MOLECULAR BASIS OF ONCOGENESIS

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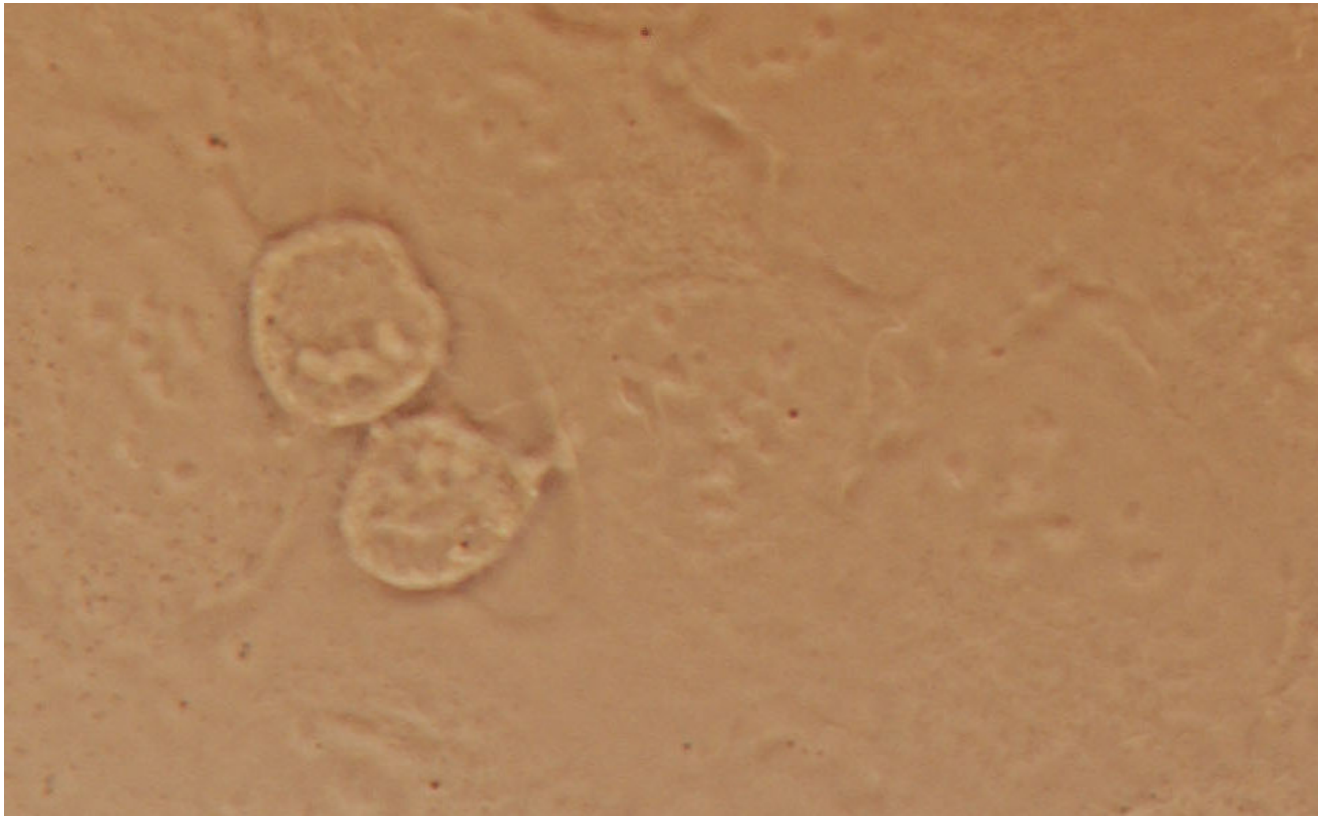
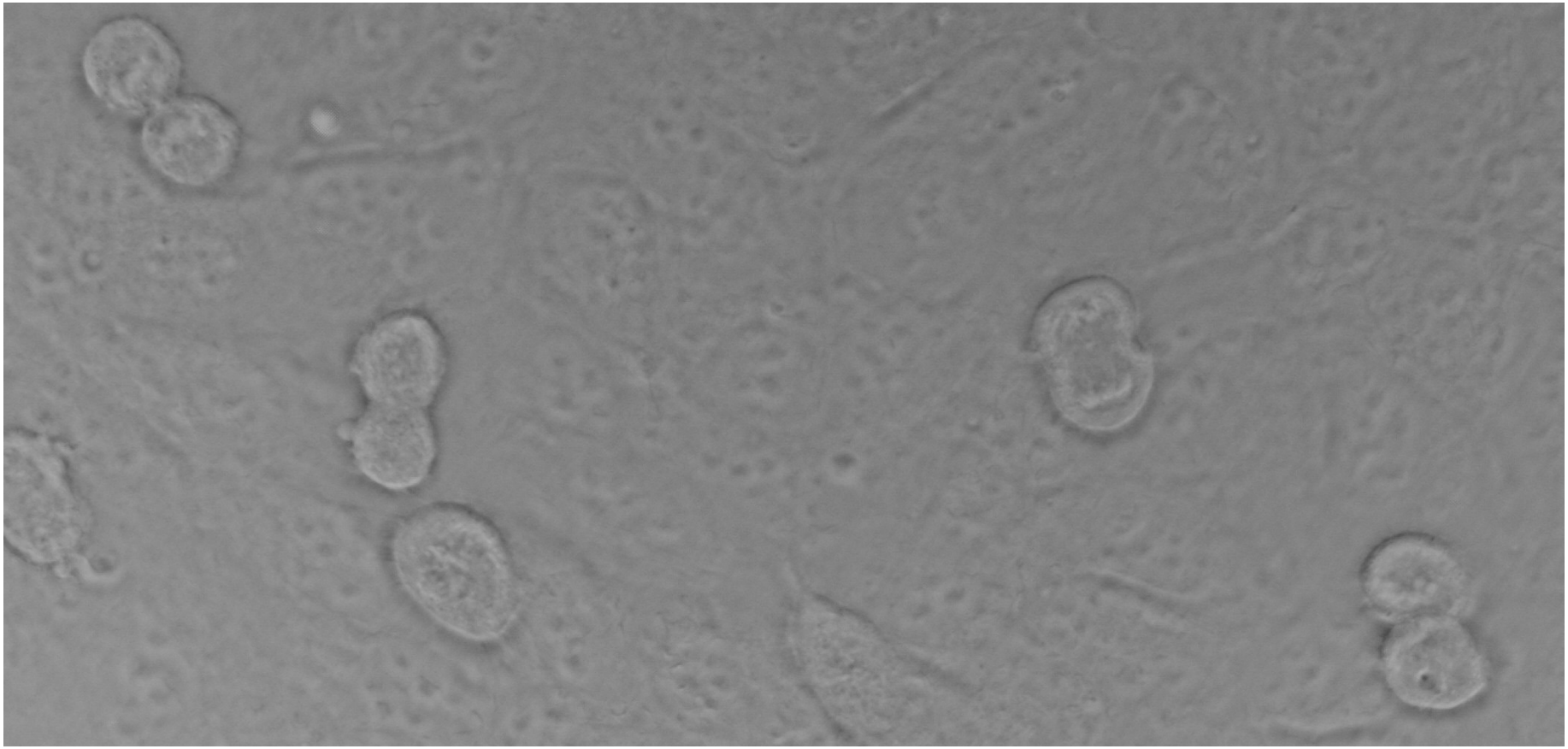
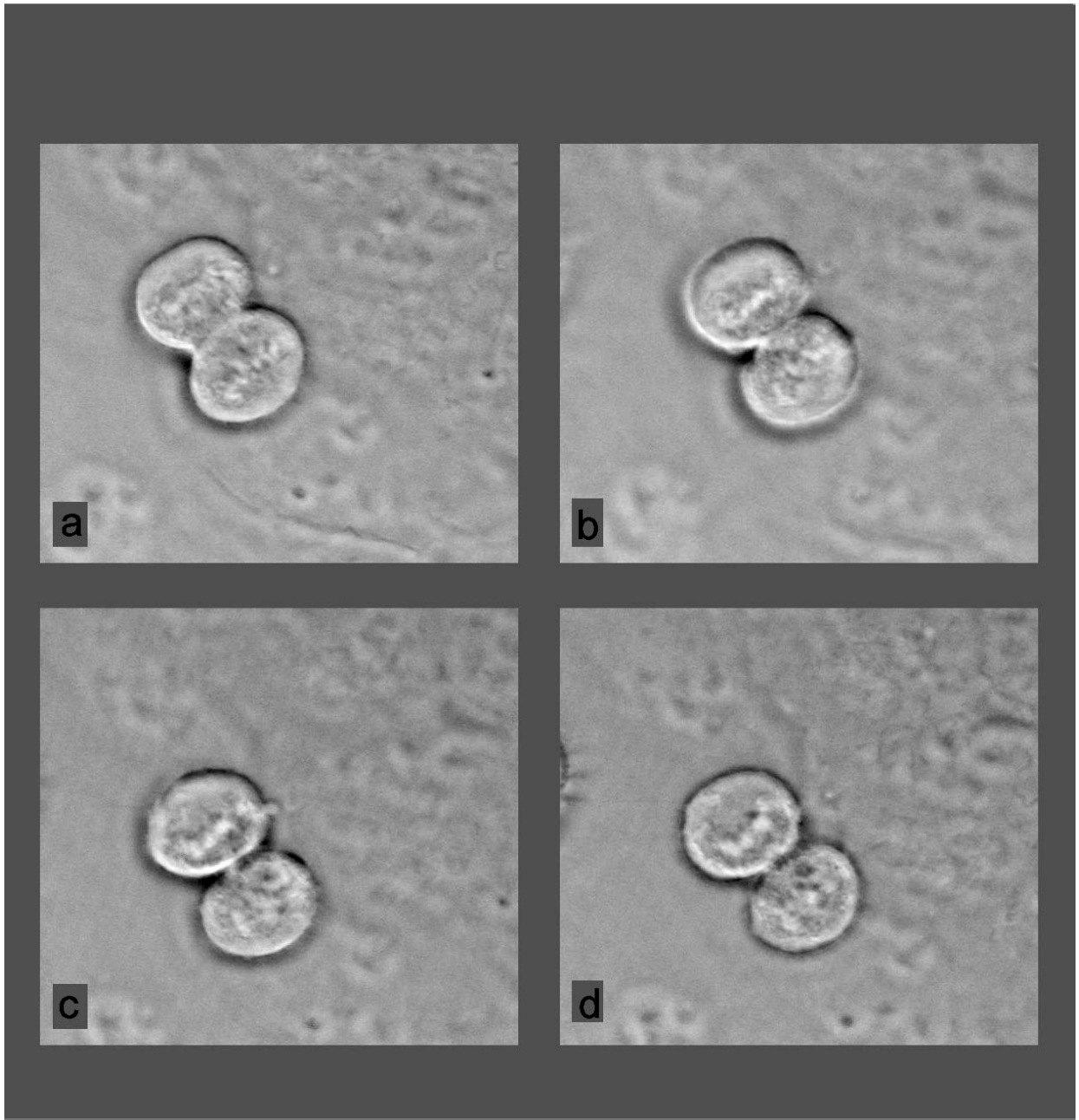






Final step of cell division - cytokinesis

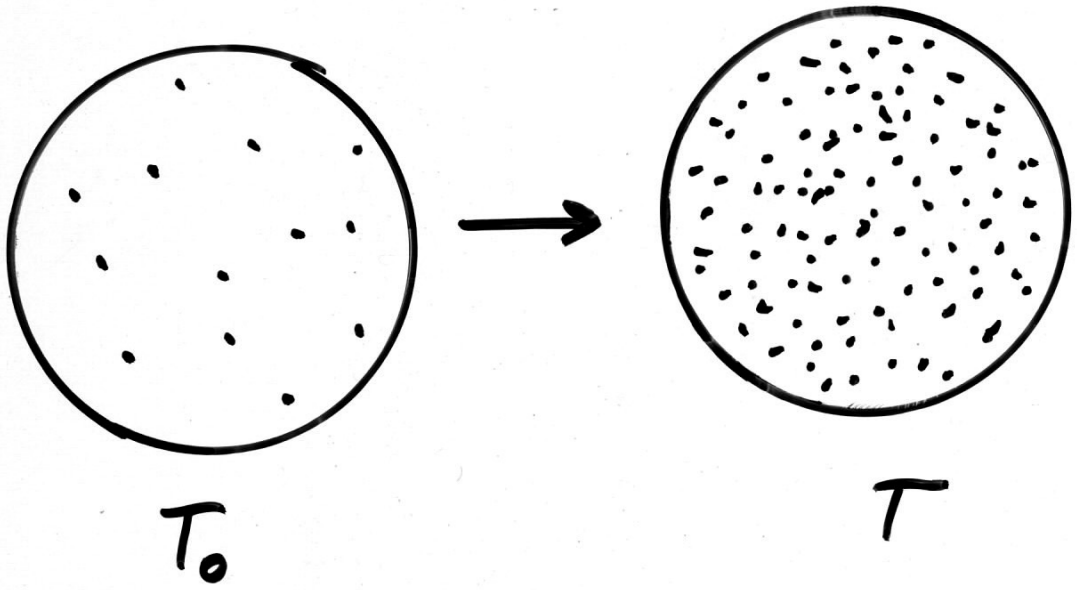
Cells in culture

doubling time ... the time interval in which the cell number doubles

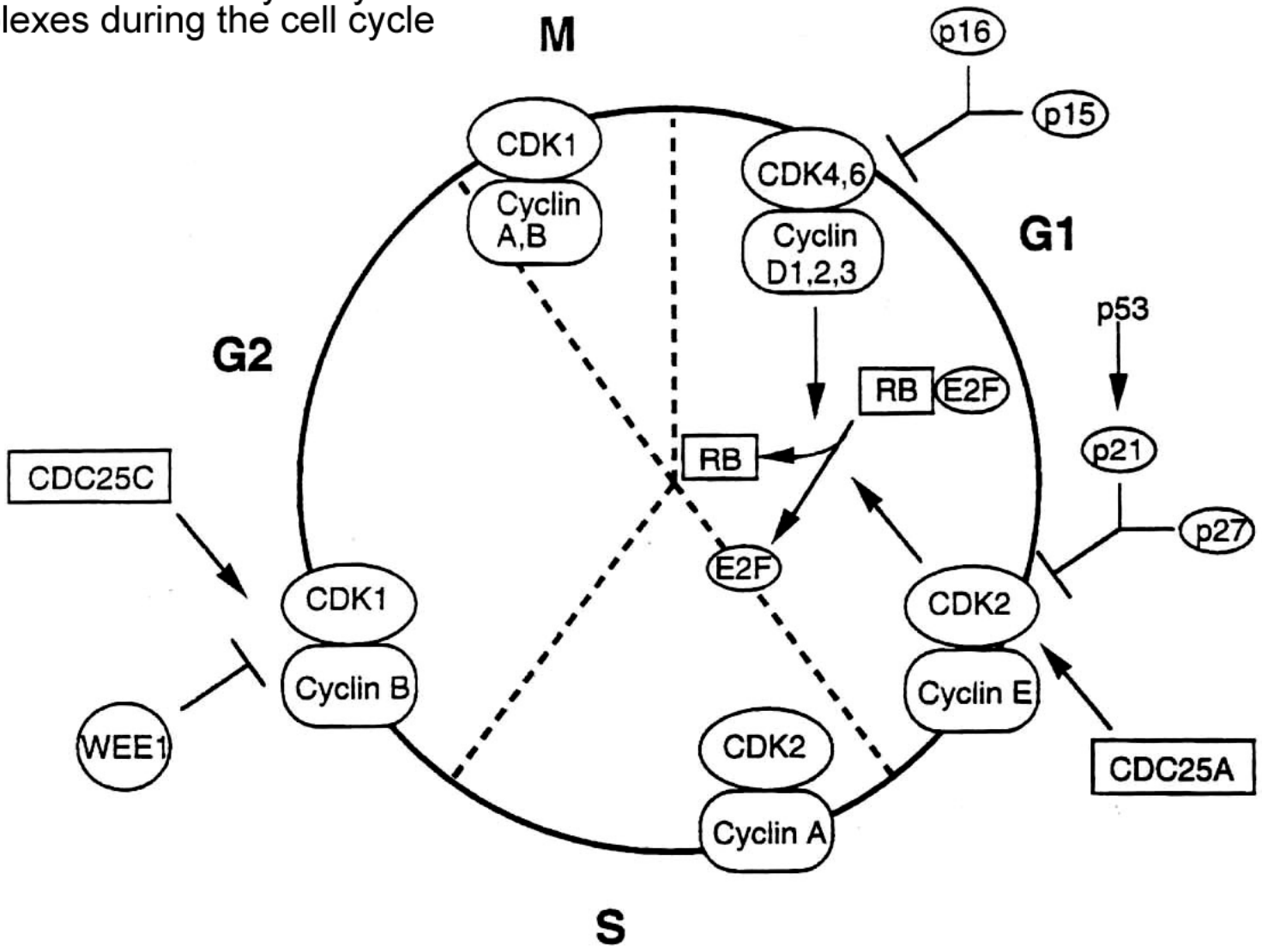
number of doublings = log N/No x 3.33

No...number of cells at time To

N....number of cells at time T



Formation and activity of cyclin-cdk complexes during the cell cycle



Summary of the regulation of cyclin/cdk complexes during celll cycle

Cell cycle phase	**Cyclin-cdk complex**	**inhibitor**	**activation**	**Substrate(s)**
G1	**Cyclin D/cdk 4,6**	p16 family, p21 family	CAK, Cdc25A	Rb protein
G1/S	**Cyclin E/cdk 2**	p21 family	CAK, Cdc25A	Rb protein, NPAT, cdc6
S	**Cyclin A/cdk 2** (Cyclin A/cdk 1)	p21 family	CAK, Cdc25	Rb protein, pre-RC, E2F
G2/M	**Cyclin B/cdk 1** (Cyclin A/cdk 1)	p21 family	CAK, Cdc25C	Several substrates required for mitosis (APC, lamins, cohesins,..)

Summary of cell cycle regulation

Basic terminology:

Cyclins – conserved proteins with homologous regions; their cellular level profoundly oscilate during the cell cycle due to transcriptional regulation and different degradation of the protein
Cyclins are catalytic subunits of active cyclin-cdk complexes,
CYCLINS A, B, D(1,2,3), E

Cyclin-dependent kinases (cdks) – kinases which require a catalytic subunit (cyclin) and their activity is regulated by phosphorylation/dephosphorylation and by cdk-inhibitors.

CDK 1,2,3,4,6,7

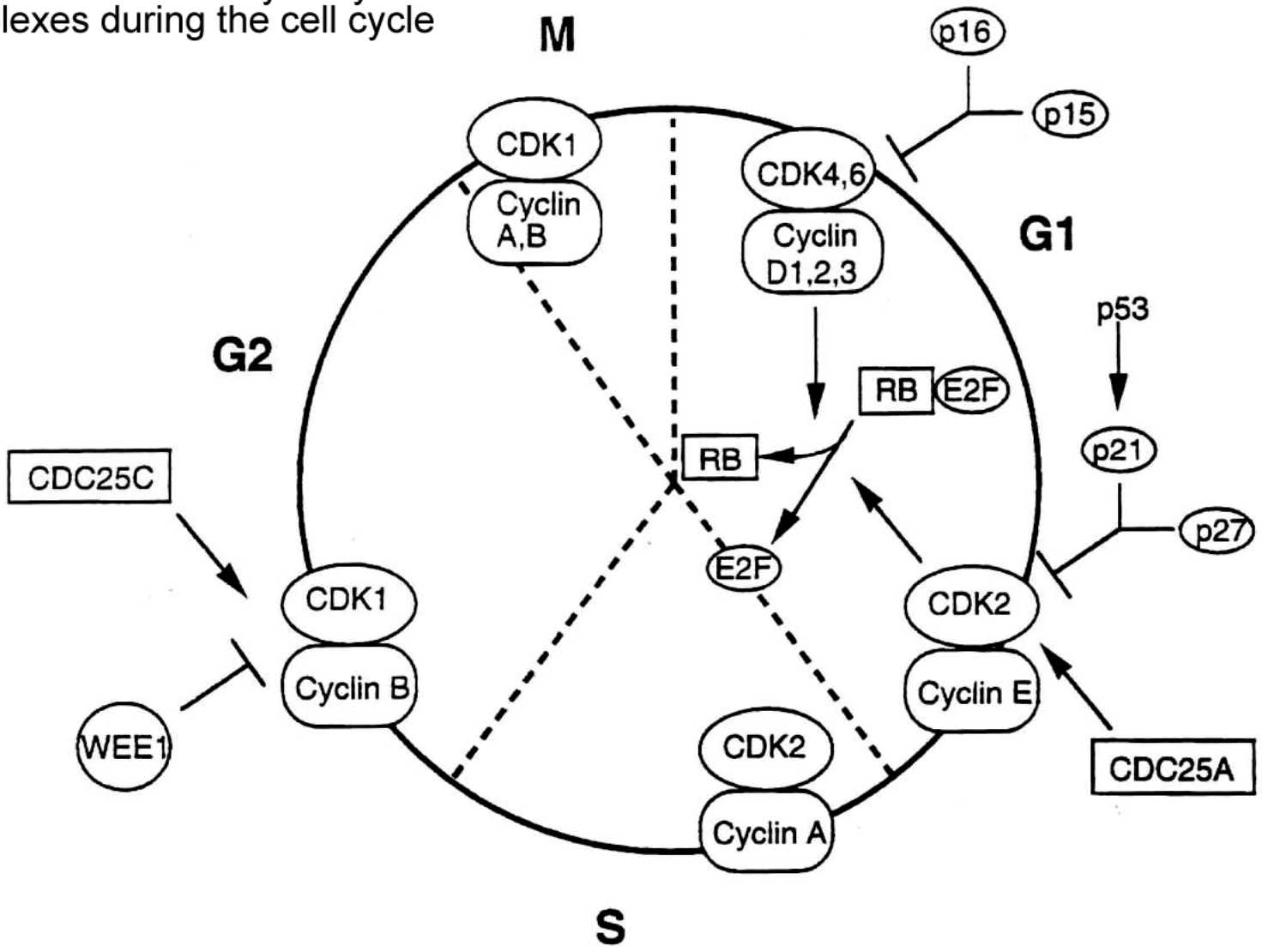
Substrates of cyclin-cdk complexes – the most important is the retinoblastoma protein (Rb).
Rb gene family: Rb, p107, p130.

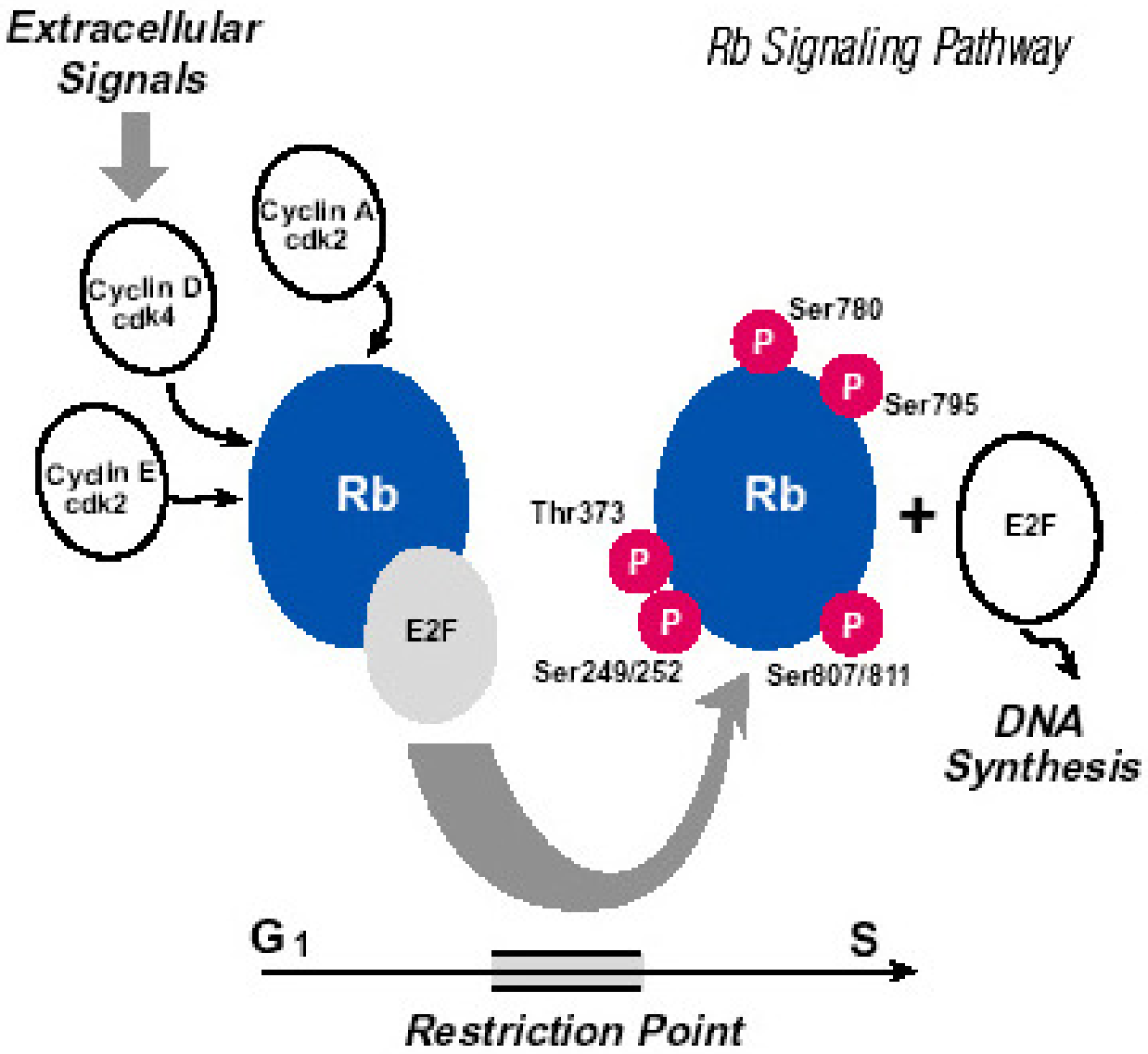
Cdk inhibitors – bind and inactivate cyclin-cdk complexes

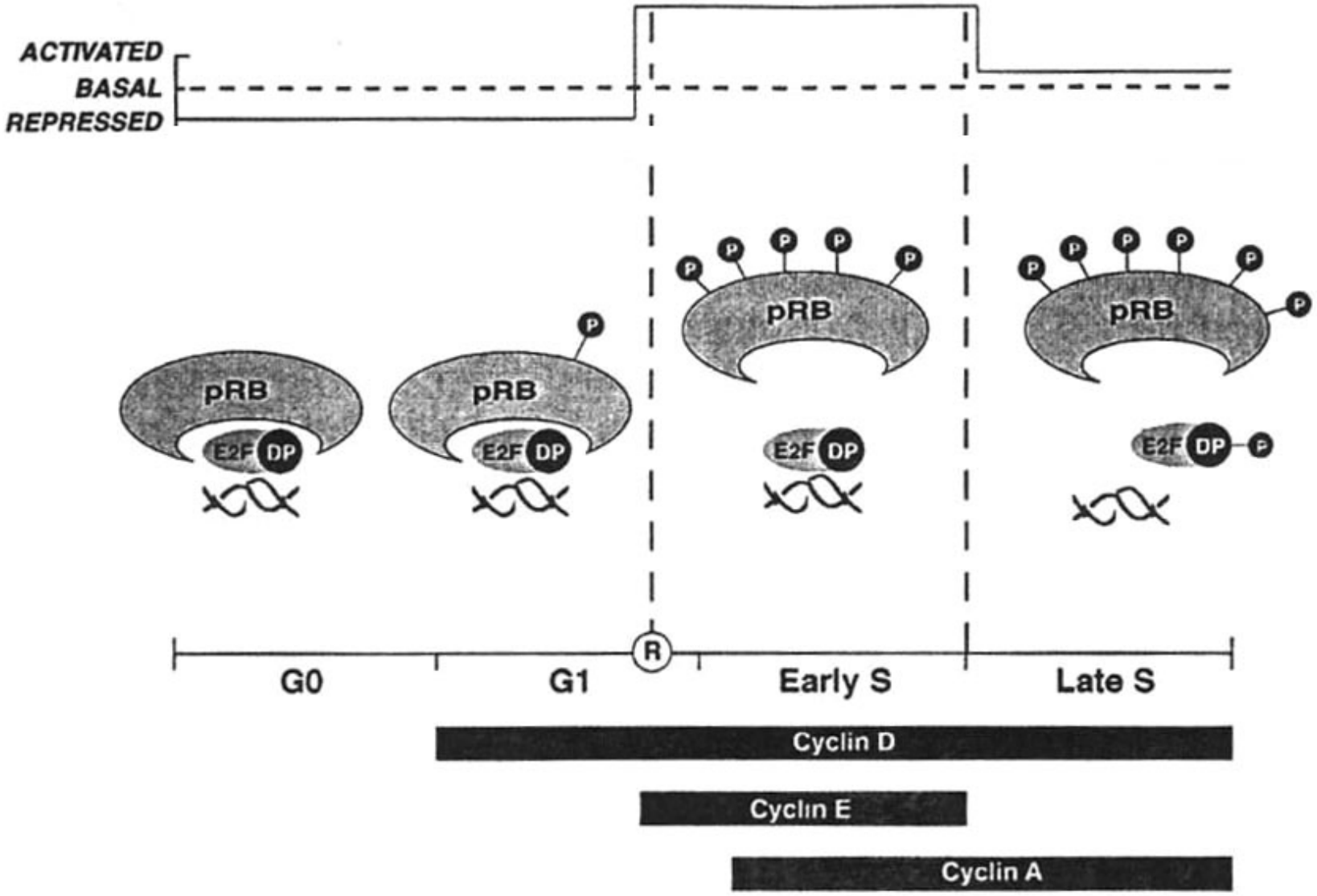
E2F transcription factors – heterodimers of E2Fs (1-5) and DPs (1,2) activate transcription of several genes important for the S-phase. Transcription by E2F is repressed by Rb protein. Only hypophosphorylated Rb protein is capable of repressing transcription. Upon phosphorylation, Rb protein becomes inactive.

E2F targets are promoters of: DNA polymerase α, dihydrofolate-reductase, thimidine kinase, Cyclin E, cyclin A, c-myc, E2F-1 (positive loop)

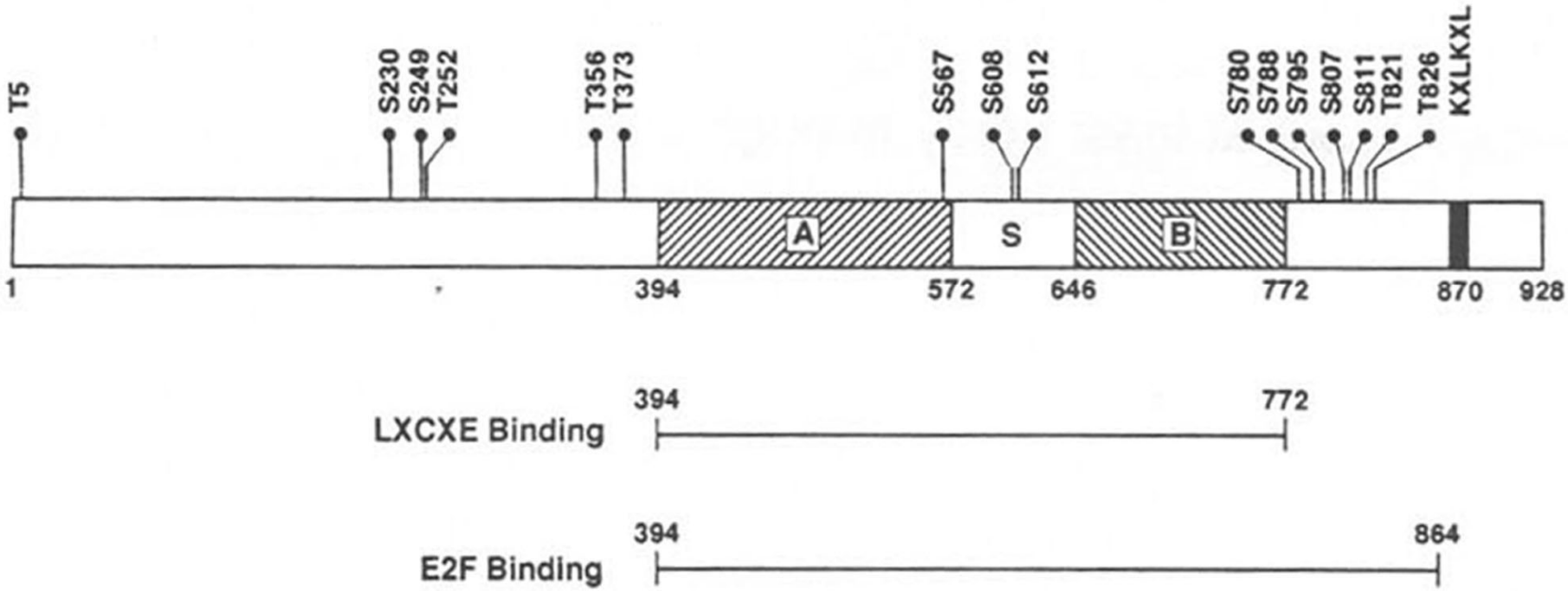
Formation and activity of cyclin-cdk complexes during the cell cycle

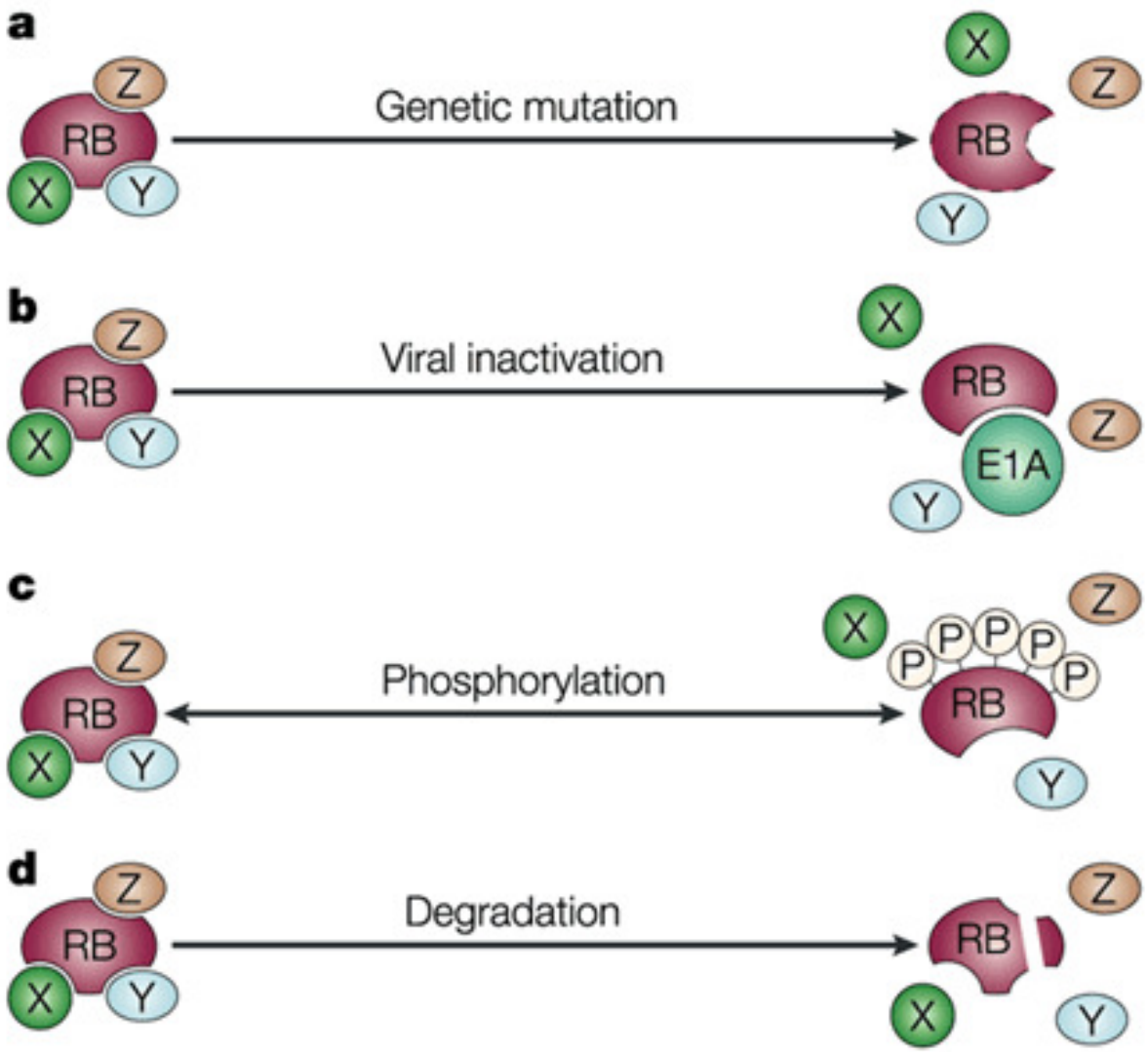



Extracellular Signals
Rb Signaling Pathway
Cyclin A cdk2
Cyclin D cdk4
Cyclin E cdk2
Rb
E2F
Ser780
P
P
Ser795
Thr373
Rb
P
P
P
Ser249/252
Ser807/811
+
E2F
DNA Synthesis
G 1
S
Restriction Point

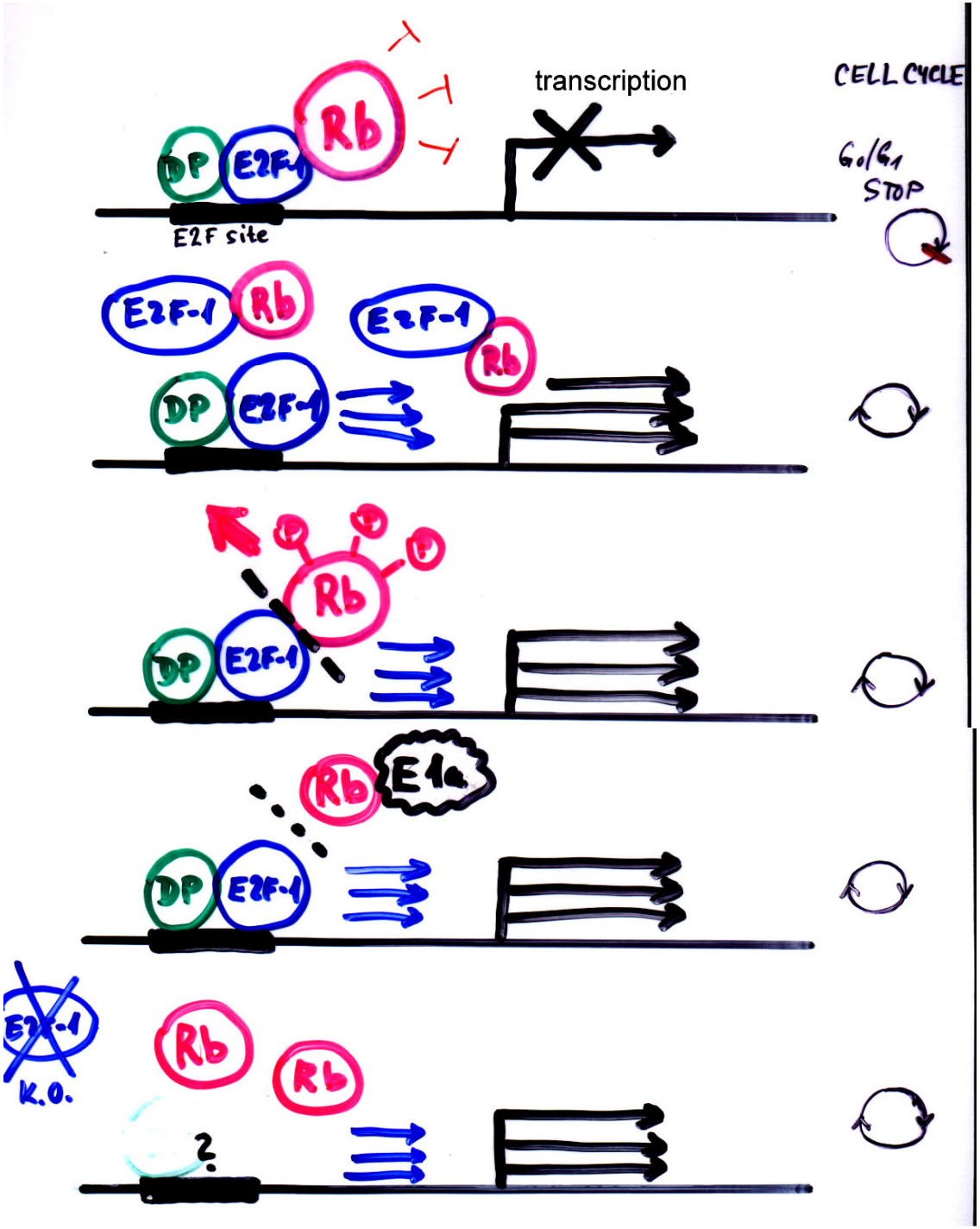

ACTIVATED
BASAL
REPRESSED
pRB
E2F
DP
P
R
G0
G1
Early S
Late S
Cyclin D
Cyclin E
Cyclin A

Structure of the retinoblastoma protein





Nature Reviews | Cancer


transcription
CELL CYCLE
G0/G1
STOP
DP
E2F-1
Rb
E2F site
E2F-1
Rb
E2F-1
Rb
DP
E2F-1
P
Rb
DP
E2F-1
Rb
E1a
DP
E2F-1
E2F-1
K.O.
Rb
Rb
?

Tumor cells in tissue culture:

1. May have different morphology
2. Can grow after confluency and pile up - foci
3. Do grow in semisolid medium (soft agar) – loss of requirement for anchorage
4. Form tumors in SCID mice

Cellular processes disrupted in cancer cells.

(Cellular processes which result also in cell cycle effects)

Differentiation.

Differentiated cells are usually in the G0 phase of the cell cycle.
Terminal differentiation (nerves, muscles) normally does not allow
the re-entry into the cell cycle. Differentiated cells have specific differentiated phenotype which includes morphology and expression of cell type-specific markers. **Differentiation program is often impaired in cancer cells.**

Senescence.

Replicative senescence results in exit from the cell cycle into G0.
Again, normally, senescent cells are unable to re-enter the cell cycle.
Senescent cells have also specific morphology and express senescent specific markers. **Cancer cells are unable to senesce (cancer cells are always immortal).**

Apoptosis.

Apoptosis (programmmed cell death) occurs after the activation of pro-apoptotic genes or inhibition of anti-apoptotic genes and in most instances it is initiated in G1. **The execution of apoptosis is impaired in cancer cells.**

Cell cycle checkpoints.

Non-physiological state:
Cell cycle control, mostly in **G1**, in **deregulated in cancer cells**.
Also, other cell cycle checkpoints are deregulated in cancer cells.

Cell transformation:

Normal cell >>>> malignant cell

Stepwise accumulation of several mutations (deletions) in oncogenes and suppressor genes

Escape from senescence (activation of telomerase)

Immortalization (cancer cells are always immortal)

Mouse cells are much more easily transformed – activation of one oncogene and inactivation of one suppressor egene is usually sufficient. Mouse cells can be easily immortalized.

Normal human cells are very difficult to transform in vitro.

Oncogenes

(activated forms of protooncogenes)

Protooncogenes are cellular genes the functions of which are required normal cells.

One activated allele (e.g. by mutation) is sufficient for oncogene activation

X

Tumor supressor genes (= “antioncogenes”)

Supppressor genes are inactivated in tumor cells

Both alleles must be inactivated (loss of the functional protein in the cell)

Mechanisms of activation of cellular oncogenes:

Point mutations (H-ras, K-ras, N-ras)

Enhanced expression

- increased (deregulated) transcription
- gene amplification
- chromosomal translocation (myc 8q24) > IgH (14q32)

  (cellular gene is transcribed from a strong promoter after translocation)

Translocation creating a new fusion protein with aberant function

Abl (9q34)+Bcr(22q11) > Abl-Bcr fusion (Philadelphia chr.)

Impaired degradation of protein (some cyclins)

Non-cellular oncogenes from DNA tumor viruses

Mechanisms of inactivation of tumor suppressors:

Point mutations: retinoblastoma (Rb) protein, p53 protein

Deletions (loss of a locus on the chromosome)

(many different losses of locuses are associated in almost all cancer cells)

Decreased expression

- decreased transcription caused by promoter methylation

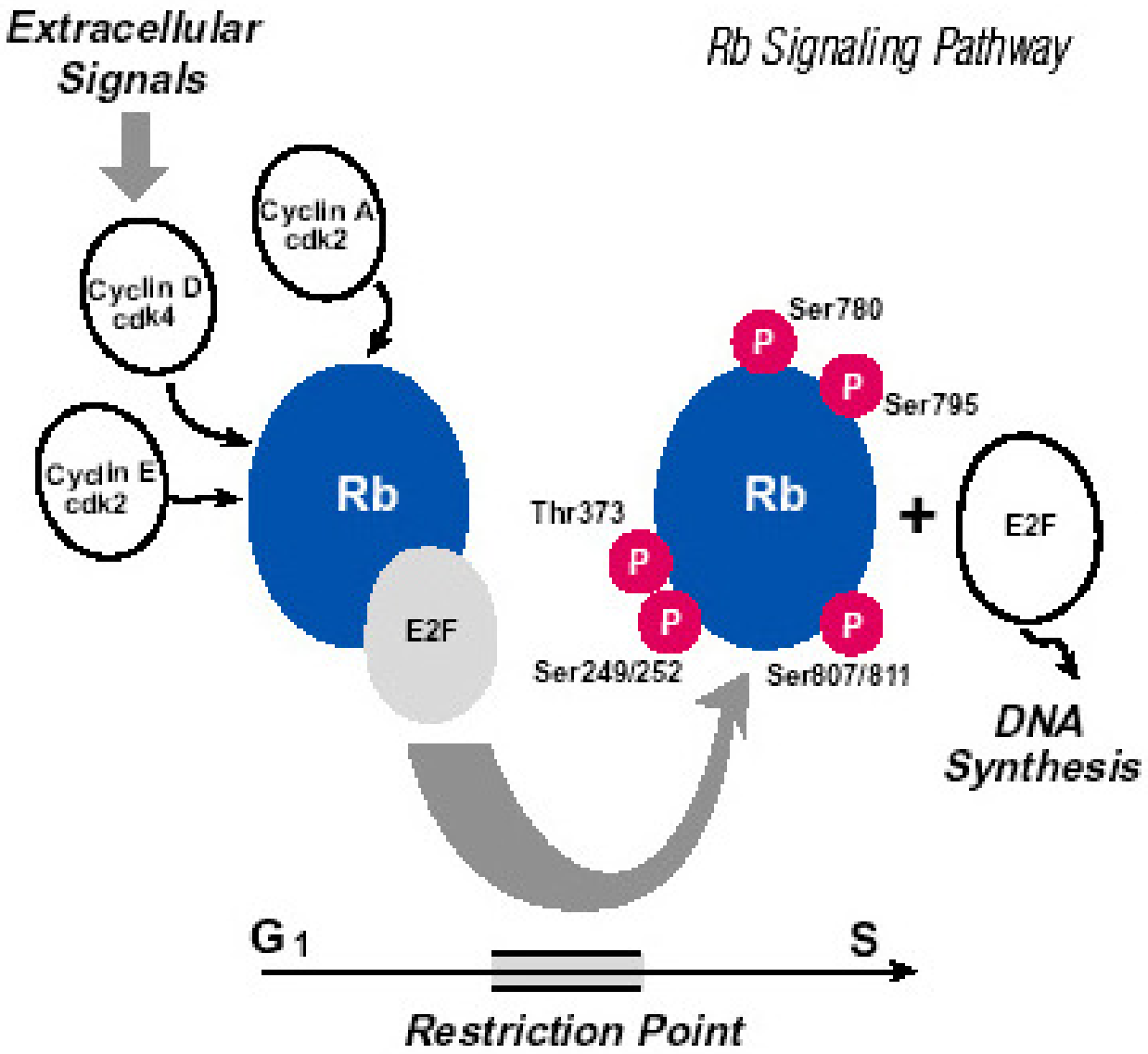
Increase protein degradation (p53 x mdm2)

Two important suppressor pathways: Rb and p53.

At least one pathway is almost invariantly impaired in cancer cells

Other tumor suppressors: p16(INK4a)-Rb pathway, and p14(ARF)-p53 pathway

Mutations in suppressor genes can be inherited – cancer syndromes)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Ser780
P
P
Ser795
Thr373
Rb
P
P
P
Ser249/252
Ser807/811
+
E2F
DNA Synthesis
G 1
S
Restriction Point

Cell cycle checkpoints deregulated in cancer

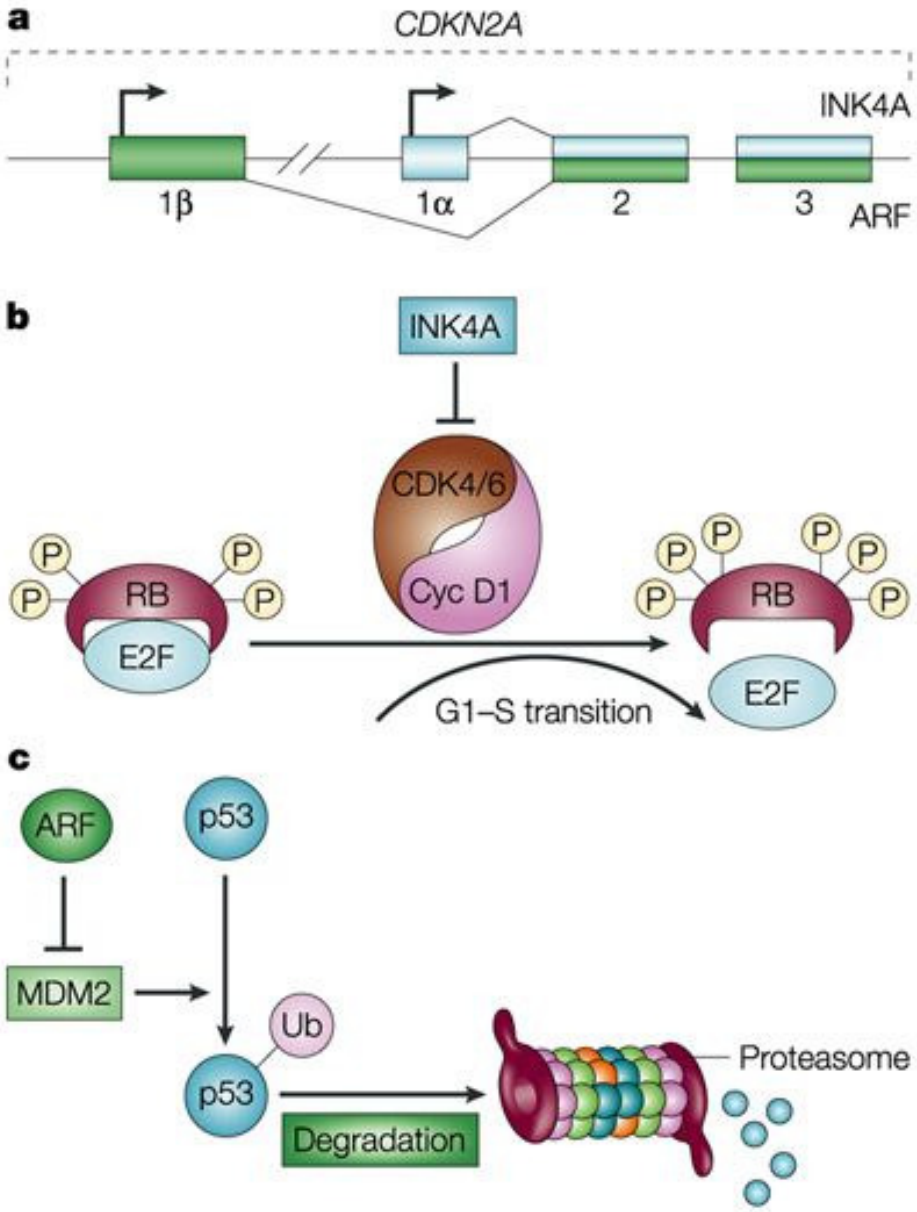
Normally, checkpoints enable cells to halt the cell cycle and repair damaged DNA or complete spindle assembly at mitosis:

- restriction point in late G1 (after this point, the cell is committed to enter the S-phase)

- DNA replication checkpoint (this checkpoint ensures that mitosis occurs only after DNA has replicated completely - DNA replicates once and only once during a single cell cycle). **Endoreduplication** (re-replication of DNA without mitosis) may occur in cancer cells.

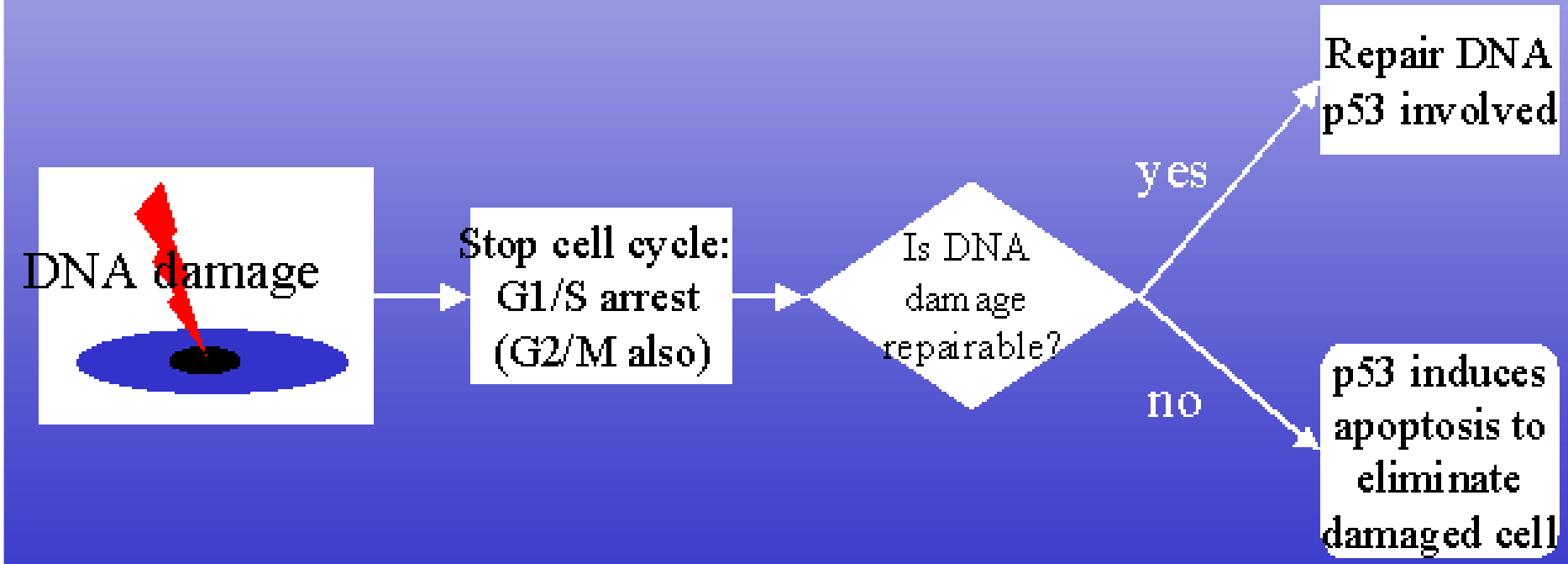
- spindle assembly checkpoint (ensures proper segregation of chromosomes during mitosis (at the metaphase to anaphase transition). Chromosomal aberations in cancer cells.

- DNA damage checkpoint(s) (cell cycle can be arrested in G1, S , or G2). Inability to arrest (and repair DNA) after DNA damage may lead to the accumulation of mutations.


a
CDKN2A
INK4A
1β
1α
2
3
ARF
b
INK4A
CDK4/6
Cyc D1
P
RB
E2F
G1–S transition
c
ARF
p53
MDM2
Ub
p53
Degradation
Proteasome

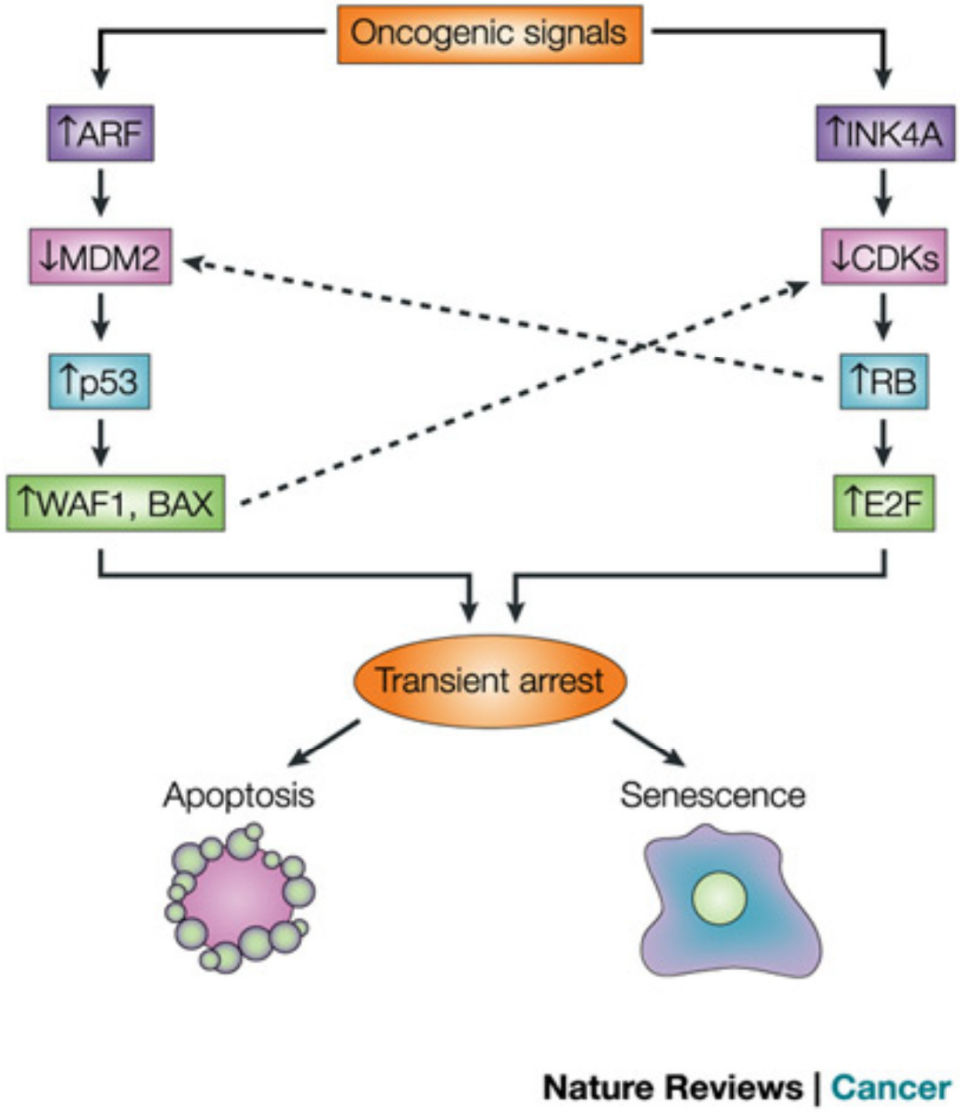
Functions of p53: simple

- One way of thinking about p53 is that it is a "guardian of the genome": it protects the cells DNA from damage



October 1998
Oliver Bogler, Ph.D.

Rb- and p53- pathways are deregulated in cancer cells



Cancer “syndromes”

Gene	locus (somatic mutations in tumors)	
Rb	13q14	Retinoblastoma,osteosarcoma, SCLC,
p53	17p13	Syndrome Li-Fraumeni
NF1,2	17q11; 22q12	Neurofibromatosis type 1,2
p16	9p21	"Familiar" melanoma
WT1	11p13	Wilms tu.

Oncogenic DNA viruses.

Virus:	Oncoprotein:	Genome size (kb):
SV-40 virus	large T Ag	5
Polyomavirus	middle T (large T)	5
Adenoviruses (Ad12)	E1a (E1b)	35
Papilomaviruses (HPV16)	E7, E6	8

Hepatitis B viruses
Herpesviruses, Epstein-Barr virus
Poxviruses

Deregulation of signaling pathways in cancer

The MAPK pathway:

encompasses a cascade of phosphorylation events involving three key kinases, namely Raf, MEK and ERK:

RAS signalling **(ras: H-ras, K-ras, N-ras – GTP-binding membrane proteins, with GTPase activity), active form: GTP-ras (constitutively active when mutated, such as in tumors)**

↓

RAF = MAP kinase kinase kinase (MAPKKK)

↓

MEK = MAP kinase kinase (MAPKK)

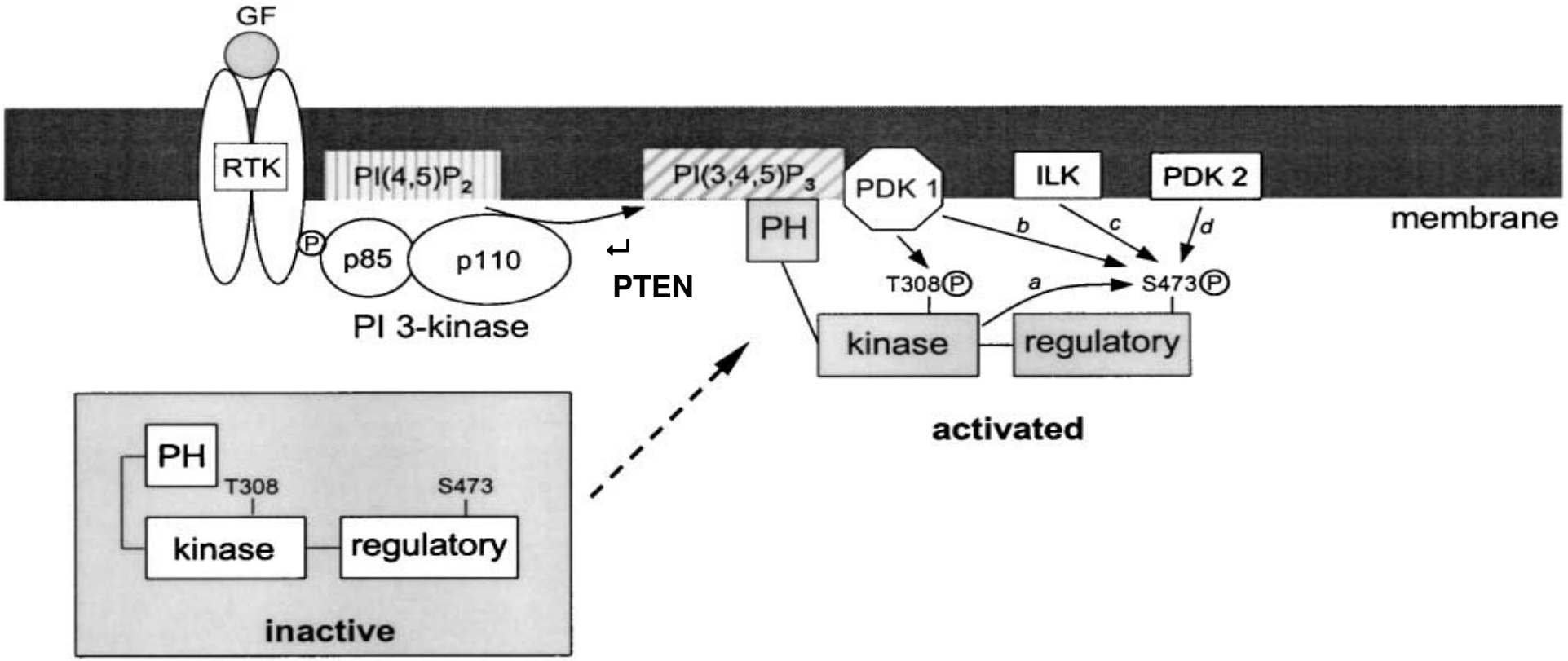
↓

ERK = MAP kinase (MAPK)

↓

protein substrates

Activation of AKT kinase by PI 3-kinase

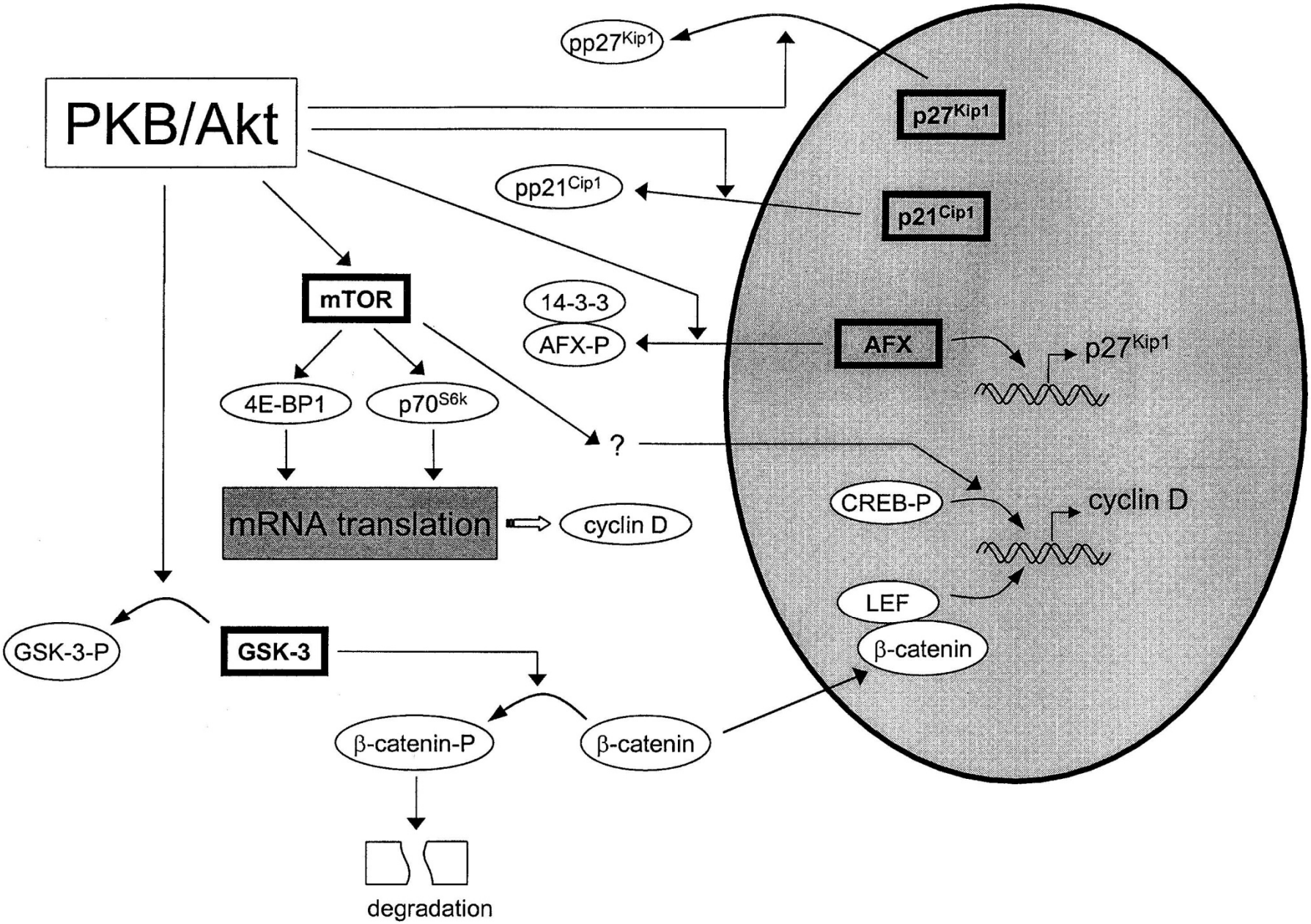


Activation of growth factor receptor protein tyrosine kinases results in autophosphorylation on tyrosine residues > **PI3K** is recruited to the membrane > this leads to allosteric activation of the catalytic subunit > activation results in production of the second messenger **phosphatidylinositol-3,4,5-trisphosphate (PIP3).**

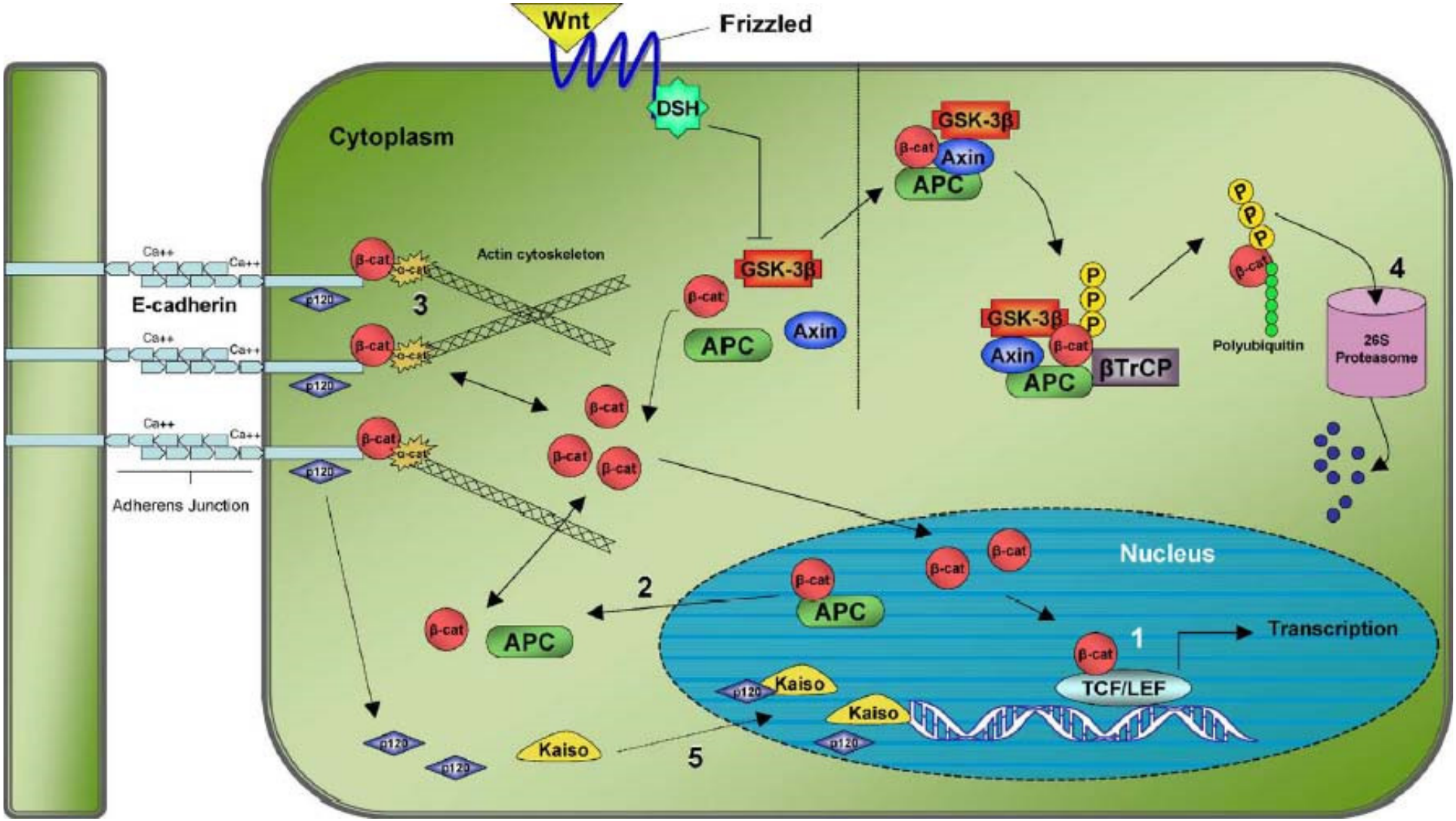
The lipid product of PI3K, PIP3, recruits a subset of signaling proteins, including **AKT (=PKB)**. (PTEN, is a PI-3,4,5-P3 phosphatase, which negatively regulates the PI3K/Akt pathway).

Once activated, **AKT mediates the activation and inhibition of several targets (by phosphorylating them)**, resulting in cellular survival, growth and proliferation through various mechanisms.

Downstream consequences of activation of the AKT pathway - cell cycle



Wnt-β-catenin signální cesta



Inactivation of GSK3β by Wnt signals, mutational activation of β-catenin and truncation of *APC* lead to the accumulation of β-catenin in the cytoplasm. TCF targets: c/myc, cyclin D1, cyclooxygenase 2,…