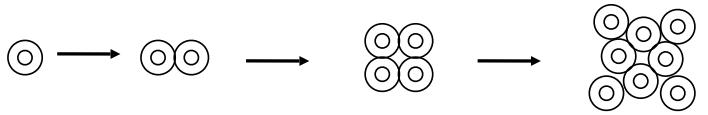
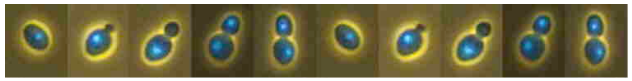
OUTLINE OF HARTWELL, CHAPTER 19
(discussed today and on Wednesday 10/22)

- The normal control of cell division
  - The cell cycle
  - Molecular signals
  - Machinery
  - Checkpoints that regulate passage through the cell cycle
- How cancer arises from defects in cell cycle control
  - General cellular phenotypes associated with all cancers
  - The clonal nature of tumors
  - Mutations in protooncogenes and tumor suppressor genes
- Comprehensive example describing the progression from low-grade brain tumors to aggressive brain cancer

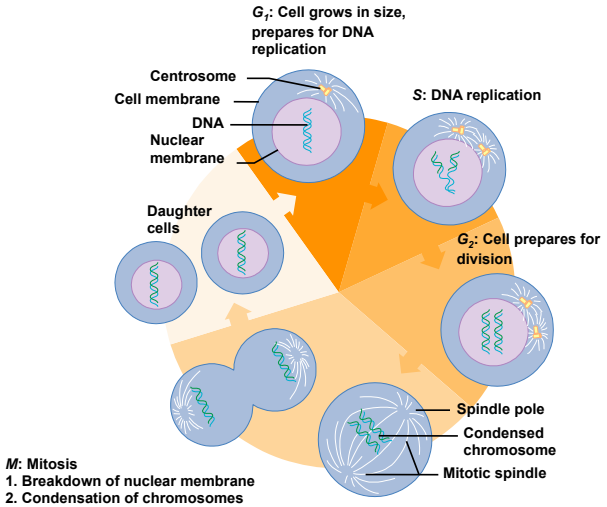
Growth and division are carefully coordinated





2 successive cell cycles in the budding yeast
Saccharomyces cerevisiae

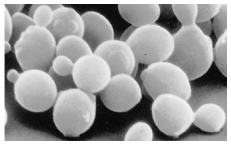
The cell cycle is the series of events that occurs between one mitotic division and the next



The molecular basis of cell cycle control was worked out using complementary approaches in different systems…

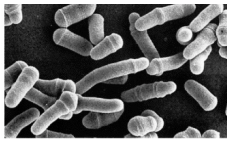
2001 Nobel Prize in Medicine: CELL CYCLE!!

Lee Hartwell



Budding Yeast

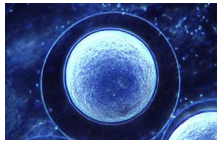
Paul Nurse



Fission Yeast

Genetic identification of molecules that regulate the cell cycle in all eukaryotes

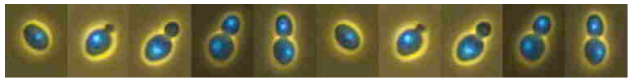
Tim Hunt



Sea Urchin Eggs

Biochemical discovery and identification of cyclin proteins

Genetic experiments in budding yeast revealed the regulatory molecules that control cell cycle progression



blue = DNA

Budding yeast cell cycle stages can be recognized by cell morphology (bud size) and nuclear division

Yeast grow as haploid or diploid organisms
Can identify recessive mutations in haploids and carry out complementation analysis in diploids

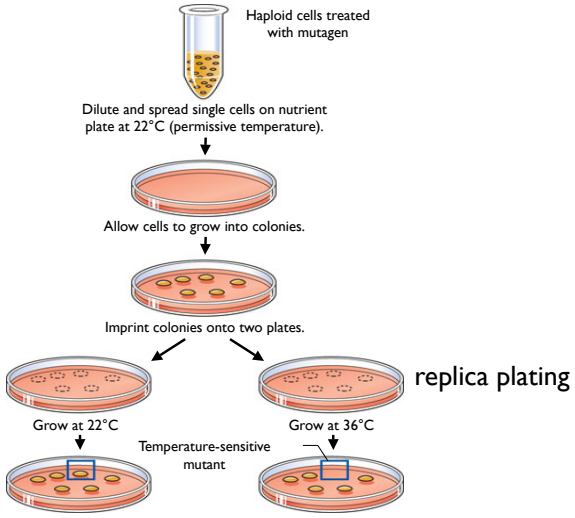
"Conditional" mutations allow you to study regulators of essential processes, like the cell cycle

Conditional mutations allow the encoded protein to function under one condition - e.g., lower temperature - while inhibiting its function under another condition, in this case, high temperature.

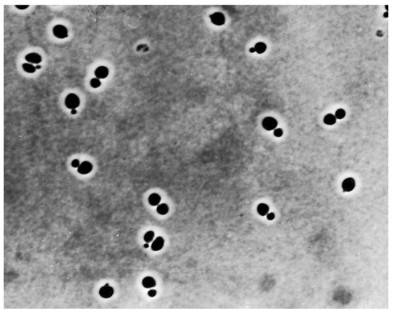
Such mutations are special alleles, often caused by missense mutations that destabilize the protein or its interaction with other proteins

Because these alleles are rarer than general loss-of-function alleles, they are most often isolated in organisms that enable rapid high-throughput screens, such as budding and fission yeast, bacteria, or phage.

Isolation of temperature-sensitive mutations in essential yeast genes (including cell cycle genes)

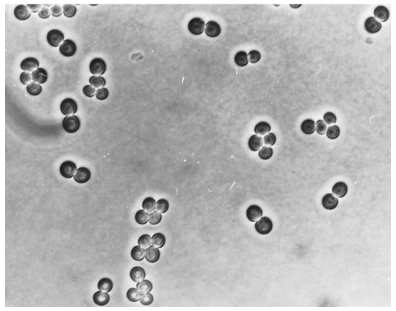


Temperature-sensitive mutations in cell cycle control genes cause all cells to arrest at a specific stage



permissive temperature (22˚C)

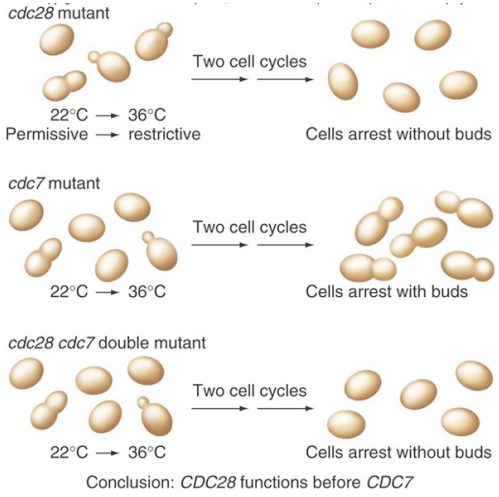
Cells grow asynchronously, and all stages of the cell cycle can be observed



restrictive temperature (36˚C)

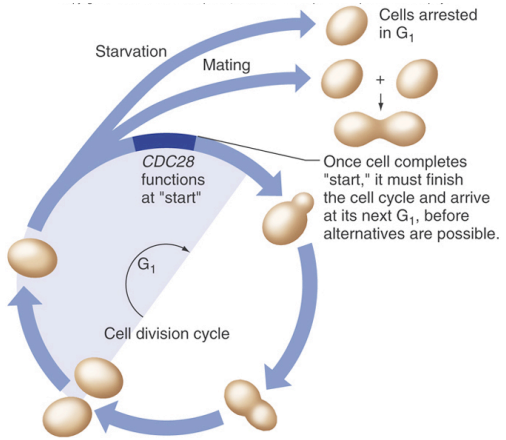
Cells arrest at a particular stage of the cell cycle. Here, they are all "large budded cells."

Epistasis experiments with double mutants make it possible to order the activities of cell cycle genes



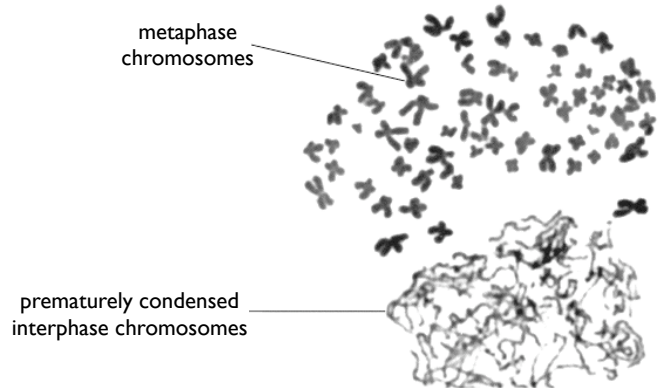
Conclusion: *CDC28* functions before *CDC7*

The budding yeast *CDC28* gene encodes an essential cell cycle regulator that controls the first key step in the cell cycle



The protein encoded by *CDC28* is a Cyclin-Dependent Kinase (CDK). These kinases control multiple cell cycle steps, and depend on cyclin proteins for their function.

Biochemical experiments had previously identified "Mitosis Promoting Factor" = MPF



Cell fusion experiments in the 1970s showed that a factor in mitotic cells can induce premature chromosome condensation in an interphase cell → **MPF**

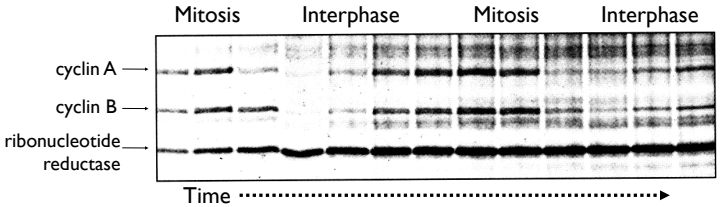
Biochemical experiments had previously identified "Mitosis Promoting Factor" = MPF

Biochemical purification of MPF revealed that it is a **protein kinase** with 2 subunits:

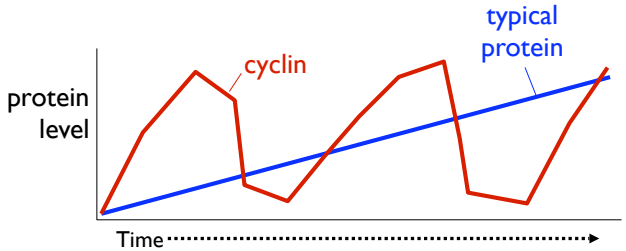
1) CDK1 = cyclin-dependent kinase 1
   = budding yeast Cdc28 = fission yeast Cdc2
   - Induces mitosis by phosphorylating specific downstream targets on serine and threonine

2) cyclin B
   = budding yeast Clb2 = fission yeast Cdc13
   - regulatory subunit that activates cdk1
   - abundance oscillates during the cell cycle

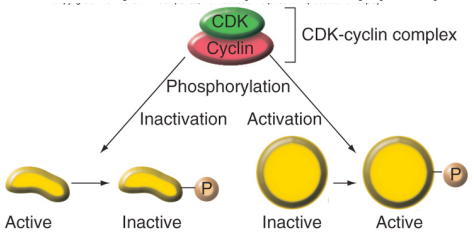
Cyclin PROTEIN synthesis and degradation drive the cell cycle



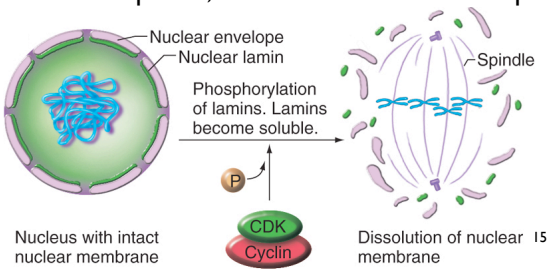
Newly synthesized proteins labeled with ^{35}S-methionine



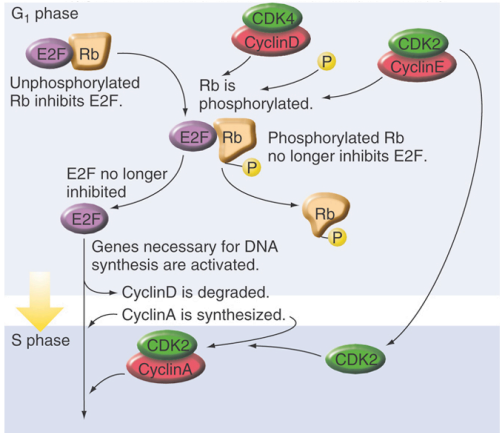
CDKs control the activity of other proteins by phosphorylating them



Phosphorylation of target proteins can change the behavior of large cellular complexes, such as the nuclear envelope



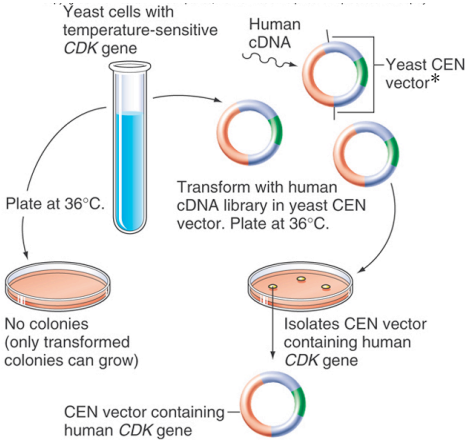
Phosphorylation of transcription factors
(or, in this case, an inhibitor of a transcription factor)
can also lead to activation or repression of other genes



This example shows how CDK4 and CDK2 control the G_1–>S transition in mammalian cells by activating DNA synthesis

Uncontrolled proliferation results from mutations in the *Rb* gene, (the E2F inhibitor shown in the previous slide), resulting in retinoblastoma

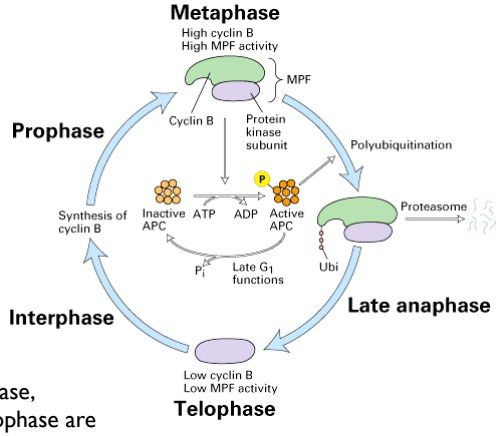
Rescue experiments identified the human versions of yeast cell cycle genes



*CEN vectors are single-copy, unlike the 2-micron plasmid I previously described that is used for high-copy suppressor screens

The cell cycle is driven by protein synthesis and degradation

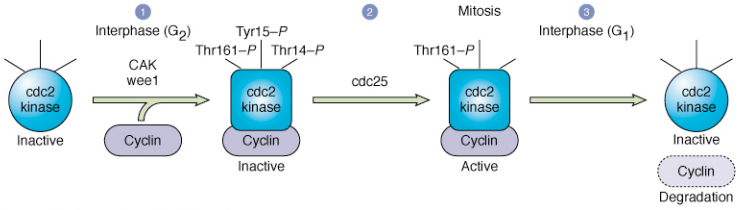
Cyclin B induces its own destruction via the Anaphase Promoting Complex (APC)



Note:
prophase, metaphase, anaphase, and telophase are stages of mitosis.

CDK activity is controlled by:

1) positive regulation by cyclin protein levels
2) inhibitory phosphorylation of the kinase subunit



Multiple levels of regulation allow more flexible control of the cell cycle and provide inputs for **checkpoint control**

Numerous "checkpoints" exist to ensure that the cell cycle does not proceed under potentially dangerous conditions

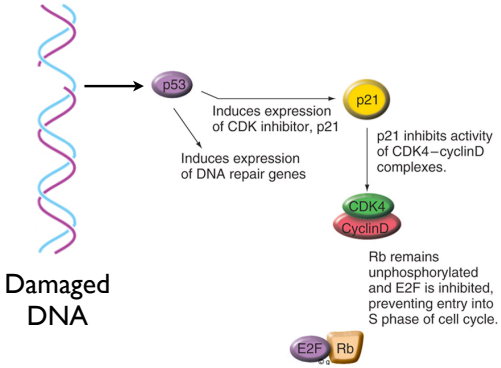
Checkpoints involve...

1. A mechanism to detect errors or problems in a cellular process (e.g., chromosome integrity, spindle attachment)
2. A reversible signal that inhibits cell cycle progression

Checkpoints slow down or arrest the cell cycle to enable cells to fix damage before proceeding

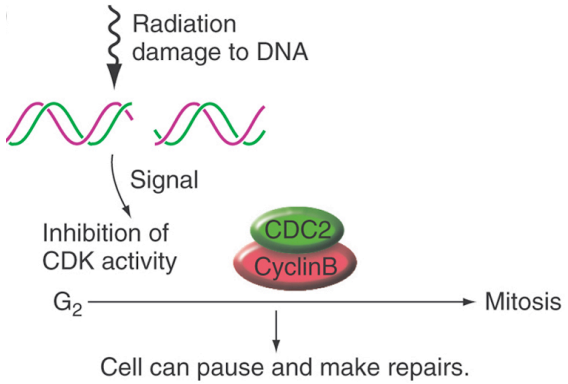
- Checkpoint mechanisms may be dispensable for a given cell division, but they are critical for the fidelity of ongoing cell division.
- If a cell is unable to fix the damage, it may undergo apoptosis
- Mutations in checkpoint genes have been linked to cancer predisposition and progression

The G_1–>S transition is inhibited by DNA damage

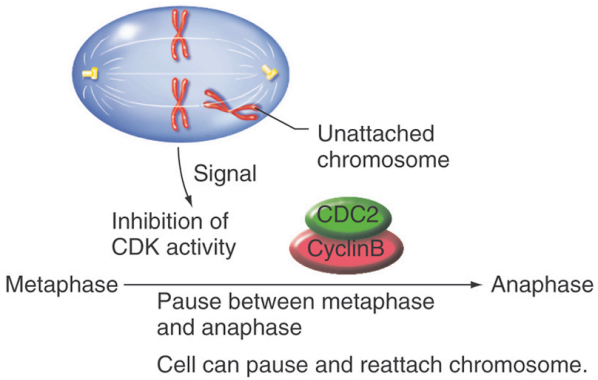


- p53: transcription factor that induces expression of DNA repair genes and CDK inhibitor p21
- p53 pathway activated by ionizing radiation or UV light (causing DNA damage) during G1 phase delays entry into S phase
- DNA is repaired before the cell cycle continues.
- If DNA is badly damaged, cells may commit suicide (programmed cell death, or apoptosis).

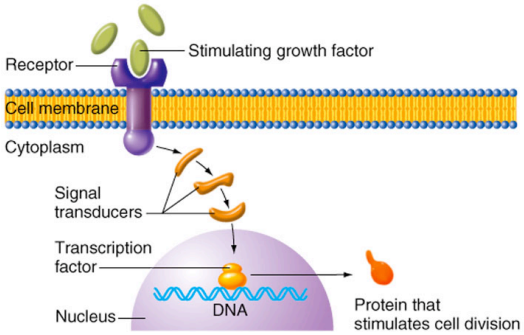
The G_2–>M transition is also inhibited by DNA damage



The Metaphase–>Anaphase transition is inhibited when one or more chromosomes fails to attach to the mitotic spindle



The cell cycle is also regulated by *extrinsic* factors that enable cells to respond to their environments



Cancer results when cells no longer regulate their growth and division appropriately