Cell Cycle – Control of Cell Proliferation

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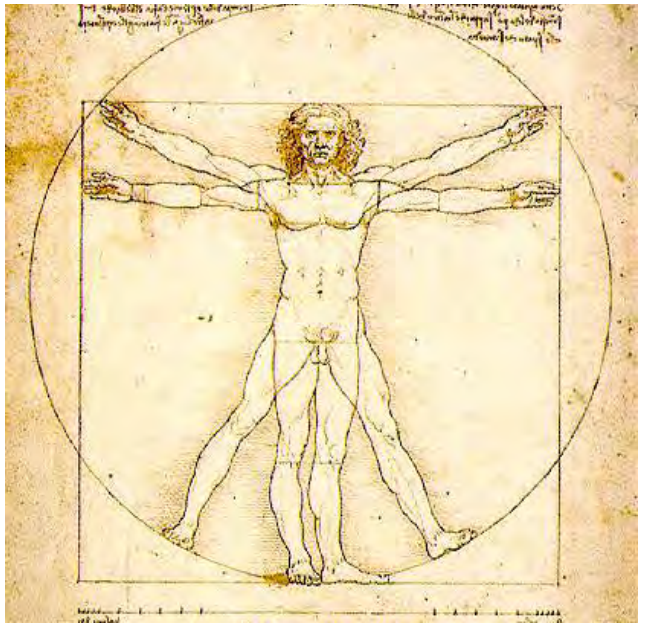


1 fertilized egg

100 000 000 000 000 cells

In Adults:

≈4.000.000 cell divisions / sec.

1. Principles of the cell cycle

Cell Cycle Checkpoints

2. The restriction point

3. CDKs - central cell cycle regulators

4. CDK inhibitors

5. The retinoblastoma protein

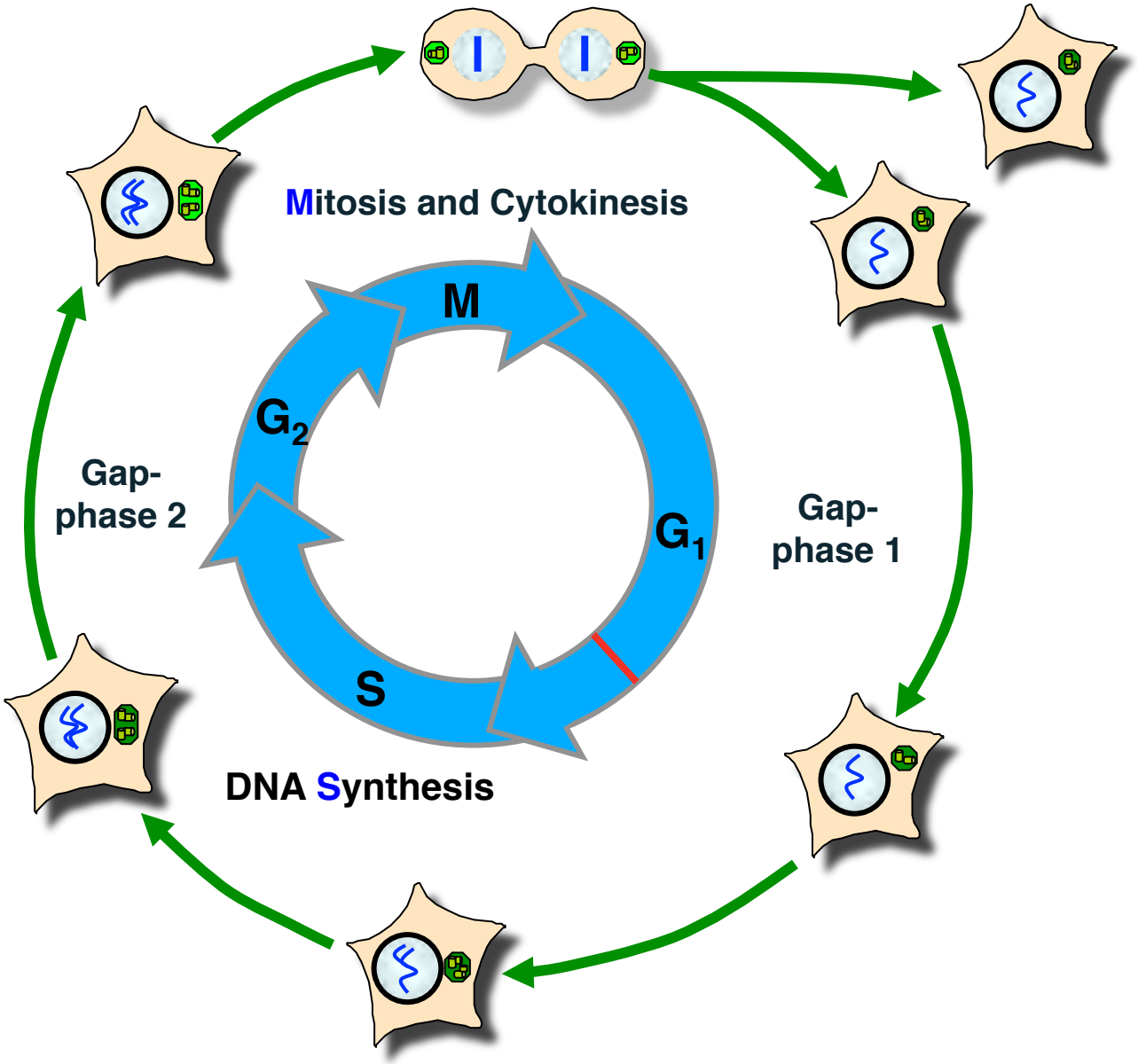
6. The RB-E2F pathway

Central Aims of the Cell Cycle

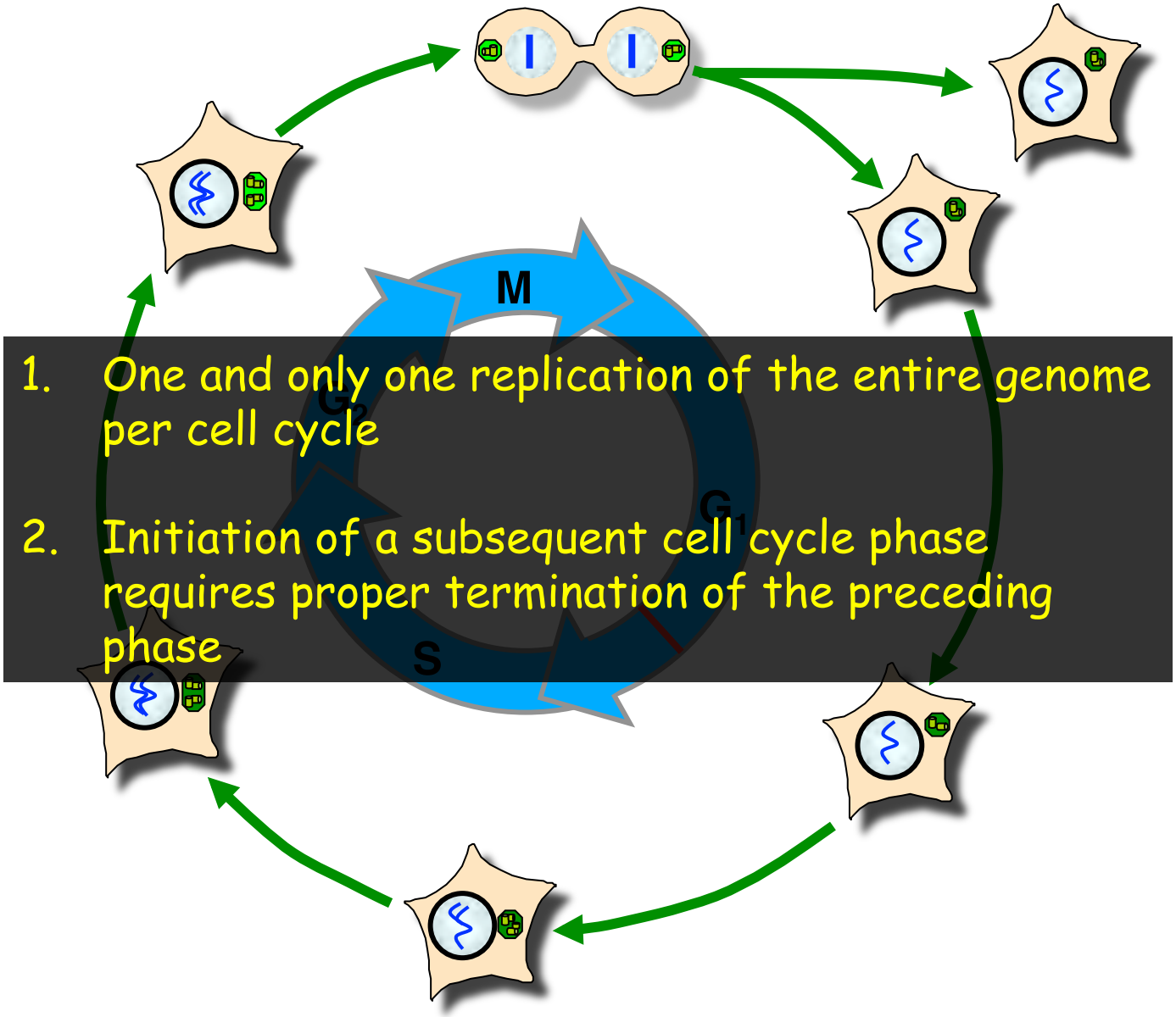
1. **Duplication of the genome**

2. **Separation of the duplicated genetic material** (and other cellular compounds) **into two daughter cells**

Four Cell Cycle Phases



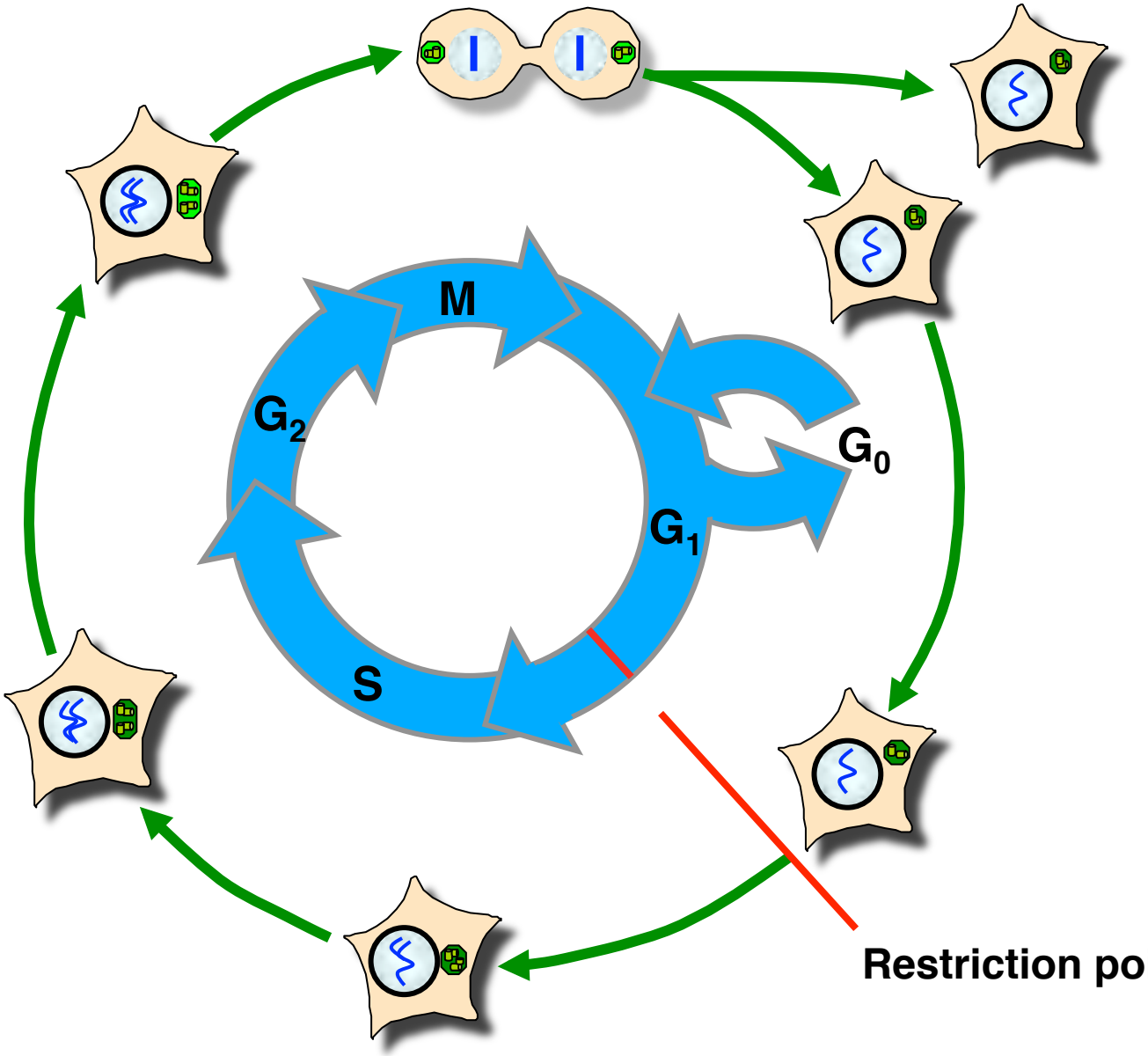
Principles of the eucaryotic cell cycle:



1. One and only one replication of the entire genome per cell cycle

2. Initiation of a subsequent cell cycle phase requires proper termination of the preceding phase

G_0-phase



Why seperate cell cycle phases?

The temporal separation of DNA replikation and mitosis permits the incorporation of control mechanisms in the eucaryotic cell cycle. These control mechanisms are called **Checkpoints**.

Checkpoints

Checkpoint: a process within the cell cycle, which controls the transition from one cell cycle state into the next state

- Checkpoints secure e.g. that DNA replication is completed before mitosis can be initiated.

- Checkpoints secure genomic stability by arresting cell cycle progression upon DNA damage.

Important Checkpoints

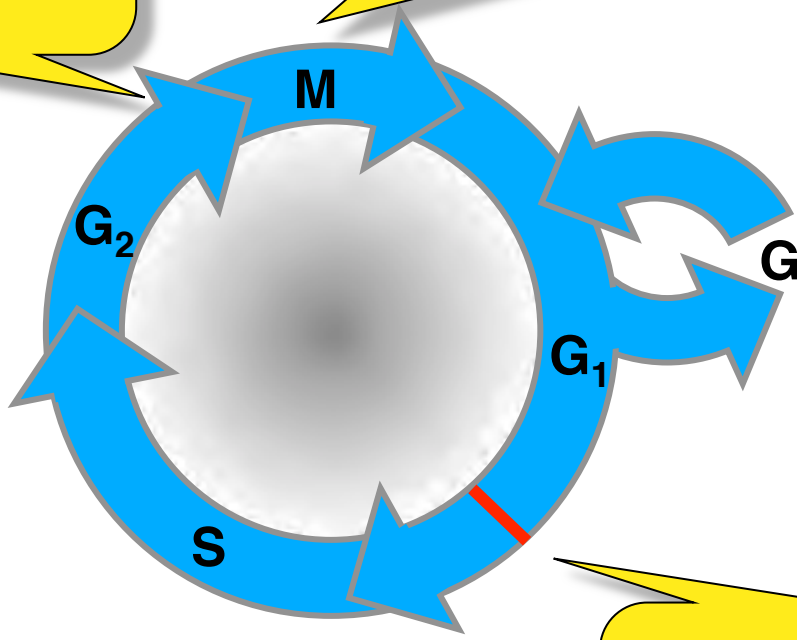
G_2 / M Checkpoint

√ DNA replicated?

√ DNA damage?

Spindel Checkpoint

√ all chromosomes attached to the spindle ?



√ mitogens anti-mitogenic signals ?

√ differentiation - signals ?

G_1/S Checkpoint

√ size ?

√ DNA damage ?

Important aims of cell cycle control mechanisms:

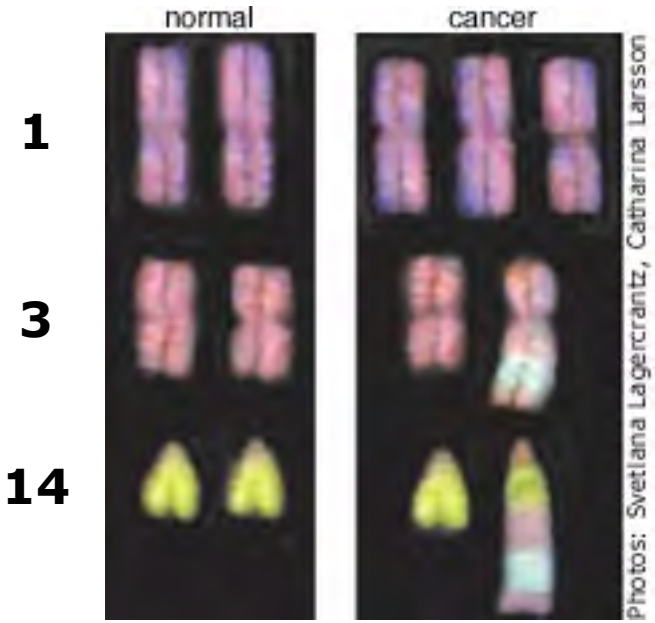
1. Restrict cell divisions to precisely the required numbers, e.g. induced divisions after wounding or cell death
   - **Avoids hypo- and hyperproliferation**

2. Warrant one, and only one DNA replication of all regions of the genome per cell cycle.
   - **Avoids genetic instability**

3. Precise segregation of the genome into both daughter cells.
   - **Avoids genetic instability**

4. Prevention of replication or segregation of damaged DNA
   - **Avoids the multiplication of damaged DNA**

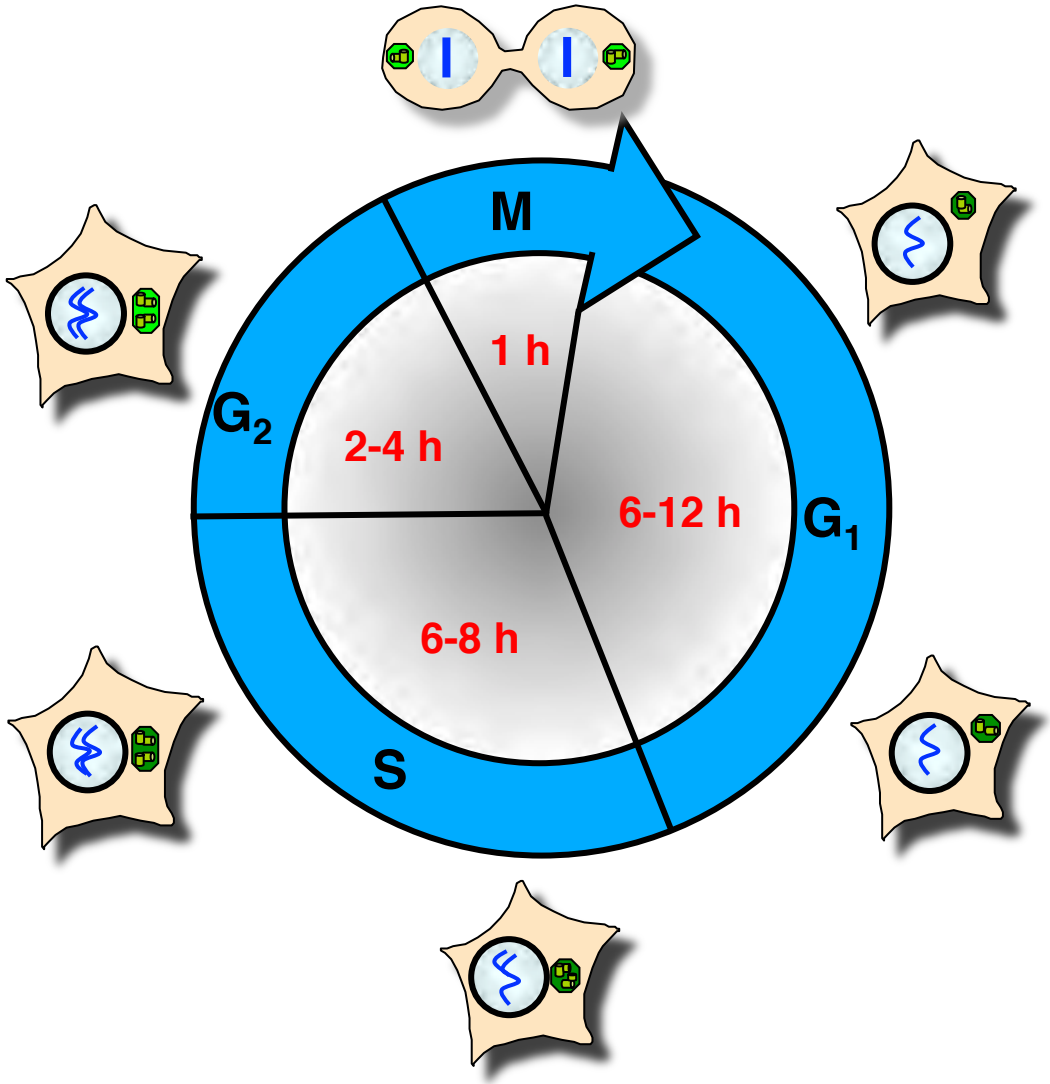
Consequences of misregulated cell cycle control: Genetic instability of tumor cells



Aneuploidy : abnormal number of chromosomes (extra or missing)

Chromosomal translocations: Rearrangements of chromosome parts between nonhomologues chromosomes

Length of cell cycle phases



Eucaryotes:

Early frog embryo cells	30 min
Yeast	1.5 - 3 h
Cells in the epithelium of the small intestine	12 h
Proliferating mammalian cells	18-24 h
Fibroblasts in culture	20 h
Human liver cells	1 year

Duplication of bacteria:

E. coli: 20 - 25 min

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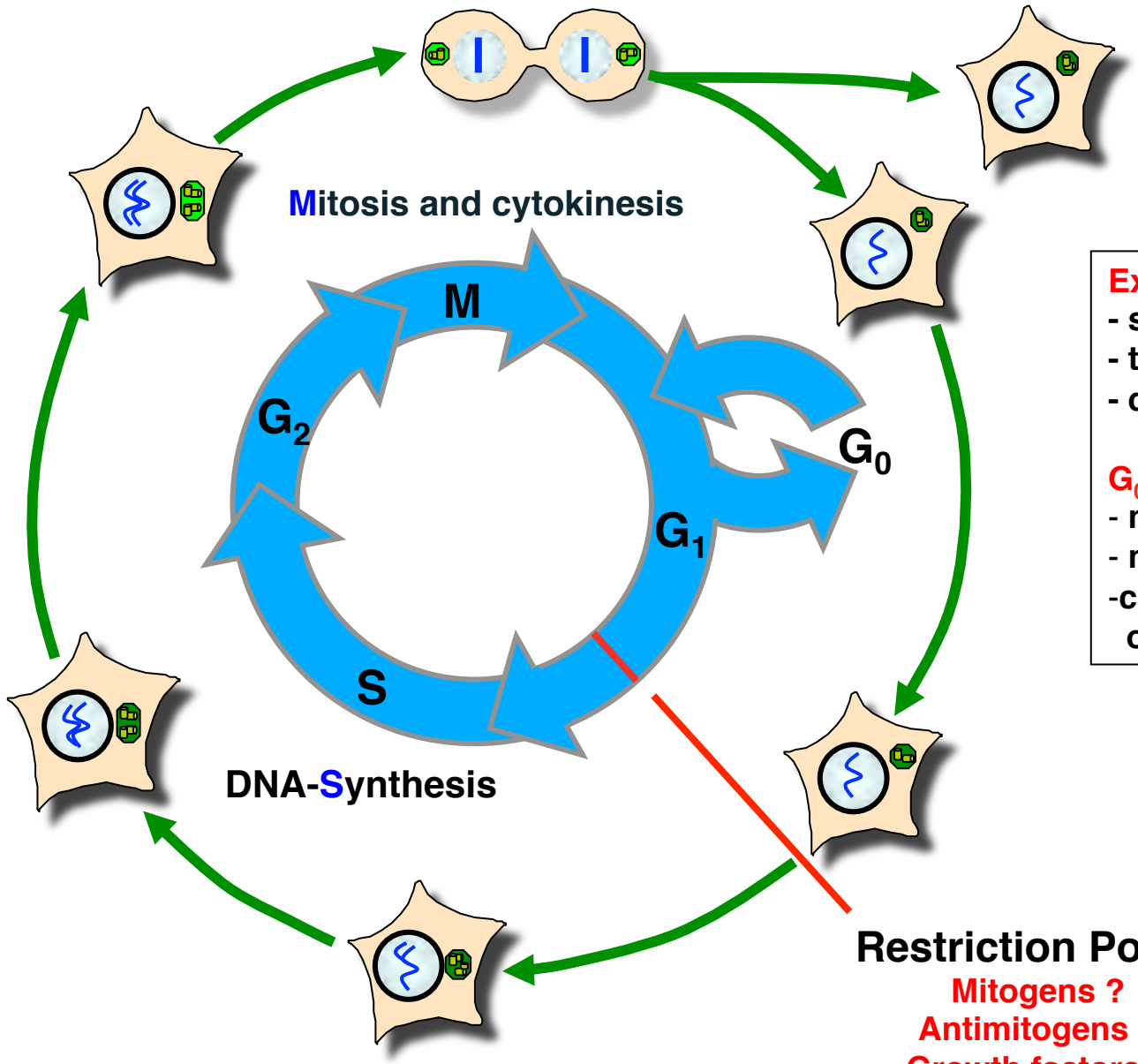
The Cell Cycle



Exit from the cell cycle:
- senescence
- terminal differentiation
- quiescence

G_0-phase / quiescence:
- no cell divisions
- no DNA-replikation
- can persist hours, days or years

Restriction Point

Mitogens ?
Antimitogens ?
Growth factors ?
Differentiation signals ?

Cell Fusions

Rao, P.N. & Johnson, R.T. (1970). Mammalian Cell Fusion: Studies on the Regulation of DNA Synthesis and Mitosis. Nature 225, 159-164.

Johnson, R.T. & Rao, P.N. (1970). Mammalian Cell Fusion: Induction of Premature Chromosome Condensation in Interphase Nuclei. Nature 226, 717-722.

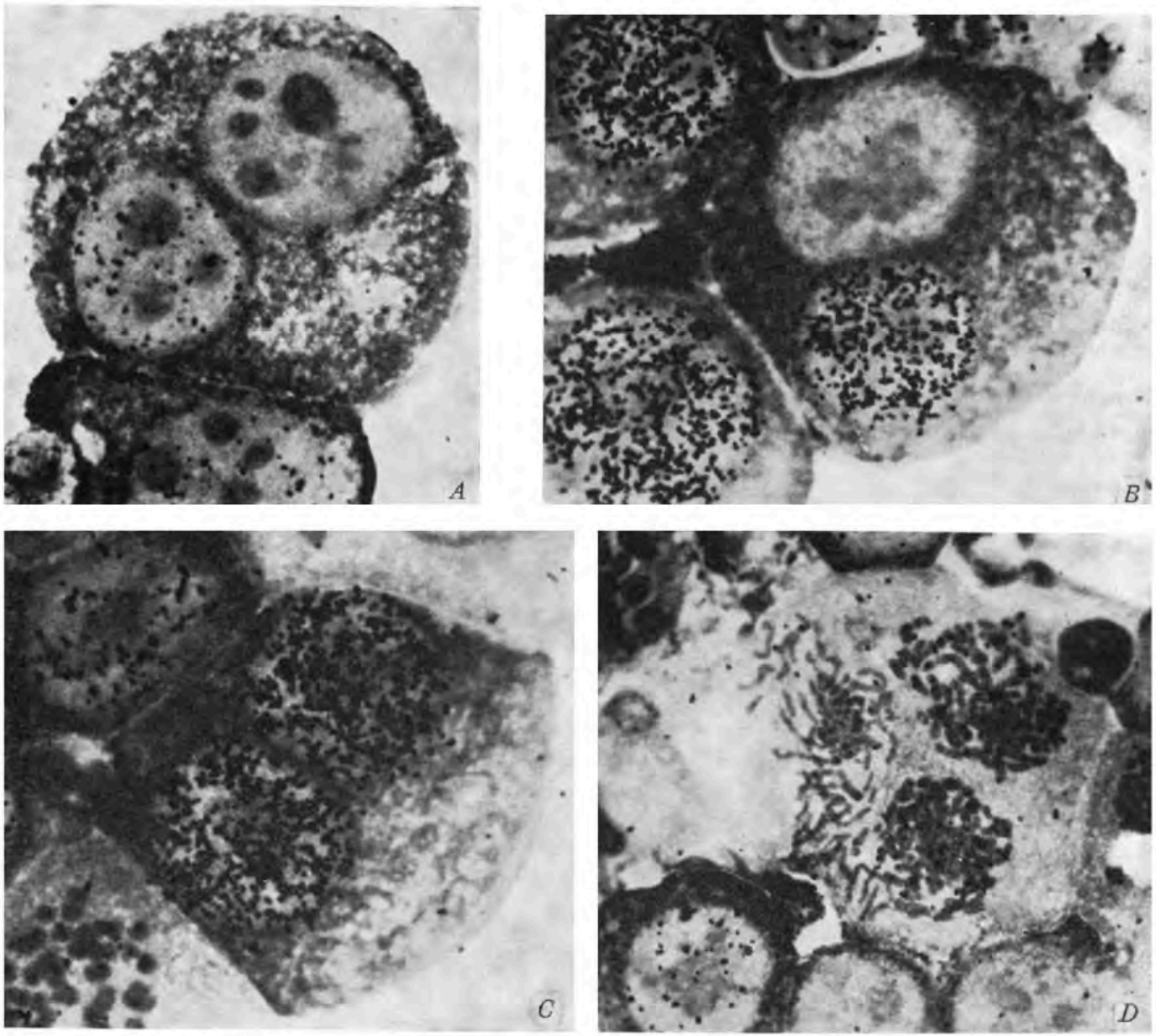
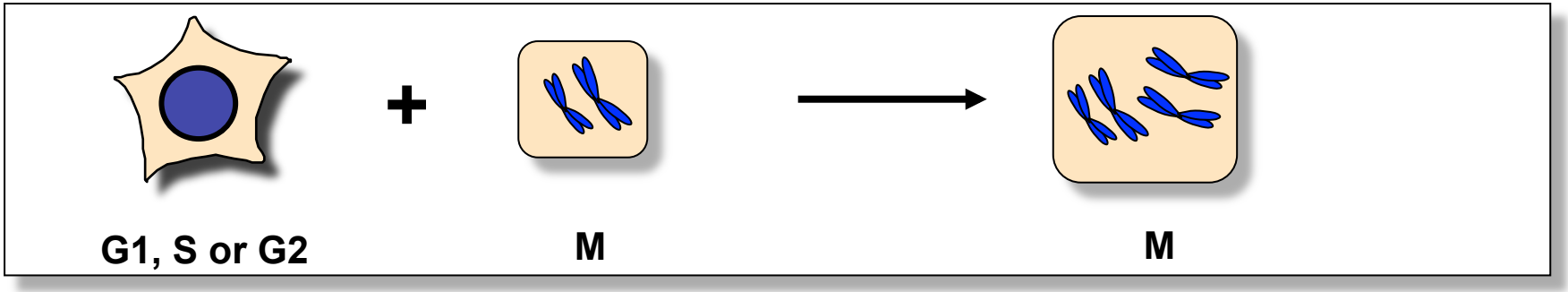


Fig. 1. *A*, Heterophasic *S*/*G*2 binucleate cell at $t = 0$ after fusion. The *S* nucleus was prelabelled with ^{3}H-thymidine. *B*, Heterophasic *S*/*G*2 binucleate cell at $t = 6$ h after fusion and incubation with ^{3}H-thymidine. The increased intensity of labelling of the *S* nucleus as compared with that in *A* arises from continued DNA synthesis after fusion. There was no uptake of ^{3}H-thymidine by the *G*2 nucleus. *C*, Homophasic *S*/*S* binucleate cell at $t = 6$ h after fusion and incubation with ^{3}H-thymidine. The intensity of labelling in each of the nuclei is comparable with that in the *S* nucleus in *B*. *D*, Heterophasic *G*1/2*G*2 trinucleate cell in synchronous mitosis (no colcemide treatment was given). *G*2 nuclei were prelabelled. Note a slightly less condensed state of the chromosomes of the unlabelled (*G*1) nucleus.

Heterocaryon experiments

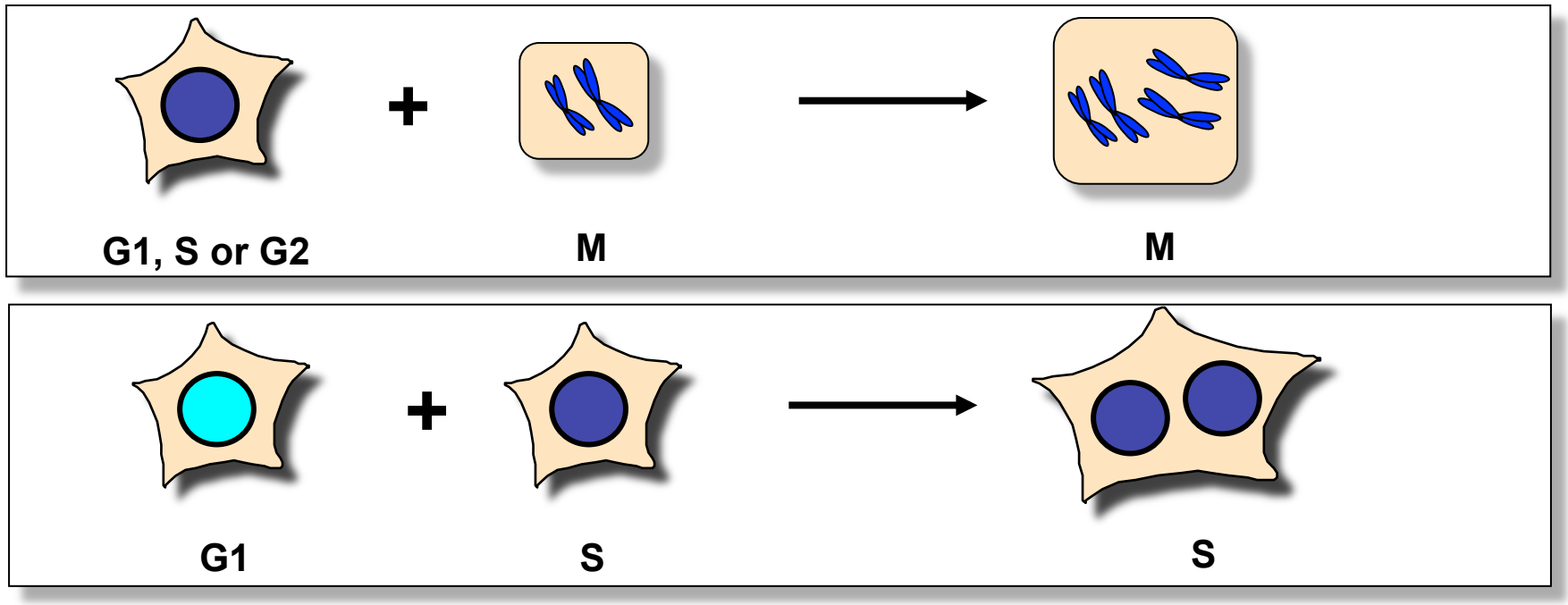


Fusion of G1, S or G2 cells with mitotic cells leads to nuclear envelope breakdown and DNA condensation in the heterocaryon.

Mitotic cells contain a factor (“MPF” - Mitosis promoting factor) which can induce mitosis in cells of other cell cycle phases.

Rao, P.N. & Johnson, R.T. (1970). Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis.Nature 225, 159-164.
Johnson, R.T. & Rao, P.N. (1970). Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei.Nature 226, 717-722.

Heterocaryon experiments

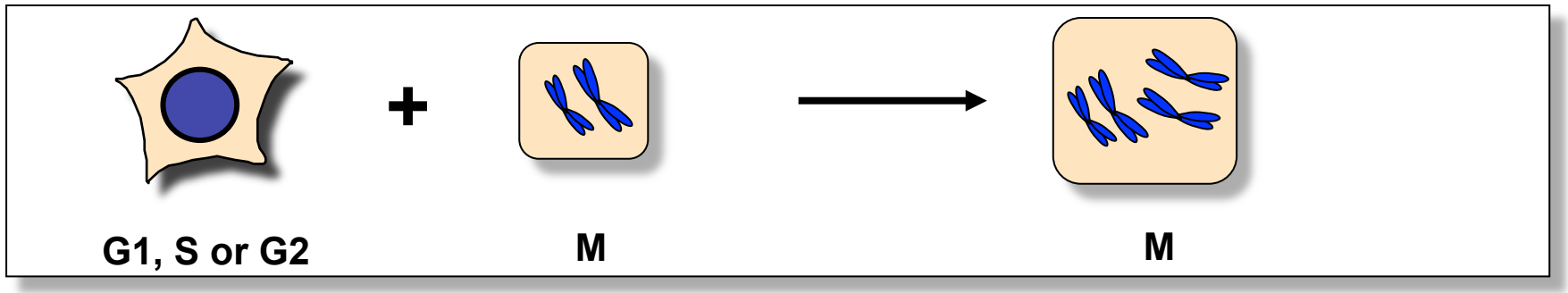


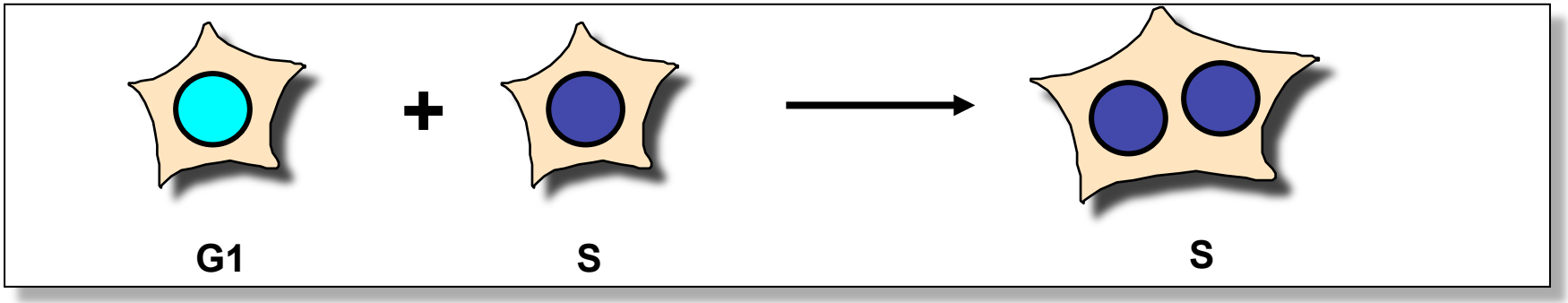
Fusion of G1 cells with S-phase cells results in heterocaryons, in which the G1 nuclei initiate DNA replikation. S-phase cells only initiate mitosis after completion of DNA replication in G1 cells.

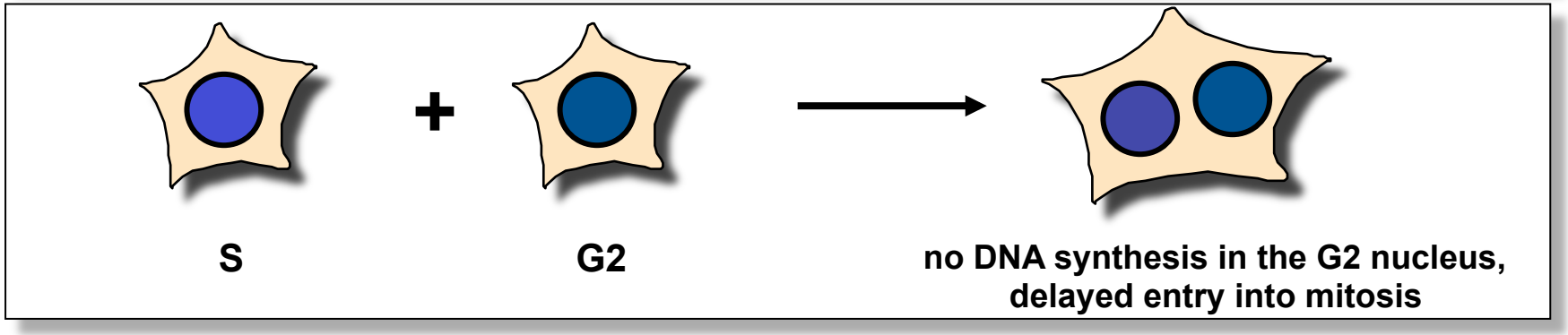
S-phase cells contain a factor which can initiate DNA replication in G1 cells. Checkpoints prevent “premature” entry into mitosis before S-phase is completed in both nuclei.

Rao, P.N. & Johnson, R.T. (1970). Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis.Nature 225, 159-164.
Johnson, R.T. & Rao, P.N. (1970). Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei.Nature 226, 717-722.

Heterocaryon experiments







G2 nuclei do not re-initiate DNA replication

Existence of a “licensing system”, which labels replicated DNA and prevents re-replication.

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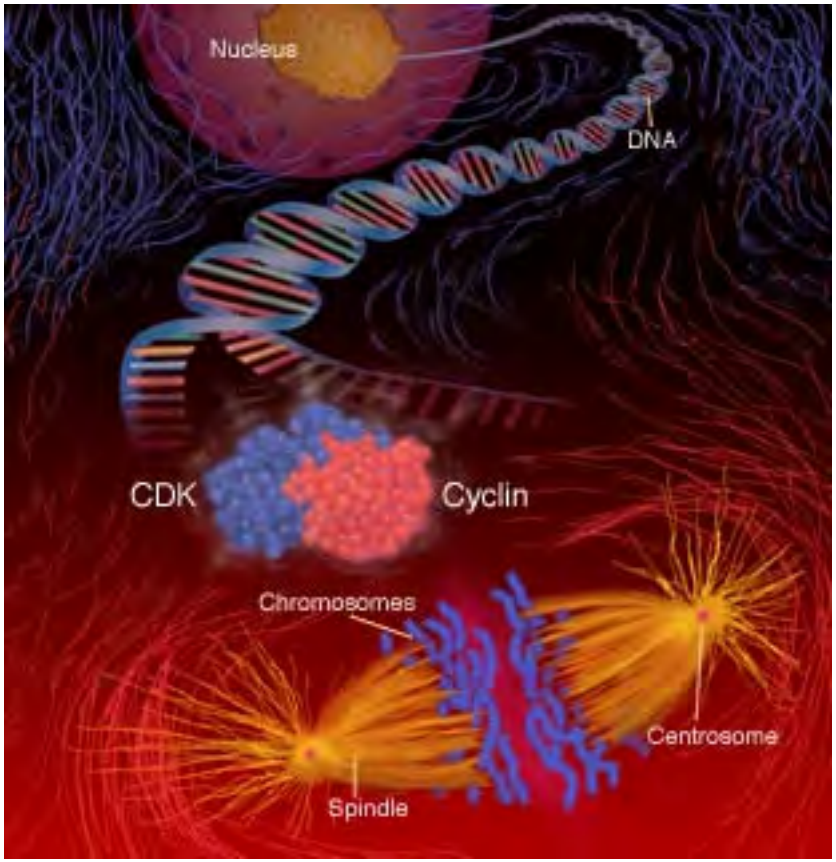
The Cell Cycle Engine

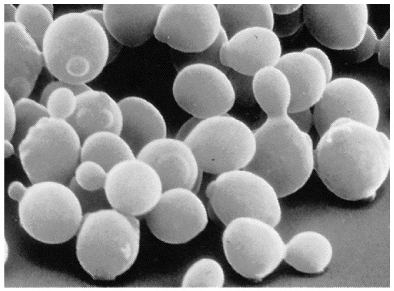
The Nobel Prize in Physiology or Medicine 2001
"for their discoveries of key regulators of the cell cycle"



Leland H. Hartwell

R. Tim Hunt

Sir Paul M. Nurse

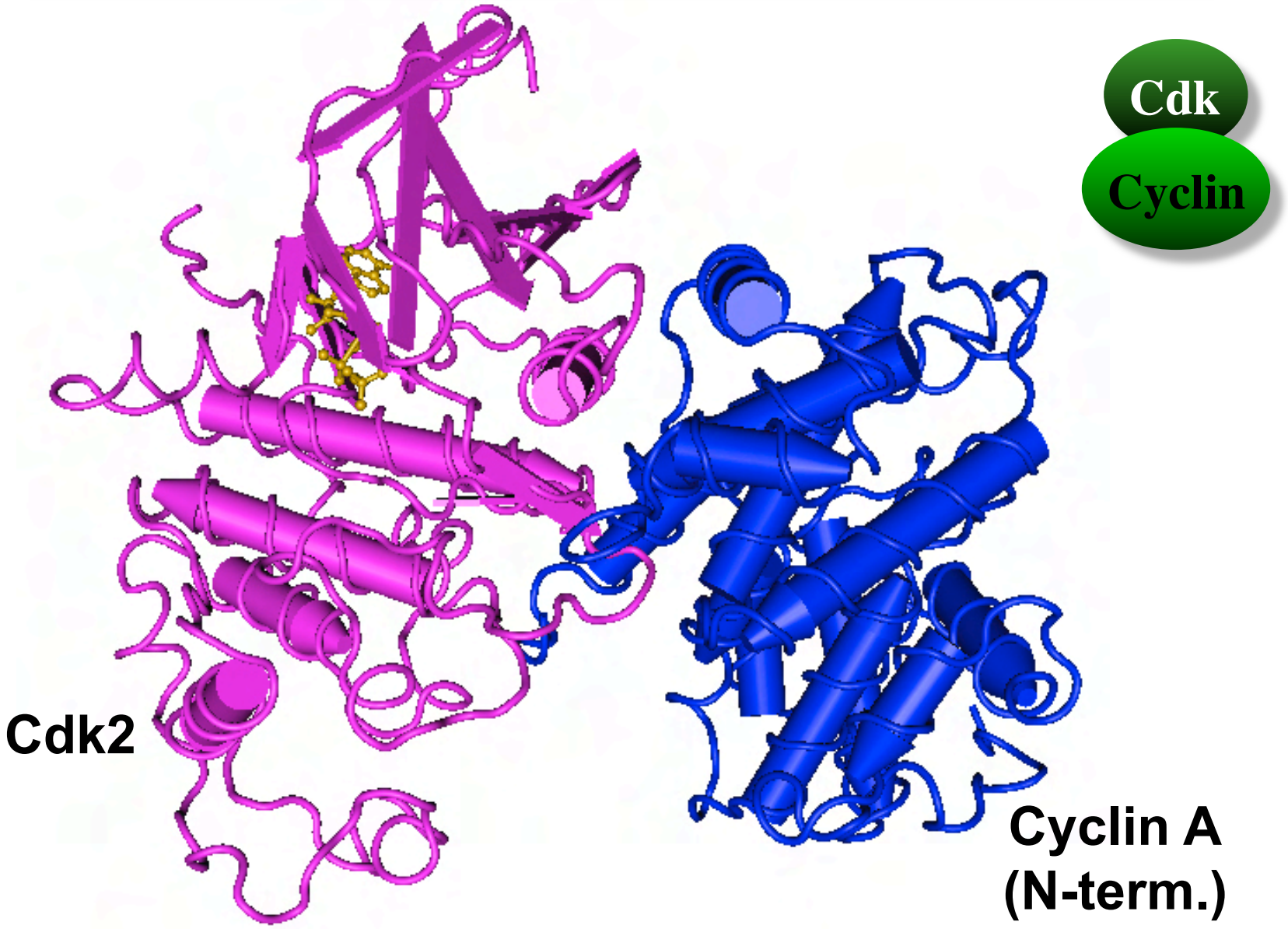


Bakers yeast

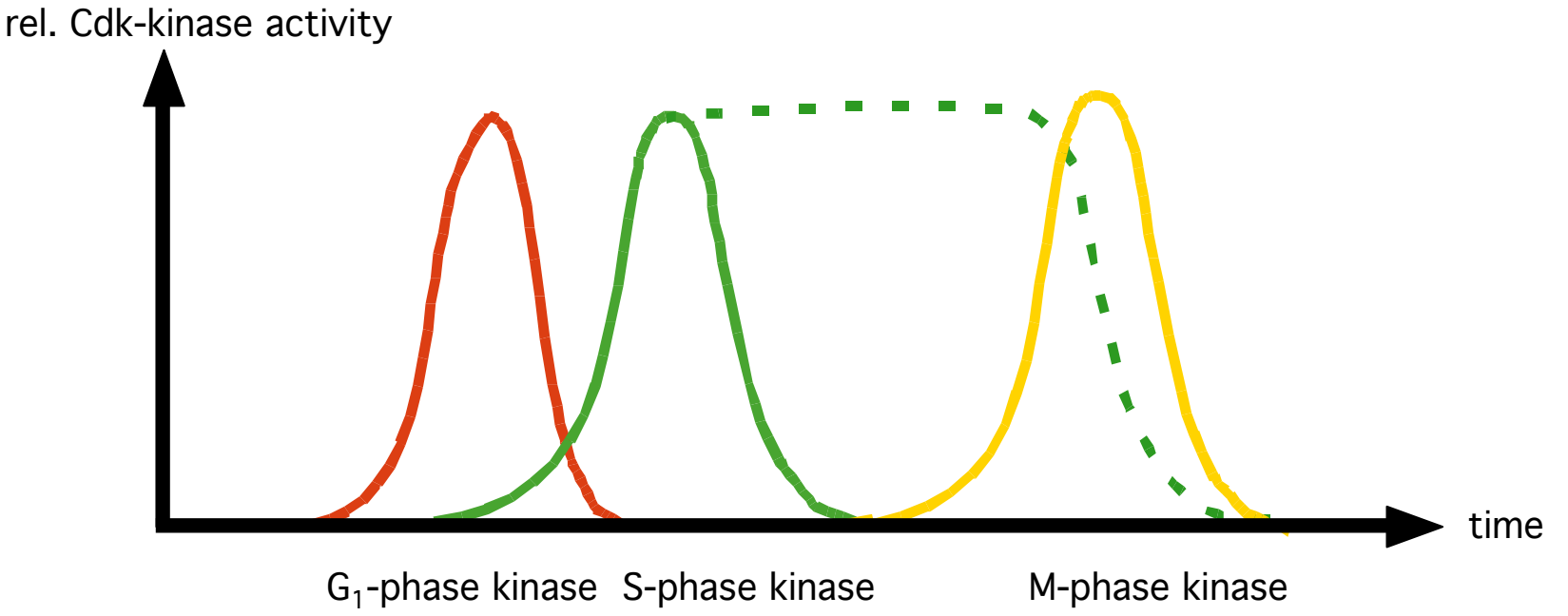
Sea Urchin

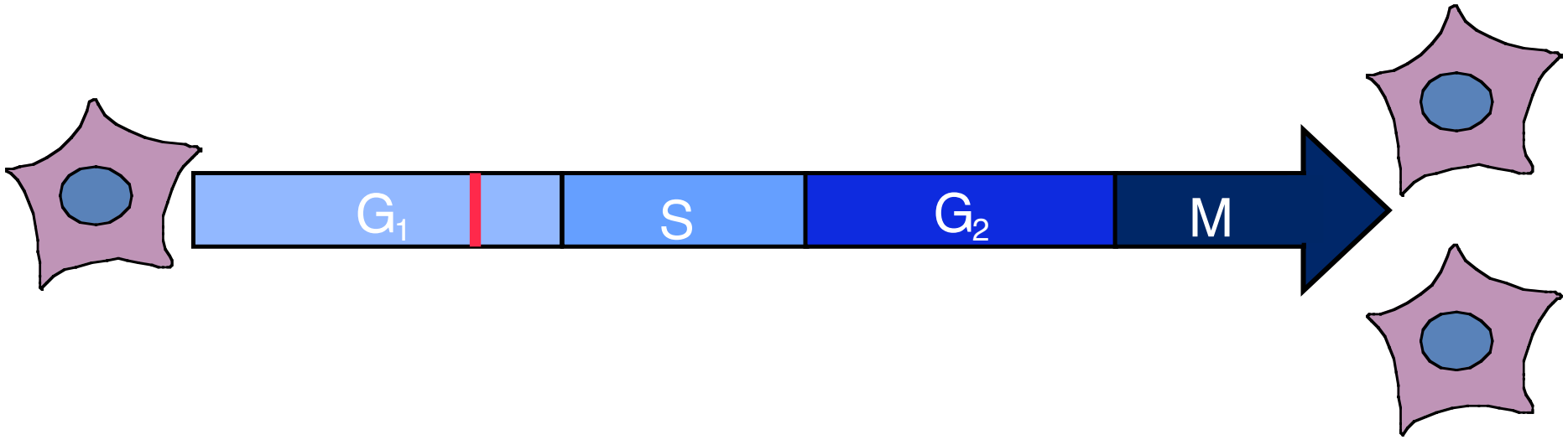
Fission yeast

The Cell Cycle Engine: Cyclin-Dependent Kinases

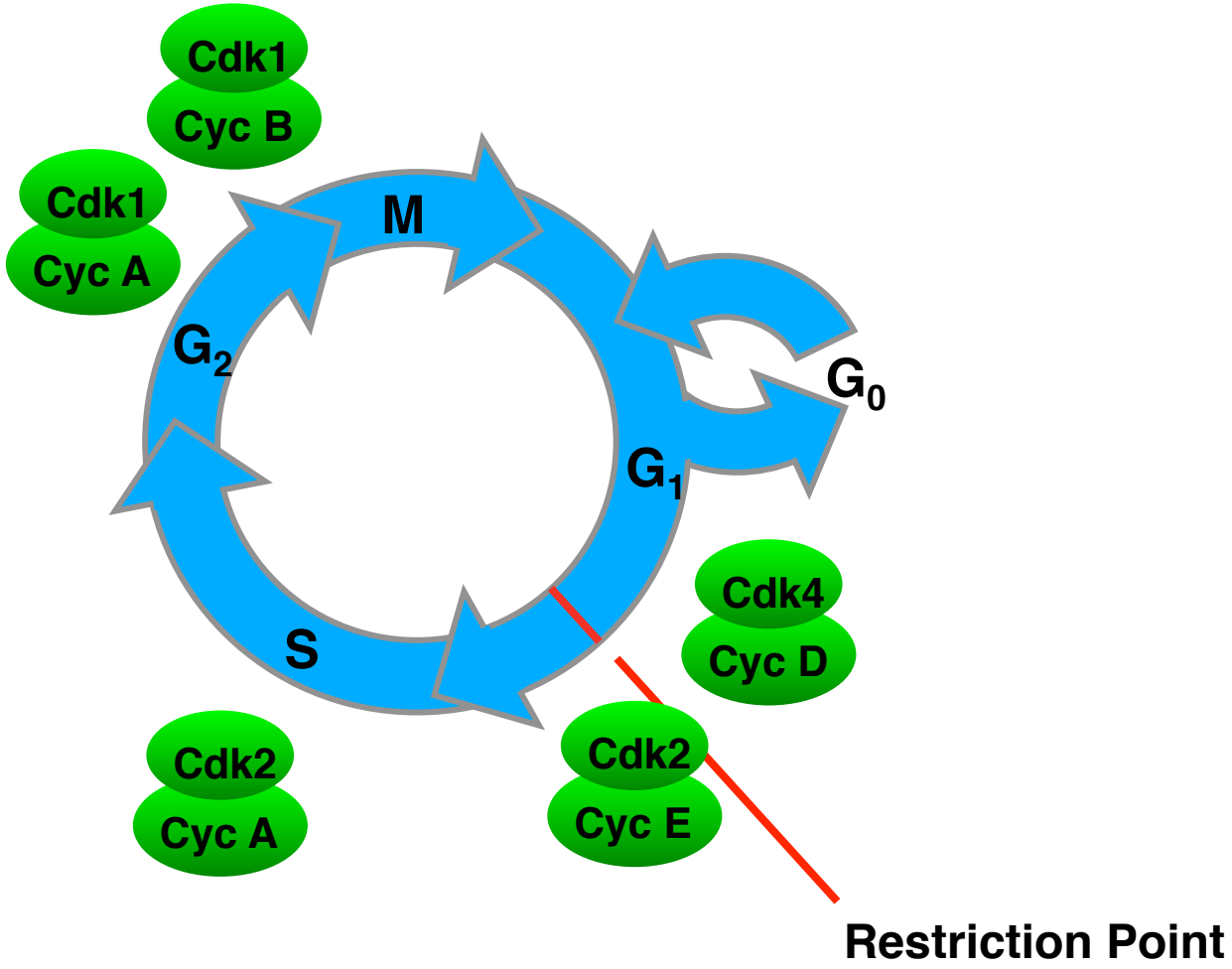


Cell Cycle Transitions are driven by the oscillating Activity of Cyclin-Dependent Kinases (CDKs)

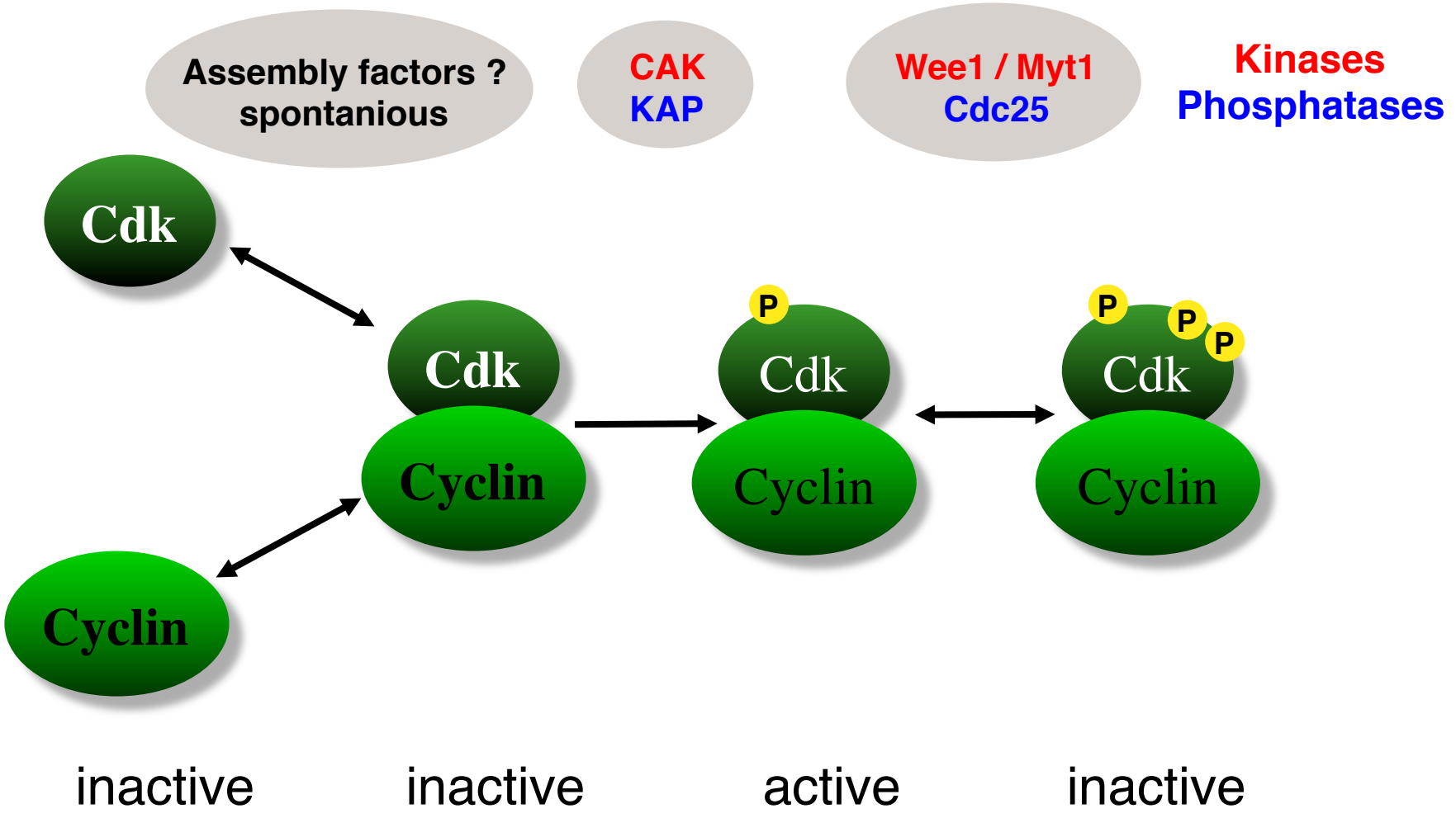




The Cell Cycle Engine: Cyclin-Dependent Kinases



CDKs are regulated by activating and inhibitory phosphorylations



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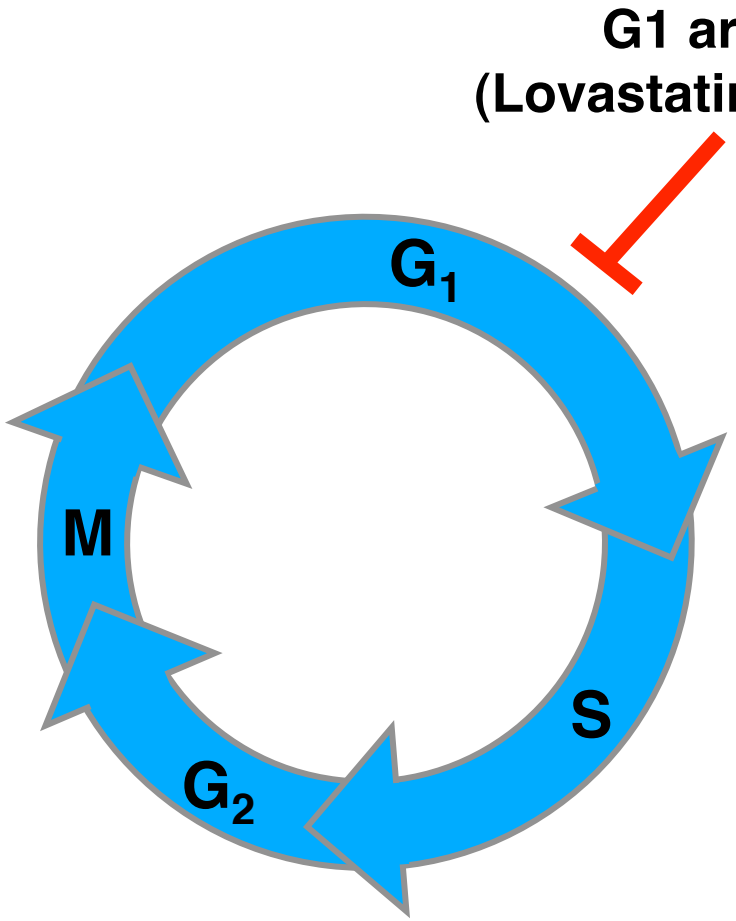
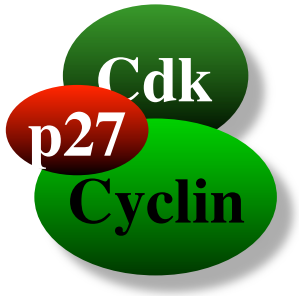
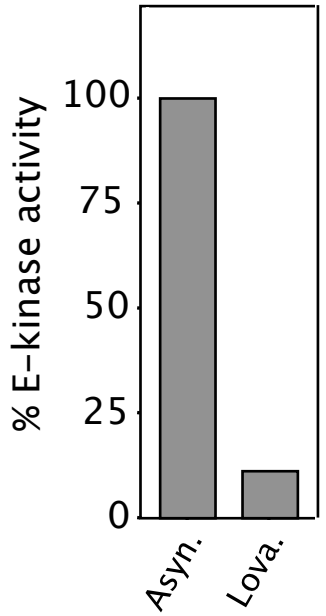
Molecular Brakes: CDK Inhibitor Proteins

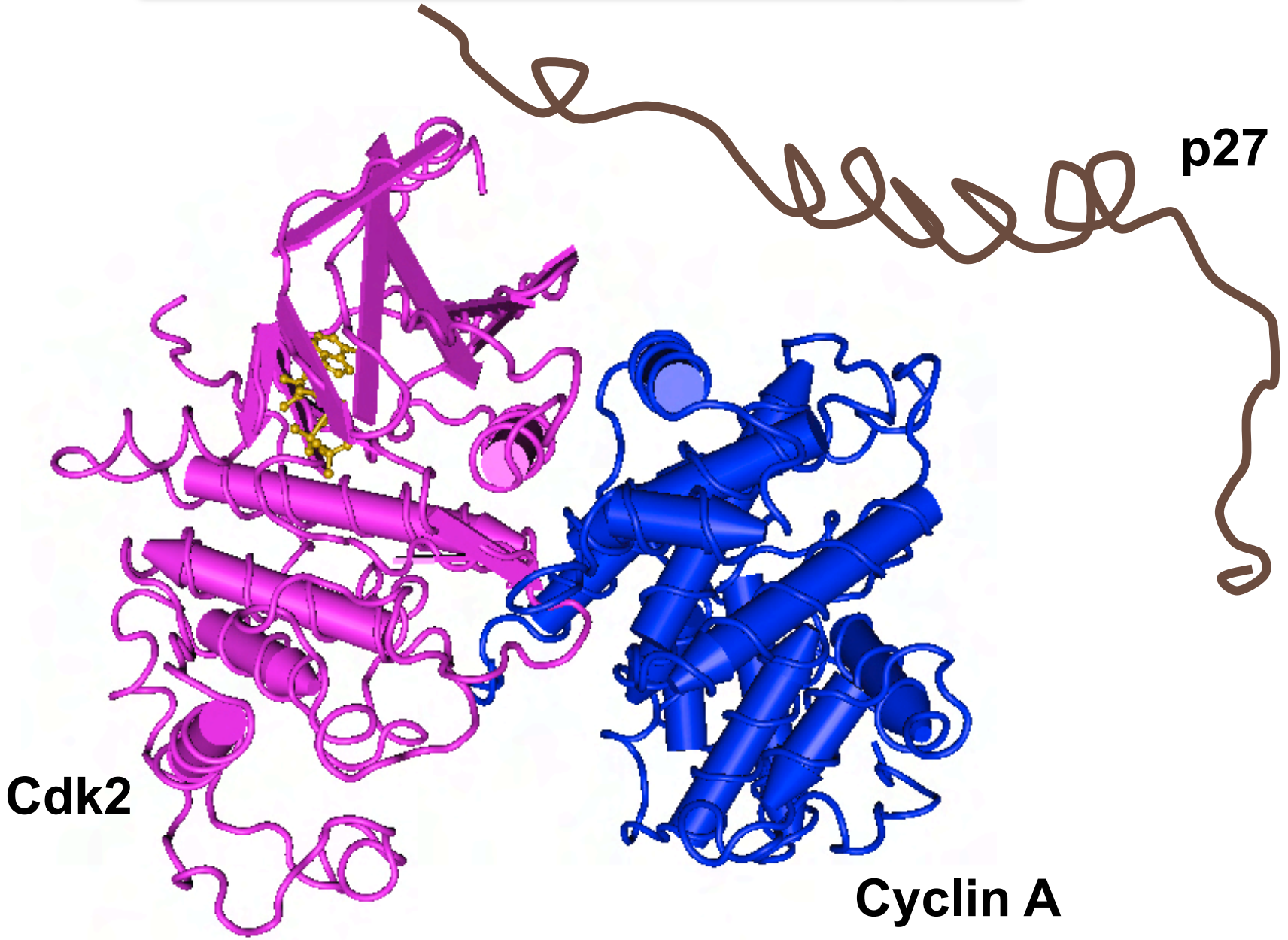






Hengst et al., *PNAS* 1994

Mechanism of CDK inhibition by p27



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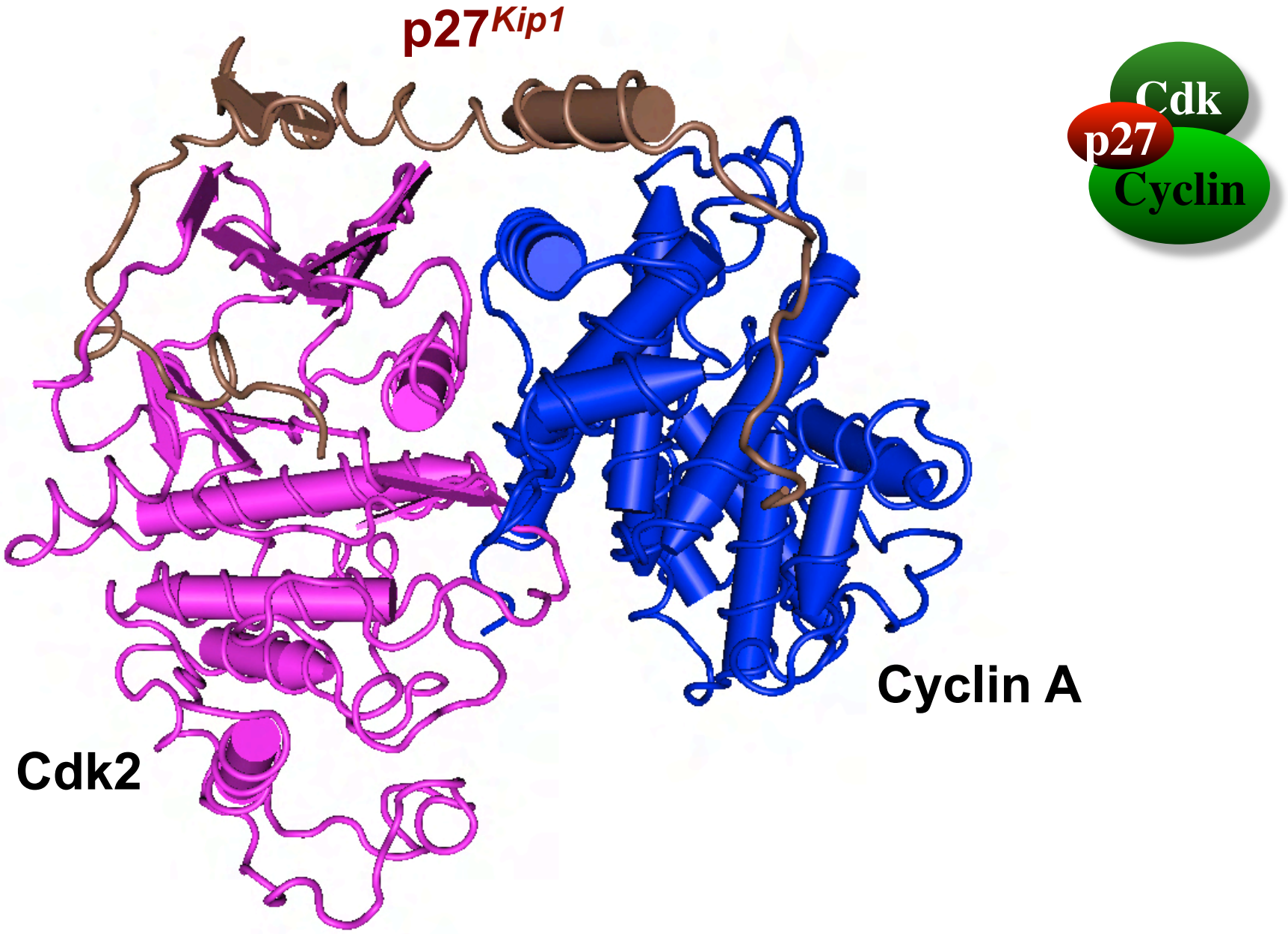
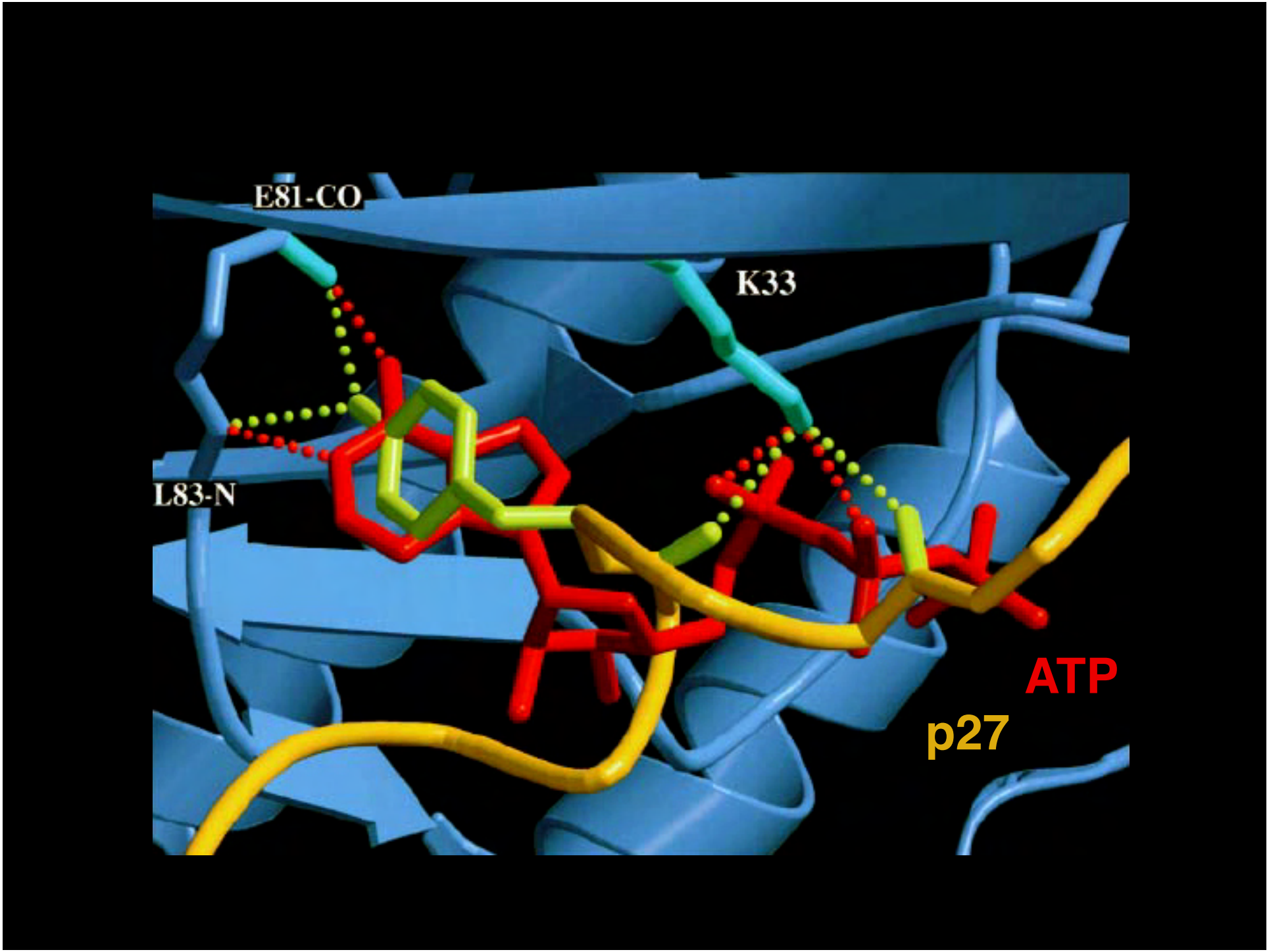
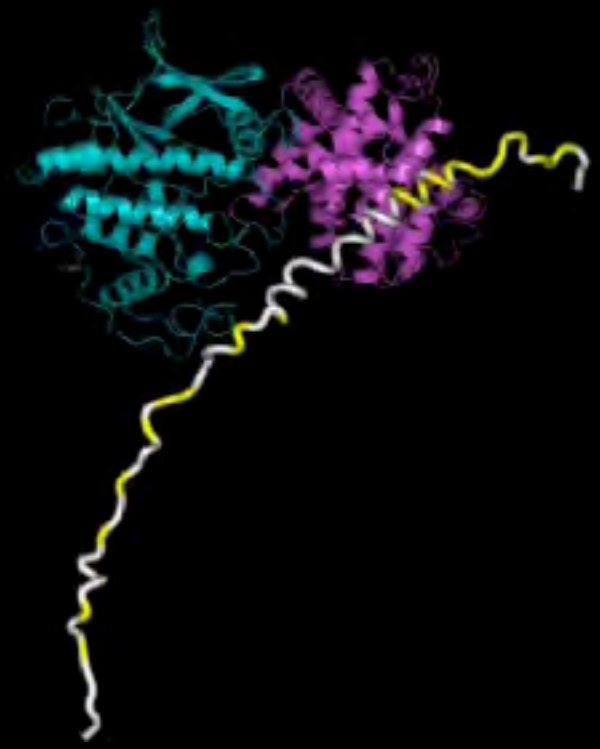


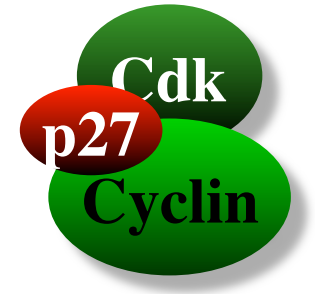

E81-CO
K33
L83-N
ATP
p27

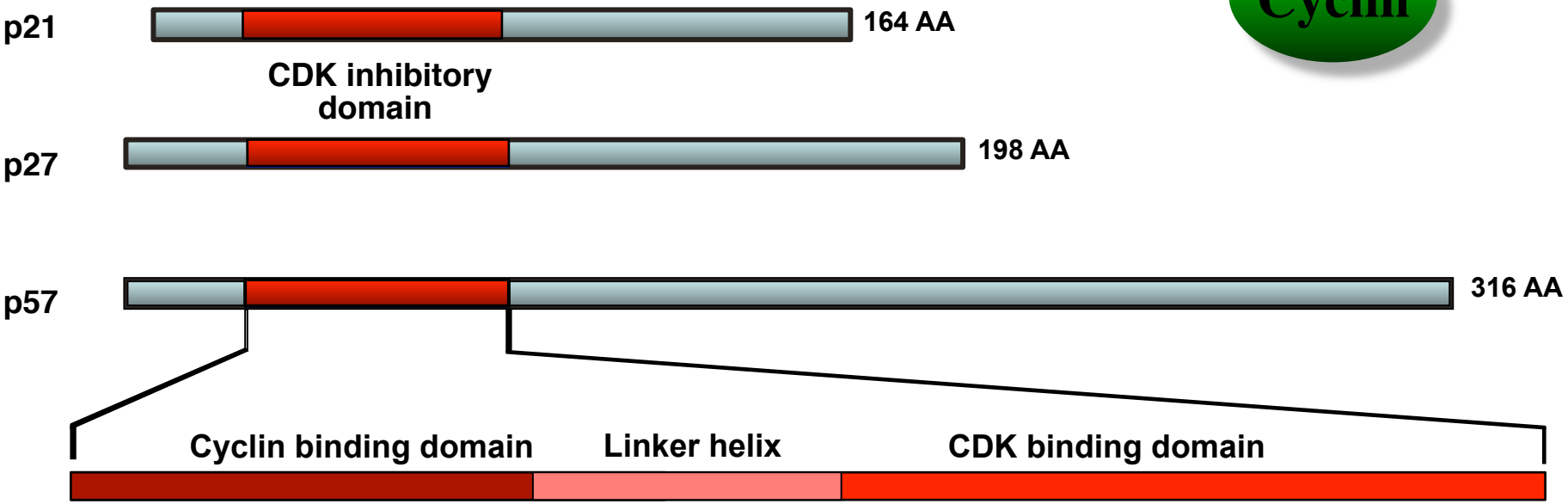
Cip/Kip Inhibitors are Intrinsically Unstructured Proteins (IUPs)



Lacy et al., *Nature Structural & Molecular Biology*. 2004

The Cip/Kip-Family of CDK Inhibitors






```
   22        30        40        50        60        70        80        90
p21 PCGSK  ACR RLFGPVD  SEQLSRDSDALM  AGCIQ EARE RWNFDFVTET  PLE--  GDFA WERVRGLG LPKLYLPTGP
p27 EHPKP SACR NLFGPVD  HEELTRDLEKHC  RDMEE AS QRK WNFDFQNHK  PLE--  GKYE WQEVEKG SLPEFYYRPPR
p57 VLVRT SACR SLFGPVD  HEELGRELRMRA  AELNA EDQNRWDFNFQQDV  PLRGP  GRLQ WMEVDSE SVPAFYRET-V
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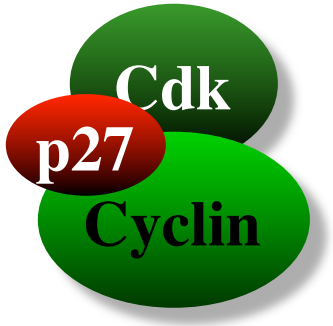
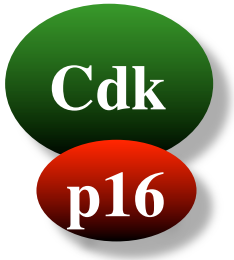
Two Families of CDK Inhibitors



Cip/Kip family
(Cdk interacting protein;
Kinase inhibitory protein)

p21, p27, p57 (Cip1, Kip1, Kip2)

- bind to a broad spectrum of CDK/ cyclins
- bind the CDK / cyclin complex
- conserved N-terminal CDK-inhibitory domain
- may act as actvators for cyclin D/Cdk4,6

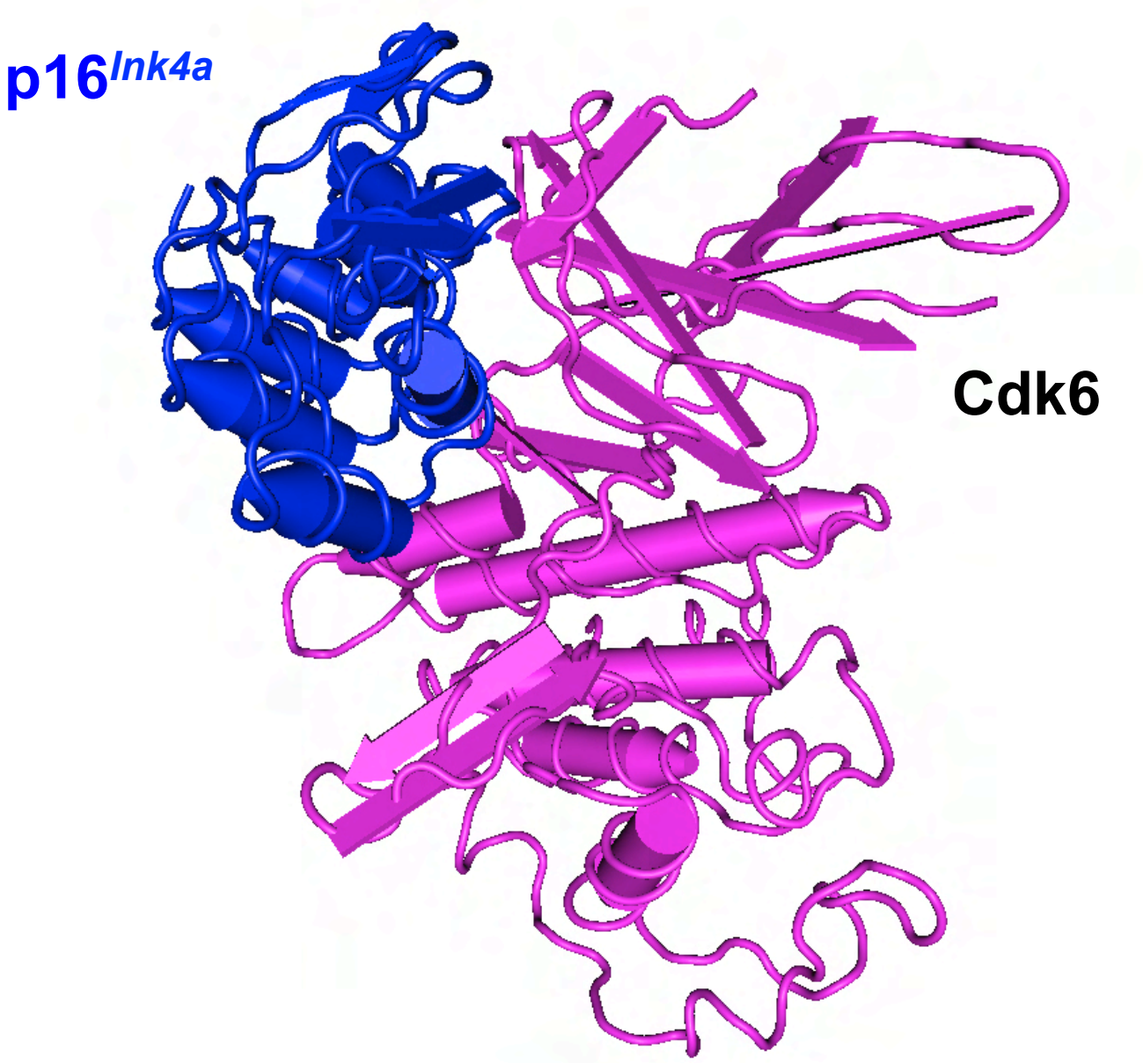


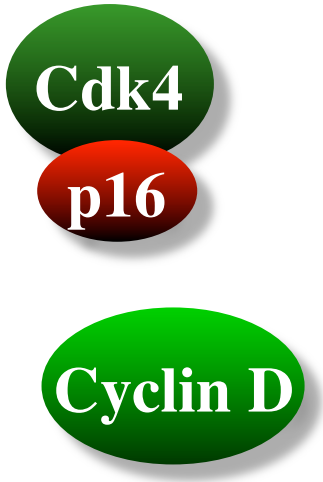
Ink4 family
(Inhibitor of Cdk4)

p15, p16, p18, p19 (Ink4 a-d)

- specific for cyclin D / CDK4,6
- bind the CDK subunit
- ankyrin repeat structure

INK4 CDK4,6 Inhibitors





What are critical CDK substrates at the G1/S transition ?

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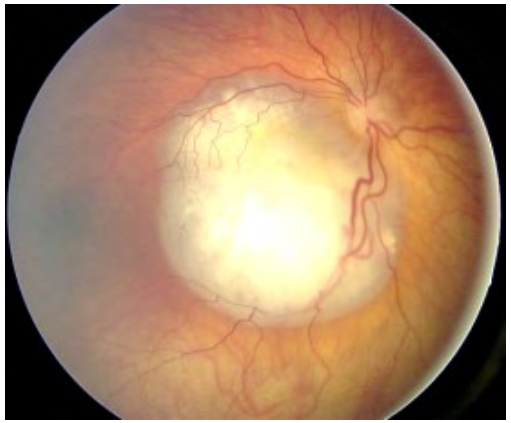
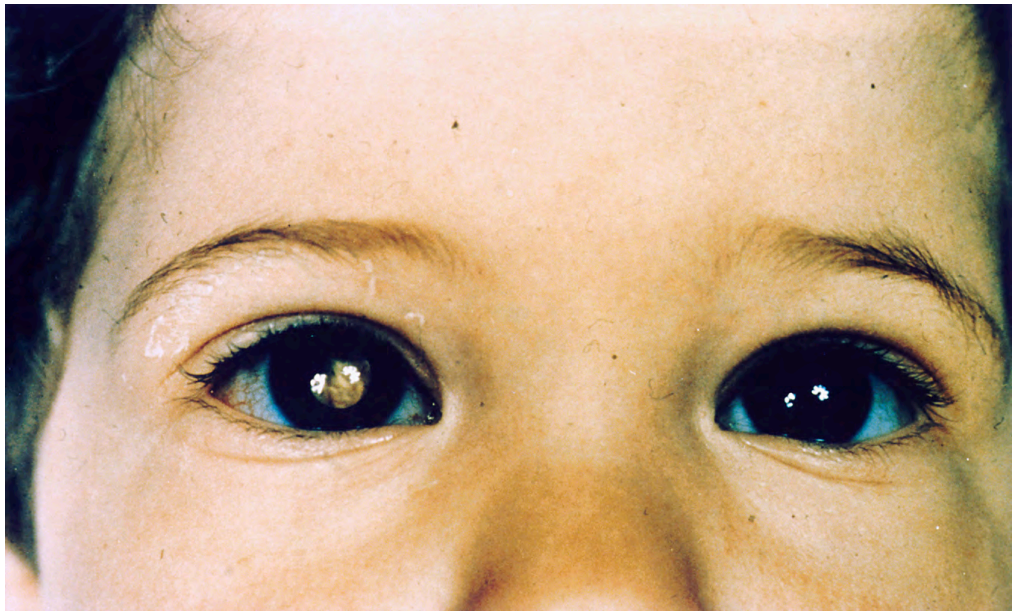
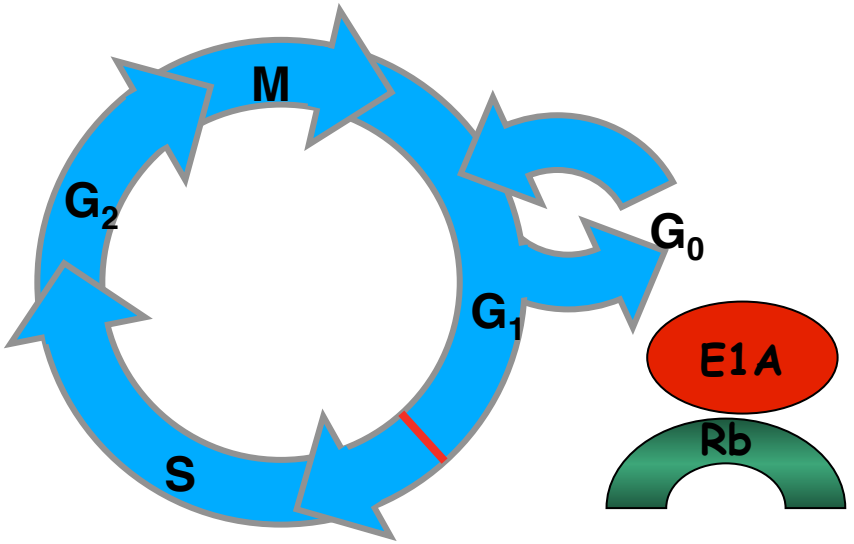
The CDK substrat retinoblastom protein (Rb) is a central cell cycle regulator

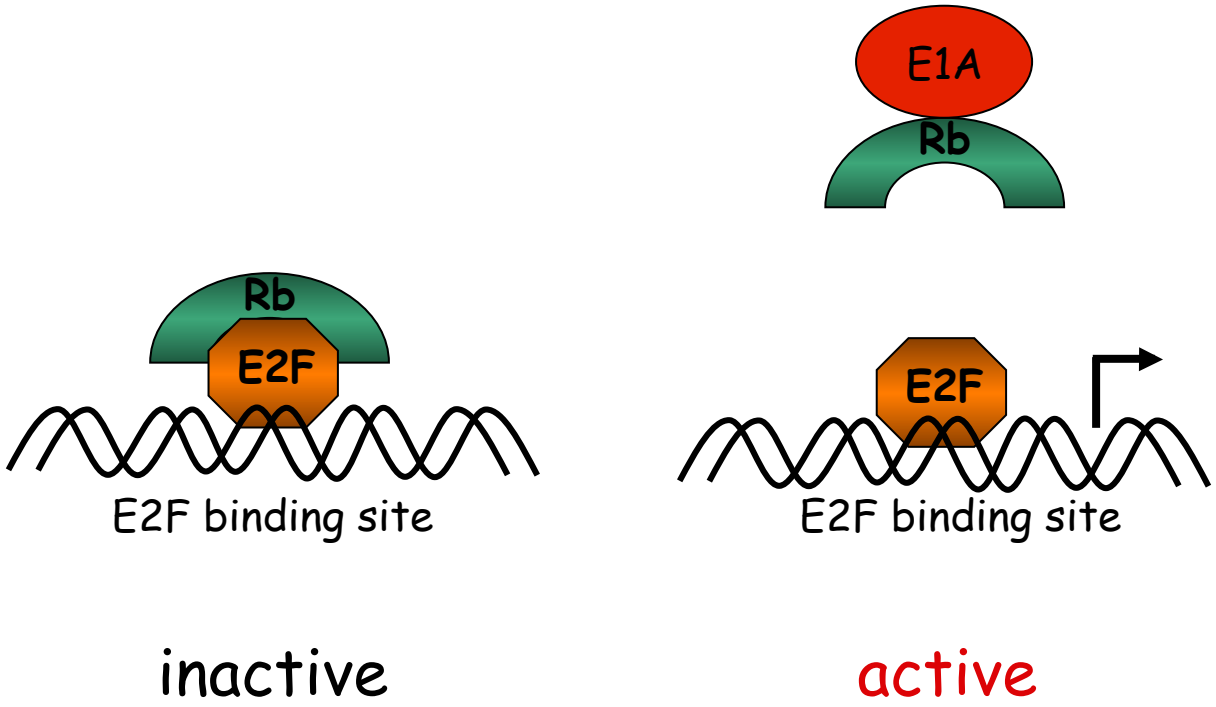


- Retinoblastoma is a rapidly developing childhood cancer (1/20.000) arising from immature retinal cells
- The Rb protein is a tumor suppressor protein; 45% of the patients carry a heterozygous mutation in Rb1
- Children with (heteroygous) mutations in Rb frequently develop tumours in other tissues (Knudsons two-hit hypothesis)
- Viral oncoproteins like E1A and HPV E7 bind Rb and induce cell division in cultured cells

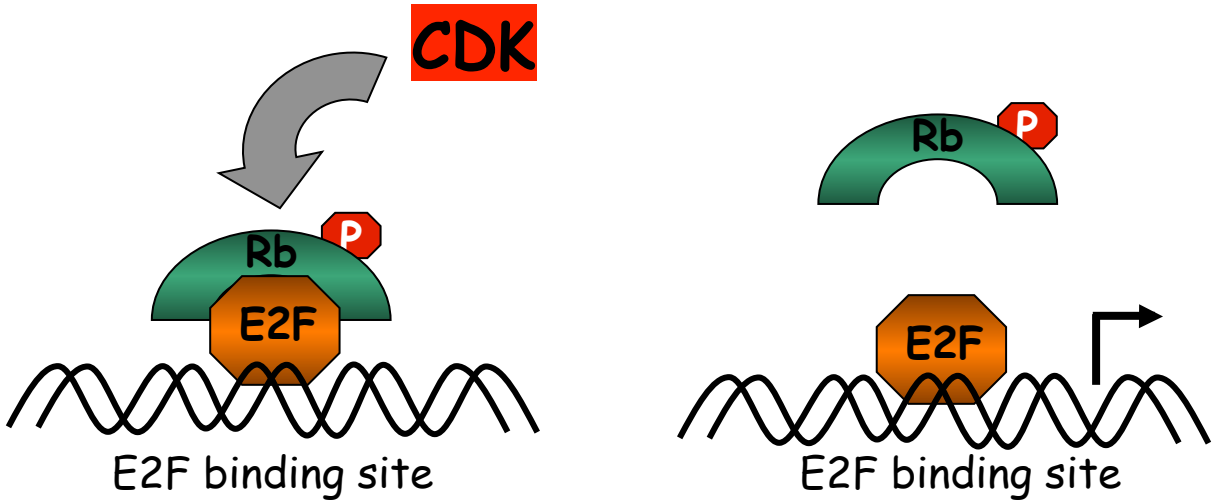




Rb binds E2F and inhibits cell cycle progression



Phosphorylation of Rb releases E2F



inactive

active

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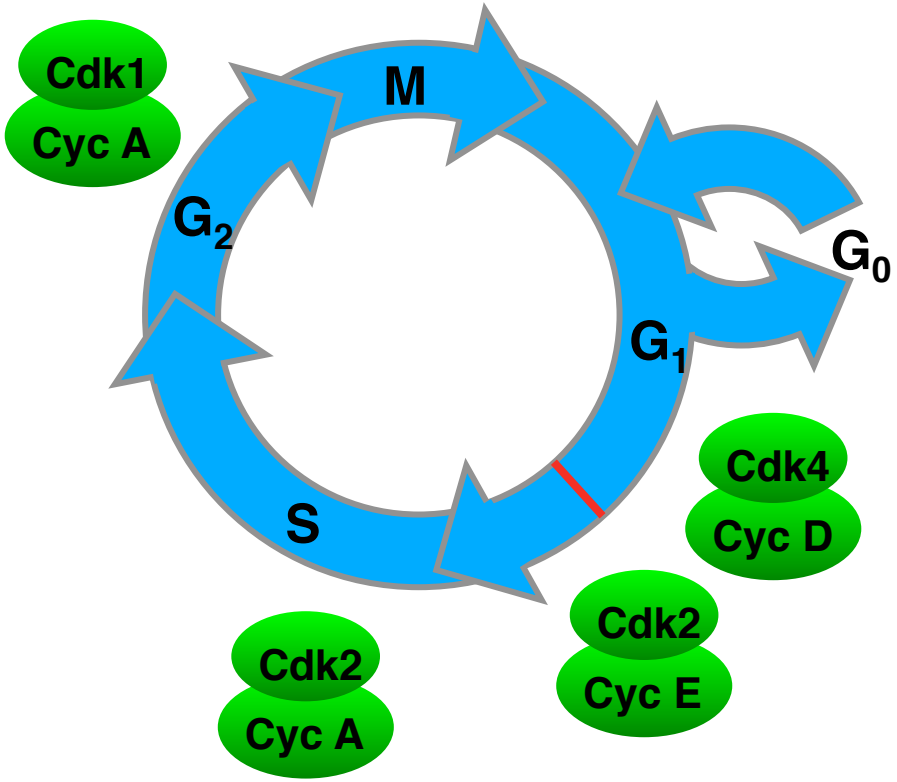
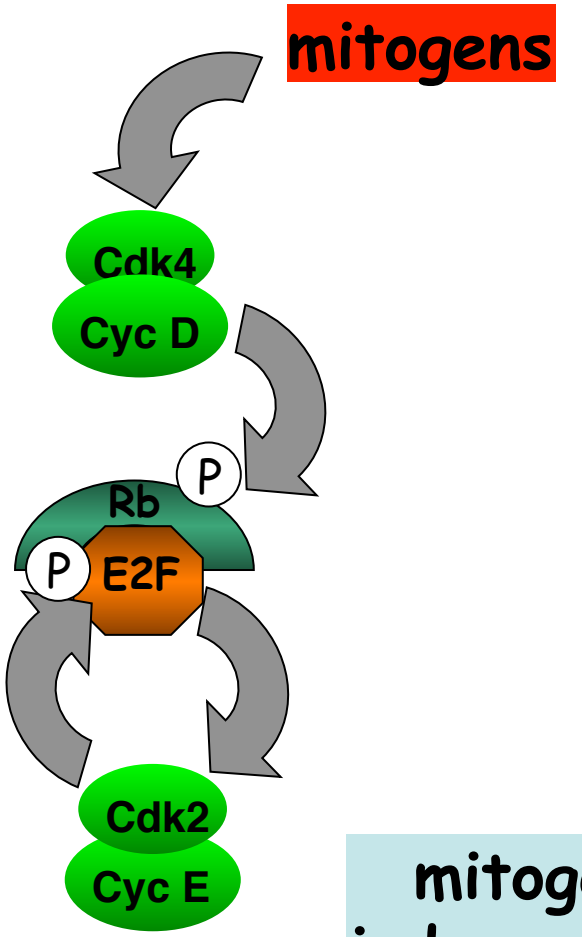
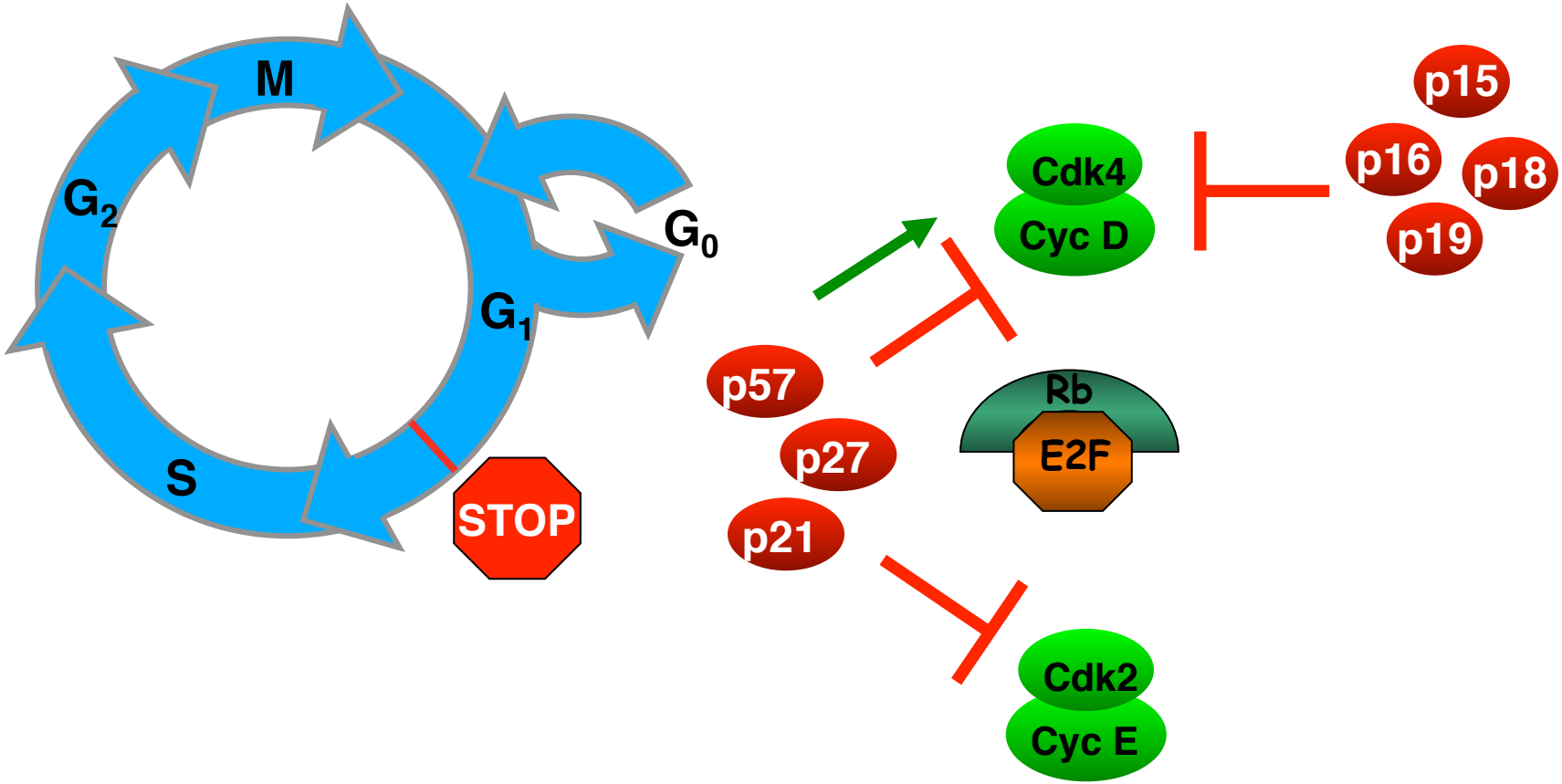
Mitogen induced expression and activation of cyclin D/ CDK initiates cell cycle progression



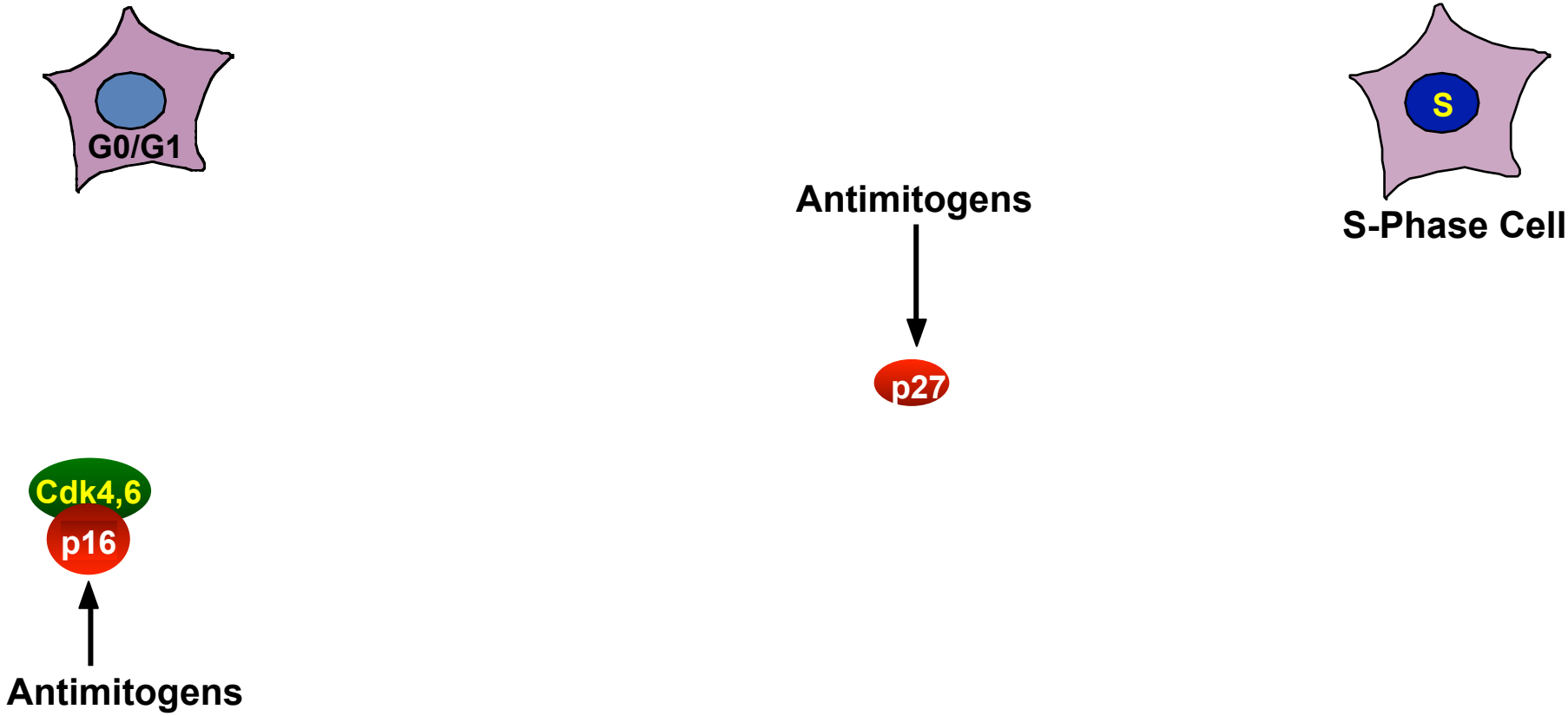


mitogen independent

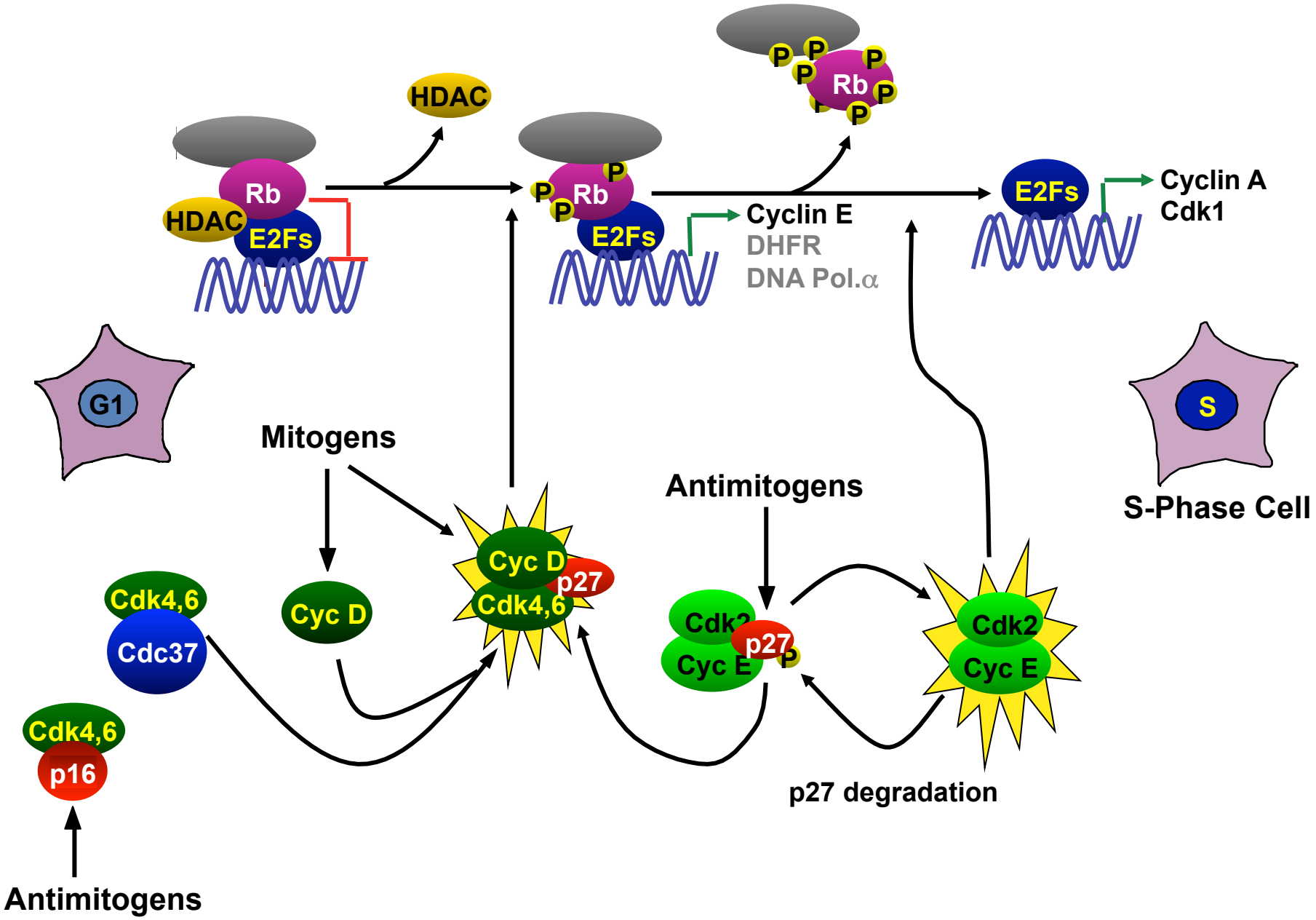
CDK inhibitors prevent CDK activation



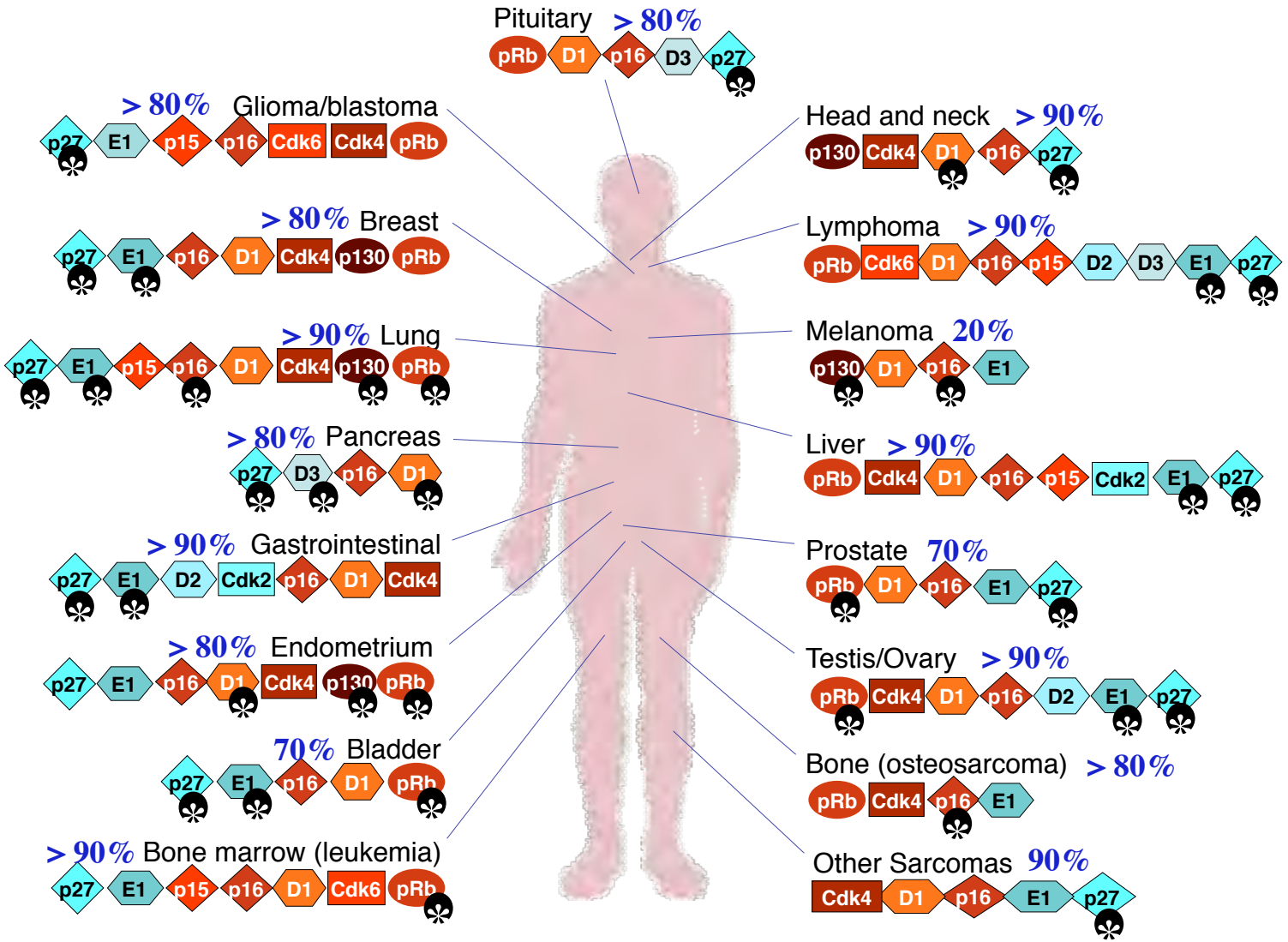
The Rb pathway during the G0/G1/S transition



Der Rb Signalweg des G0/G1/S Übergangs



Cancer is a disease of the cell cycle



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Questions

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