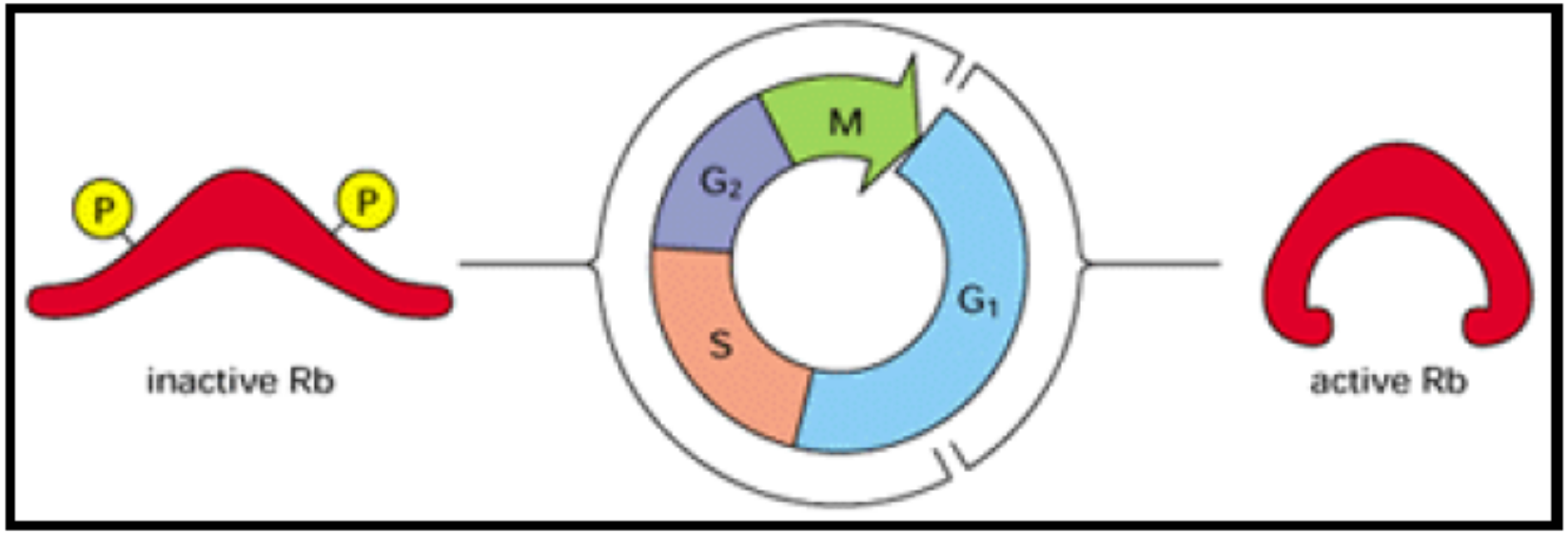
GROWTH FACTORS STIMULATE CdK AND CYCLIN SYNTHESIS – ALSO ROLE OF RETINOBLASTOMA PROTEIN (pRB)

- The third mechanism for changing CdK activity is regulation of the rate of synthesis of the cyclin or CdK or both
- Extracellular signals such as growth factors and cytokines (developmental signals that trigger cell division) activate, by phosphorylation, the nuclear transcription factors Jun and Fos, which promote the synthesis of many gene products, including transcription factor E2F
- In turn, E2F stimulates production of several enzymes essential for the synthesis of deoxynucleotides and DNA, CdK and cyclins allow the cell to enter the S phase

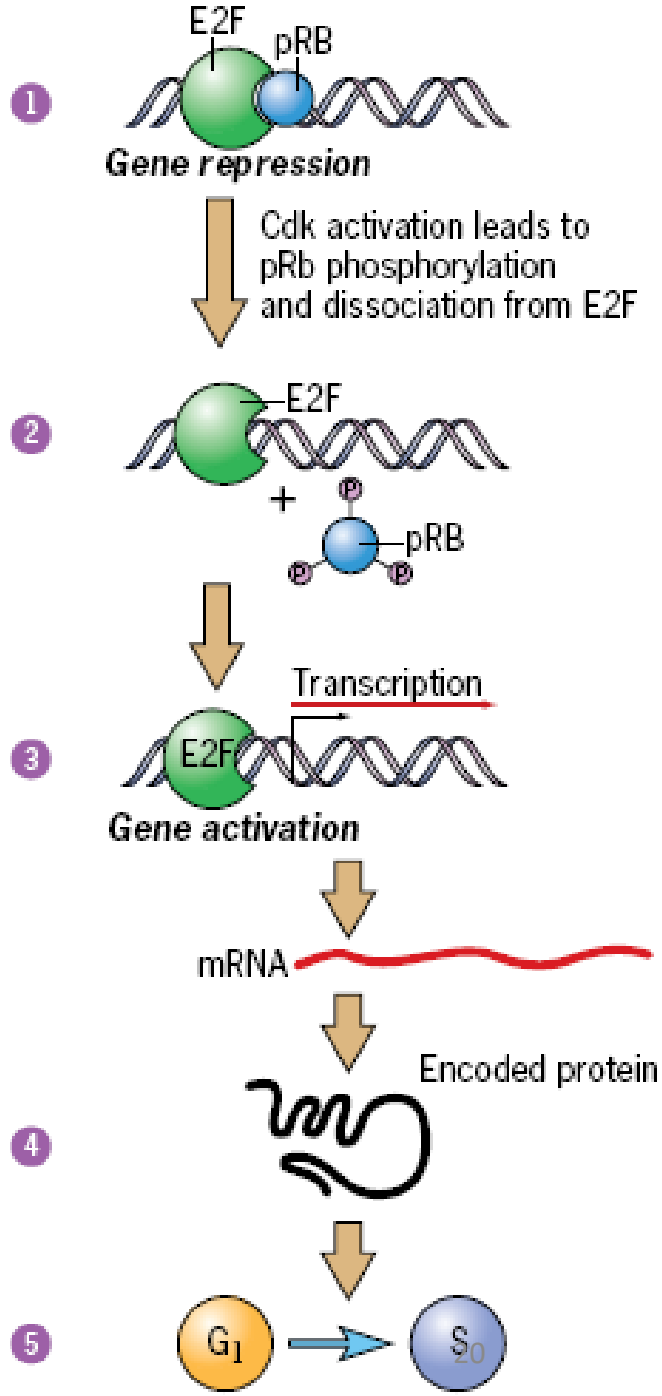
Changes of pRb phosphorylation in a cycling cell

- In proliferating cells the phosphorylation of the pRb increases and decreases in every cycle
  - It rises late in G1
  - Remains high in S and G2
  - Falls back to a dephosphorylated state as the cell exits mitosis
- pRb becomes dephosphorylated as the cell exits from mitosis
- pRb is rephosphorylated late in G1, as the cell prepares to go past Start



Role of pRB in controlling transcription of genes required for progression of cell cycle

- During most of the G1, the unphosphorylated pRB is bound to the E2F protein (step 1)
  - The E2F-pRB complex binds to regulatory sites in the promoter regions of numerous genes involved in cell cycle progression (e.g., proteins required for S phase entry including G1/S cyclins and S-cyclins), acting as a transcriptional repressor that blocks gene expression
- Activation of CdK leads to the phosphorylation of pRB, which can no longer bind the E2F protein (step 2)
- Loss of the bound pRB converts the DNA-bound E2F into a transcriptional activator, leading to expression of the genes being regulated (step 3)
- The mRNA in translated into proteins involved in the progression of cells from G1 to S phase (step 4)
- Progression of cells from G1 into S phase of the cell cycle (step 5)



- This transcriptional control system sharpens G1/S transition by

- Positive feedback loop
  - Liberated E2F increases transcription of its own gene
  - E2F-dependent transcription of G1/S cyclin (cyclin E) and S-cyclin (cyclin A) genes leads to
    - Increased G1/S-Cdk and S-Cdk activities, which increase pRb phosphorylation and promote further E2F release

- Increase in G1/S-Cdk and S-Cdk activities enhances phosphorylation of Hct-1 and p27, leading to their inactivation or destruction
  - **Hct-1**
    - As Hct1 is phosphorylated, APC is inhibited, Cyclin is not degraded, G1/S-Cdk and S-Cdk active
      - Hct1 maintains APC activity after anaphase and throughout G1
      - It is inhibited by phosphorylation by CdKs
  - **p27**
    - As p27 s phosphorylated and inactivated, G1/S-Cdk and S-Cdk are not inhibited
      - It is Cdk inhibitor CKI in mammals
      - It is inhibited by phosphorylation by CdKs

Regulation of CdK at the time of DNA damage – Role of pRb and p53

- When DNA damage is detected, pRb participates in a mechanism that arrests cell division in G1
- Braking action is regulated by phosphorylation and dephosphorylation

- **Cell cycle arrest due to DNA damage at G1**
  - Damage (DNA breaks) to the cell's DNA triggers a series of events that inactivate CdK2, blocking cell division
  - **MRN complex** (MRE11-RAD50-NBS1) detects damage to the DNA (double-strand break site)
  - Binding of MRN to DNA damage activates two protein kinases, **ATM and ATR**
  - ATM and ATR phosphorylate and activate the transcription factor **p53**
  - Active p53 promotes the synthesis of another protein, **p21**, an inhibitor of CdK2
  - Inhibition of CdK2 stops pRb phosphorylation
  - Phosphorylated pRb continues to bind and inhibit E2F
  - With E2F inactivated, genes essential to cell division are not transcribed
  - Cell division is arrested at G1

Ataxia-telangiectasia mutated and ataxia telangiectasia and Rad3 related proteins are key regulators of DNA damage response (DDR) and maintain genome integrity in eukaryotic cells

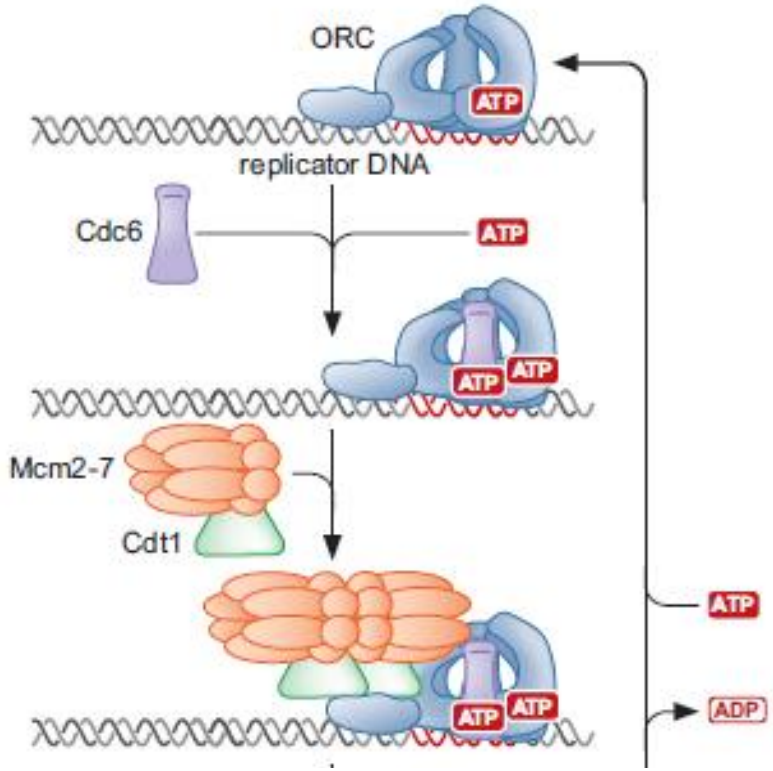
Cell Cycle Control System contd. – Role of cyclin dependent kinase (CdKs) - Proteins phosphorylated by CdKs or Substrates of CdKs

CdKs regulate cell division by phosphorylating critical proteins

- Few proteins that are phosphorylated by activated Cdk protein (CdK targets) are
  - DNA replication proteins – ORC, MCM2-7, Cdc6, Cdt1
  - Proteins required for DNA repair
  - ATP-driven contractile machinery acting during cytokinesis
    - CdK phosphorylates a small regulatory subunit of myosin, causing dissociation of myosin from actin filaments and inactivating the contractile machinery
  - Proteins that regulate microtubule behavior (influence assembly and dynamics of mitotic spindle)
  - Hct-1
  - P27
  - Cdc20 (activating subunit of APC)
  - Retinoblastoma protein, pRb
  - Protein tyrosine phosphatase (PTP)
  - Destruction box recognition protein (DBRP)
  - Lamin
  - Condensin II

Eukaryotic helicase loading

- Loading of the eukaryotic replicative DNA helicase is an ordered process that is initiated by the association of the ATP-bound **origin recognition complex (ORC)** with the **replicator (origin of replication called autonomously replicating sequence)**
- Once bound to the replicator, ORC recruits ATP-bound **Cdc6** and two copies of the **Mcm2-7 helicase** bound to a second helicase loading protein, **Cdt1**



- This is the formation of **pre-replication complex (pre-RC)** – It represents **replicator selection**
- Phosphorylation of above proteins by CdK marks **origin activation or firing**

- **Replicator selection occurs in G1 phase when CdK level is low**
- **Origin activation occurs in S phase when CdK levels are high**