Lecture 3: The Cell Cycle and DNA Damage

Biochemistry of Cancer

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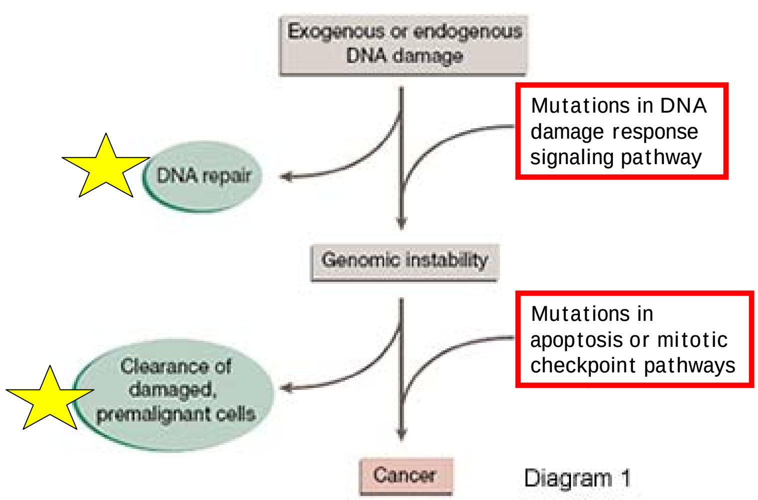


Diagram 1

Before we get started....
What IS normal?

- Dependence on growth factors.
  - Cell and tissue specific signals.
  - Loss of these signals leads to *apoptosis*.
- Anchorage dependant proliferation.
  - Requires interaction with transmembrane proteins (integrins) with components of the ECM.
- Contact inhibition.
  - Contact with like cells inhibits cell proliferations and movement.
- Limited proliferation capacity.
  - Normal somatic cells have a limited number of divisions before entering a *senescent state*.

Therefore… Cellular Transformation (in the culture dish) is…

- Immortalization and *aneuploidy*.
  - Diploid cells surviving past the point of senescence will give rise to *clonal populations* that often have chomosomal rearrangements and aneuploidy.
- Partial or complete loss of growth factor dependence.
- Loss of contact inhibition of growth and movement.
- Loss of anchorage dependant growth.

Hallmarks of tumorigenicity and cancer

- Failure to respond to signals to stop proliferating or to differentiate.
- Self-generation of factors that promote growth.
- Sustained proliferative capacity.
- Evasion of apoptosis.
- Motility and invasiveness
- Angiogenesis

Cell Cycle Control is Regulated by a Balance Between Growth Promoting and Growth Inhibiting Signals

- STOP Signals

DNA damage/repair

Cell-cell contact

Loss of mitogenic signal

Terminal differentiation

Senescence (aging)

- GO Signals

Mitogens

Transformation (viral or genetic alterations)

CKIs, tumor suppressors

CDKs, cyclins, oncogenes

Mitosis

- The cell cycle is controlled by a complex pattern of synthesis and degradation of cell cycle regulators as well as control of their cellular localization.

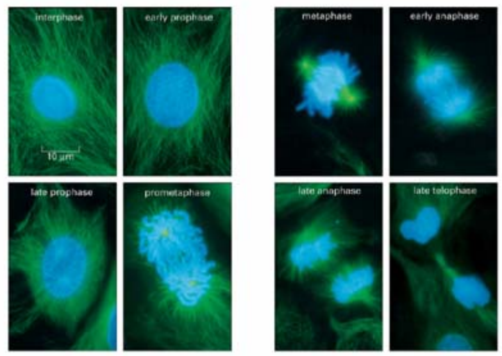
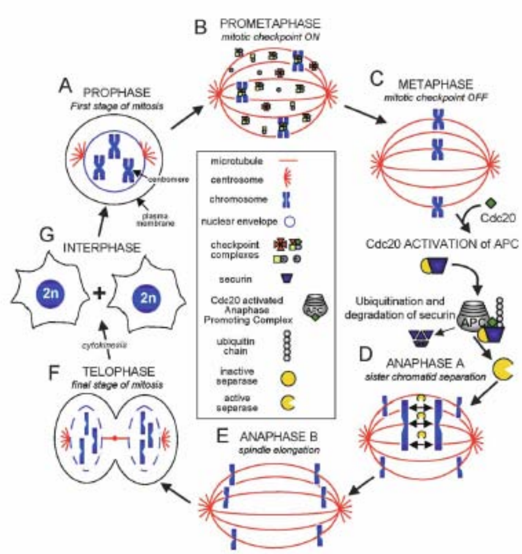


Figure 18–8 part 1 of 2. Molecular Biology of the Cell, 4th Edition.Figure 18–8 part 2 of 2. Molecular Biology of the Cell, 4th Edition.



Weaver and Cleveland (2005) Cancer Cell 8:7-12.

Phases of the Cell Cycle

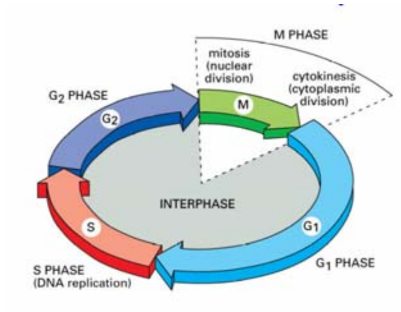


Figure 17-3. Molecular Biology of the Cell, 4th Edition.

- Chromosomes are replicated (S), condensed (M), segregated and decondensed.
- Spindle poles must duplicate (S), separate, and migrate.
- Nuclear membrane is disassembled and reasssembled (M).
- Mitotic spindle is disassembled and reasssembled (M).
- Cell membranes invaginate to complete cytokinesis.

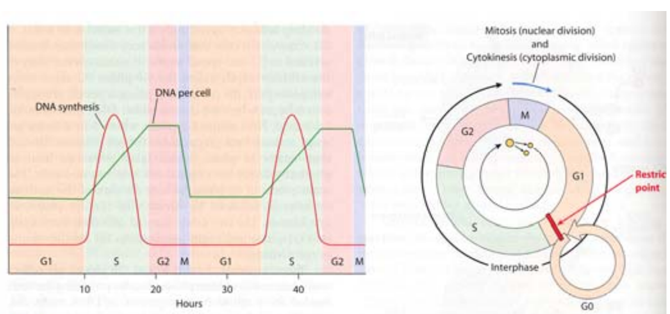
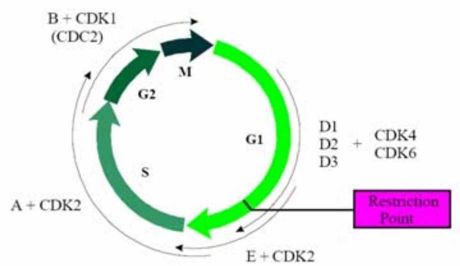


Figure 2-7 The Cell Cycle. The graph on the left shows how the four phases of the cell cycle (G1, S, G2, and M) are defined by two variables: DNA synthesis and the amount of DNA per cell. S phase is defined as the time during the cell cycle when DNA synthesis is taking place, leading to a doubling of the amount of DNA per cell. M phase is the time when the amount of DNA per cell drops in half as cells divide. G1 is defined as the interval between M phase and S phase, and G2 is defined as the interval between S phase and M phase. The cell cycle is commonly represented with a circular diagram like the one shown on the right. The restriction point is a control point near the end of G1 where the cell cycle can be halted until conditions are suitable for progression into S phase. Under normal conditions, the ability to pass through the restriction point is governed mainly by the presence of growth factors.

Three Classes of Cell Cycle Control Proteins



- Cyclin-dependant kinases (CDK)
  - Serine/threonine kinases
- Cyclins
  - Heterodimeric partners with CDKs, regulate activity and localization
- Cyclin-dependant kinase inhibitors (CKIs)
  - Inhibit CDK activity by physically blocking activation or by blocking substrate/ATP access.

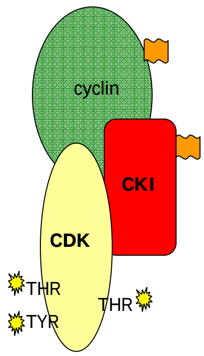
Cyclins and CDKs

Cyclin	CDK	Stage
A1-2	CDK1,2,3	S, G1, M
B1-3	CDK1	M
C	CDK8	G1
D1-4	CDK4,6	G1
E1-2	CDK2,4,5,6	G1,S
F	?	G2/M
G1-2	GAK	G1, S
H	CDK7	all
K	?	?
T1-2	CDK9, CDH10	?

Cyclin Dependant Kinases (CDKs)

- Two subunits, a kinase and cyclin.
- Serine/threonine *kinases* phosphorylate multiple substrates involved in the cell cycle.
  - RB and p53
- Binding to cyclins confer partial activity, which is enhanced by phosphorylation at specific residues.

CDK Regulation



1. Interaction with cyclin
2. Phosphorylation at Thr-160
3. Interaction with CKIs
4. Phosphorylation at Thr-14 and Tyr-15
5. Ubiquinization of CKI and cyclin for targeted degradation.

Both interaction with cyclin and phosphorylation are needed for CDK activity

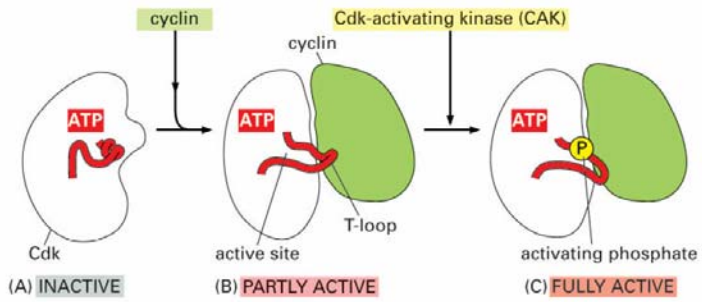


Figure 17–17. Molecular Biology of the Cell, 4th Edition.

Phosphorylation can both activate *and* inhibit CDK activity

CAK=cyclin activated kinase

Wee1 kinase- inhibitory kinase (cell size)

Cdc25 phosphatase- removes inhibitory phospate

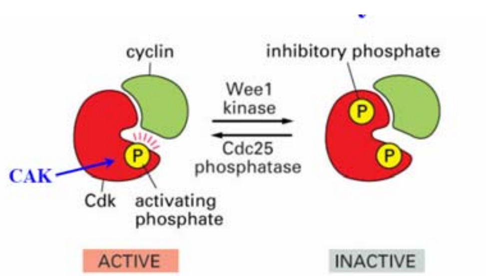


Figure 17–18. Molecular Biology of the Cell, 4th Edition.

Upstream events indicating availability of nutrients and cell size regulate CDK phophorylation

4886 Journal of Cell Science 116 (24)

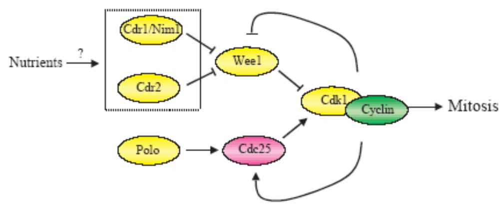


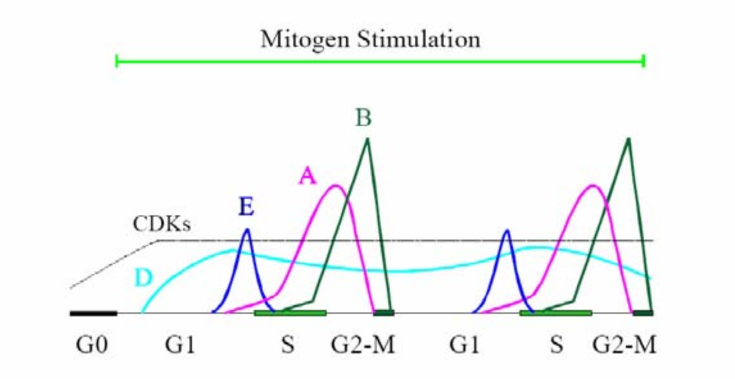
Fig. 3. A model for entry into mitosis based on work in fission yeast and *Xenopus*. Work in *Xenopus* suggests that Polo kinase might be required for activation of Cdc5 and entry into mitosis; however, work in fission yeast suggests that Polo kinase might not be required for entry into mitosis (see text). Kinases are shown in yellow and phosphatases are shown in pink.

- Wee-1 phosphorylates a conserved Tyr residue in the N-terminus of CDK1.
- Wee-1 and Cdc25 are both hyperphosphorylated during mitosis.
  - Wee1 is *inhibited*
  - Cdc25 is *activated*

Cyclins are critical for CDK and cell cycle regulation

- Interact with conserved cyclin box in CDKs.
- Regulate by changing cyclin abundance and localization.
- Cyclin degradation is critical for inactivation of CDKs.
- G1 and mitotic cyclins
  - G1-relatively short lived (30 min), dependant on mitogenic signals, regulated by assembly and synthesis
  - Mitotic- longer lived, degraded at the end of mitosis by the proteosome (ubiquinated by APC)

Cyclin availability alters during the cell cycle



Sherr CJ. Cancer Cell Cycles. Science 274:1672-1677,1996.

Proteolysis of Cyclins by Anaphase Promoting Complex (APC)

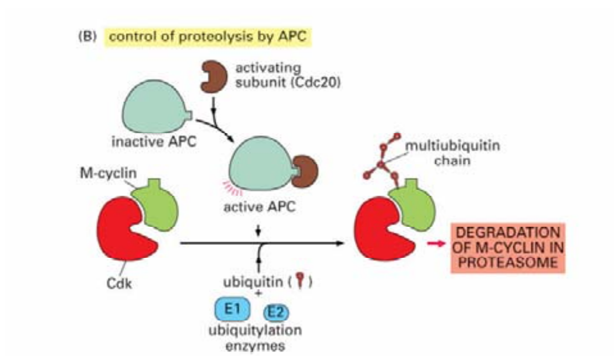


Figure 17–20 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Proteolysis of CKIs

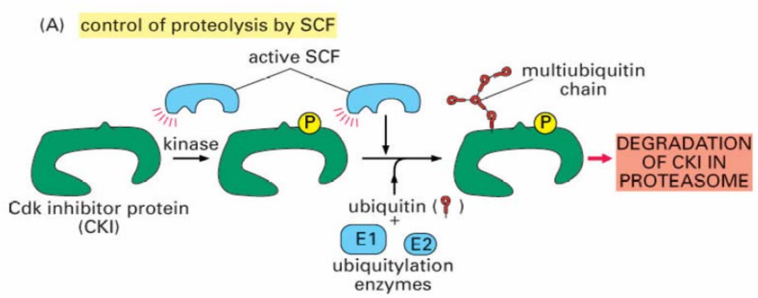


Figure 17–20 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Cell Kinase Inhibitors (CKIs)

- Two main groups
  - CIP/KIP
  - INK4a
- Normal expression low/undetectable
- Expression is induced by growth inhibitory signals
  - Mitogen withdrawal, contact inhibition, DNA damage
- Block CDK activity and growth

CIP/KIP CKIs

- p21 CIP1, p27 KIP1, p57 KIP2
- Universal inhibitors of G1 CDKs (except CDK 4)
- Overexpression cause G1 and G2 arrest; differentiation in some cell types.
- Bind to both CDKs and cyclins.
- Highly expressed in differentiated tissues, and are induced by DNA damage.

CIP/KIP proteins contact both CDK and cyclin

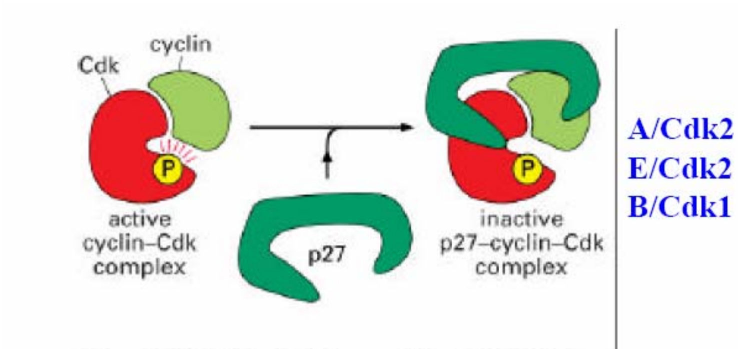


Figure 17–19. Molecular Biology of the Cell, 4th Edition.

INK4A Family

- Inhibitors of CDK4 and CDK6
- Overexpression leads to G1 arrest, differentiation, senescence
- Interact with CDKs, not cyclins.

Cell Cycle Checkpoints

- Defects in spindle pole duplication will block mitosis.
- Nicks in DNA will delay the onset of replication.
- Arrested DNA replication and double strand breaks can block anaphase.

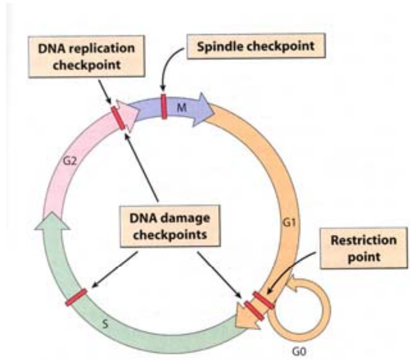


Figure 2-11 Control Points in the Cell Cycle. The ability of growth factors to promote passage through the restriction point illustrates how the cell cycle is regulated by external conditions. Another type of control involves checkpoint pathways that monitor conditions inside the cell and transiently halt the cell cycle at various points if conditions are not suitable for continuing. Checkpoint pathways monitor for DNA damage, DNA replication, and chromosome attachment to the spindle, transiently halting progression through the cell cycle at various points if conditions are not suitable for continuing.

Checkpoints in M Phase

DNA damage checkpoint

CDK1 activation, PLK1, NEK2

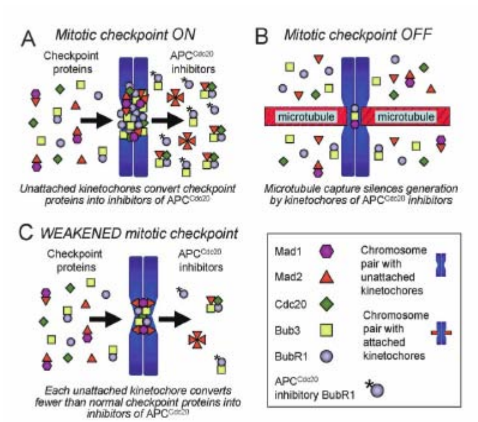
CDK1

CDK1, PLK1, BUB1

Spindle assembly checkpoint

Any mutation that removes or modifies a checkpoint inhibitor (ie. p53) will lead to a loss of cell cycle regulation and to cancer.

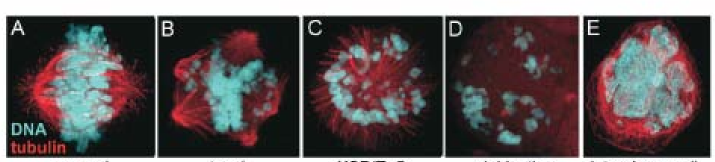
Kinetochore and tumor production



- Unattached kinetochores are the signal generators for the mitotic checkpoint.
- Once kinetochores have made attachment to spindle micotubules, signal is turned off.
- Problems with this signaling process produces a "weak" checkpoint which is associated with tumor production.

Weaver and Cleveland (2005) Cancer Cell 8:7-12.

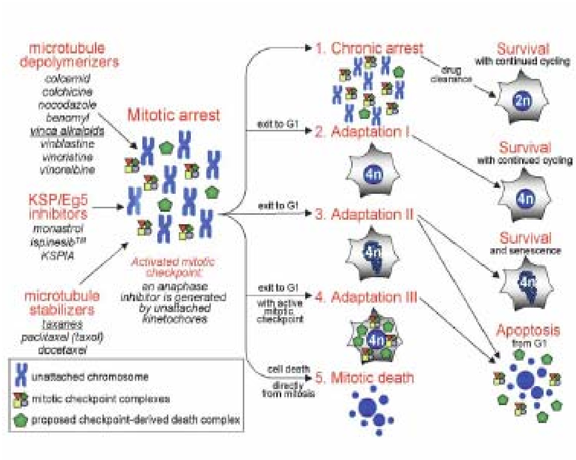
chronic activation of the mitotic checkpoint is a common chemotherapeutic strategy!!!



normal metaphase spindle | taxol treated spindle | KSP/Eg5 inhibited spindle | vinblastine treated spindle | interphase cell after adaptation

Weaver and Cleveland (2005) Cancer Cell 8:7-12.

Possible outcomes of prolonged treatment with antimitotic drugs



Weaver and Cleveland (2005) Cancer Cell 8:7-12.

Role of p21 in the DNA damage checkpoint response

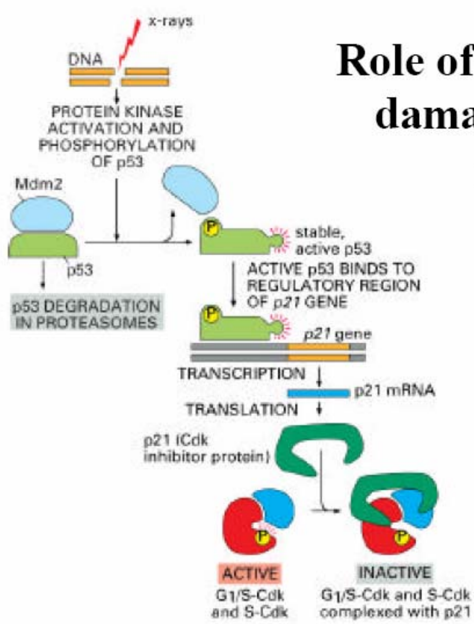
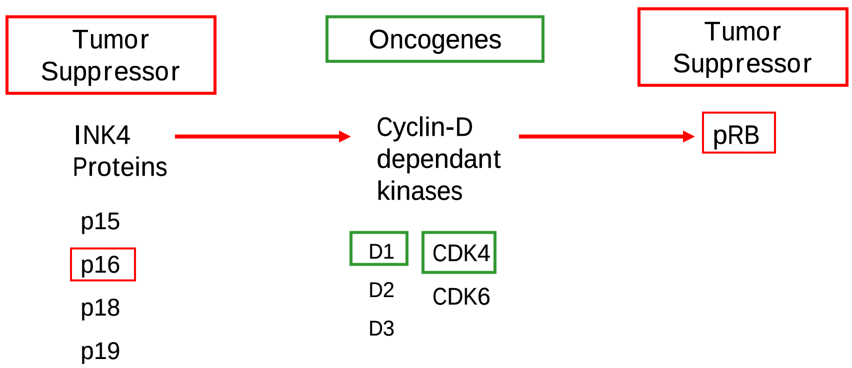


Figure 17-33. Molecular Biology of the Cell, 4th Edition.

Cell Cycle, Oncogenes and Tumor Suppressors



G1 Regulators in Cancer

Gene	Alteration	Phenotype	Cancers
Cyclin D	Activation-gene amplification, gene expression, translocation	Shorter G1, harder to growth arrest, require less stimulation	Breast, head and neck, pituatary, esophageal
CDK4	Activation-mutation, gene amplification	Shorter G1, require less stimulation to grow, not arrested by p16	Gliomas, sarcomas, melanomas
pRB	Inactivation-mutation, loss of gene	No restriction point control, sensitive to genetic alterations	Retinoblastoma, small cell lung carcinoma
p16 (CKI)	Inactivation-mutation or gene silencing	Reduced checkpoint control, sensitive to genetic alterations	Melanoma, head and neck, glioma, bladder, ovarian, leukemia, etc.