Cell cycle and cancer

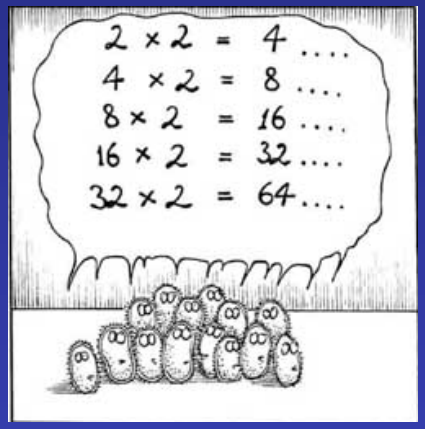
Dr Gerardo Ferbeyre
Département de biochimie, E515
g.ferbeyre@umontreal.ca

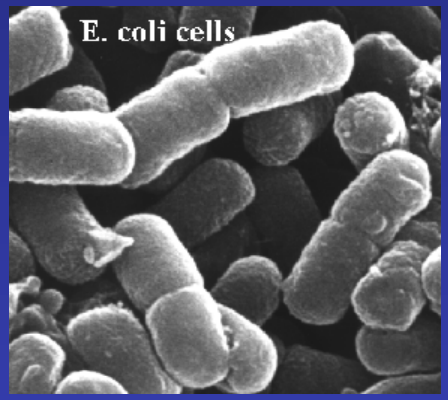
Summary

- Introduction to the Cell Cycle: CDK, cyclins and CKIs
- E2F and retinoblastoma protein
- Checkpoints
- P53 and protein post-translational modifications (PTM)

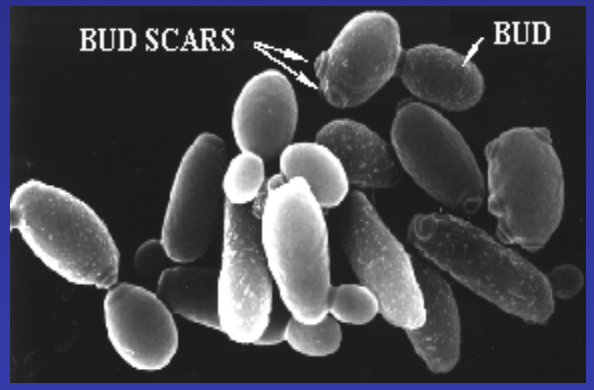
The concept of a cell cycle

- Mechanism of cell reproduction
- One cells give rises to two cells





Binary



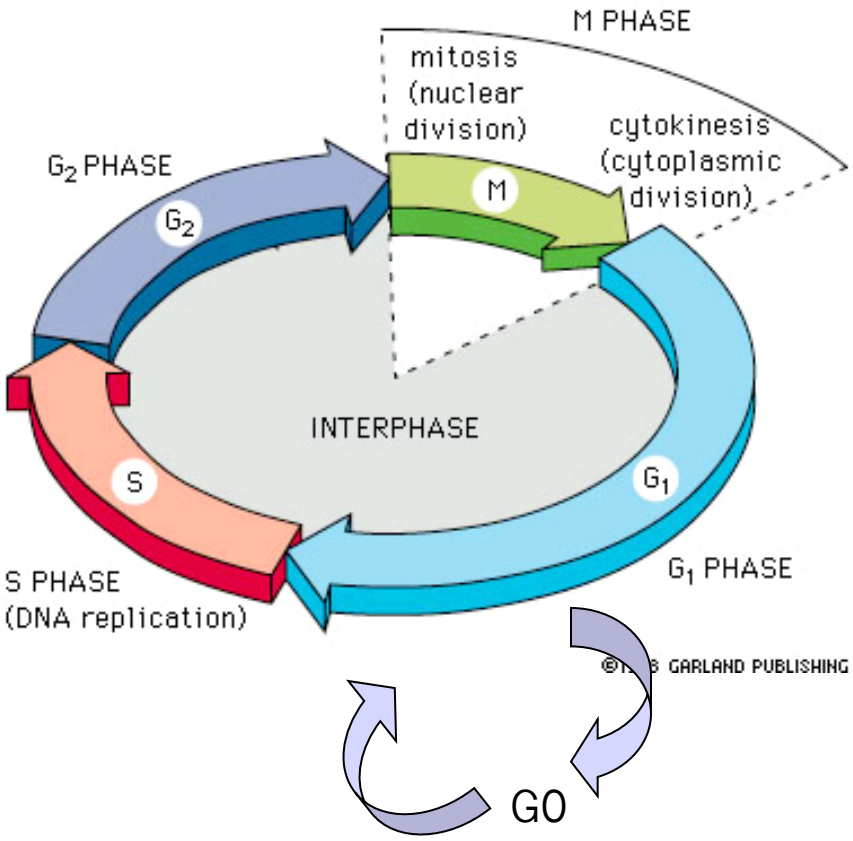
Budding

The hallmarks of the cell cycle

- Replicate genetic information (DNA replication)
- Separate genetic information (Chromosome segregation)
- Divide the cell in two (Cytokinesis)

Le rêve de toute cellule: devenir deux cellules
Francois Jacob

Cell cycle stages



G0

- Cells exit the cell cycle (reversible)

G1 et G2

- Cell growth (in mass)
- Length variable
- Cells must ensure everything is ready for DNA replication and chromosome segregation

S

- DNA replication (8 hours)
- G1, S and G2 also known as interphase (24 hours)

M

- Chromosome segregation and cytokinesis (1 hour)

Methods to study the cell cycle

- Counting mitotic cells
- 3H-thymidine incorporation
- BrdU incorporation
- PCNA
- Flow cytometry

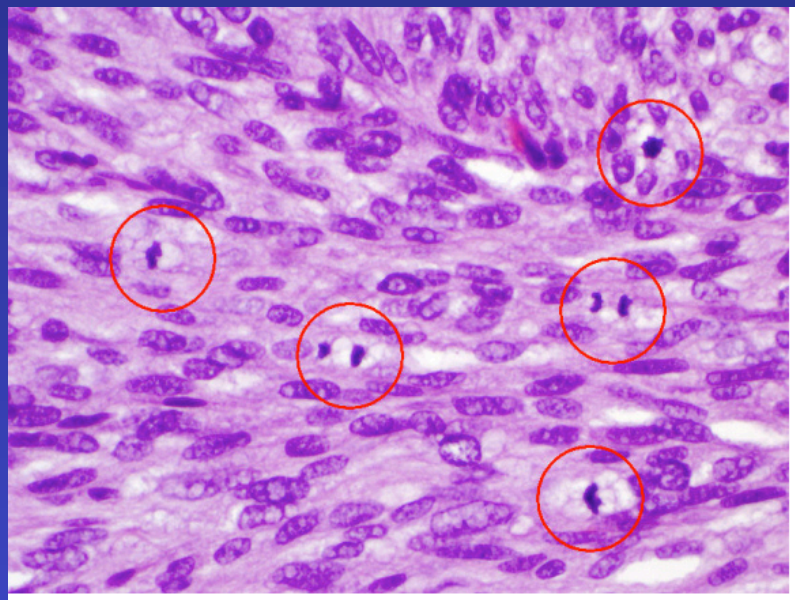
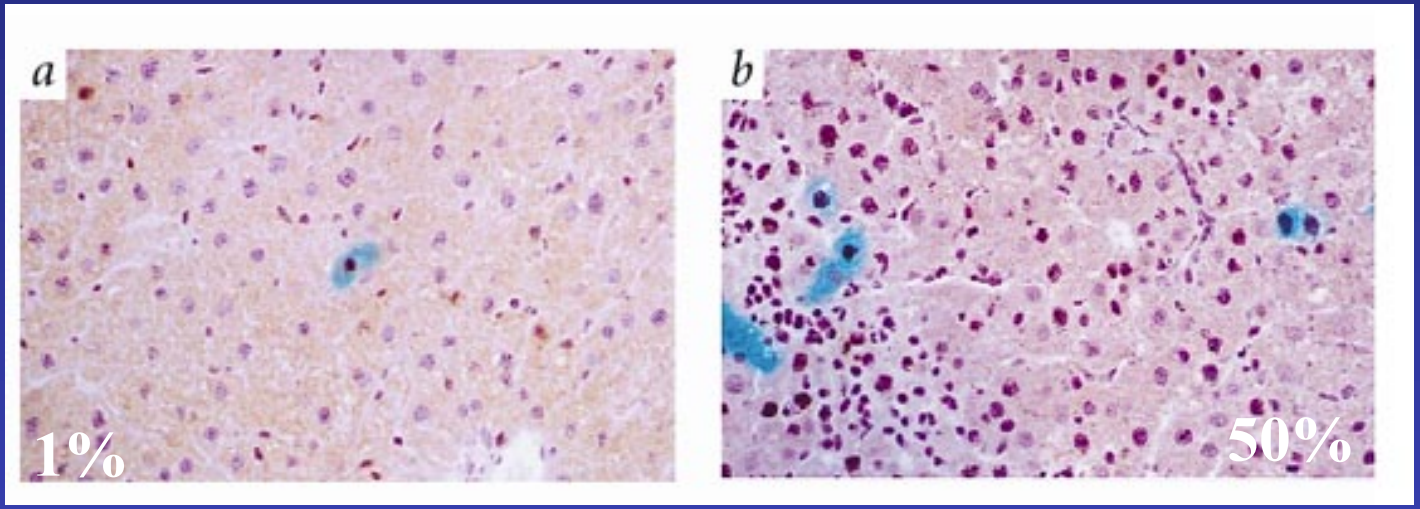


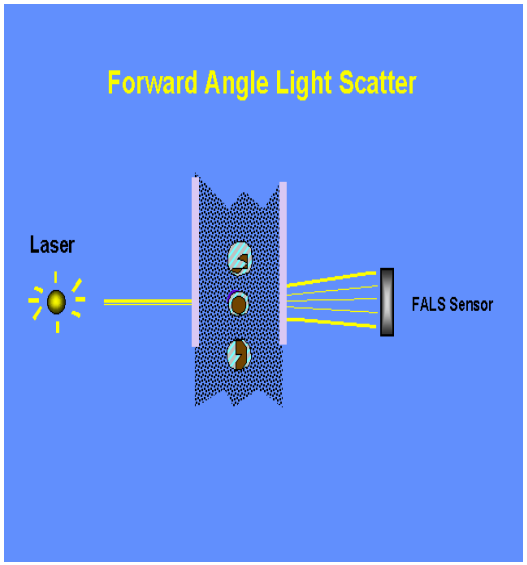
FIGURE 6. GIST CELLS AT 400X MAGNIFICATION WITH MITOTIC FIGURES CIRCLED, ILLUSTRATING WHAT THE PATHOLOGIST LOOKS FOR TO COUNT THE MITOTIC RATE.

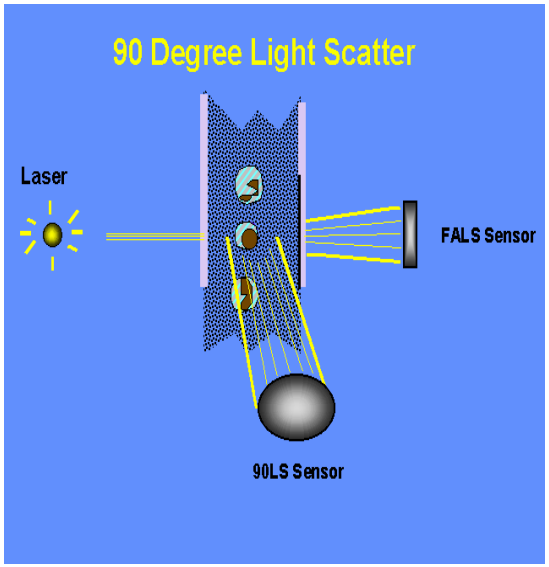
Looking at S phase cells

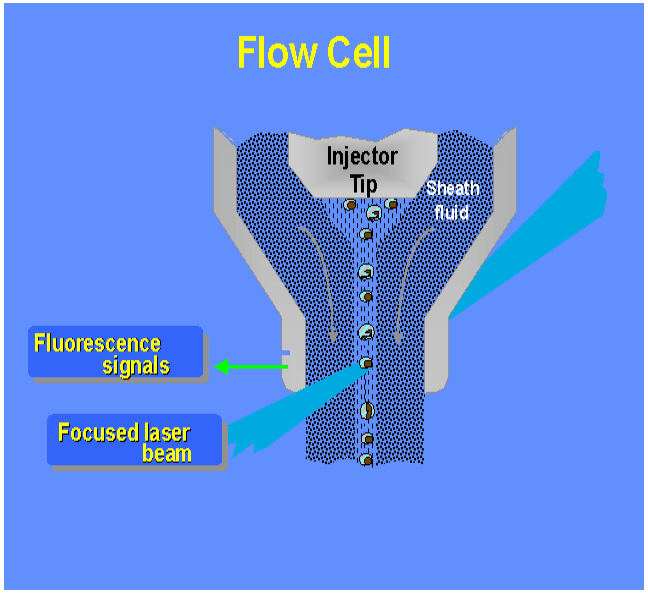


- BrdU (thymidine analogue)
- Antibodies against BrdU coupled to peroxydase
- Antibodies against PCNA or KI67
- Cell proliferation is asynchronous

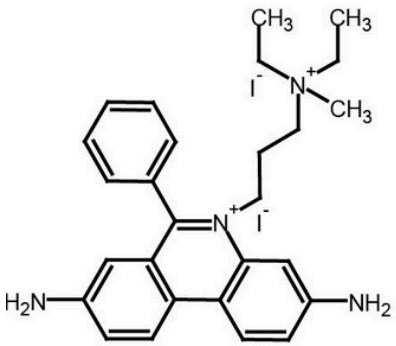
Flow cytometry

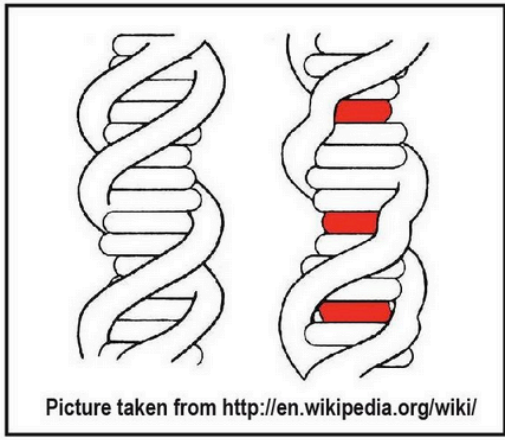






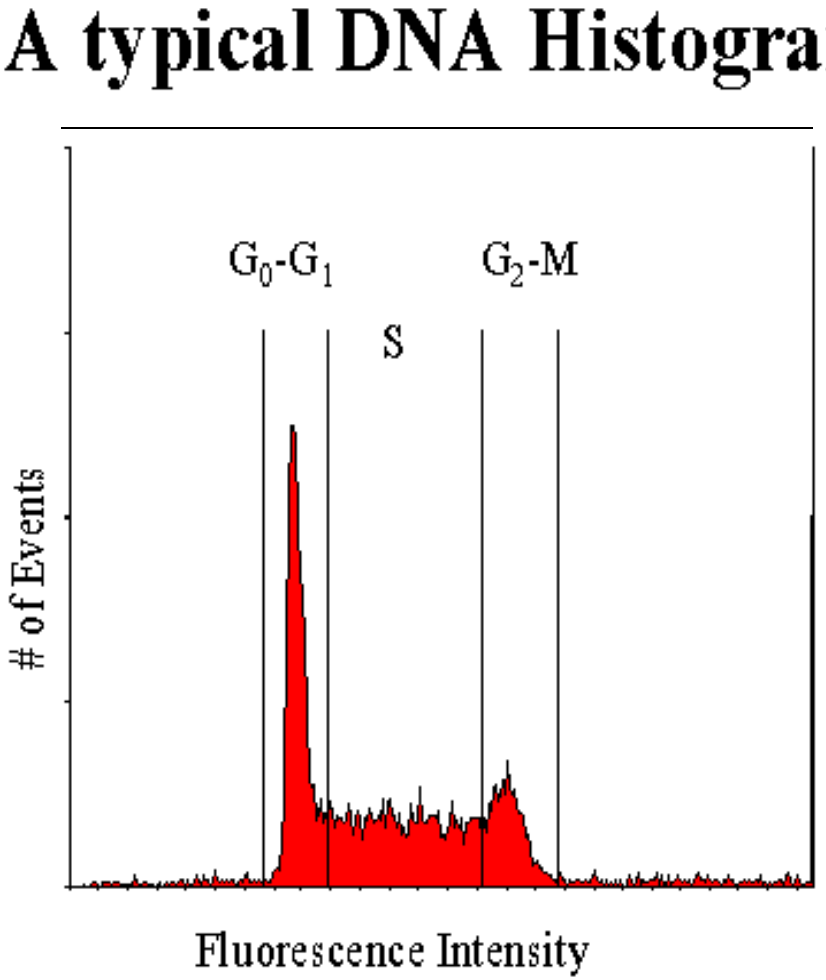
Propidium iodide





Red bars on the left panel represents intercalating agent between DNA base stacks

DNA content analysis by flow cytometry



Fluorescent intensity
is proportional to DNA content

- The binding of growth factors to specific receptors on the plasma membrane is usually necessary for cell division

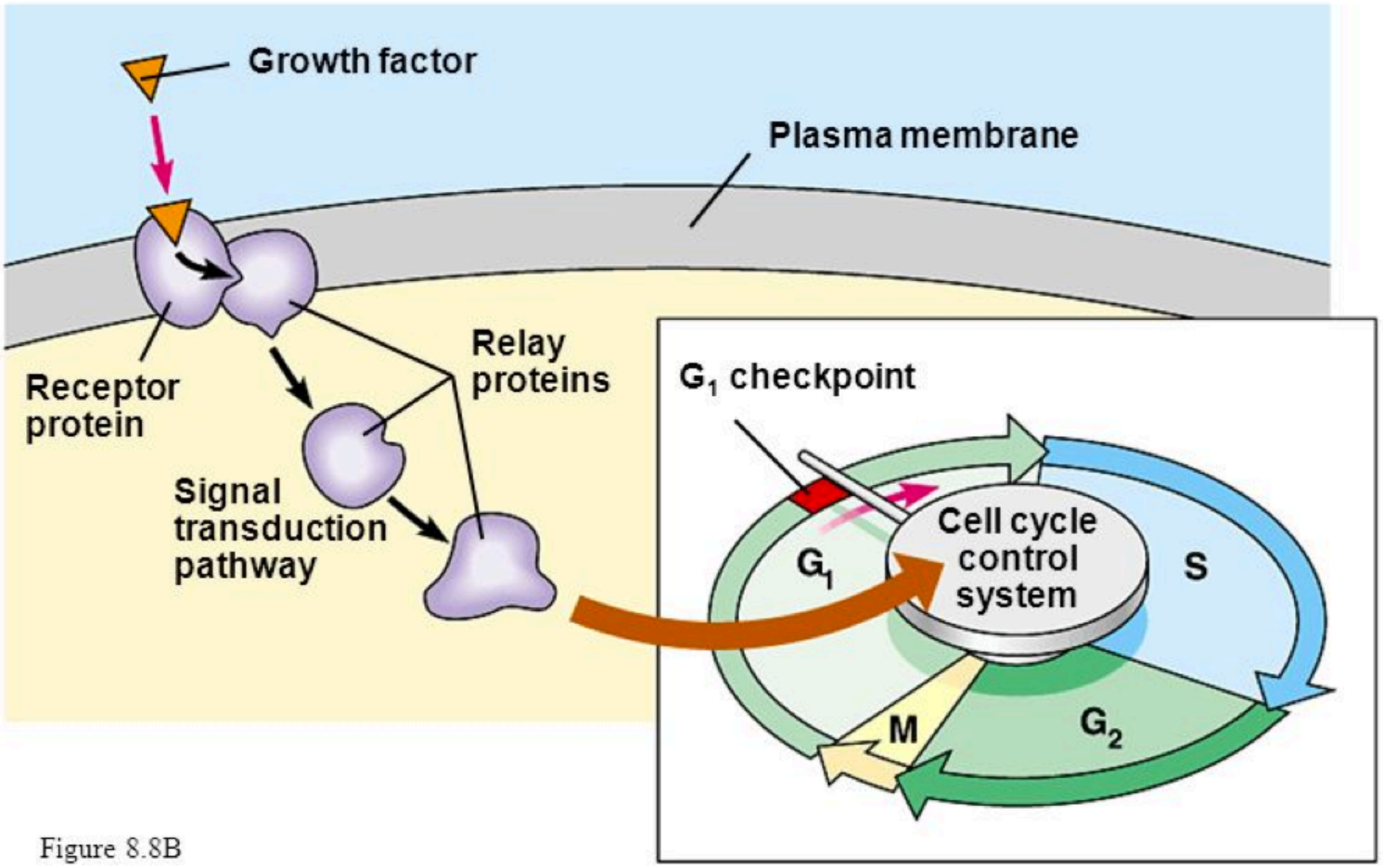
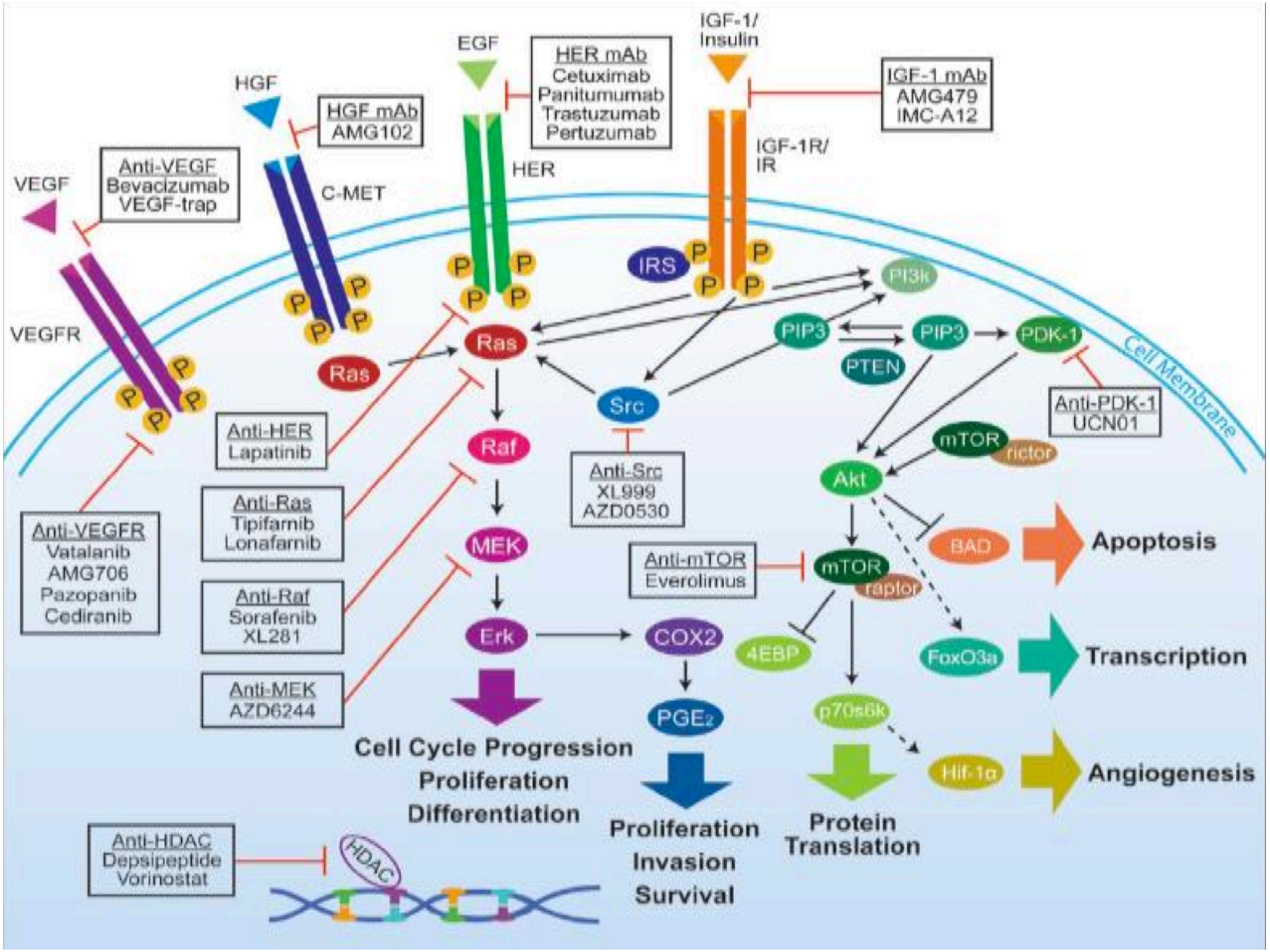


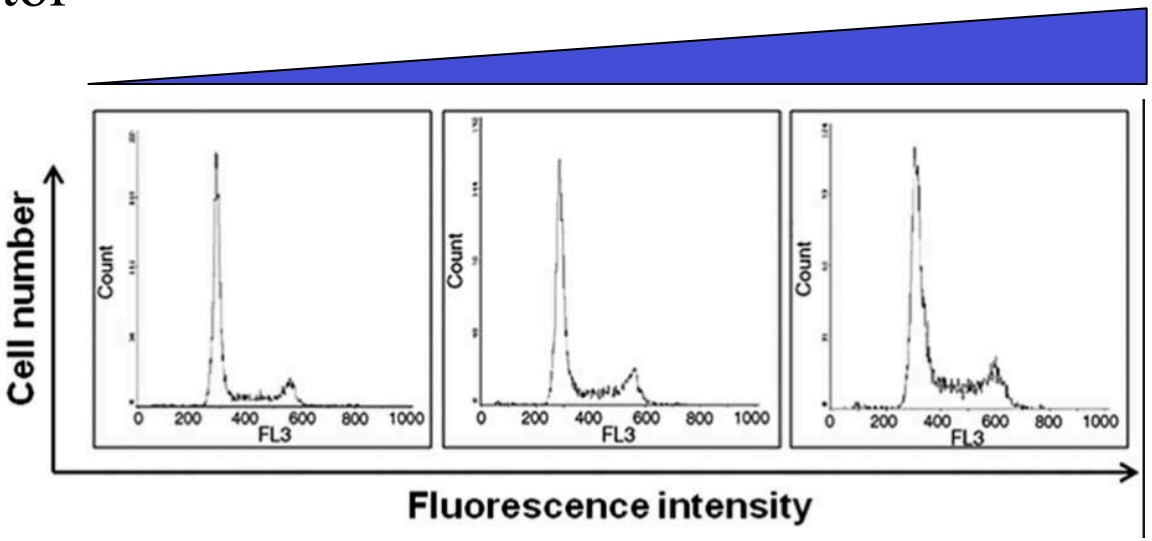
Figure 8.8B

Targeted therapy against GF receptors



Quiz#1 cell cycle

1- Cells were treated with a) growth factor b) growth inhibitor

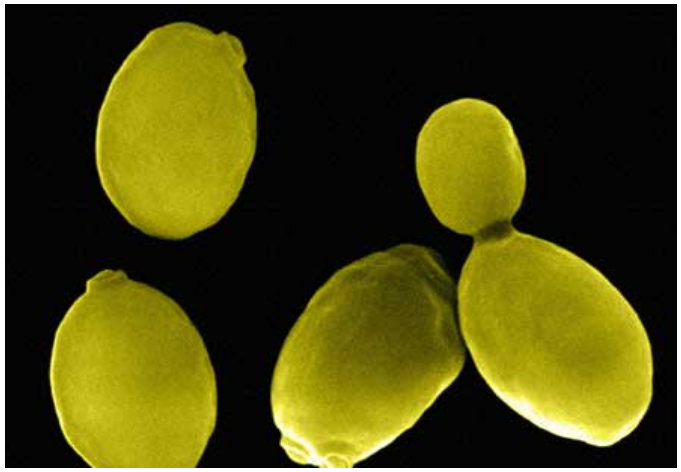


2- What is false?: cancer cells become independent of external growth factors by:
a) Autocrine stimulation, b) Mutations in GF receptors c) Stimulating their microenvironment to produce GF d) Eliminating G1

Cell cycle engine: Prix Nobel 2001!

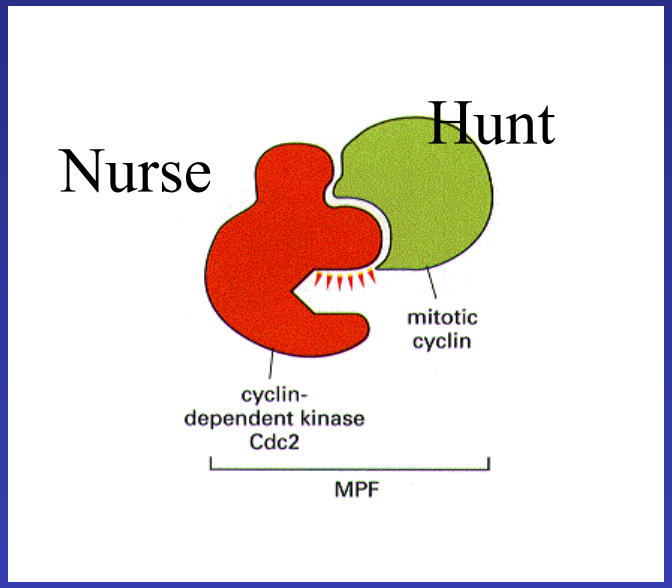


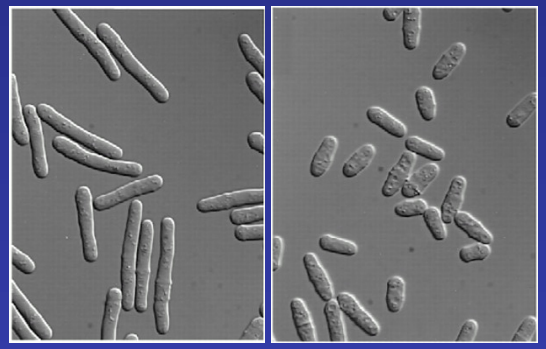




Lee Hartwell Paul Nurse Tim Hunt

The cell cycle is controlled by a family of protein kinases





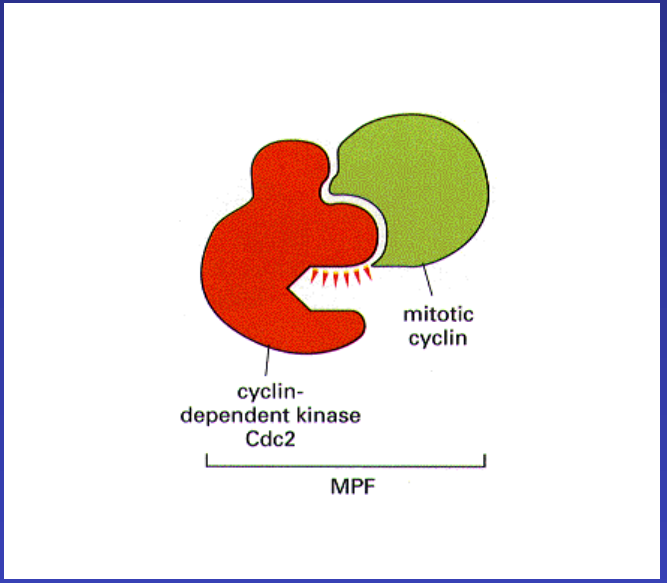
cdc2^{ts} *cdc2*^{ts} *hCDC2* plasmid

Lee, M. G. & Nurse, P. Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*. *Nature* **327**, 31-35 (1987)

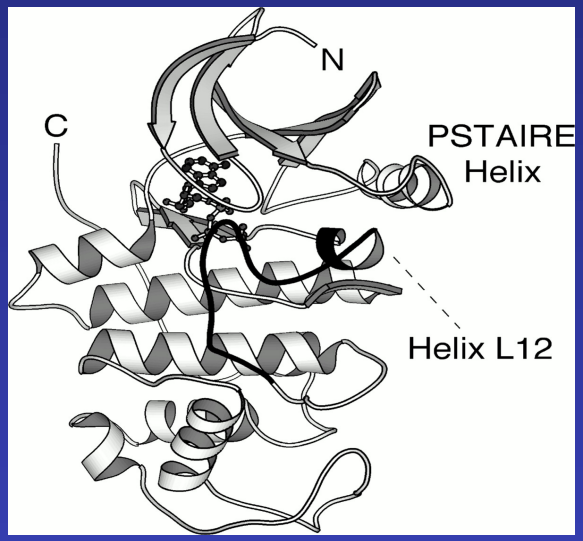
- CDK = cyclin dependent kinases
- MPF = maturation promoting factor
- MPF = CDK + cyclin

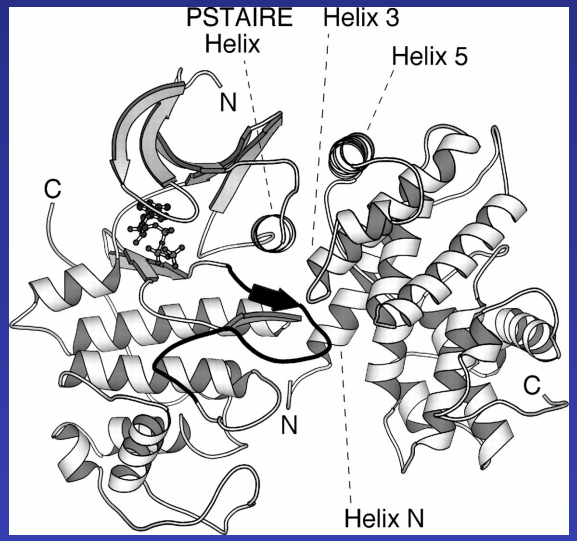
Regulation of CDKs

- Cyclins
- Phosphorylation
- Dephosphorylation
- Inhibitors (CKI)



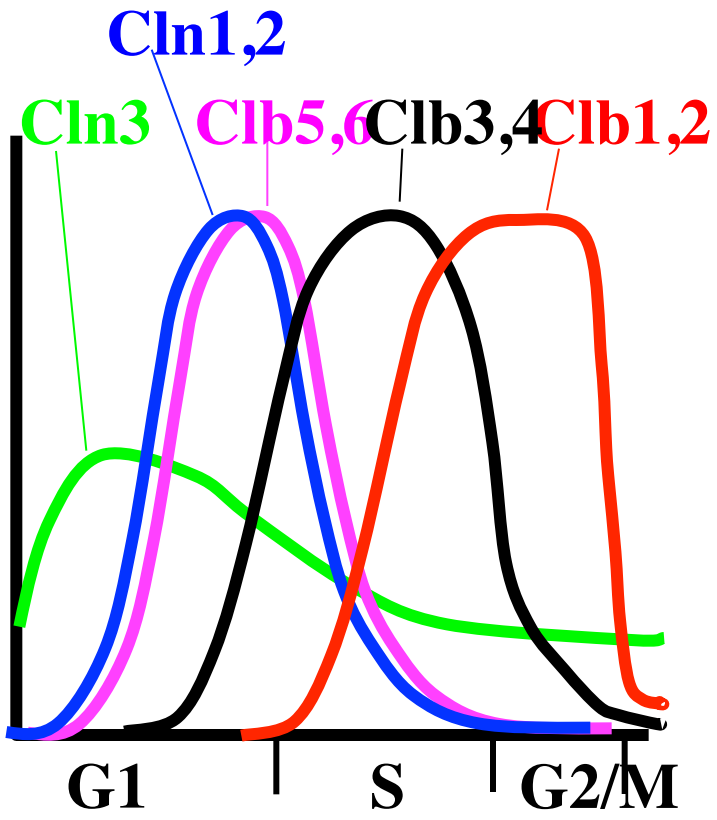
Activation of CDKs by cyclins

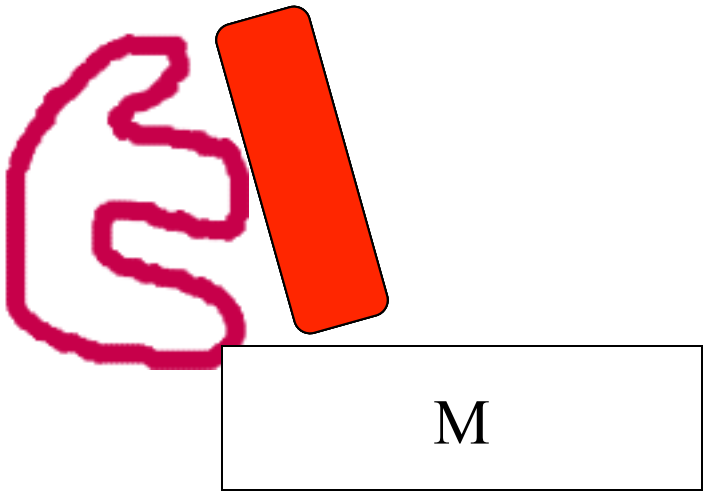




- T loop inhibits CDKs
- Cyclin binding displaces the T loop from the active center

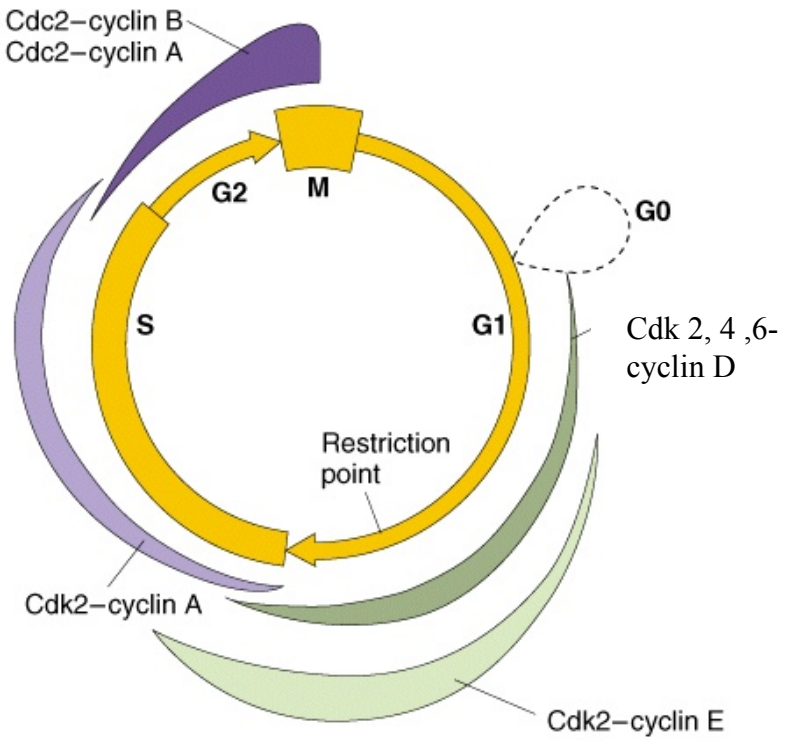
Cell cycle stages are controlled by specific CDK complexes





Substrats des CDK-cyclines

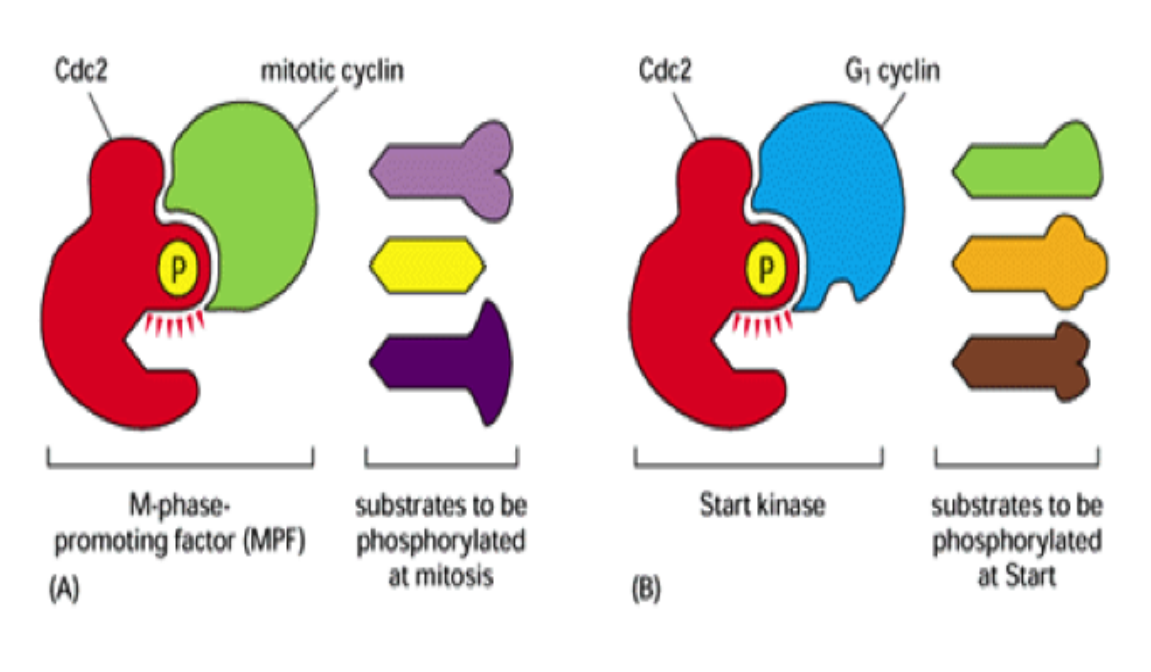
Mammalian CDK complexes



The levels of the CDKs are generally constant throughout the cell cycle.

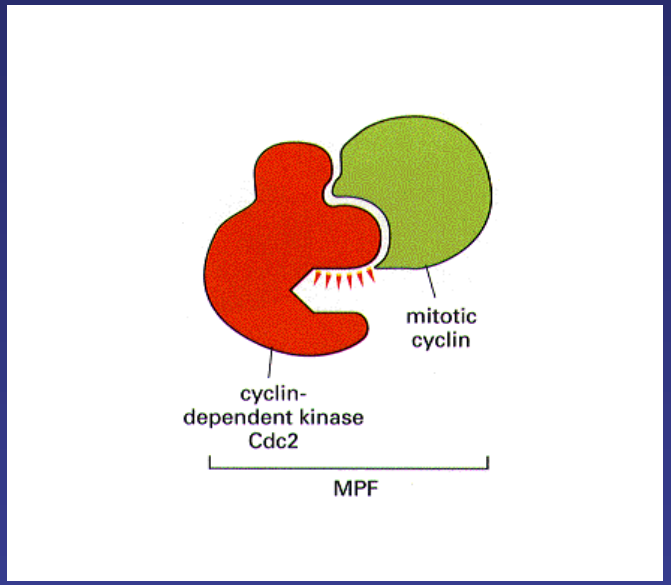
	Mammifères		S. cerevisiae	
G1CDK	***Cyclin D***	***Cdk4, Cdk6***	***cln2,3***	***cdc28=cdc2***
G1SCDK	***Cyclin E***	***Cdk2***	***cln1,cln3***	***cdc28***
S-CDK	***Cyclin A***	***Cdk2***	***Clb5,6***	***cdc28***
M-CDK	***Cyclin B***	***Cdk1= cdc2***	***Clb2,3,4***	***cdc28***

CDK-cyclins activate their targets by phosphorylation

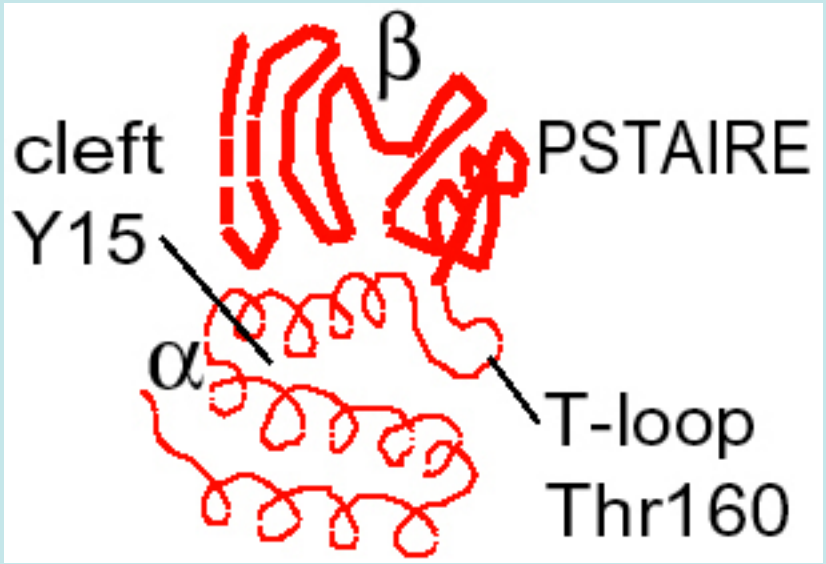


- Phosphorylation site: (S/TPXK/R)
- CDK4 and 6 exhibit narrow specificity and CDK1 has broad specificity (does not require K/R)
- Motif Cy (ou RXL): interacts with the cylcin

- *Association des cyclines*
- Phosphorylation
- Dephosphorylation
- Inhibitors (CKI)



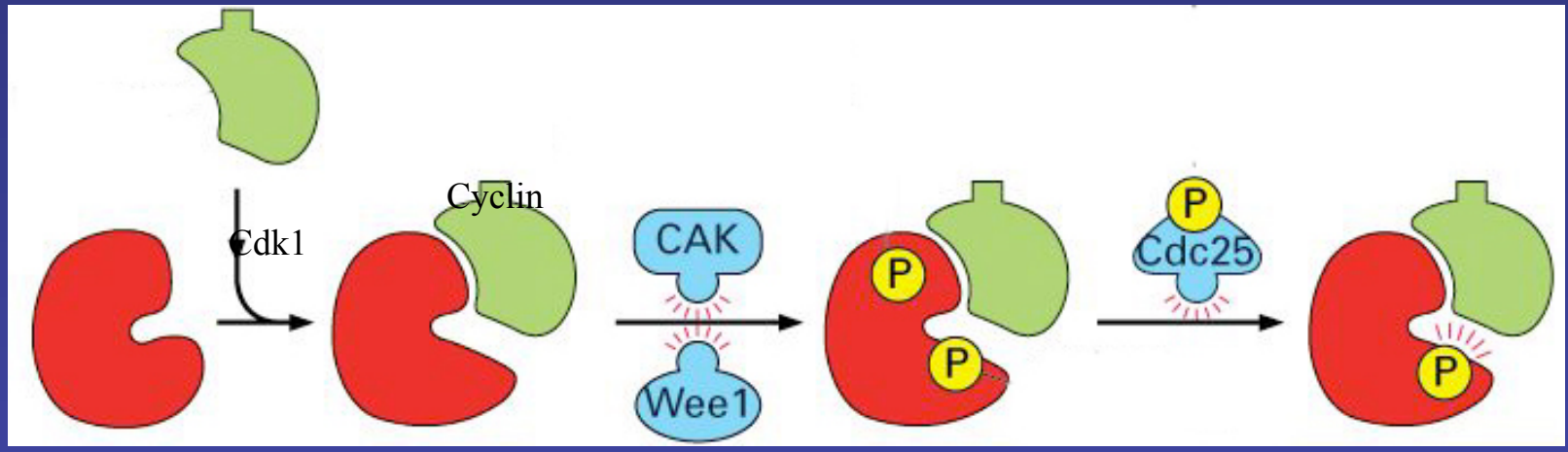
CAK: CDK phosphorylation at Thr 160



CAK (Cdk7/CyclinH/Mat1 in metazoos, Cak1 in S. cerevisiae)

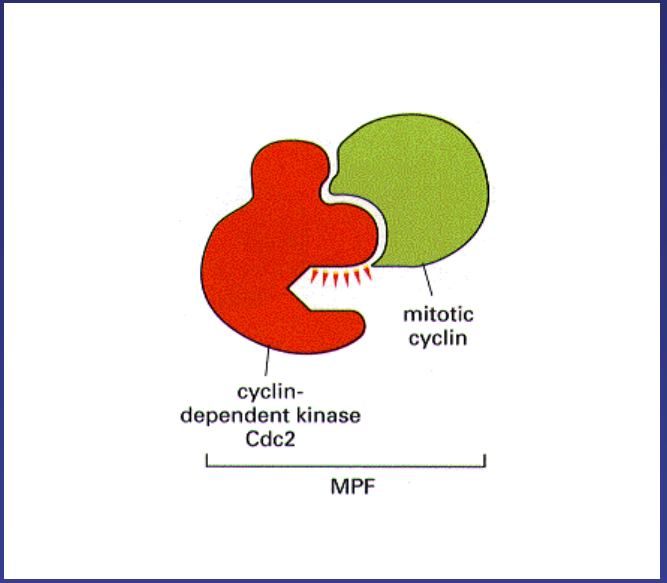
Inhibitory phosphorylation: Wee1 kinase and Cdc25 phosphatase

- Wee1 phosphorylates tyrosine 15 at the active center and inhibit CDK activity
- Cdc25 A, B, C phosphatases dephosphorylates CDKs at Wee1 site

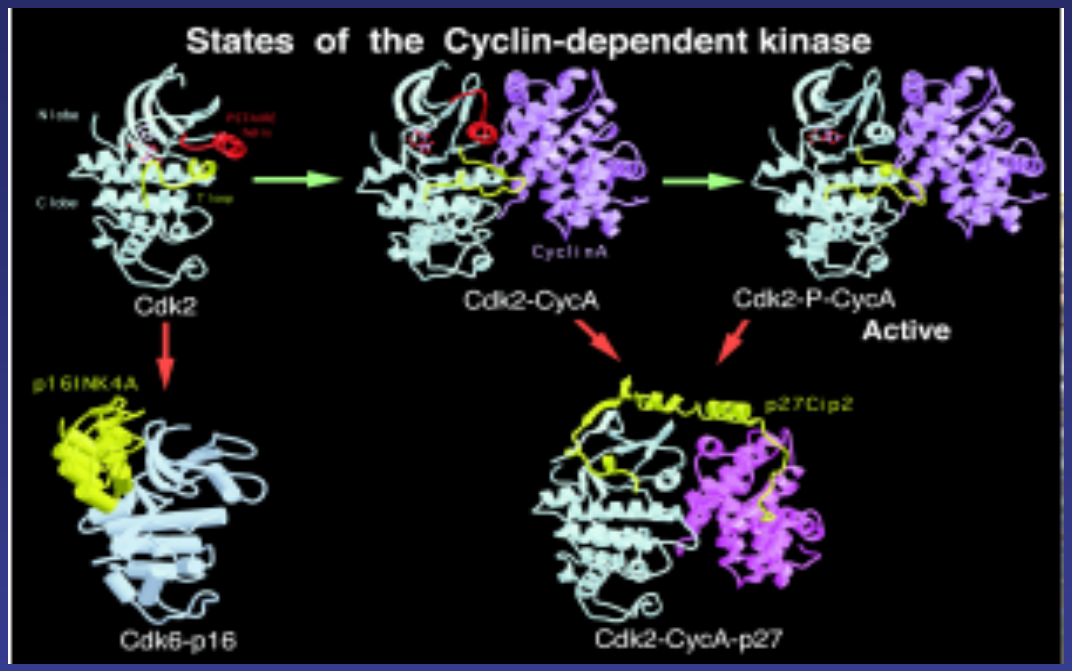


Regulation of CDKs

- Cyclins
- Phosphorylation
- Dephosphorylation
- Inhibitors (CKI)



Cip/Kip vs. INKs



Two CKI families:

- Cip/Kip: p21, p27, p57: inhibit Cdk2, Cdk4/6 in complexes with G1/S cyclins
- Ink: p15, p16, p18, p19: inhibit the interaction of Cdk4/6 with cyclin D

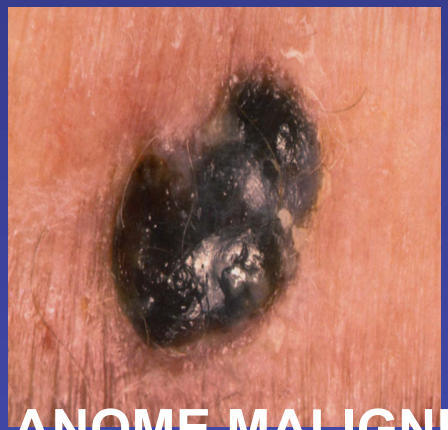
SIC1: yeast CKI

Mutations in CKIs

BECKWITH-WIEDEMANN SYNDROME

BECKWITH-WIEDEMANN SYNDROME

- 13.5 pounds at birth
- Big tongue
- Mutations in p57
- high risk of cancer



MELANOME MALIGNE

Malignant melanoma

- Mutations in $p16^{INK4a}$

Quiz#2 cell cycle

1- Which one of the following statements best describes the mechanism by which the "cell cycle control system" regulates events of the cell cycle?

A. Ca^{++} and cAMP are released into the nucleus at particular times.

B. Protein activity is regulated through phosphorylation and dephosphorylation.

C. Specific hormones signal when it's time to move to each stage of the cell cycle.

D. Changes in membrane potential signal progress of the cell cycle.

2- Which of the following describe(s) cyclin- dependent kinase (Cdk)?

A) Cdk is inactive, or ″ turned off,″ in the presence of cyclin.

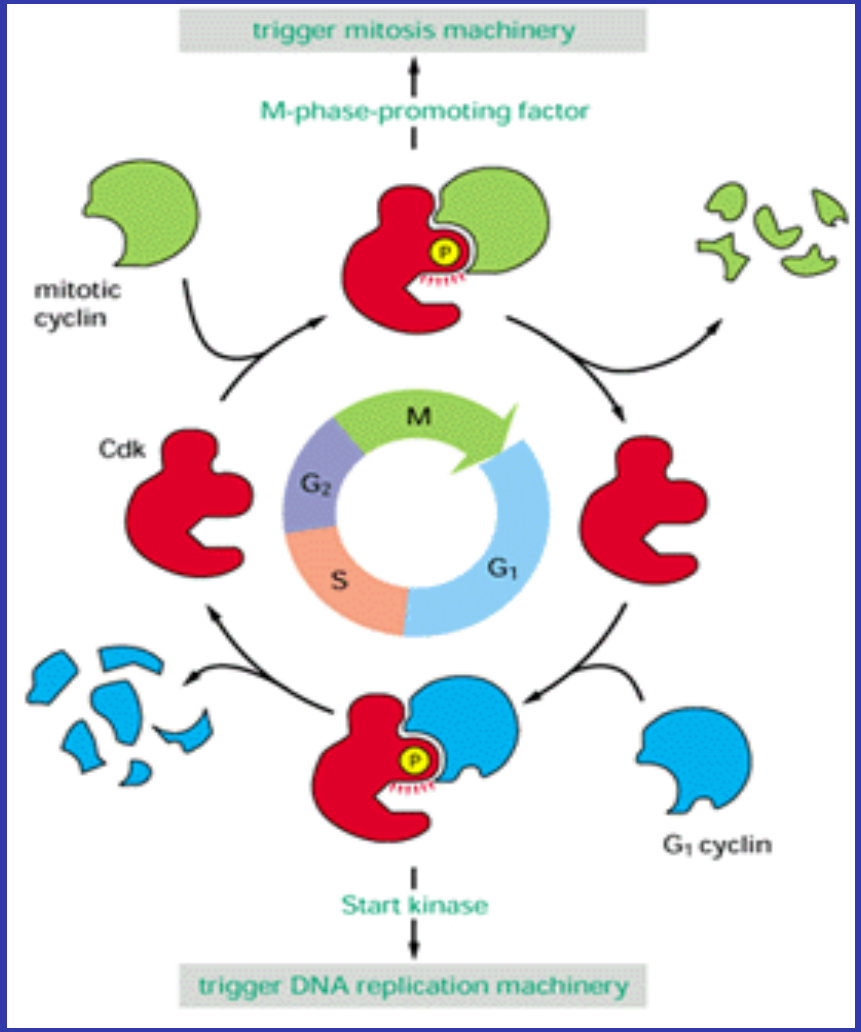
B) Cdk is present throughout the cell cycle.

C) Cdk is an enzyme that attaches phosphate groups to other proteins.

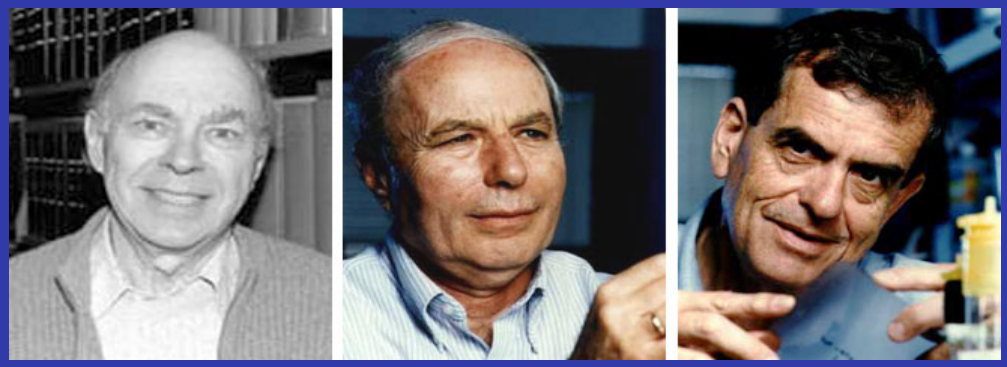
D) Both A and B are true.

E) Both B and C are true.

Controlling the cyclins

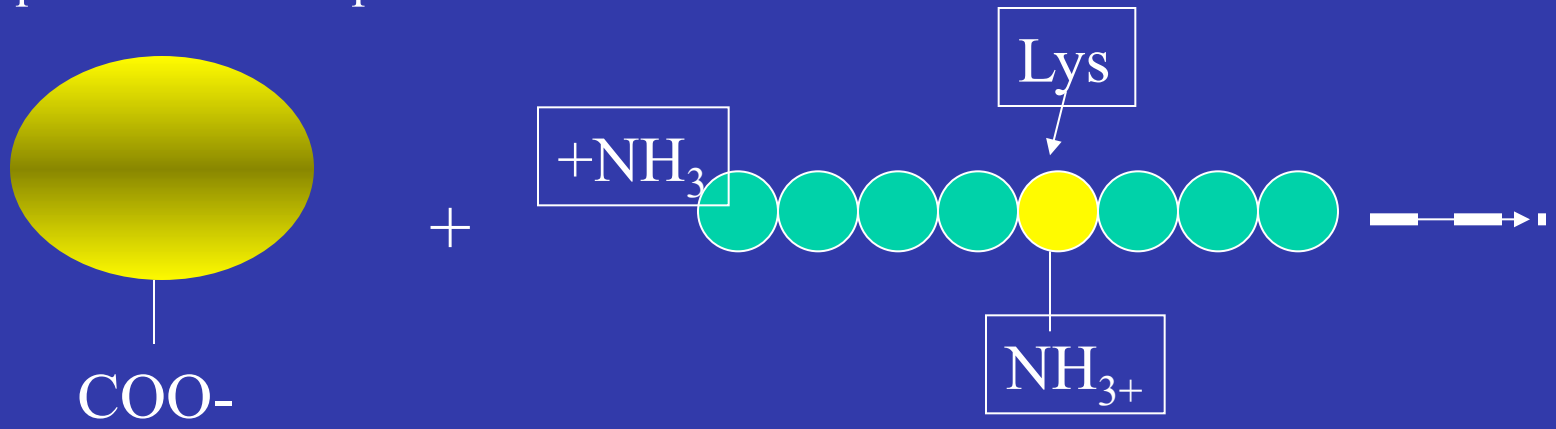


Ubiquitin labels proteins for degradation



Rose, Hershko and Ciechanover: Prix Nobel 2004

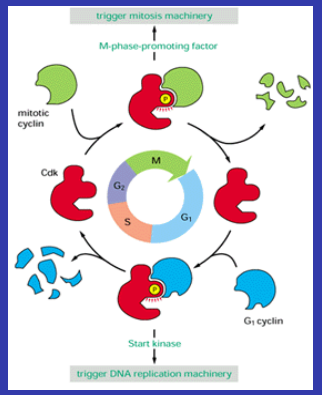
Ubiquitin is a 76 aa protein



C-terminal glycine of ubiquitin is attached to
The epsilon amino group of one lysine
in the target protein

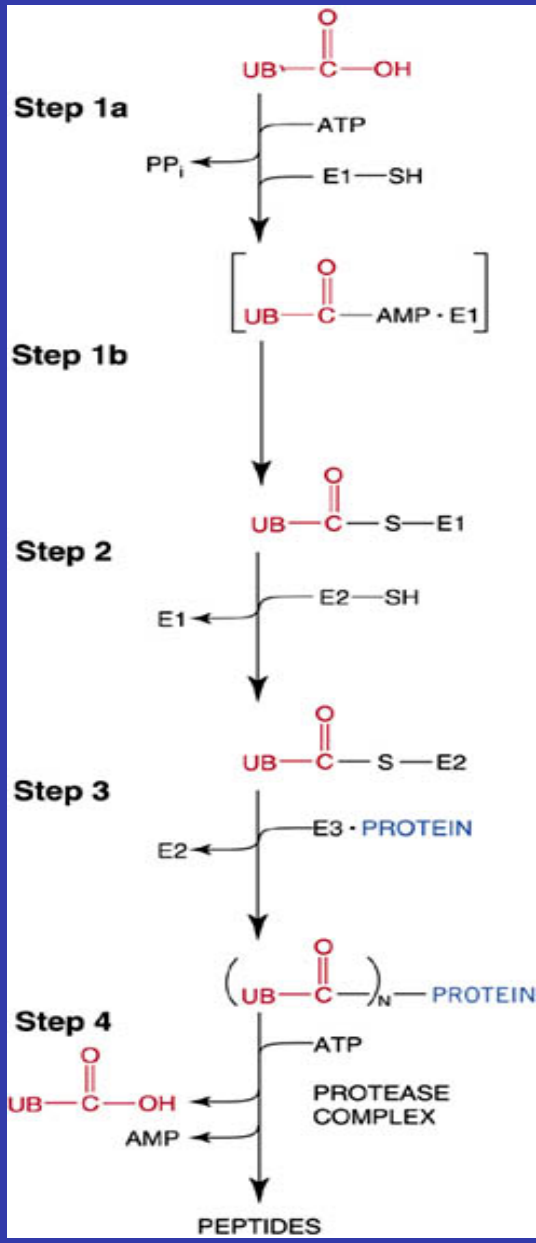
E3 ubiquitin ligases control cyclin degradation during the cell cycle

- Two kinds of E3 ligases
- APC: The recognition subunit is CDC20 in mitosis and CDH1 in G1
- SCF: The recognition subunits are F-box proteins
  - G1/S: Ubiquitine ligase SCF (constitutive)

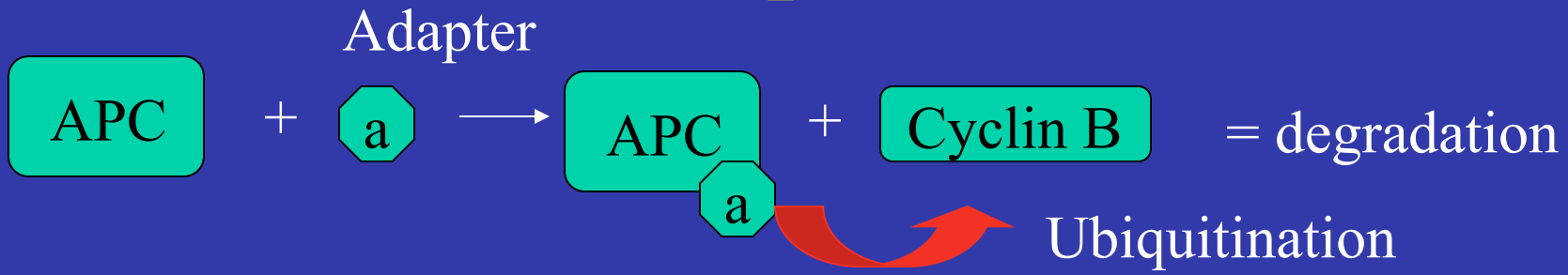


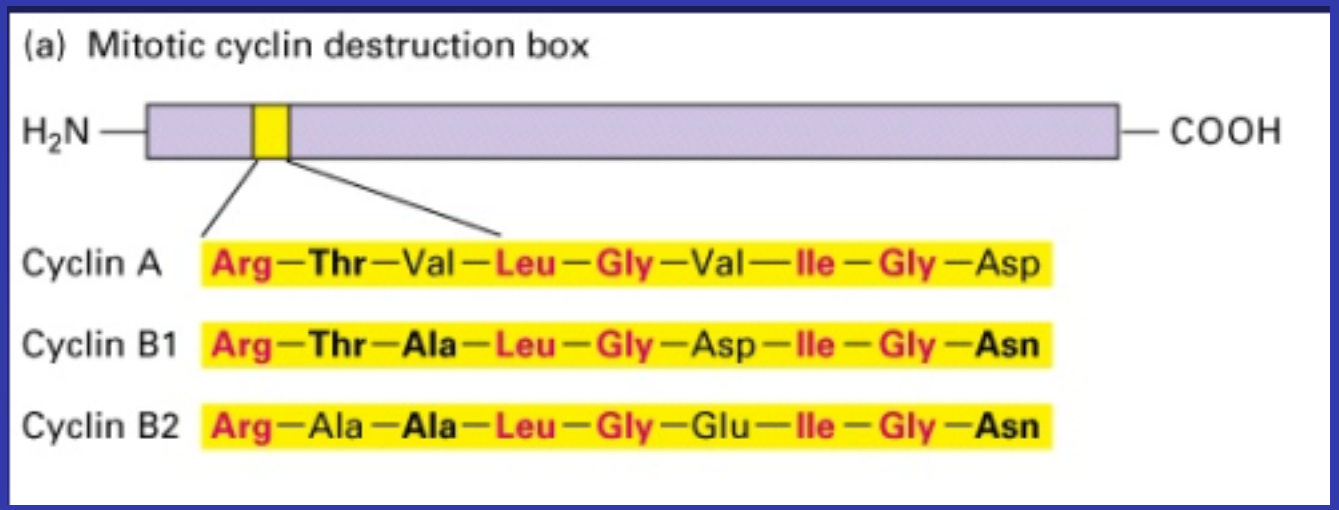
APC:CDC20

SCF



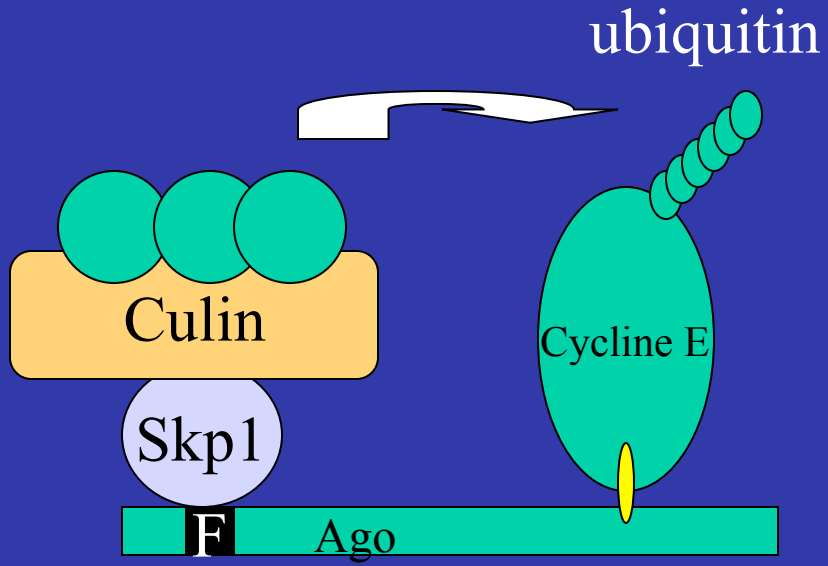
APC: anaphase promoting complex





Adaptors CDC20 and CDH1 decide their targets by binding to the cyclin destruction box

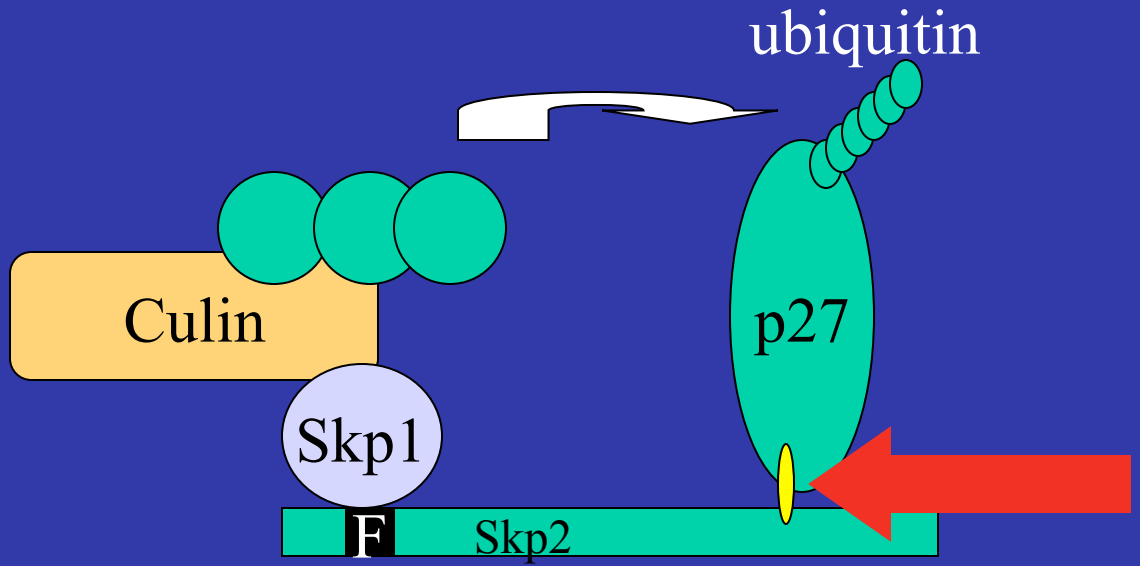
SCF complexes recognize phosphorylated proteins

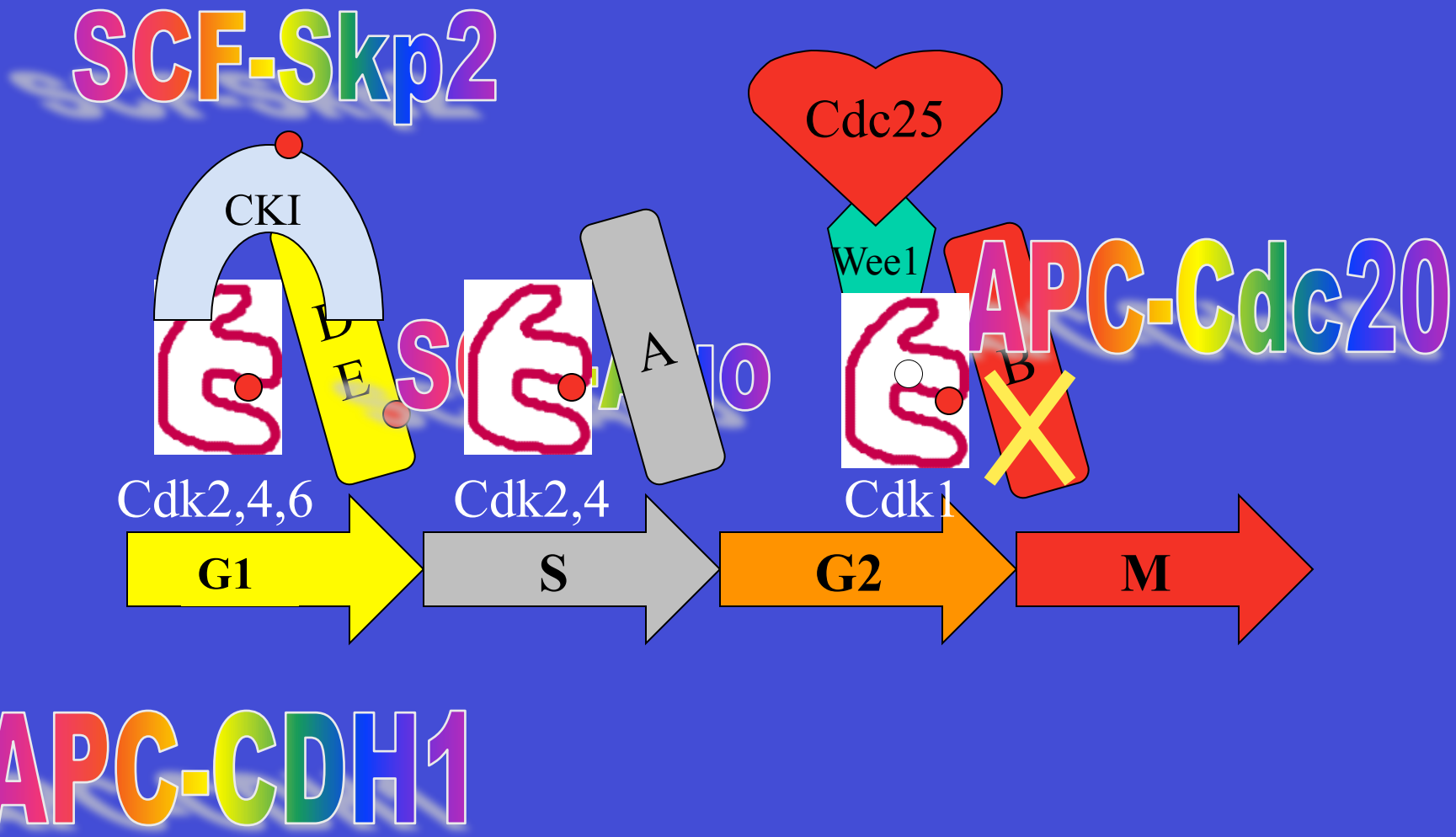


- SCF = Skp + Culins + F box
- F box recognizes the phosphorylated target (Ago for cycline E, Fbx4 for cycline D)
- Around 60 F box proteins in the human genome

Substrate phosphorylation is the signal for degradation

CKIs are also degraded via SCF complexes




SCF-Skp2
CKI
D
E
Cdc25
Wee1
APC-Cdc20
A
B
Cdk2,4,6
Cdk2,4
Cdk1
G1
S
G2
M
APC-CDH1

Quiz#3 cell cycle

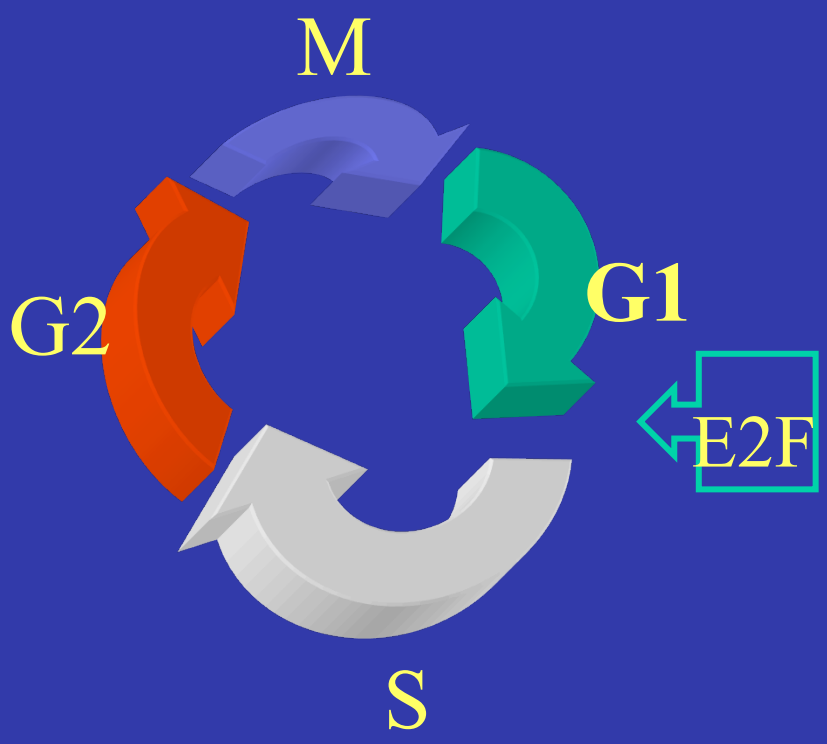
1- Which of the following types of mutations would prevent a cell from progressing into the S phase of the cell cycle?

A. a cell that has a loss of function mutation in SCF.
B. a cell that has a loss of function mutation in wee1.
C. a cell that has a loss of function mutation in p27.
D. a cell that has a loss of function mutation in Cdk1

2- SCF complexes catalyze directly

A- protein degradation
B- protein phosphorylation
C- protein ubiquitination
D- protein dephosphorylation

Transcriptional control of the cell cycle



E2F family

E2F1, 2, 3a
- Activators
- G1-S and G0 exit

E2F 3b, 4, 5
- Repressors:
- G0 and cell differentiation

E2F6: Polycomb dependent repressor

E2F7: S and G2 repressor, senescence

E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis.
Muller H et al. Genes Dev 2001 Feb 1;15(3):267-85

E2F target genes

Cell cycle
- Cdk1
- Cyclin D and E
- E2F 1, 2, 3
- Cdc25

DNA replication
- Initiation: Orc1, Cdc6, Mcm 3, 5 , 6
- Replication factors: RPA, RFC, PCNA, DNA ligase, DNA polymerase alpha, Topoisomerase II alpha.

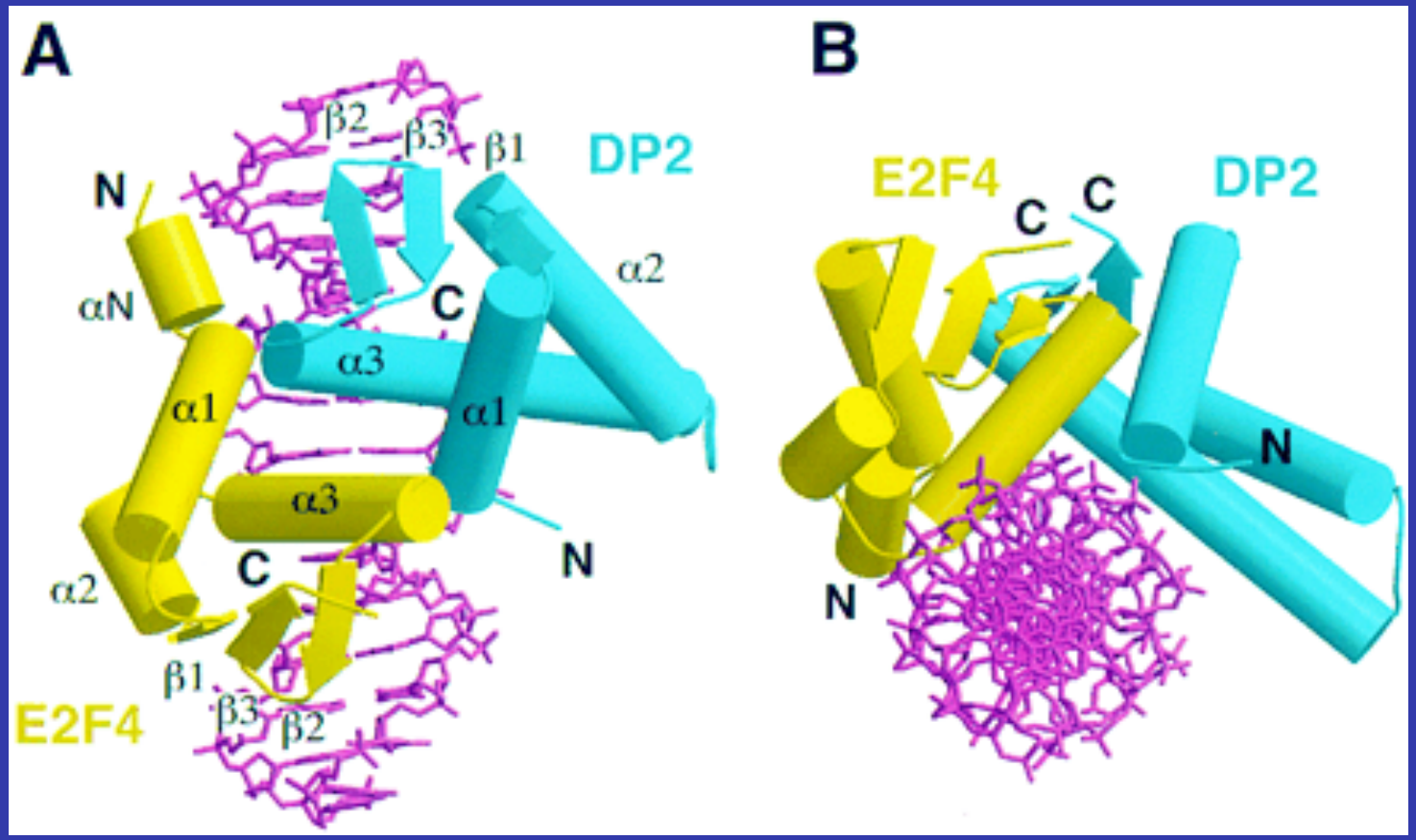
Centrosome duplication:
- RanBPM

Metabolism
- Dihydrofolate réductase
- Thymidylate synthase
- Ribonucleotide réductase

Mitotic activities.
- Cyclin A, B
- Cdc2
- Bub1
- Cdc20

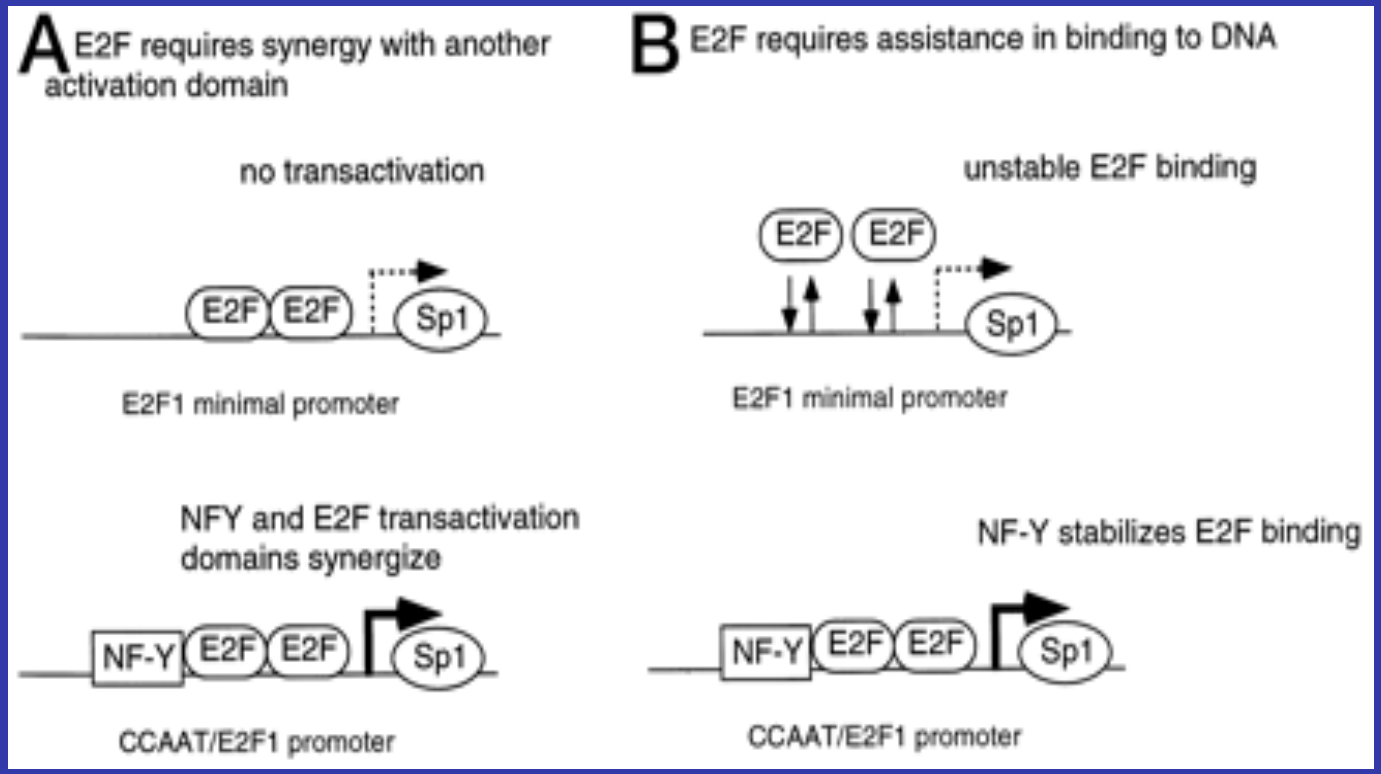
E2F-DP heterodimers

E2Fs bind DNA as heterdimerswith their partner the DP proteins.

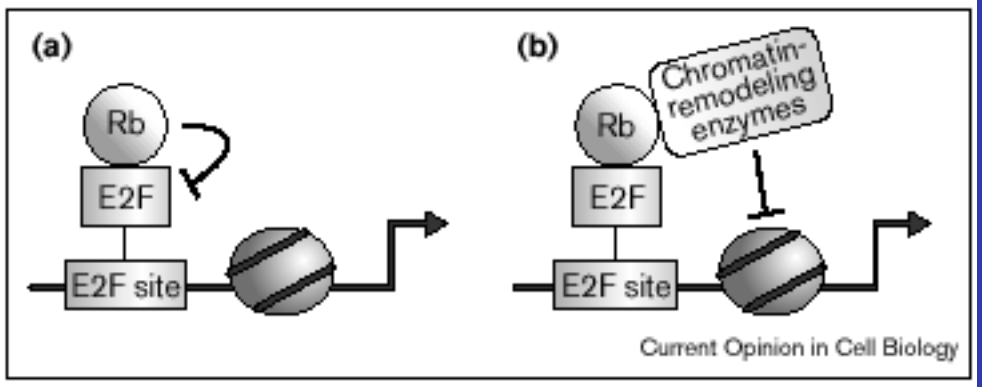


Winged-helix

E2Fs cooperate with other transcription factors to regulate gene expression



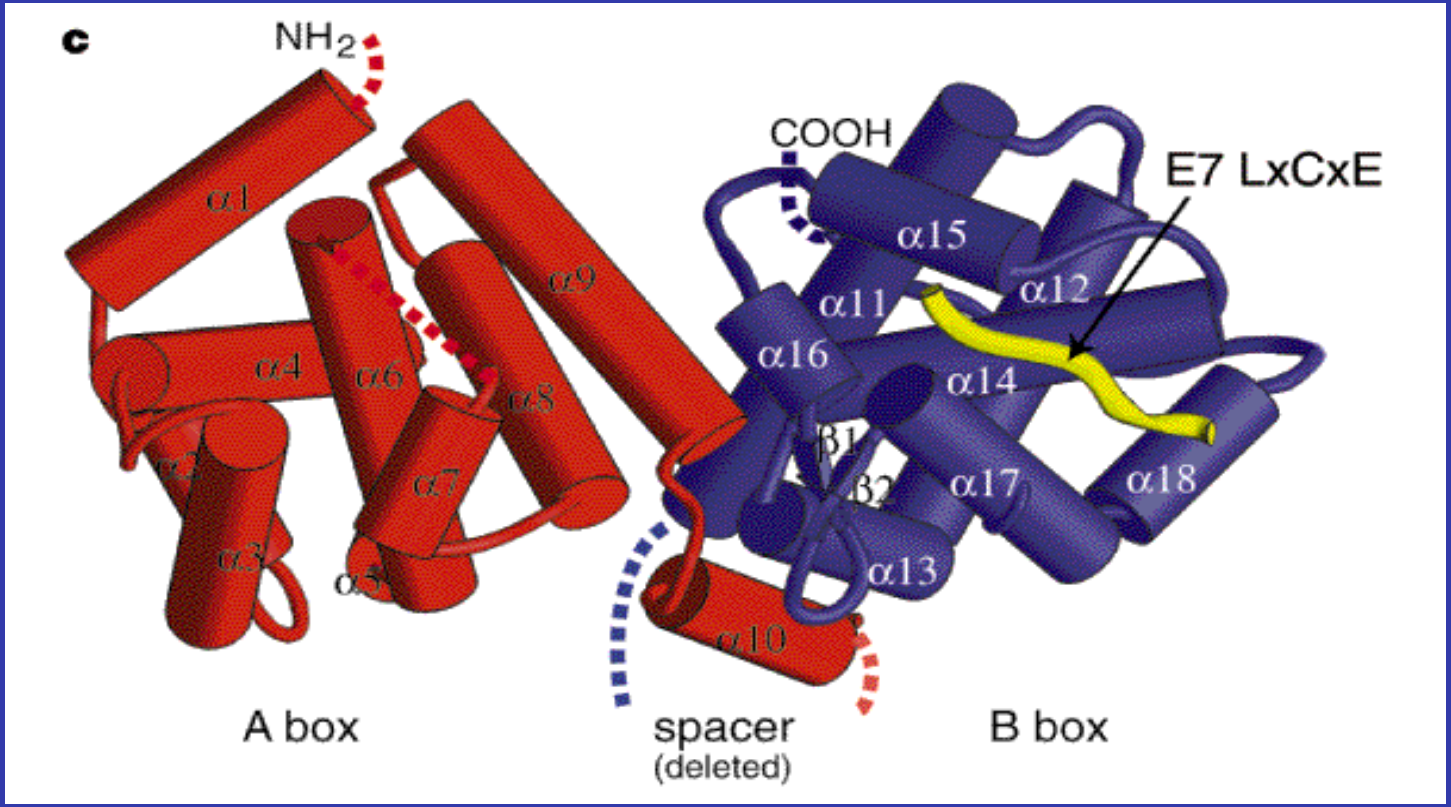
The retinoblastoma family of repressors



Rb can regulate E2F-dependent transcription by two distinct mechanisms. **(a)** Rb binds to the transactivation domain of E2F family members and blocks their ability to activate transcription. **(b)** Rb can interact with chromatin remodeling enzymes such as HDACs and SWI/SNF, and interaction of Rb with E2F allows these chromatin remodeling enzymes to be targeted to promoters where they can promote nucleosome assembly. This second mechanism of repression is the focus of this review.

Rb family = pocket proteins (Rb, p107, p130)

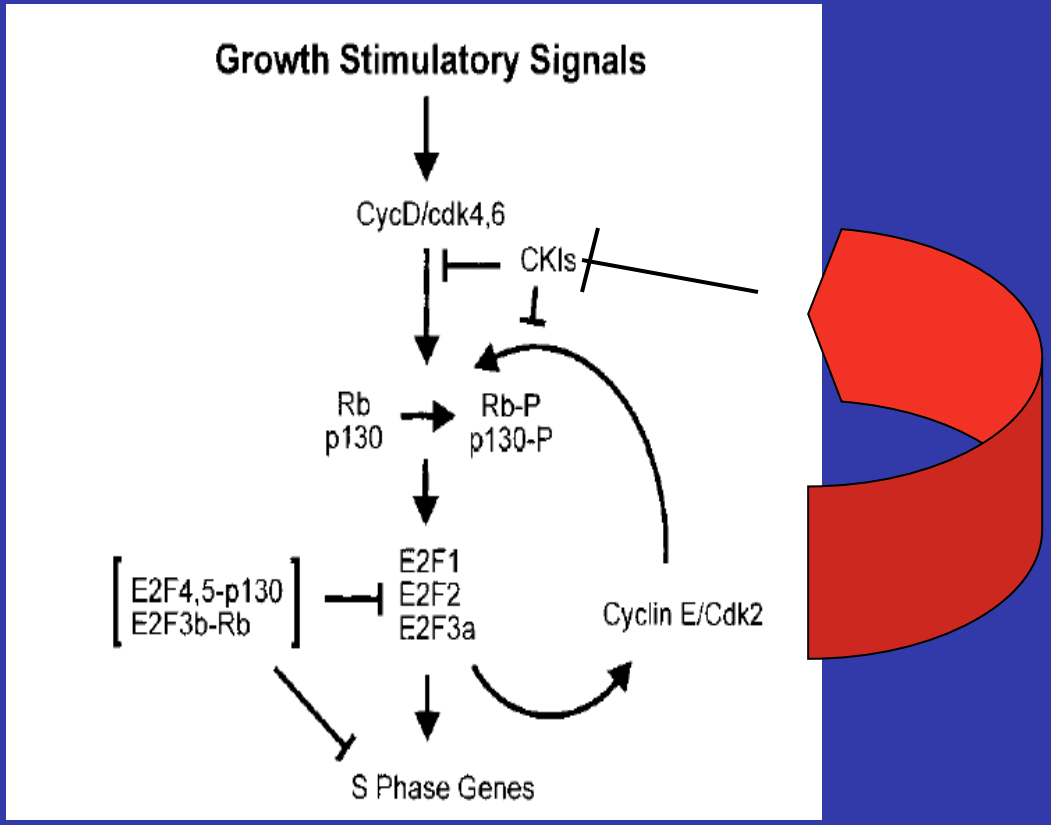
The "pocket" and the LxCxE motif



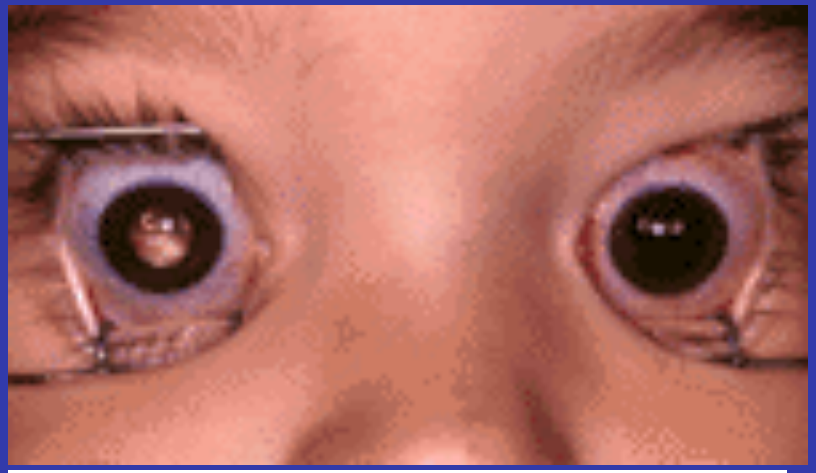
The Rb-E2F pathway

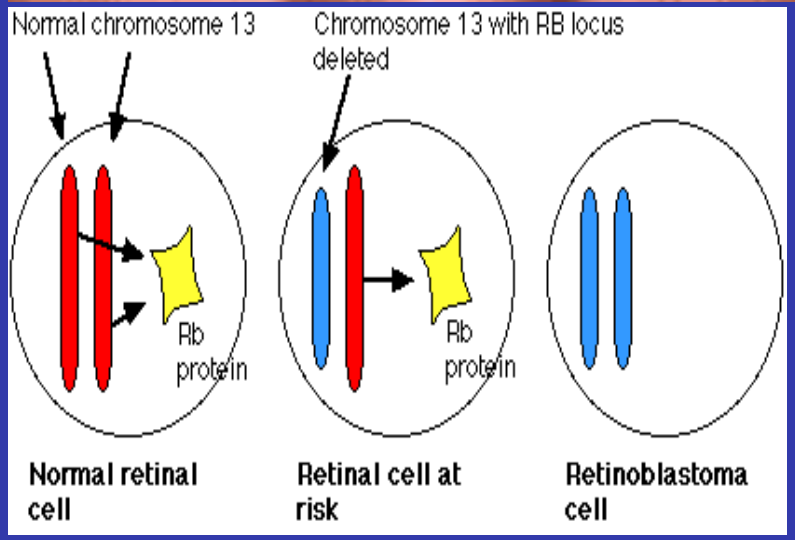
Positive feedbacks

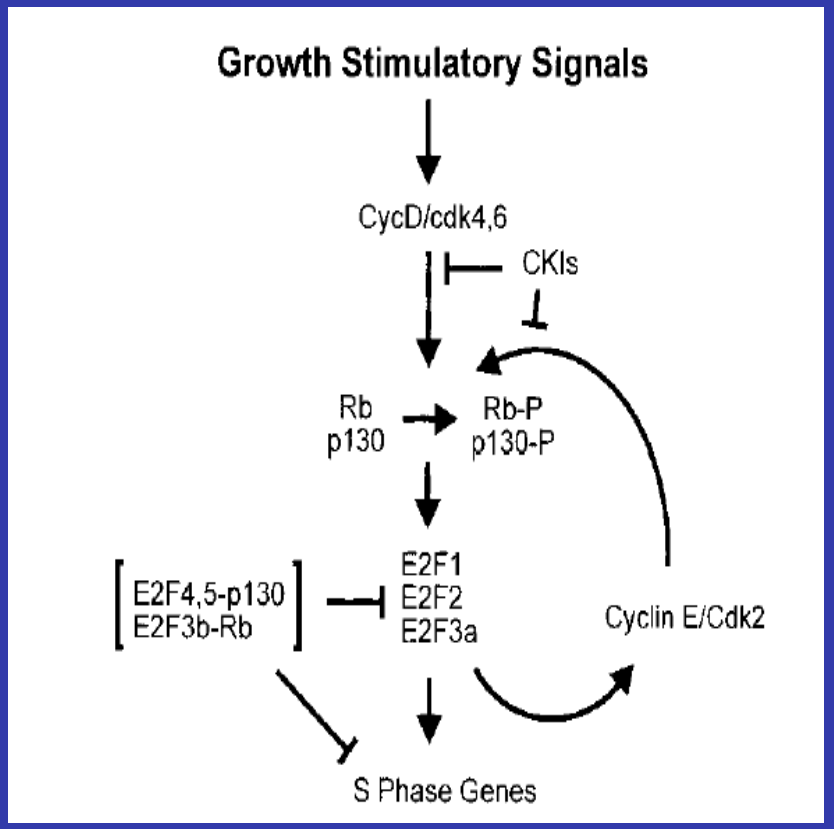
1. Induction of cyclin E
2. Induction of E2F
3. Induction of cyclin A
4. Phosphorylation of CKI and Cdh1 par CDK-cyclines complexes



Tumor suppress genes: retinoblastoma

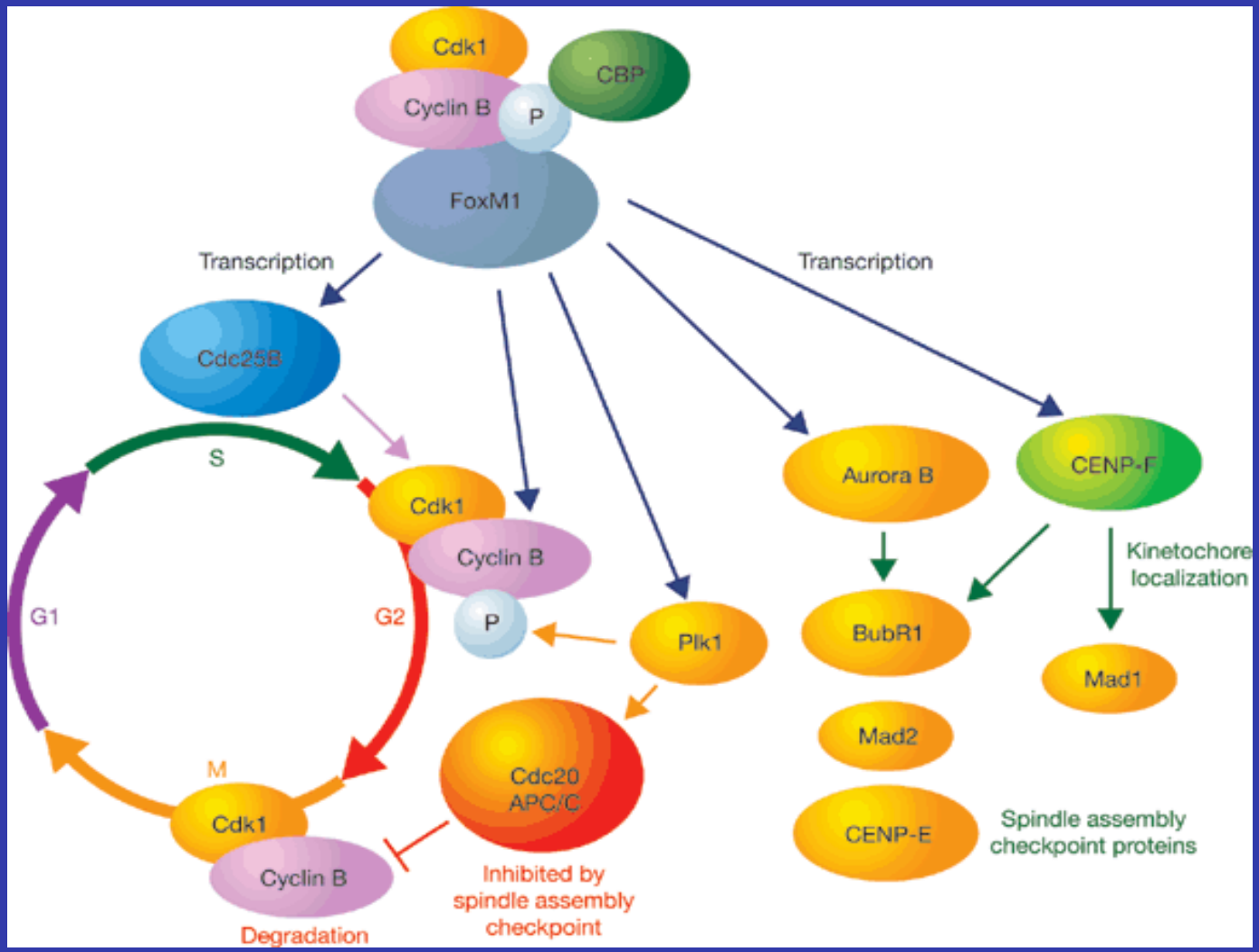




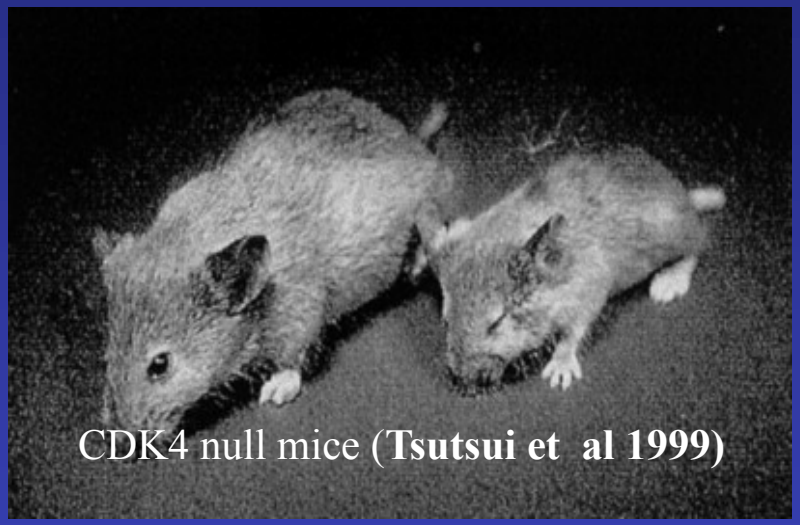


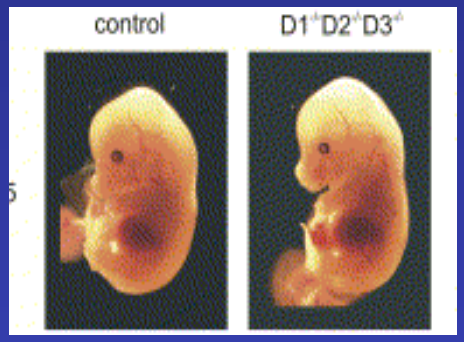
All cancers have a deregulation of the RB-E2F pathway

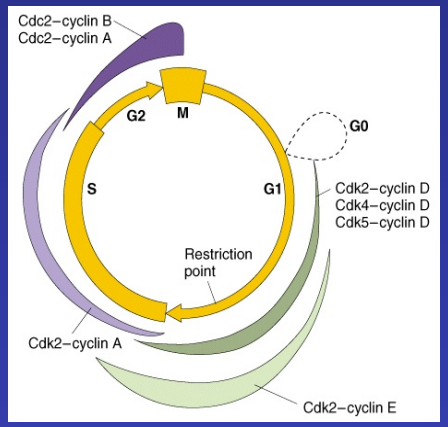
FoxM1 the transcription factor of G2/M



Cyclin D, Cdk2, Cdk4 and Cdk6 knockout mice: viables!


CDK4 null mice (**Tsutsui et al 1999**)



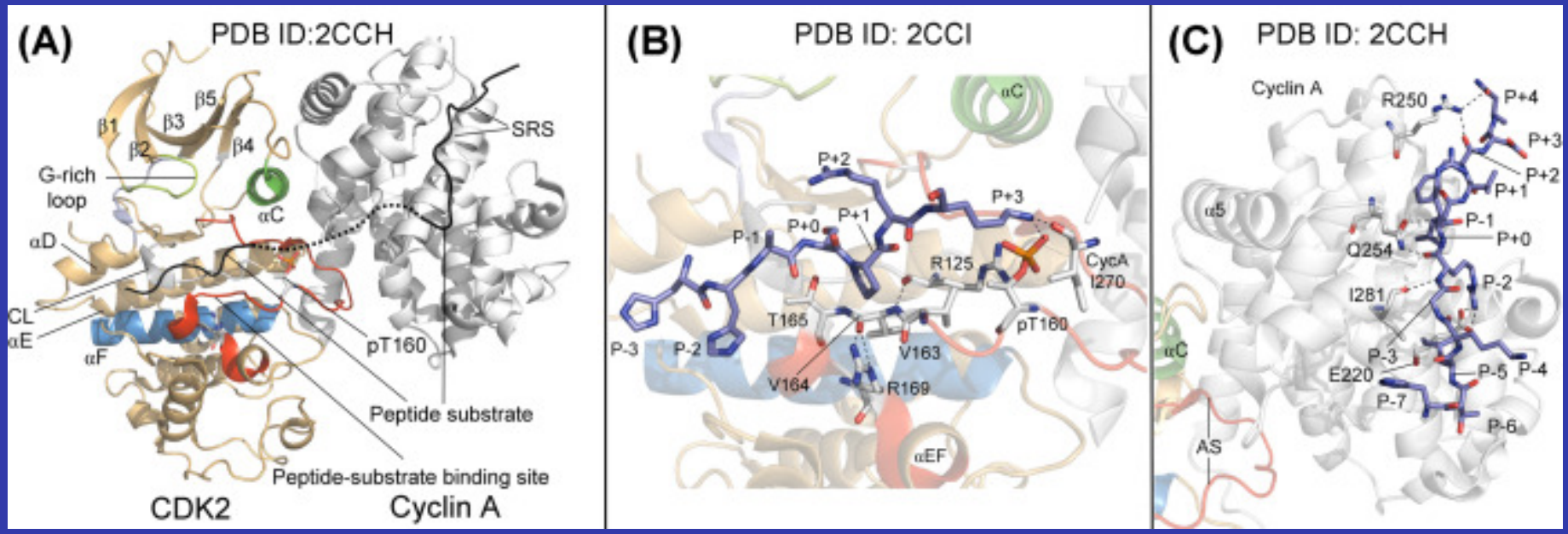


Cyclin D, E, A or B amplification and overexpression

Loss of CKI (p16INK4a, p21, p27 or p57)

CDK1, 2, 4, 6 overexpression, CDK4 amplification

CDK-cyclin substrate recognition

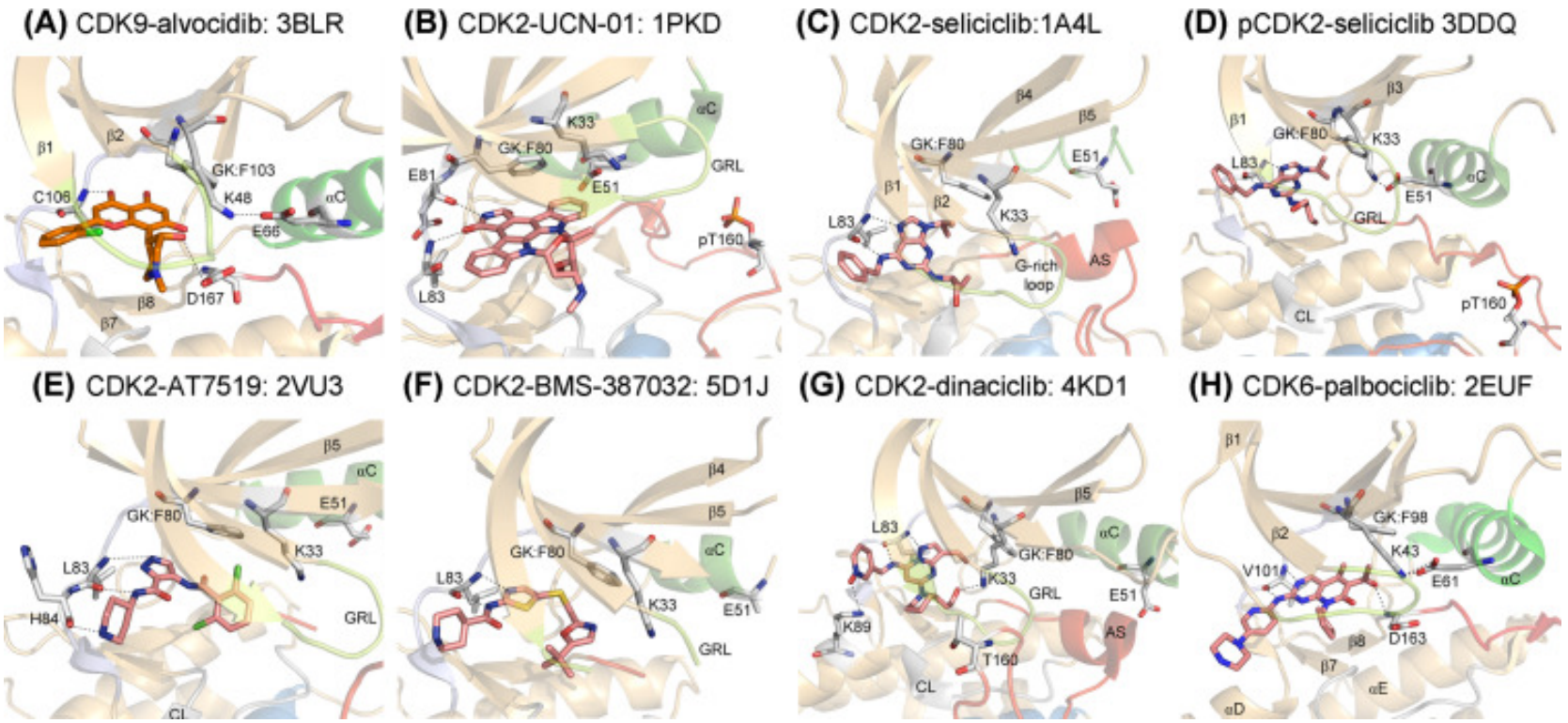


Overview of pCDK2-cyclin A interacting with a peptide substrate (black) at both the enzyme peptide substrate binding site and the cyclin substrate recruitment site (SRS).

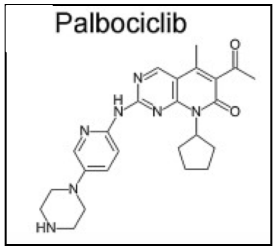
Cyclin-dependent protein kinase inhibitors including palbociclib as anticancer drugs

Robert Roskoski Jr. Pharmacological Research, 2016, Available online 16 March 2016

Selected CDK inhibitors in clinical trials.



Palbociclib (Ibrance): CDK4/6 inhibitor (IC50 11nM)
Effective against breast cancer
Clinical trials for many other cancers



Robert Roskoski Jr. Pharmacological Research, 2016, Available online 16 March 2016

Quiz#4 cell cycle

1- What is the normal interaction between the Rb protein and the E2F transcription factor?

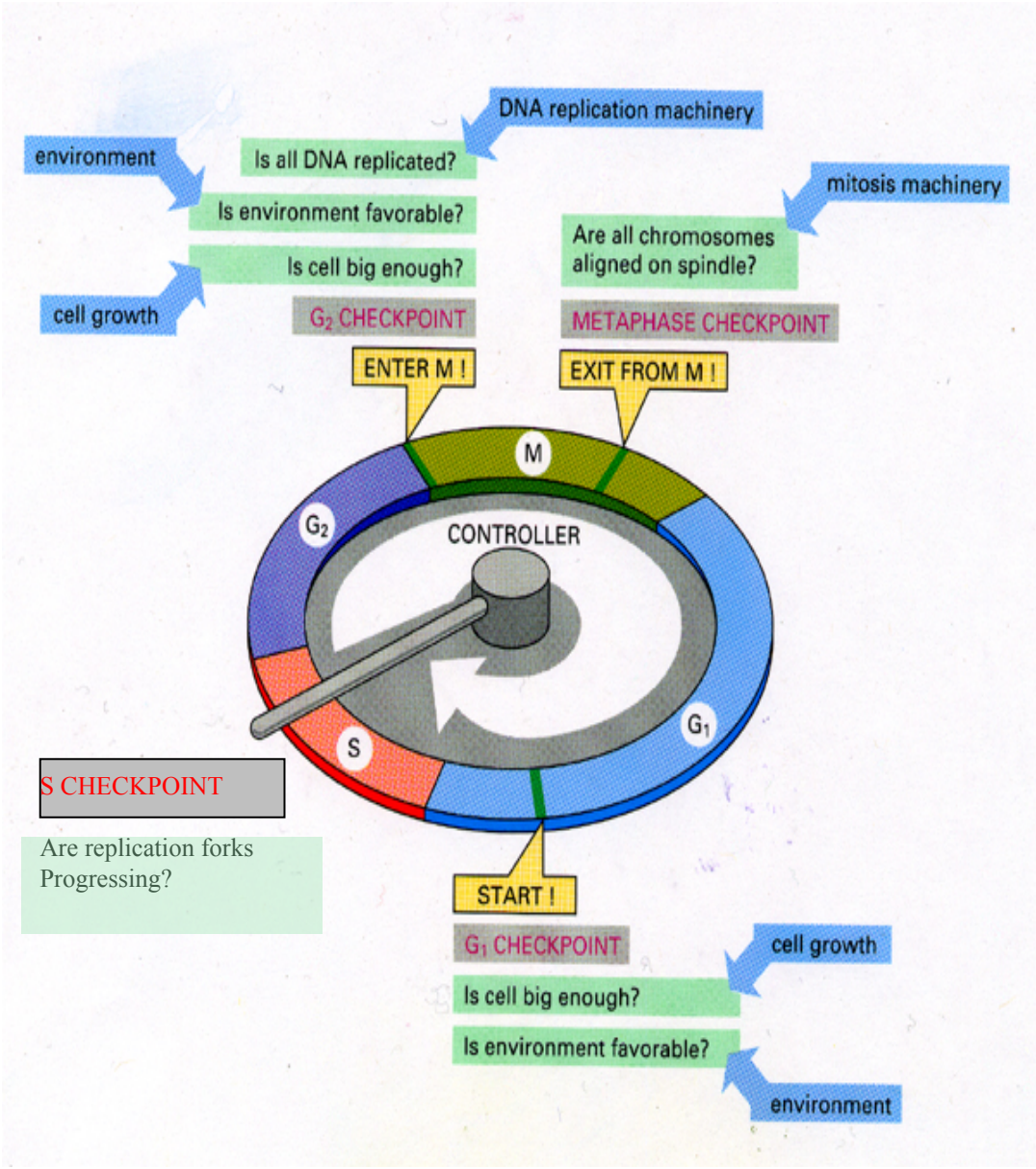
A) Rb inhibits E2F activity, effectively preventing cell division.
B) Rb enhances E2F activity, effectively preventing cell division.
C) Rb inhibits E2F activity, increasing the rate of cell division.
D) Rb enhances E2F activity, increasing the rate of cell division.

2- CDK4/6 targeted therapy works by

A- restoring E2F activity
B- inhibiting E2F activity
C- activating CDK-cyclins
D- inhibiting the retinoblastoma protein

« Checkpoints »

- Mechanisms that arrest cell cycle progression to ensure completion of current cell cycle events
- Allocate time for error repair



General checkpoint mechanism: the DNA damage checkpoint

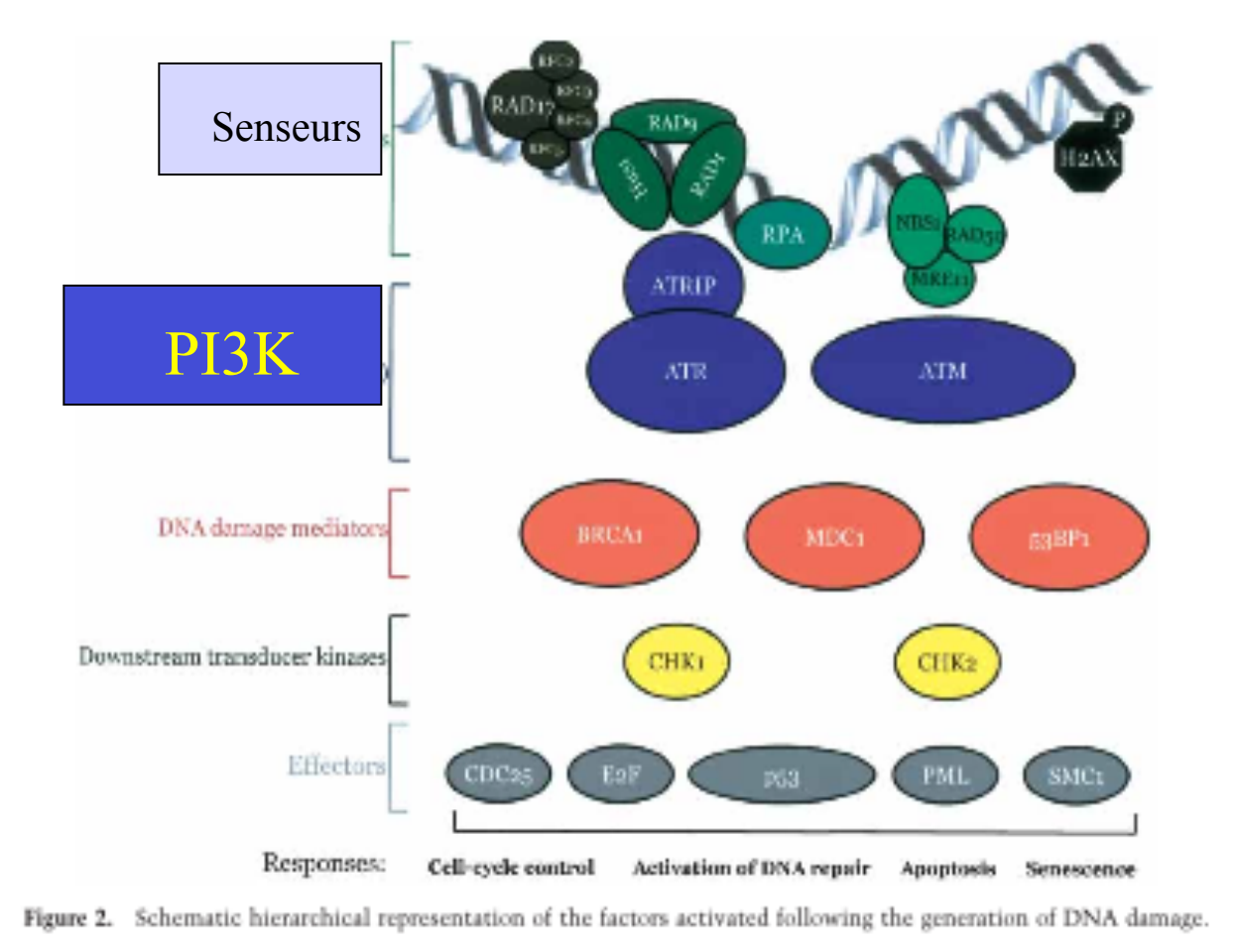
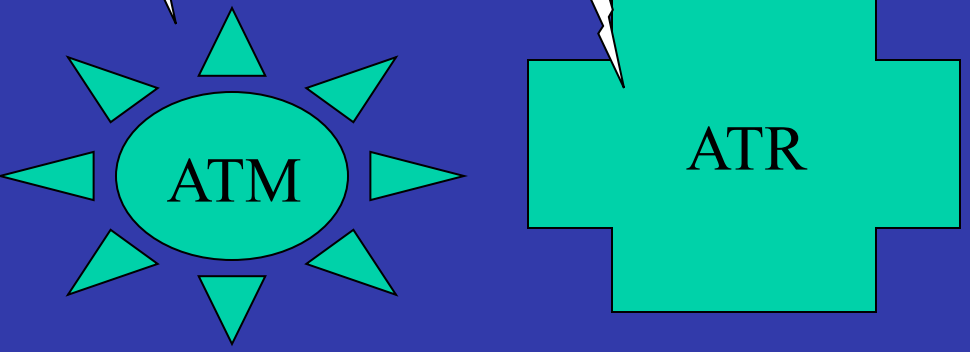


Figure 2. Schematic hierarchical representation of the factors activated following the generation of DNA damage.

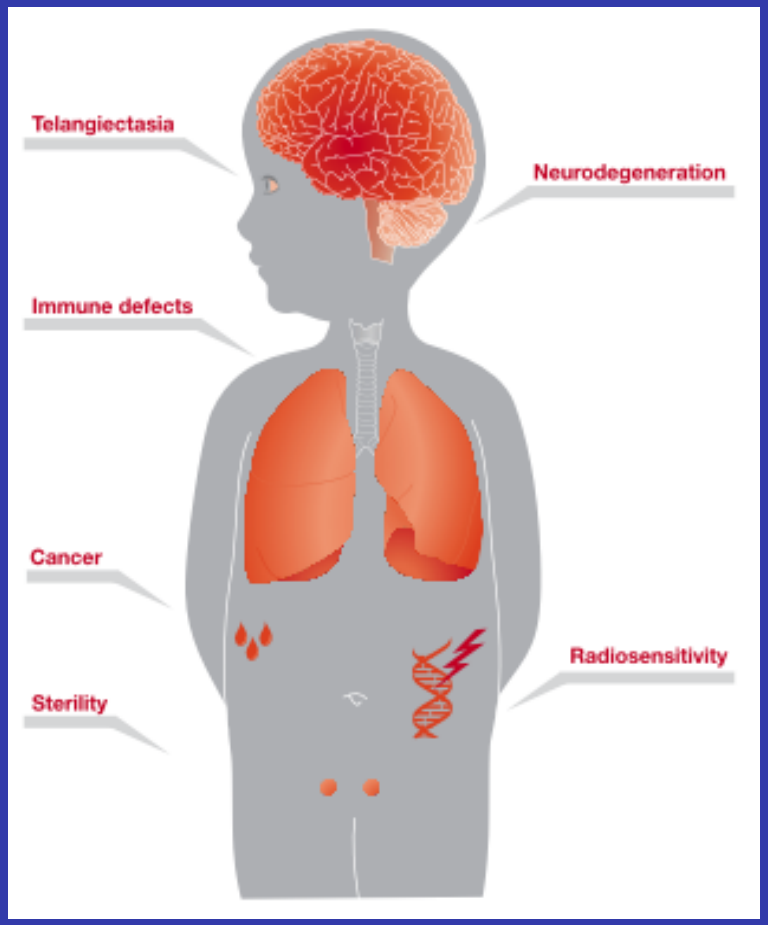
PI3K ATM and ATR

Radiation

UV light
Replication stress

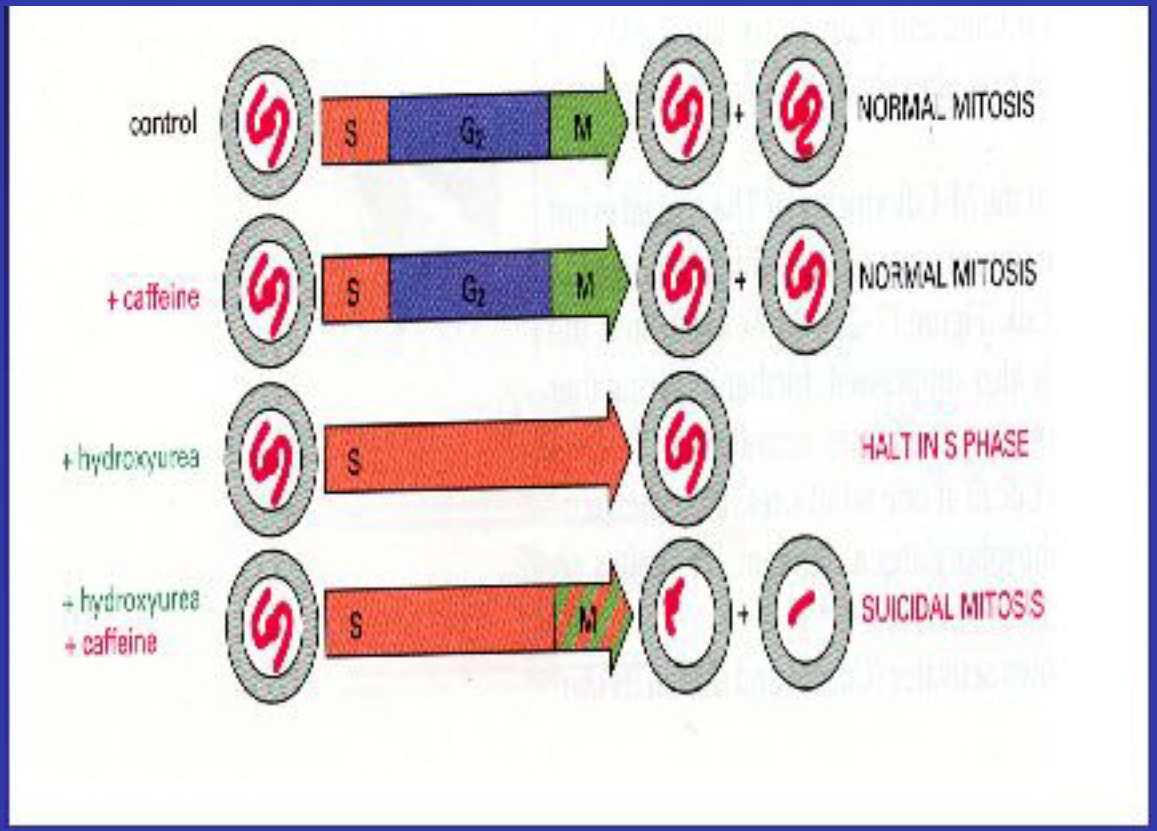


- Phosphorylation at Ser/Thr-Gln



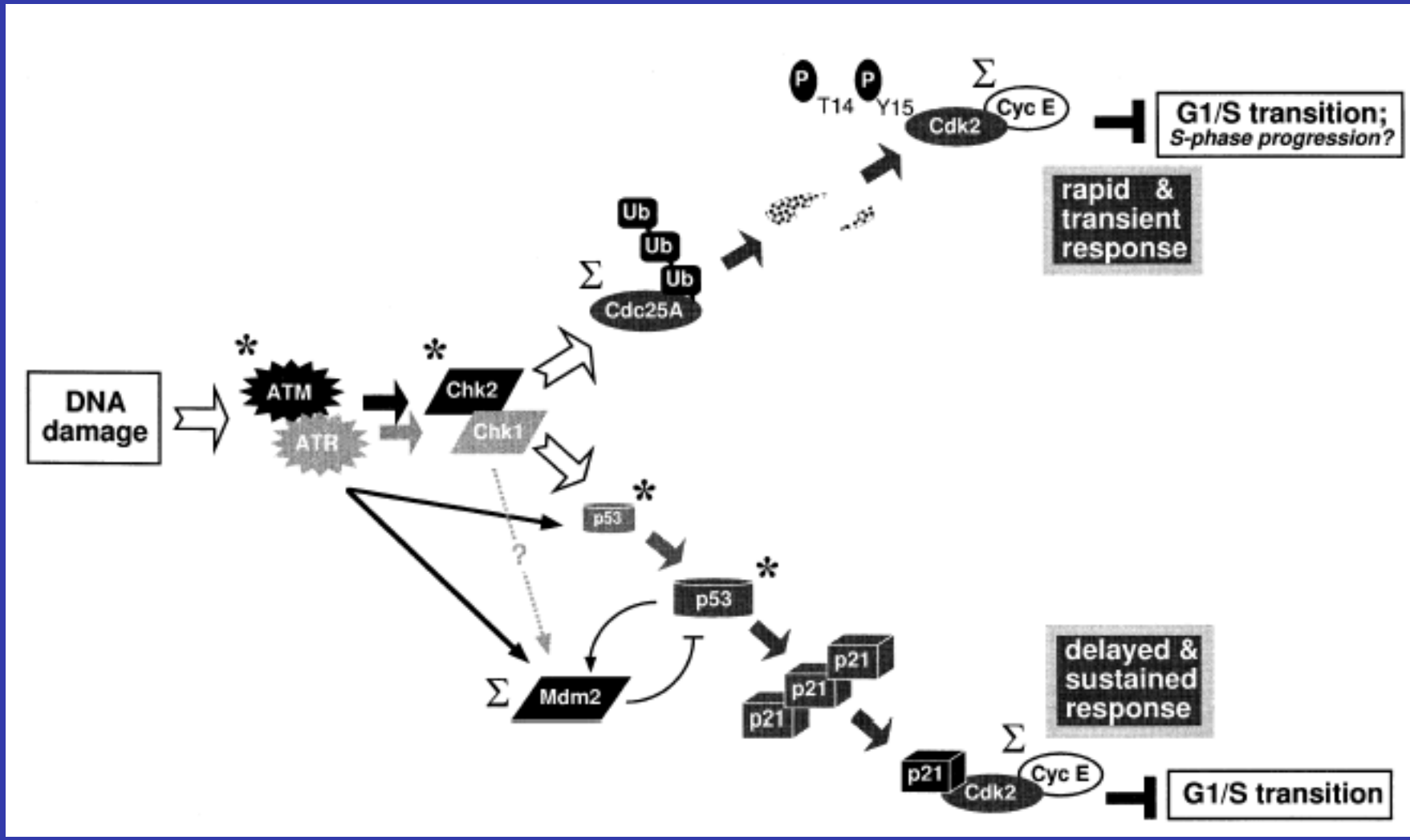
Ataxia teleangiectasia

Importance of checkpoints

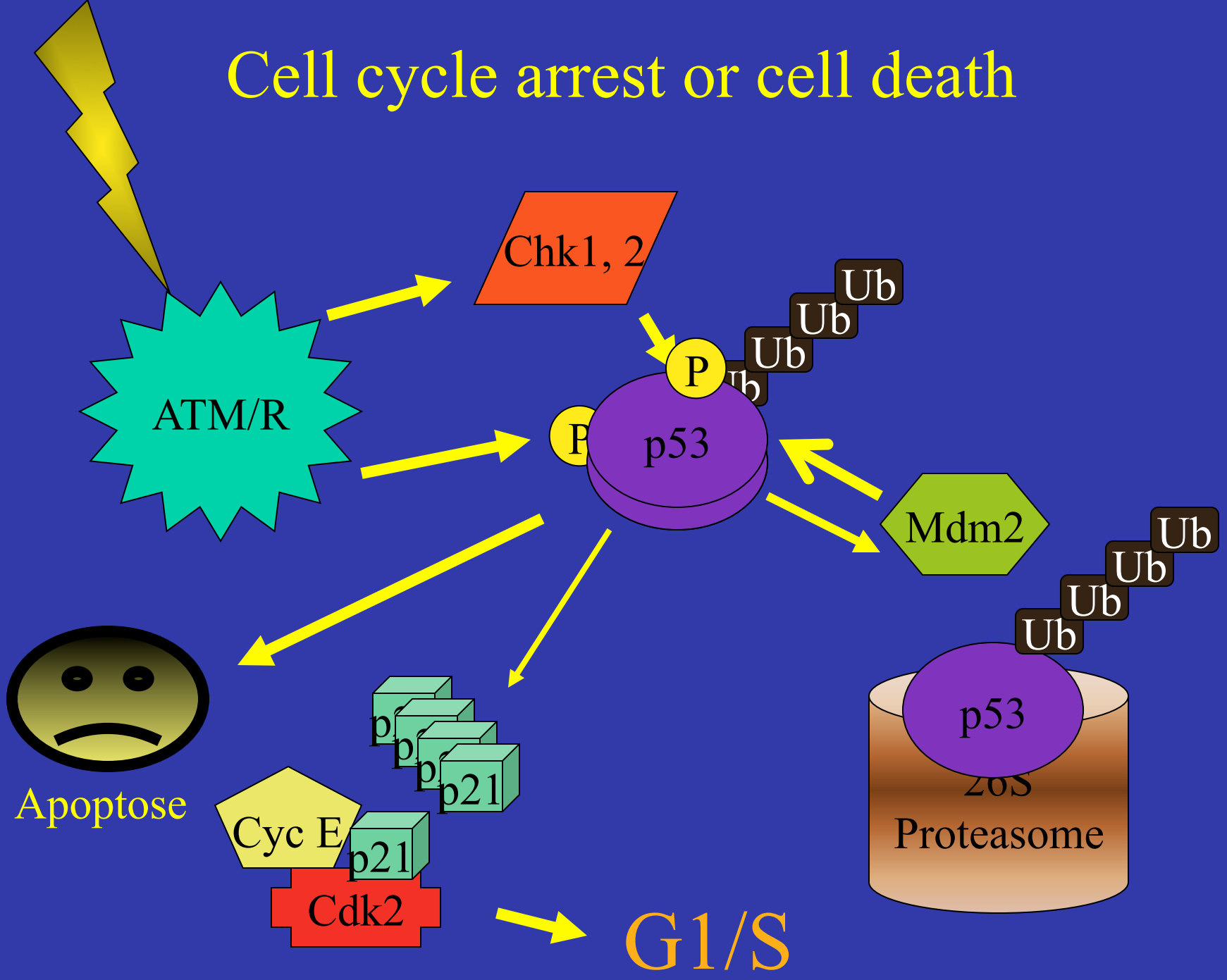


- Ensure that cell cycle events are correctly finished
- Mutations in checkpoint proteins lead to cell death or accumulation of mutations

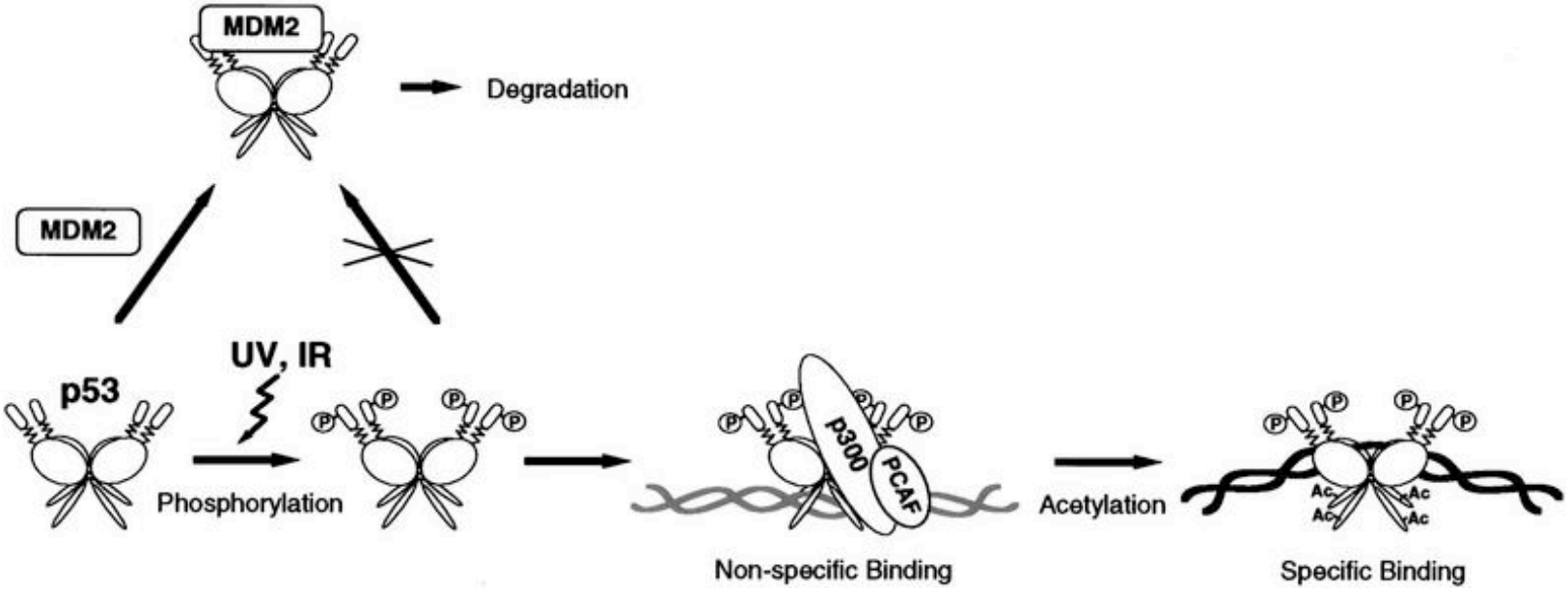
G1 DNA damage checkpoint: p53



Cell cycle arrest or cell death



Phosphorylation-acetylation signaling cascade



Genes Dev. 1998 Sep 15;12(18):2831-41.
DNA damage activates p53 through a phosphorylation-acetylation cascade.
Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, Vassilev A, Anderson CW, Appella E.

Fully acetylated the C-terminus completely loses DNA binding capacity

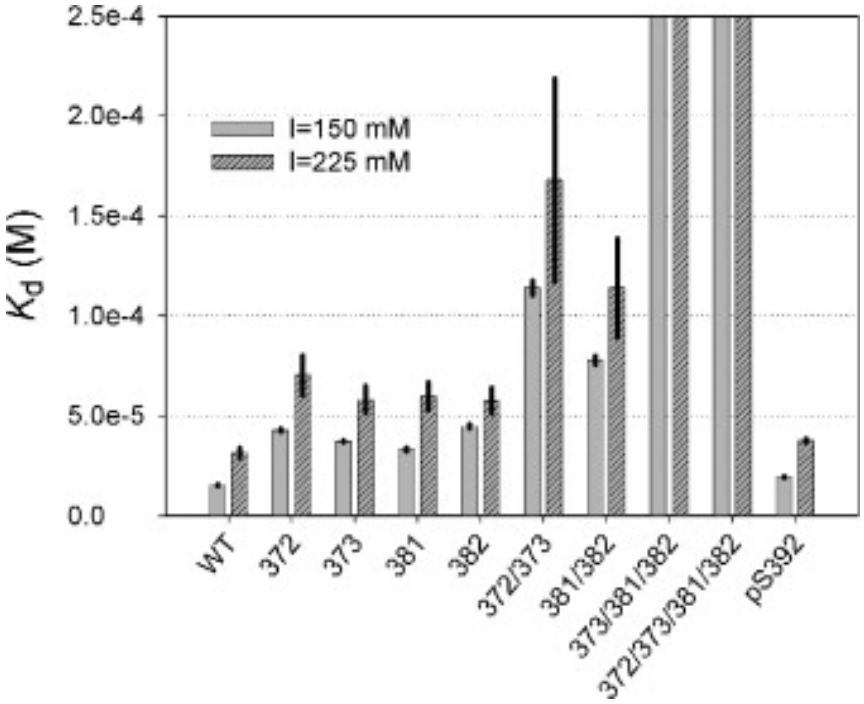


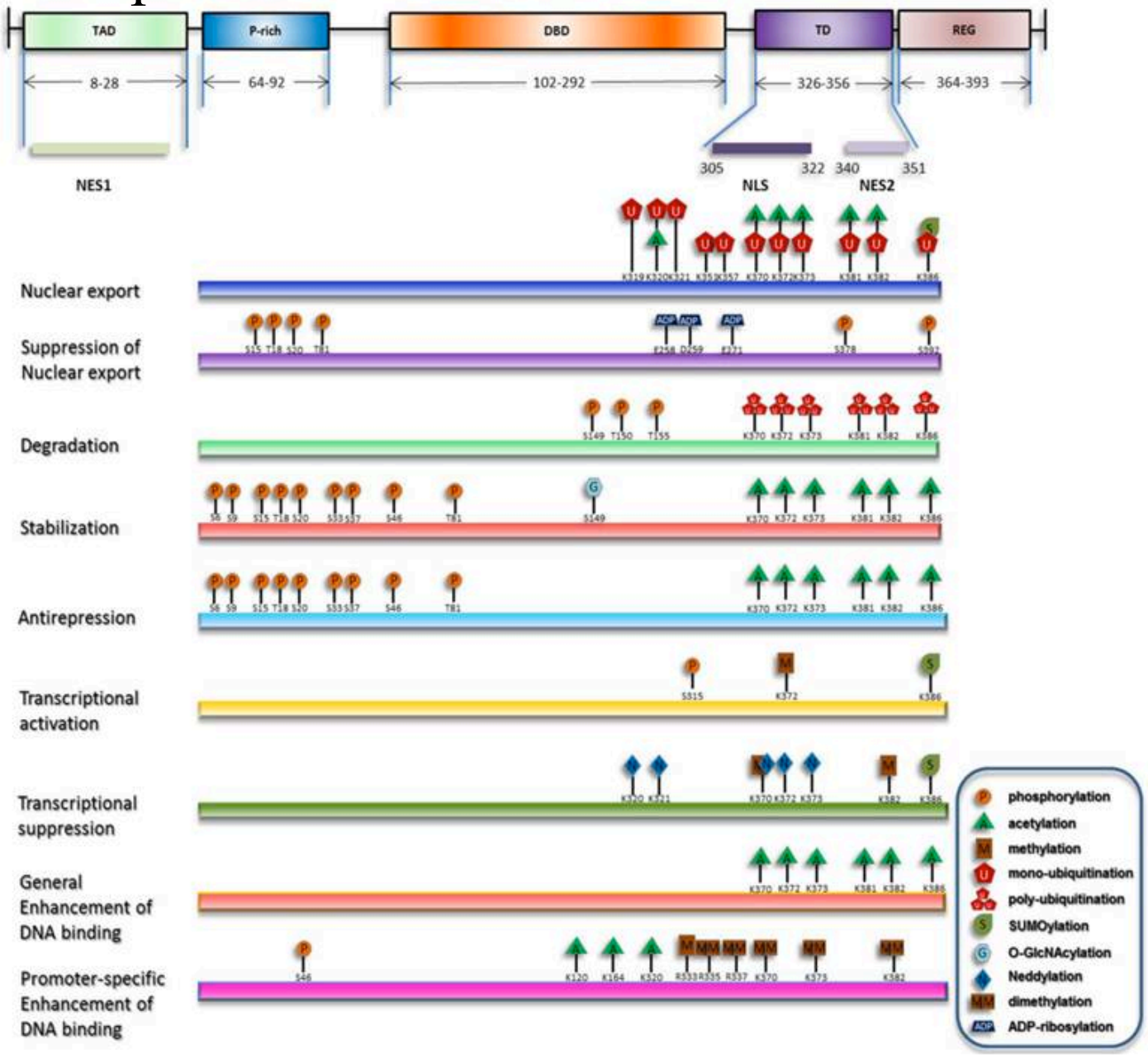
Figure 3. Binding of Sheared Herring Sperm DNA to Fluorescein-Labeled p53 C-Terminal Peptides, at Ionic Strengths of 150 mM and 225 mMThe dissociation constants were determined using AUC. The peptides with three and four acetylations (K372/373/381Ac and K372/3...

Assaf Friedler, Dmitry B. Veprintsev, Stefan M.V. Freund, Karoly I. von Glos, Alan R. Fersht

Modulation of Binding of DNA to the C-Terminal Domain of p53 by Acetylation

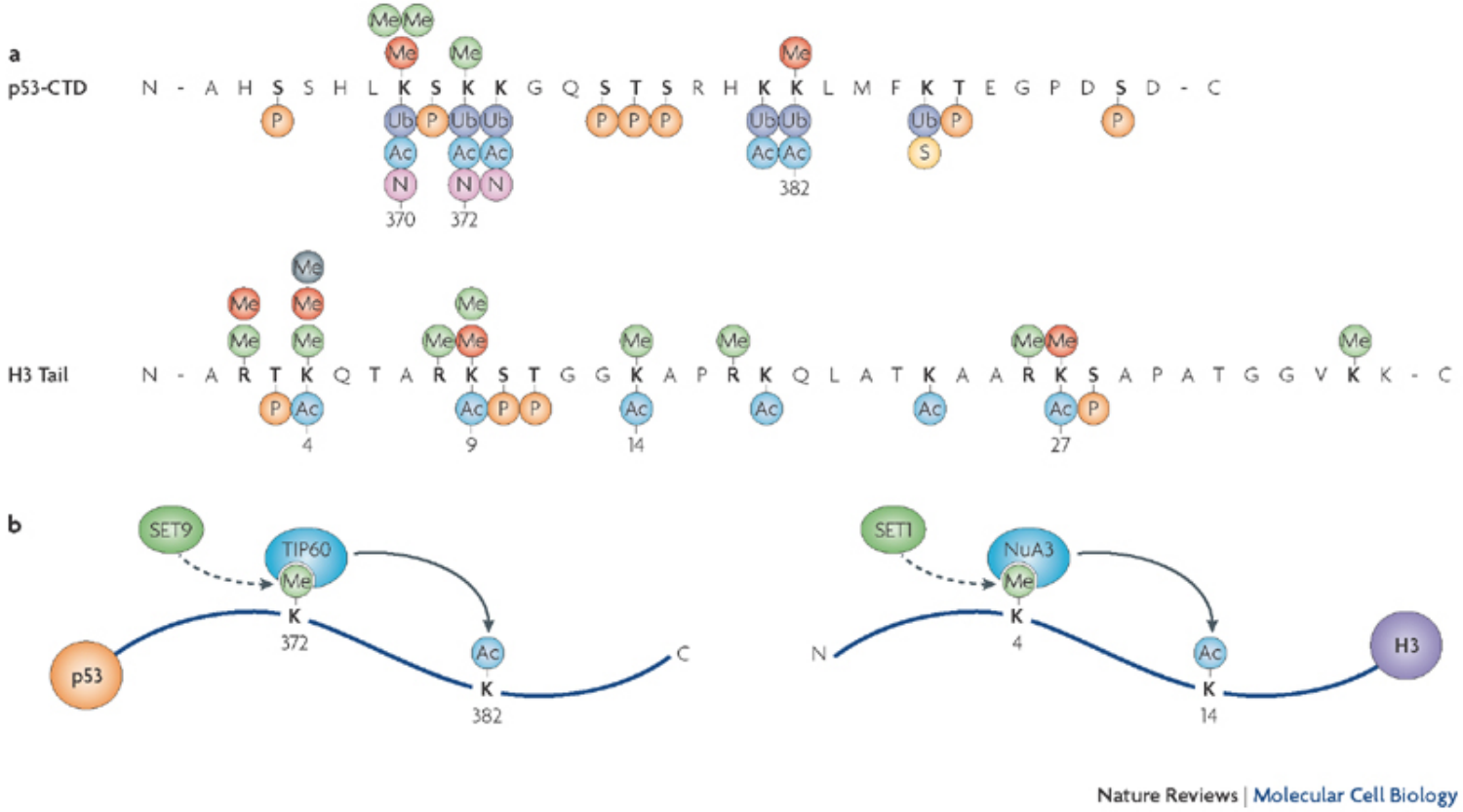
null, Volume 13, Issue 4, 2005, 629–636

p53: a postranslational modification network



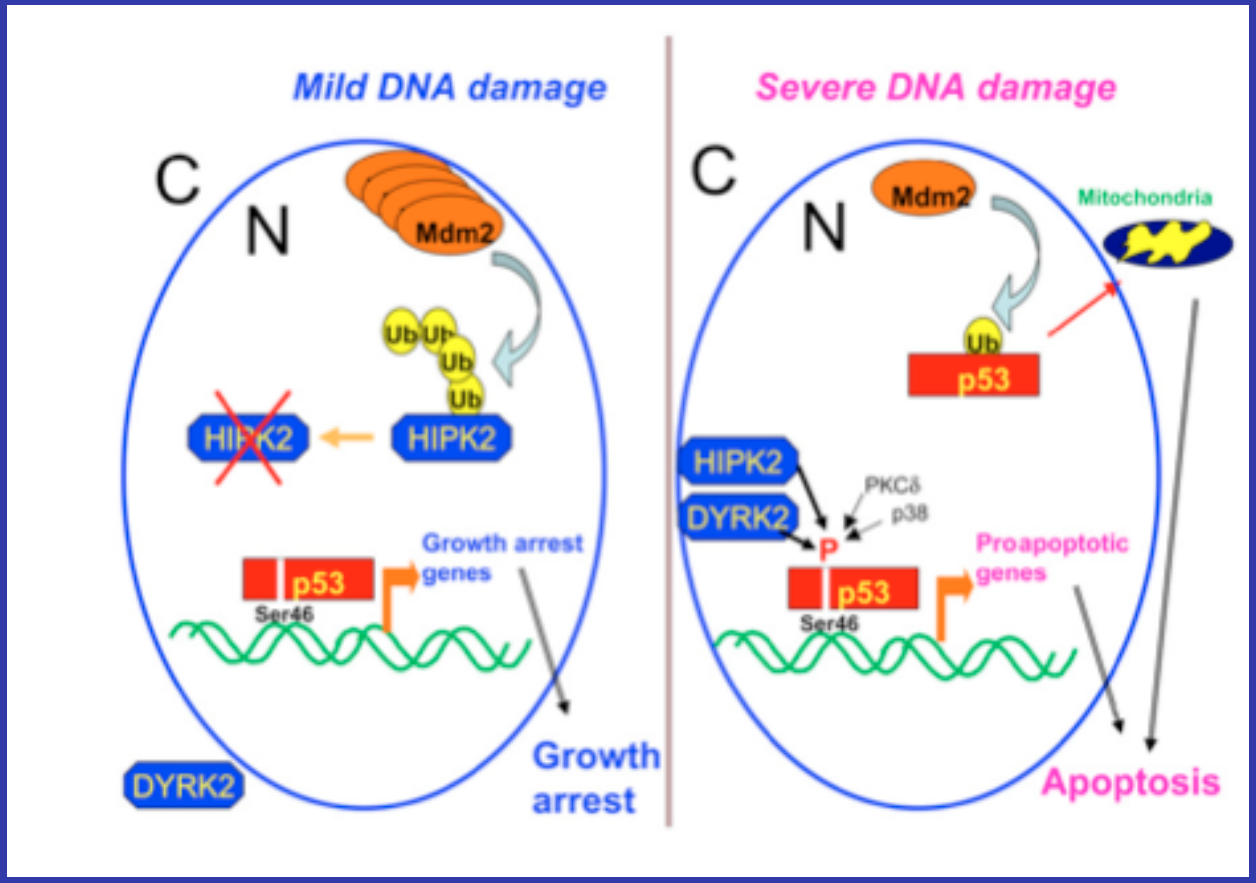
Gu Int J Biol Sci 2012; 8(5):672-684. doi:10.7150/ijbs.4283

The functional interplay between methylation and acetylation that occurs in p53 and histones.



Nature Reviews Molecular Cell Biology 9, 815-820 (October 2008)

Serine 46 phosphorylation and apoptosis

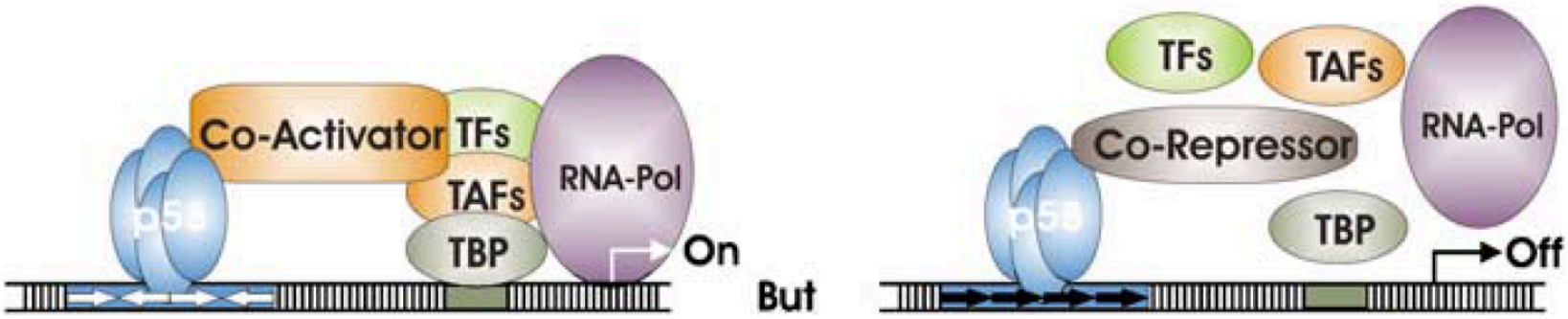


DNA binding by p53

- The consensus p53 DNA RE consists of two pairs (half-sites) of head-to-head arranged pentamers,

  5'-PuPuPuCA/TA/TGPyPyPy-3'

  (Pu is purine, Py is pyrimidine)
- Half sites are separated by 0–13 nucleotides

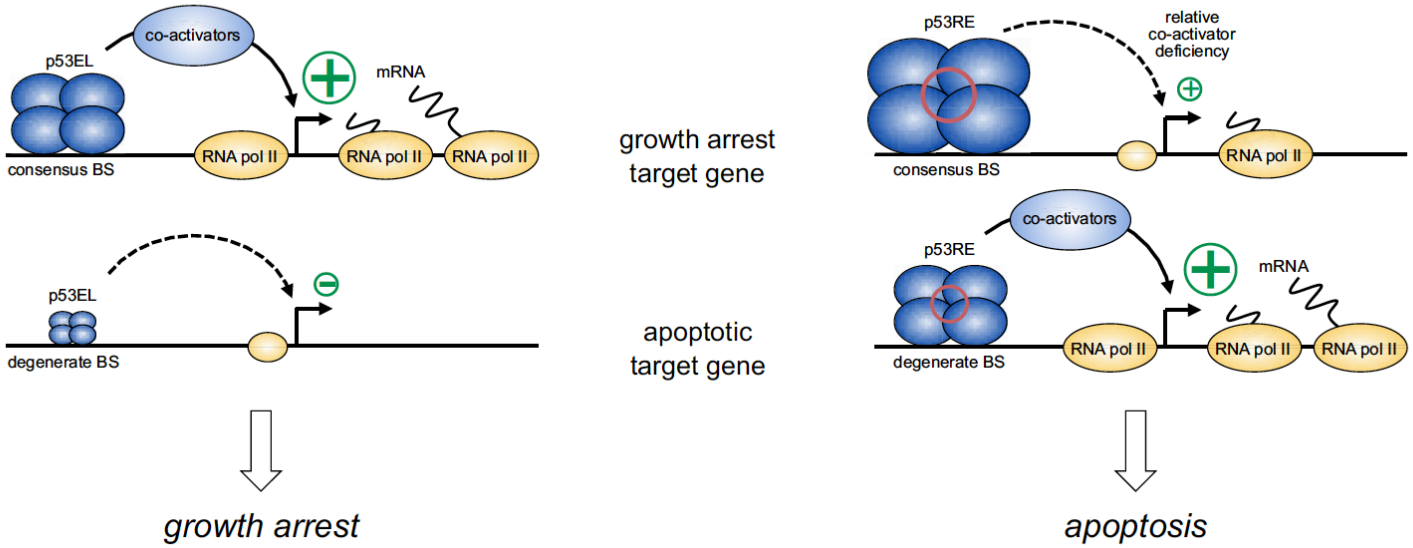


Cell Death and Differentiation (2006) 13, 951–961

Molecular Cell 38, 356–368, May 14, 2010

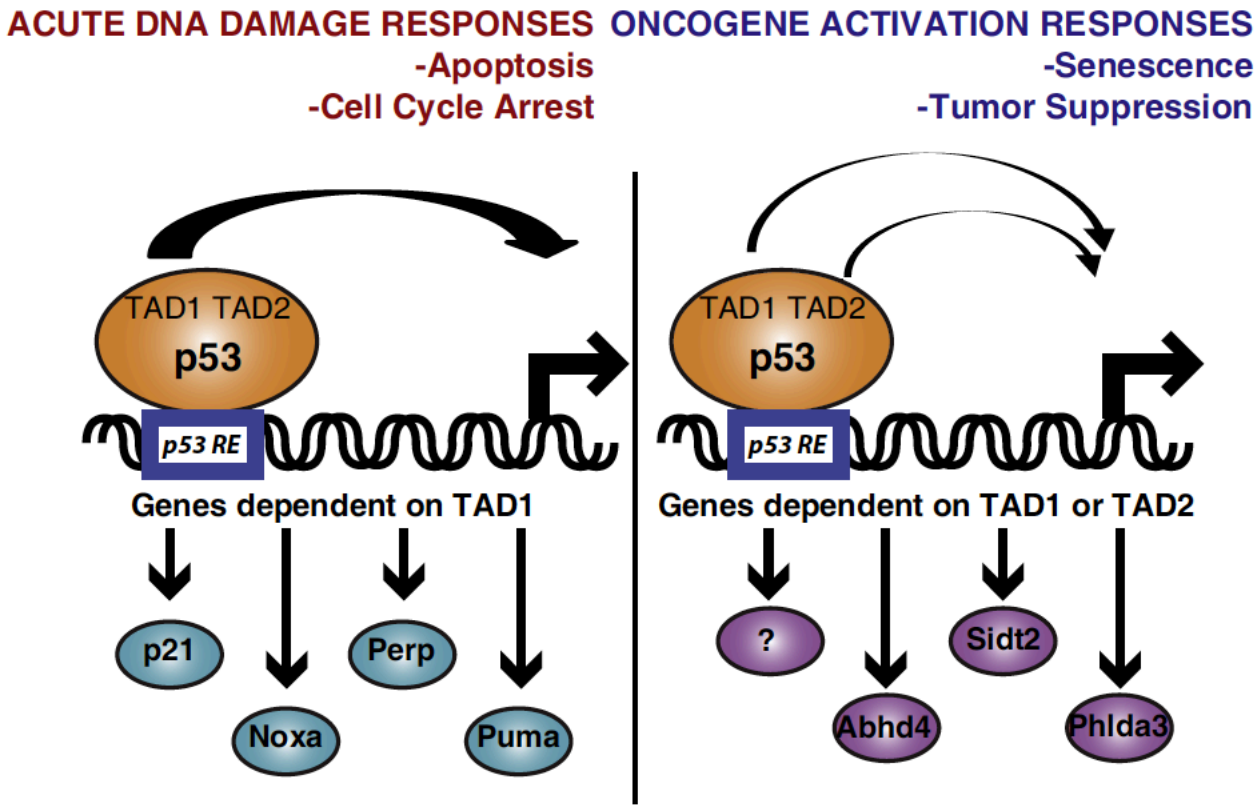
DNA binding cooperativity

low cooperativity (e.g. EL mutant)		*high cooperativity* (e.g. RE mutant)
low	DNA complex stability *in vitro*	high
small	target genes spectrum *in vivo*	large
high	binding sequence specificity	low
growth arrest target > apoptotic target	transactivation	apoptotic target > growth arrest target
growth arrest > apoptosis	cellular outcome	apoptosis > growth arrest



DNA binding cooperativity: Hill coefficient= 1.8, J. Mol. Biol. 341, 1145–1159.
PTM may change DNA binding cooperativity
High cooperativity implies low specificity and less coactivator available

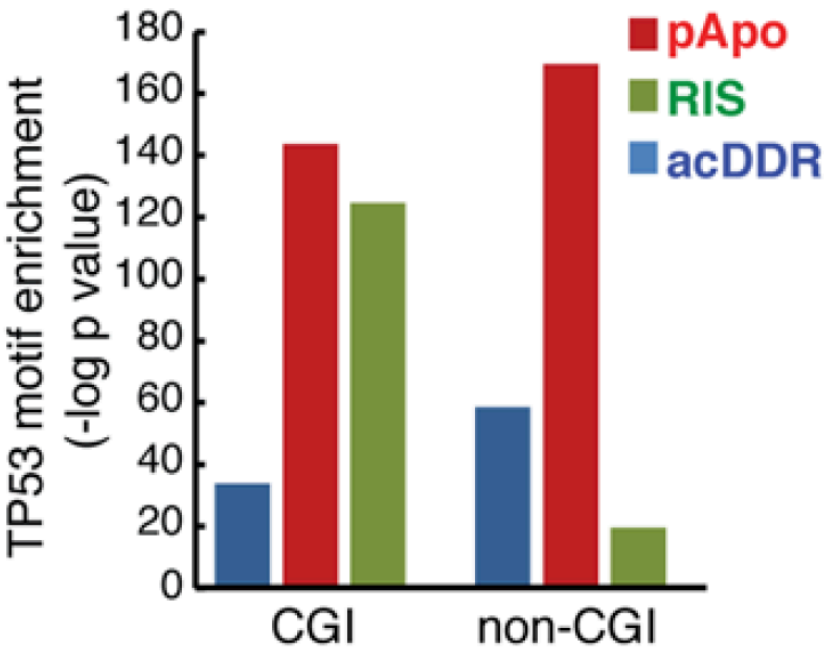
Distinct p53 Transcriptional Programs Dictate Acute DNA-Damage Responses and Tumor Suppression



Acute vs. chronic DNA damage: perhaps not the same

Attardi lab: Cell 145, 571–583, May 13, 2011

Chronically Activated p53



- Generally, CGIs are ‘ open’ , enriched for the binding sites of many TFs, including Sp1.
- CGI-promoters contain multiple ‘ weak’ p53REs (including many half-sites), which somehow favor persistent accumulation of p53

‘Chronic’ p53 preferably associates with CpG islands and regulates a different set of genes in comparison with ‘Acute p53’,
RIS= Ras-induced senescence, pApo= Oncogene induced apoptosis,
acDDR= acute DNA damage

Narita’s lab PLOS Genetics | DOI:10.1371/journal.pgen.1005053

Quiz #5 cell cycle

1- Which statement about p53 is false

A. Its half life is very short: 6-20 min.
B. Levels are mainly regulated by proteolytic turnover.
C. Most cancer have normal p53 gene
D. P21, a CKI, is a p53 target gene that mediates cell cycle arrest
E. DNA binding cooperativity modulates p53 target gene selection

2- Which of the following genes are activated by p53

A- p21 and BAX
B- GADD45 and BAX
C- BAX, APAF1 and MDM2
D- All of above

3- Propose a molecular model for the specificity of the chronic p53 response