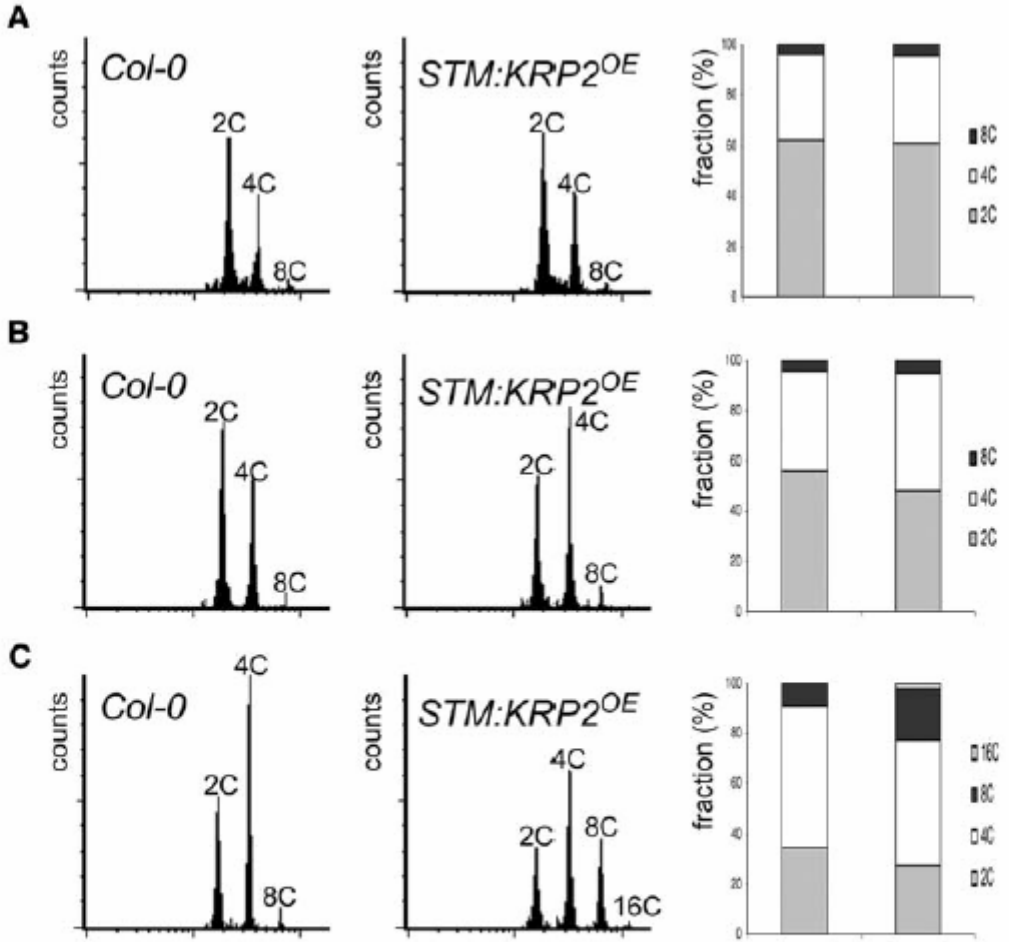


Figure 6. Ploidy Level Distribution of the First Leaves of Wild-Type (Col-0; Left) and *STM:KRP2^{OE}* (Line 5; Right) Plants during Development as Measured by Flow Cytometry.

(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

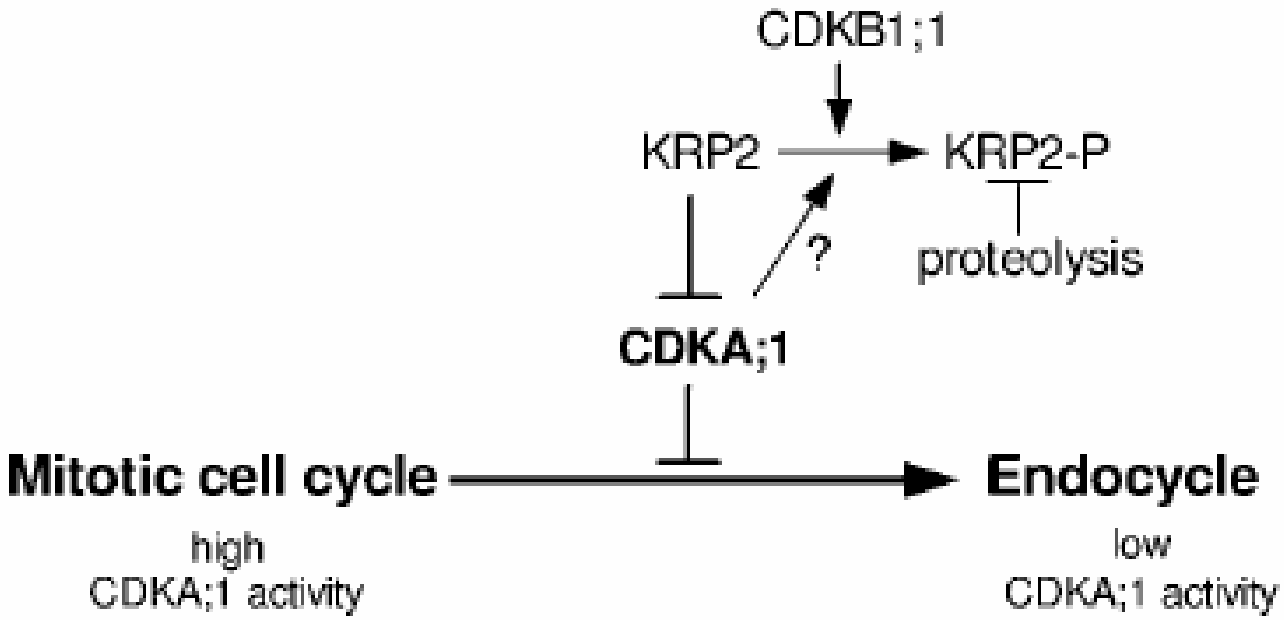
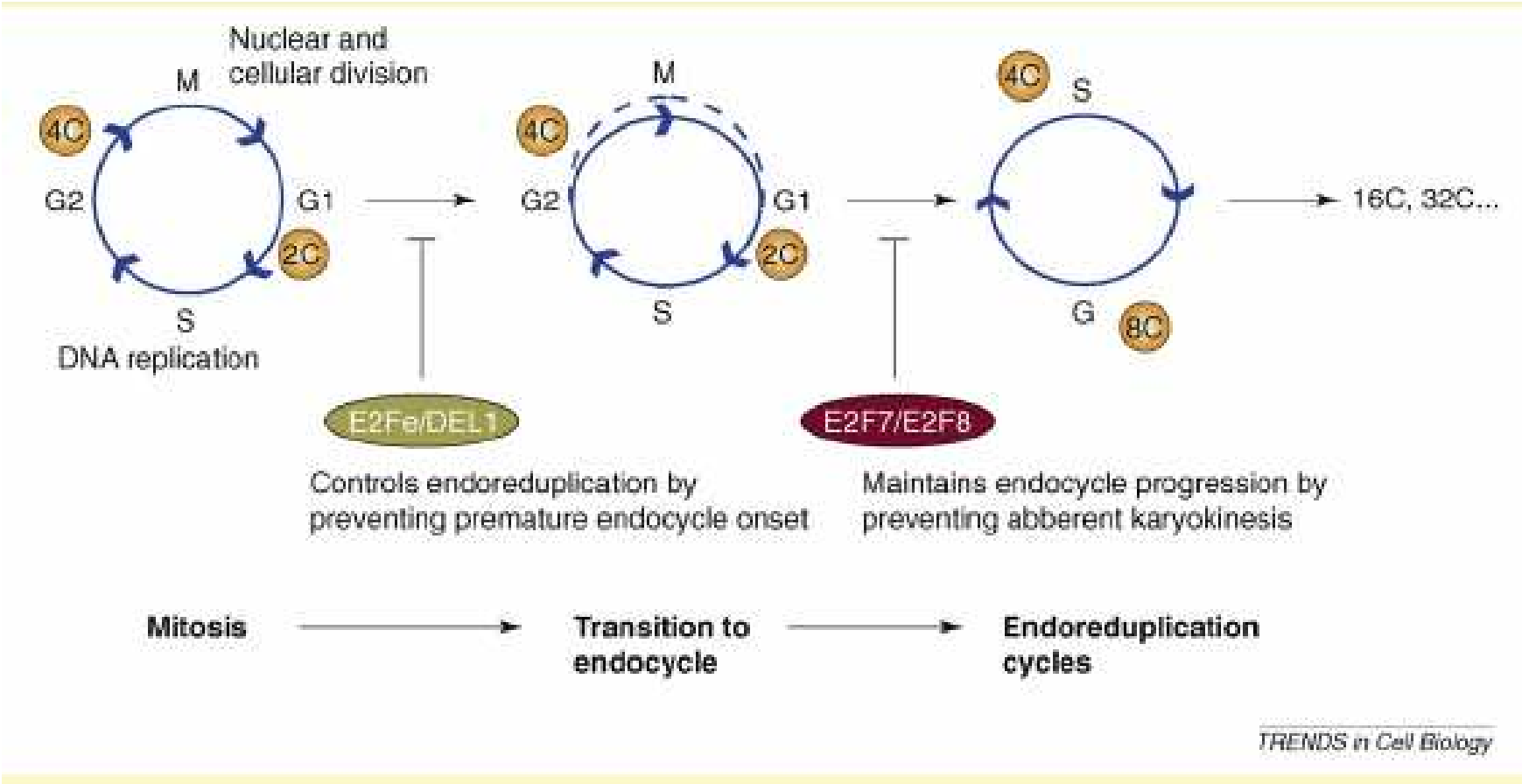


Figure 9. Model Illustrating the Role of CDK Activity in Controlling the Onset of Endoreduplication.

„Atypické E2F pro endoreduplikaci?



… např. kontrola exprese aktivátorů APC…

FZR2/CCS52

Arabidopsis:

FZR2/CCS52

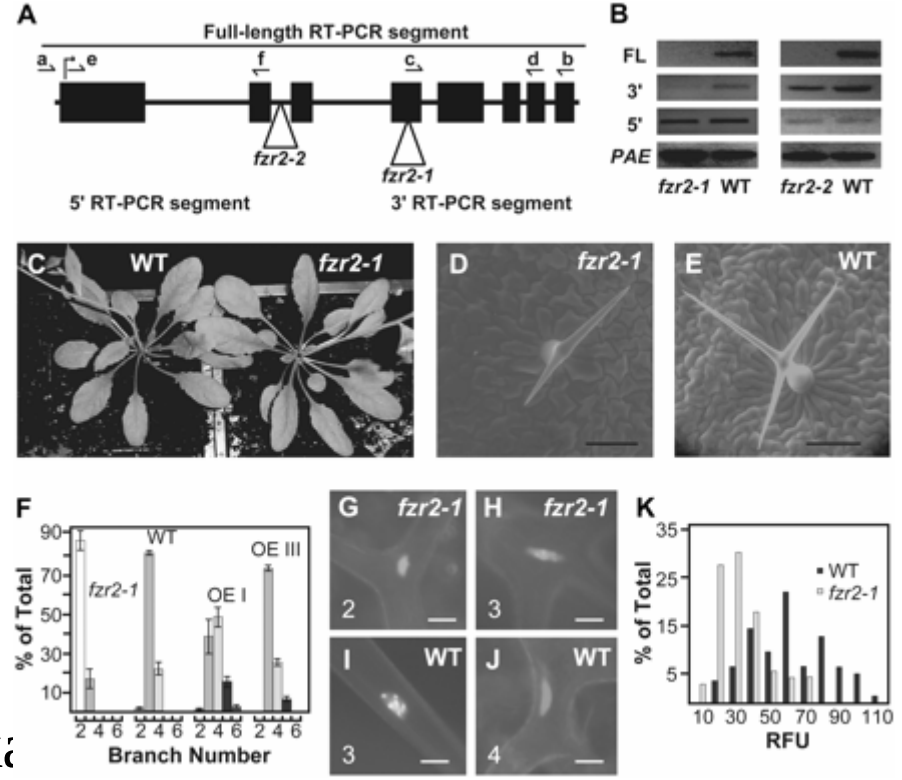
↓

endocycle

specif. APC aktivátor

mutace inhibuje endoreduplikaci

(degradace cyklinů???)



(Larson-Rabin,2009)

A, Diagram of the *FZR2* locus depicting. B,. C, Flowering wild-type (WT; left) and *fzr2-1* (right) plants. D and E, ESEM micrographs of leaf trichomes of *fzr2-1* (D) and wild-type (E) plants. Scale bar = 100 μm. F, Summary of leaf trichome branch production. Error bars represent SEs. G and J, Representatives of DAPI-stained trichome nuclei of *fzr2-1* (G and H) and wild type (I and J). The branch number for each trichome is given in the lower left of each picture. Scale bar = 15 μm. K, Summary of in situ fluorescence measurements of DAPI-stained trichome nuclei, given in relative fluorescence units (RFU).

Rozhoduje i poměr isoforem CDK!

Arabidopsis:

CDKB1;1 +CYCA2;3

⊥

endocycle

dominant neg. cdkb1:1
indukuje endored.

- suprese ox CYCA2;3

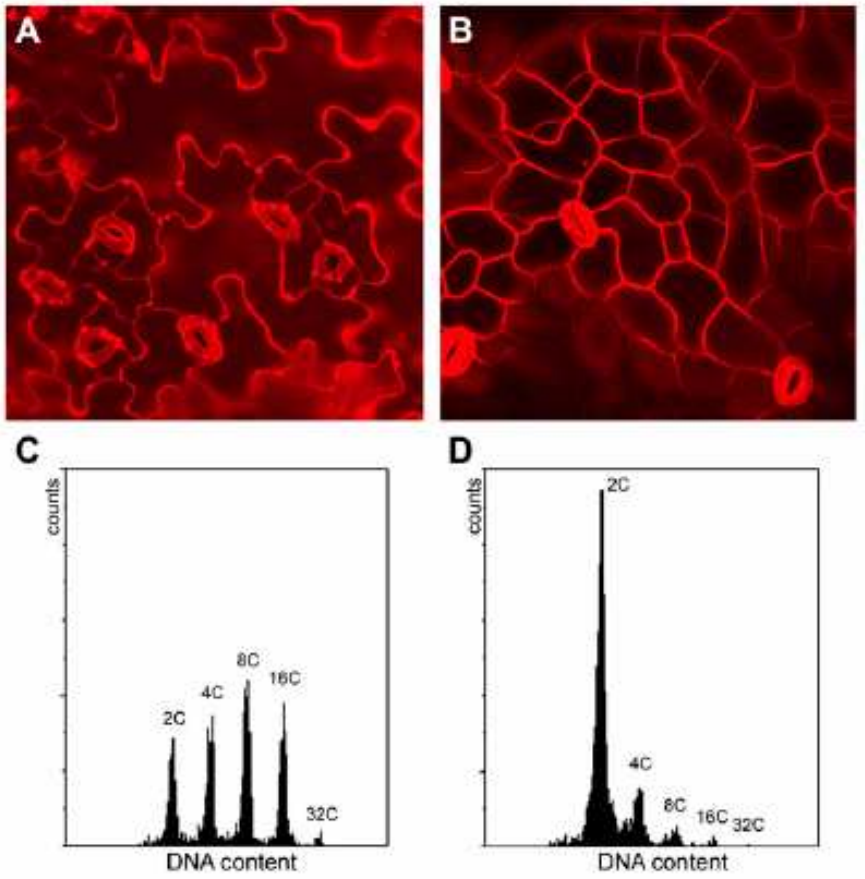


Figure 2. Effect of coexpression of *CYCA2;3-nGFP* and *CDKB1;1-cGFP*. Col-0 seedlings (A and C) and plants coexpressing *CYCA2;3-nGFP* and *CDKB1;1-cGFP* (B and D) were grown for 12 DAG on agar medium, and cotyledons were harvested. The cotyledons were stained with PI and observed with a confocal laser-scanning microscope (A and B) or subjected to flow cytometry (C and D).

Plant Physiol. Vol. 150, 2009

(Boudolf et al. 2004,2009)

Další faktory ovlivňující endoreduplikaci

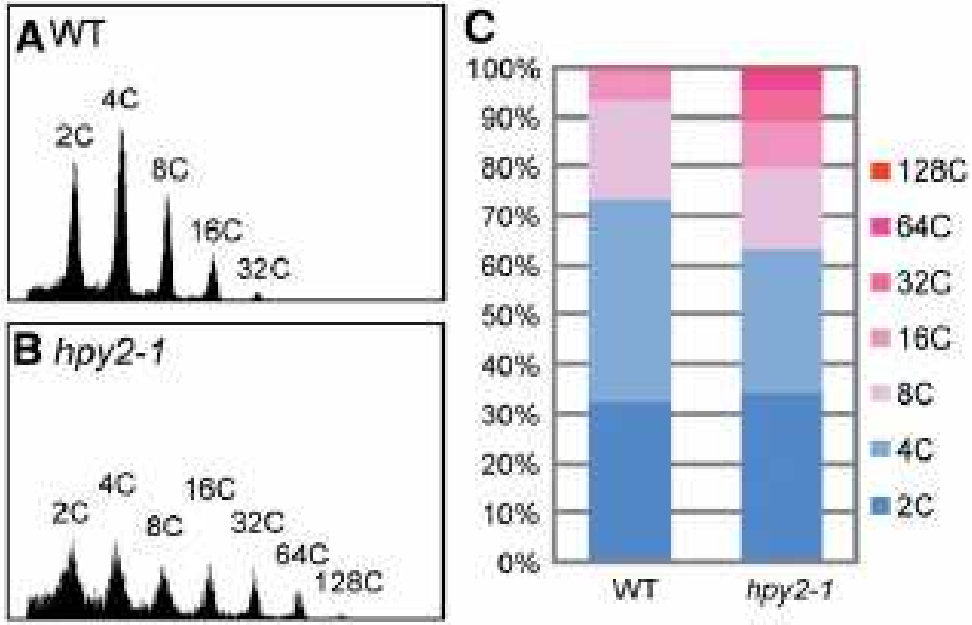
The Plant Cell, Vol. 21: 2284–2297, August 2009, www.plantcell.org © 2009 American Society of Plant Biologists

SUMO E3 Ligase HIGH PLOIDY2 Regulates Endocycle Onset and Meristem Maintenance in *Arabidopsis*

Takashi Ishida,[a] Sumire Fujiwara,[a] Kenji Miura,[b] Nicola Stacey,[c] Mika Yoshimura,[a] Katja Schneider,[a] Sumiko Adachi,[d] Kazunori Minamisawa,[d] Masaaki Umeda,[d] and Keiko Sugimoto[a,c,1]

hpy2:
méně CYCB1, CDKB1, CDKB2!

exprese pHPY2:GUS
- indukována NAA
- potlačena antagonisty auxinu



Research Article

Auxin modulates the transition from the mitotic cycle to the endocycle in *Arabidopsis*

Takashi Ishida[1], Sumiko Adachi[2], Mika Yoshimura[1], Kohei Shimizu[2], Masaaki Umeda[2] and Keiko Sugimoto[1,*]

+ Author Affiliations

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Summary

Amplification of genomic DNA by endoreduplication often marks the initiation of cell differentiation in animals and plants. The transition from mitotic cycles to endocycles should be developmentally programmed but how this process is regulated remains largely unknown. We show that the plant growth regulator auxin modulates the switch from mitotic cycles to endocycles in *Arabidopsis*; high levels of TIR1-AUX/IAA-ARF-dependent auxin signalling are required to repress endocycles, thus maintaining cells in mitotic cycles. By contrast, lower levels of TIR1-AUX/IAA-ARF-dependent auxin signalling trigger an exit from mitotic cycles and an entry into endocycles. Our data further demonstrate that this auxin-mediated modulation of the mitotic-to-endocycle switch is tightly coupled with the developmental transition from cell proliferation to cell differentiation in the *Arabidopsis* root meristem. The transient reduction of auxin signalling by an auxin antagonist PEO-IAA rapidly downregulates the expression of several core cell cycle genes, and we show that overexpressing one of the genes, *CYCLIN A2;3* (*CYCA2;3*), partially suppresses an early initiation of cell differentiation induced by PEO-IAA. Taken together, these results suggest that auxin-mediated mitotic-to-endocycle transition might be part of the developmental programmes that balance cell proliferation and cell differentiation in the *Arabidopsis* root meristem.

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January 1, 2010
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137, 63-71.

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K cyklu (volně) patří i „poslední věci buňky“

Není smrt jako smrt

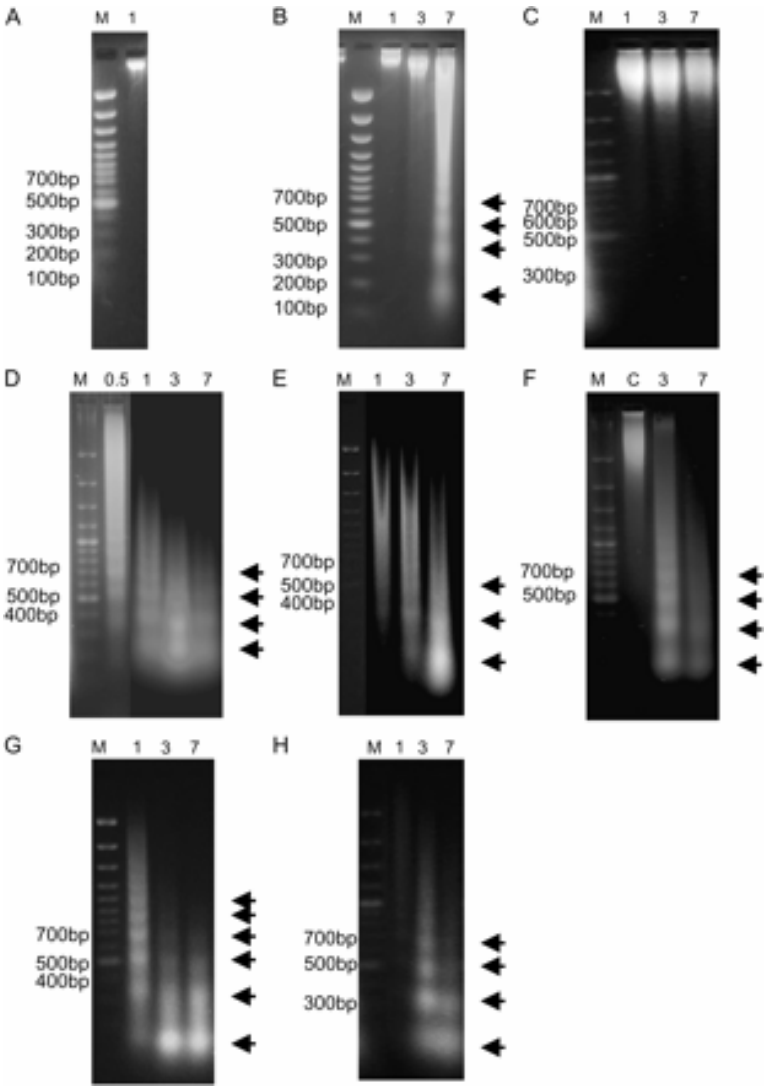
Comparison of morphological features of apoptosis and necrosis.

Apoptosis	Necrosis
Single cells or small clusters of cells	Often contiguous cells
Cell shrinkage and convolution	Cell swelling
Pyknosis and karyorrhexis	Karyolysis, pyknosis, and karyorrhexis
Intact cell membrane	Disrupted cell membrane
Cytoplasm retained in apoptotic bodies	Cytoplasm released
No inflammation	Inflammation usually present

„Kritéria apoptózy“ - DNA žebřík, fragmentace (TUNEL), aktivita kaspáz...

... ale toto pro (fagocytující mnohobuněčné) živočichy
... tedy otázka obecnosti ...

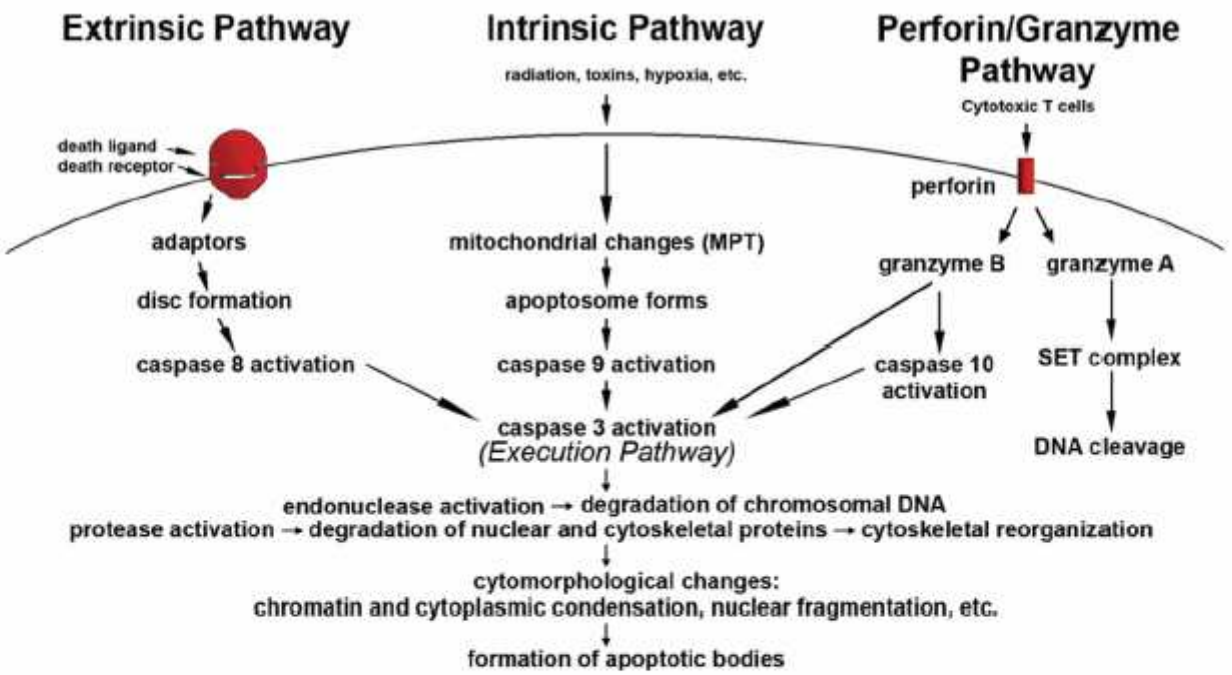
U rostlin to může být i jinak ...



Internucleosomal fragmentation of tobacco genomic DNA during 7 d after different treatments. (A) Untreated control BY-2 cells in the exponential phase of growth, (B) BY-2 cells treated with 50 µM $CdSO_4$, (C) BY-2 cells treated with 1 mM $CdSO_4$, (D) BY-2 cells killed by freezing in liquid nitrogen and further cultivated after thawing at 25 °C, (E) BY-2 cells killed by liquid nitrogen and further cultivated at 25 °C in the presence of 1 mM $CdSO_4$, (F) tobacco leaves frozen in liquid nitrogen and cultivated at 25 °C, (G) BY-2 cells killed mechanically by homogenization in a mixer mill and further cultivated at 25 °C, (H) BY-2 cells treated with 5% Triton X-100. DNA isolated from the cells ... was separated in 1.8% agarose gel. Line markers: M, molecular mass marker; C, untreated control leaves; 0.5, 1, 3, 7, d of culture (exposure in case of $CdSO_4$ and Triton X-100 treatments).

(Kuthanová, Opatrný, Fischer - J. Exp. Botany 2008)

Cesty k apoptose ... u živočichů



(Elmore 2007)

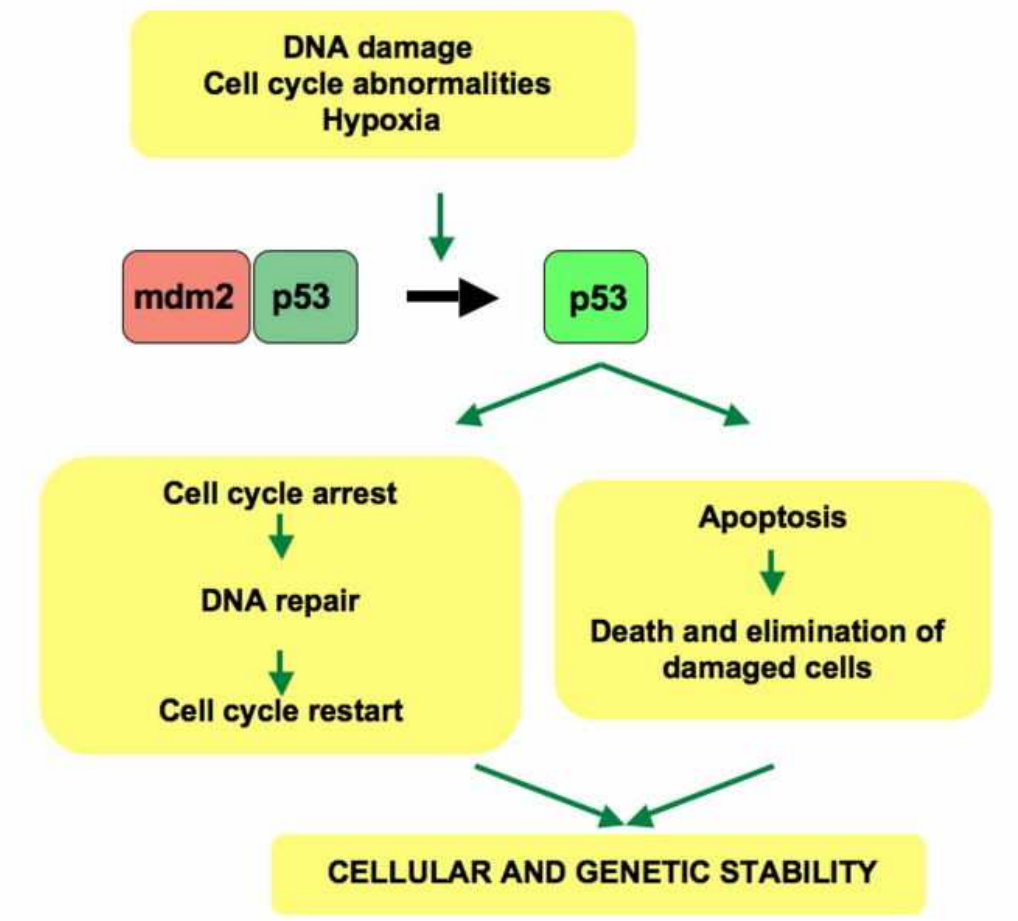
Figure 3.
Schematic representation of apoptotic events. The two main pathways of apoptosis are extrinsic and intrinsic as well as a perforin/granzyme pathway. Each requires specific triggering signals to begin an energy-dependent cascade of molecular events. Each pathway activates its own initiator caspase (8, 9, 10) which in turn will activate the executioner caspase-3. However, granzyme A works in a caspase-independent fashion. The execution pathway results in characteristic cytomorphological features including cell shrinkage, chromatin condensation, formation of cytoplasmic blebs and apoptotic bodies and finally phagocytosis of the apoptotic bodies by adjacent parenchymal cells, neoplastic cells or macrophages.

Napojení na regulaci cyklu

- Cdk1 indukuje apo T-lymfocytů (inhibitory chrání)
- Ox Cyc B1 dtto (u rostlin endoreduplikace!)
- Bad1p (pro-apo) fosforylován CDK1/CycB
- ALE Cdk2 ox chrání vlas. folikly před apoptosou
- CDK2 downstream od kaspázy? (dnCdk2 v HeLa - kondenzace chrom. bez aktivace kaspáz)
- 2-hybrid interakce CycD3-kaspáza 2

Napojení na checkpointy

- p53 antionkogen: mismatch signalling/repair



... a ještě detektivka na závěr: rostlinný p53?

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A tormentor in the quest for plant p53-like proteins

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Proč by rostliny mohly/měly mít 53?

- MkAb Pab240 x 5 aa motivu myšího p53
- Rozpoznává 53–100 kDa u *Zea mays* a *Pisum sativum*
- Antigen koreluje s DNA repair enzymy v klíčících semenech
- 53, 73, 110 kDa v ječmeni ... purifikace

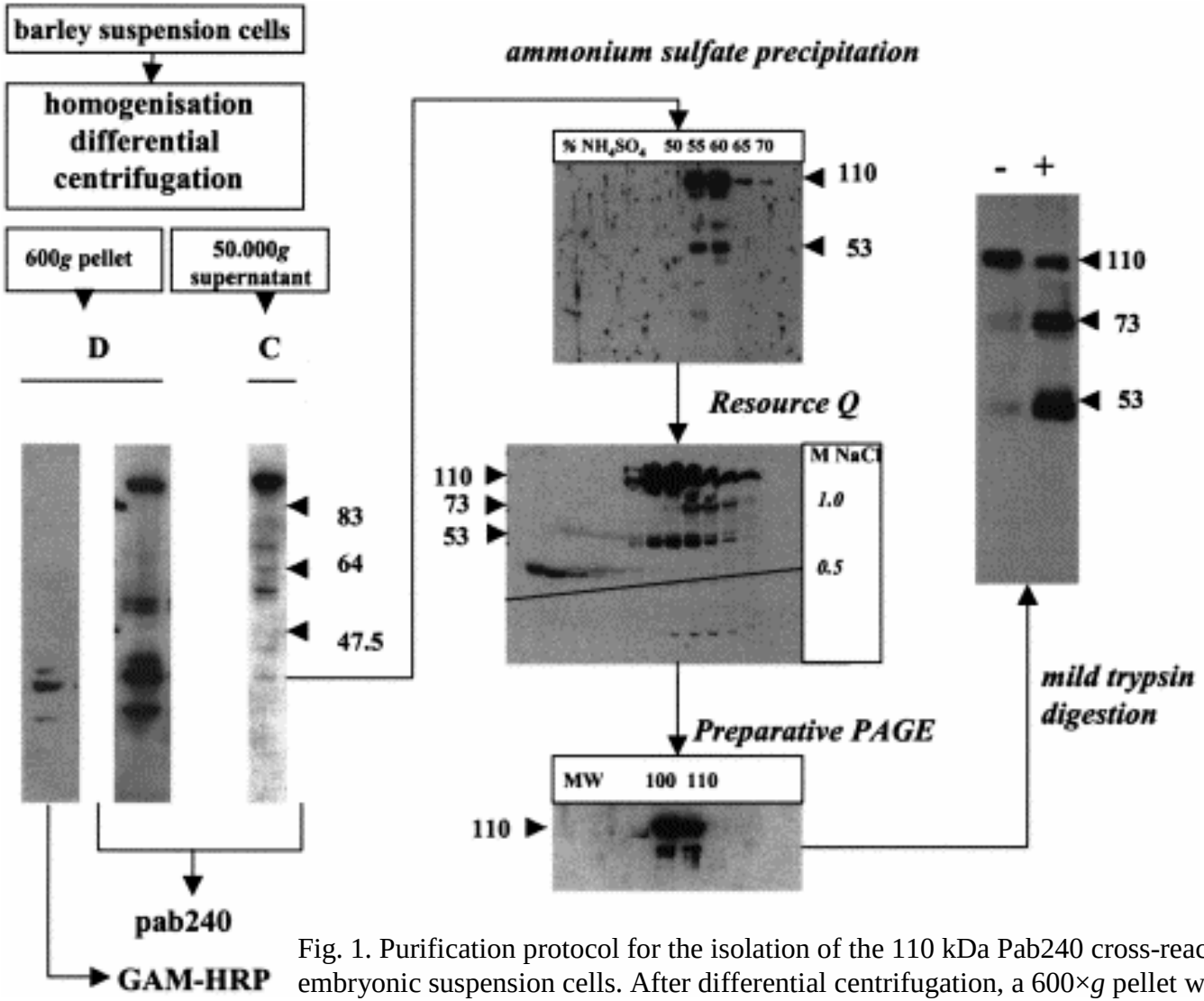

Fig. 1. Purification protocol for the isolation of the 110 kDa Pab240 cross-reactive polypeptide from barley embryonic suspension cells. After differential centrifugation, a 600×*g* pellet was obtained consisting of cellular debris (D; organelles and nuclei) and a 50 000×*g* supernatant containing the cytosolic fraction (C). The cytosolic fraction was subjected to a purification protocol ... ammonium sulphate precipitation, anion exchange (Resource Q) and Preparative PAGE. The 110 kDa polypeptide (−) was subjected to mild trypsin digestion (+) and the 73 and 53 kDa bands were excised from gel and used for sequence analysis. The antibody ... was Pab240. Non-specific cross-reaction caused by the secondary antibody (GAM-HRP) was found mainly to be present in the cellular debris.

Sekvenování:

2-oxoglutarate dehydrogenase E1 subunit !

... a má epitop i na další MkAb ...

...degradační produkty 53 a 73 kDa

... korelace s klíčením dává smysl

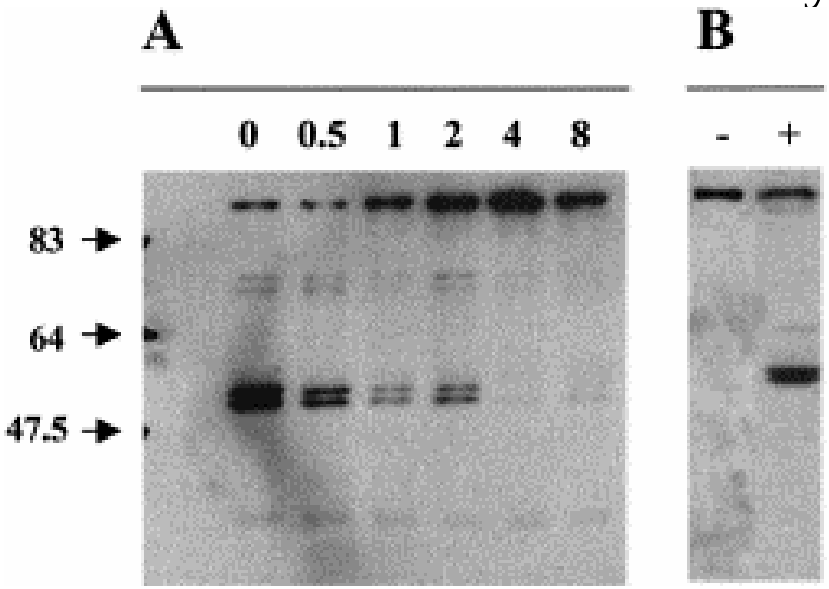

Fig. 4. A: Behaviour of the Pab240 cross-reactive polypeptides in cytosolic extracts of barley embryonic suspension cells after heat shock (10 min, 42°C). Cytosolic extracts were made prior to heat shock (0) and after 0.5, 1, 2, 4 and 8 h recovery at 28°C. B: Trypsin treatment of a cytosolic extract obtained from heat-shocked cells after 4 h recovery; (−) before trypsin treatment, (+) after 50 min incubation with 20 µg/ml trypsin at 23°C. Both Western blots were probed with the Pab240 antibody.