CHAPTER 14

CELLULAR REPRODUCTION

OBJECTIVES

- Outline and define the stages in the cell cycle: M, G_1, S, G_2 and, occasionally, G_0.
- Describe the methods by which the lengths of the various stages were determined.
- Describe the control of the cell cycle, emphasizing the participation of protein kinases and cyclins in the process.
- Discuss the role of maturation-promoting factor and its associated cyclin(s) and describe some of the experiments that have clarified its composition and function.
- Emphasize the idea of checkpoints in the cell cycle and their importance to cell survival.
- Describe mitosis and cytokinesis pointing out the distinguishing features of each stage.
- ~~Summarize what is known about the generation of forces required to move chromosomes during mitosis and meiosis.~~
- ~~Compare and contrast the mechanisms of cytokinesis in plant and animal cells.~~
- ~~Describe meiosis emphasizing the differences between the various stages.~~
- ~~Discuss the functional reasons for meiosis, pointing out the reasons for the reduction division of Meiosis I.~~
- ~~Describe what is known about the mechanisms of genetic recombination during meiosis.~~
- Emphasize the similarities of and differences between mitosis and meiosis.

LECTURE OUTLINE

Cellular Reproduction: Background

I. The 3rd tenet of Cell Theory – new cells originate only from other living cells; happens via cell division

II. Multicellular organisms – countless divisions of a single-celled zygote produce an organism of astonishing cellular complexity & organization
   A. Cell division does not stop with formation of mature organism, but continues throughout its life
   B. Tens of millions of cells undergo division at any given moment in an adult human
   1. This amount of division is needed to replace cells that have aged or died
   2. Old, worn-out blood cells are removed and replaced at the rate of ~100 million/minute
      3. Importance of cell replacement becomes apparent after exposure to high radiation levels, which interfere with cell division & cause death by radiation poisoning (like those who sealed Chernobyl)

III. Each dividing cell is called a mother cell & its descendants are called daughter cells
   A. Mother cell transmits copies of its genetic information to its daughter cells, which become the next cell generation
   B. Daughter cells then become mother cells to their own daughter cells, etc. – thus the process is called cellular reproduction

IV. Cell division occurs in all organisms, but is different in prokaryotes & eukaryotes; this chapter concentrates on eukaryotes – it is used to reproduce cells & also reproduces new organisms through cellular gametes

A. Cell division links parents to their offspring, living species to their extinct ancestors & humans to the earliest, most primitive cellular organisms

The Cell Cycle: Introduction

I. Cycle starts with cell division & ends with daughter cell formation or death – the stages through which a cell passes from one cell division to the next constitute the cell cycle

II. Two major cell cycle phases - based on cell activities readily visible in light microscope

A. M phase – M for "mitotic"; this stage includes mitosis (duplicated chromosomes are separated into 2 nuclei) & cytokinesis (entire cell & its cytoplasm divide into 2 daughter cells)

1. Only a small percentage of cells in a tissue or cell culture are seen to be in mitosis at any given time, suggesting that cells spend the majority of their time in interphase
2. In mitosis, the cell is focused on activities necessary for cell division; usually lasts only ~1 hour
3. Macromolecular synthesis during mitosis is relatively inactive & largely shut down
4. Length of M = % of cells in population in mitosis or cytokinesis **x** cell cycle length

B. Interphase - occupies bulk of cycle: may extend for days, weeks or longer, depending on cell type & conditions; divided into G_1 (first gap), S (synthesis) & G_2 (second gap)

1. Numerous preparations for upcoming mitosis occur during interphase, including replication of cell's DNA; cell often grows in volume during interphase
2. Does active metabolic functions (glucose oxidation, replication, transcription, translation)

III. When does replication occur? – originally, it was thought that replication occurred throughout interphase; in the early 1950s, studies proved this idea wrong

A. Studies were done on asynchronous cultures, whose cells are randomly distributed throughout cell cycle

1. Briefly pulse (30 min) cultured cells with ^{3}H-thymidine —> fix a sample of the cell population, dry it on a slide, examine it by autoradiography
2. Only a certain percentage of cells have radioactive nuclei, but no cell nuclei that were mitotic at the time of fixation are labeled; these cells were not engaged in DNA synthesis during the labeling period
3. These mitotic cells replicated their DNA before the labeling period; this same result is observed even if labeling is allowed to continue one to a few hours before sampling
4. Conclude that there is a definite time period (G_2; second gap) between end of DNA synthesis & the start of M phase
5. Duration of G_2 = the time between start of pulse & the appearance of labeled mitoses; first labeled mitotic figures contain DNA in last stages of replication at start of ^{3}H-thymidine incubation

B. DNA replication occurs during a period of cell cycle called **S phase** - can measure its length directly; labeled mitoses numbers rise to plateau, then fall off

1. S phase is also the period when the cell makes additional histones that will be needed as the cell doubles the number of nucleosomes in its chromosomes
2. In an asynchronous culture, the percentage of cells engaged in a particular activity is an approximate measure of the percentage of time that this activity occupies in the cells' lives

3. If one knows the length of the entire cell cycle, the S phase length can be calculated directly from the % of cells whose nuclei are radioactively labeled during a brief pulse with ^{3}H-thymidine

C. M phase length – can be calculated from the percentage of cells in the population that are seen to be engaged in mitosis or cytokinesis

D. If one adds up G_2 + S + M lengths, it is apparent that there is an additional period in cell cycle yet to be accounted for, G_1 (first gap) phase; its length = cell cycle length - (M + S + G_2); wedged between M & S

The Cell Cycle: Cell Cycles *in Vivo*

I. Three broad categories of cells

A. Cells that are highly specialized & lack the ability to divide (nerve cells, muscle cells, RBCs) - once differentiated, they remain in that state until they die

B. Cells that normally do not divide but can be induced to begin DNA synthesis & divide when given an appropriate stimulus

1. Liver cells can be induced to proliferate by the surgical removal of part of the liver
2. Lymphocytes can be induced to proliferate by interaction with an appropriate antigen

C. Cells that normally possess a relatively high level of mitotic activity - tissues subject to continual renewal by continual production of new cells

1. Spermatogonia that give rise to the male gametes
2. Hematopoietic stem cells that give rise to red & white blood cells
3. Cells at the base of epithelia that line body cavities & the body surface
4. The relatively unspecialized cells of apical meristems located near the tips of plant roots & stems

II. Lengths of cell cycles are highly variable, particularly at different stages of development

A. <30 min in rapidly dividing cells of cleaving embryos (other than mammals, which cleave slowly)

B. Several months in slowly growing tissues, like mammalian liver

C. Rapidly growing human cells typically divide every 12 - 36 hours

III. Of the 3 interphase stages - G_1 is most variable, although S & G_2 can exhibit major differences

A. With a few exceptions, cells that have stopped dividing (temporarily or permanently; in culture or in the body) are present in a stage preceding the initiation of DNA synthesis

1. Cells arrested in this state (majority of cells in the body) are usually said to be in G_0 state (a special G_1; name distinguishes it from typical G_1 cells that may soon enter S phase

B. A cell must generate an internal signal to go from G_1 to S; once the signal is generated, the cell invariably completes the round of DNA synthesis & then continues through mitosis

Control of the Cell Cycle: Background and the Fusion of Mitotic Cells

I. Study of cell cycle is important in basic cell biology & has enormous practical implications in combating cancer, a disease that results from a breakdown in a cell's ability to regulate its own division

II. Potu Rao & Robert Johnson (1970, Univ. of Colorado) - fused cells in different stages of cycle & asked does cytoplasm contain regulatory factors that act on nucleus? —> began to explain cell cycle regulation

A. G_1 cells + S cells —> G_1-donated nucleus starts replication —> S cell cytoplasm has diffusible factors (replication signal or signals) that stimulate the initiation of DNA synthesis in G_1-phase nuclei
B. G_2 cells + S cells —> G_2-phase nuclei did not initiate another round of DNA synthesis; G_2-nuclei have already replicated their DNA & can no longer respond to initiation factors present in S-cell cytoplasm
    1. The basis for this finding is that the initiation of replication requires the assembly of a prereplication complex, which can only occur during early G_1

III. Other cell fusion experiments suggest that transition from G_2 to M was also induced by cytoplasmic factors
A. Fusion of mitotic cells with cells in other cell cycle stages —> mitotic cells induced chromatin compaction in nucleus of nonmitotic cell as if mitotic phase was dominant over other cell cycle phases
B. G_1 cells + M cells —> G_1-donated nuclei undergo **premature chromosomal condensation** to form a set of elongated compacted chromosomes
C. G_2 cells + M cells —> G_2-donated chromosomes also did premature chromosome compaction, but, unlike G_1 nuclei, the compacted chromosomes were visibly doubled reflecting fact that replication had occurred
    1. However, G_1 & G_2 packing ratios were never as high as that of true mitotic chromosome
D. S cells + M cells —> S-phase chromatin compacted, but since replicating DNA is very sensitive to damage, S-phase nuclear compaction led to pulverized chromosomal fragment formation
    1. Did not get intact, compacted chromosomes
E. Concluded that both transitions (G_1 —> S & G_2 —>M) were under positive control (both were induced by the presence of some stimulatory agent)

Control of the Cell Cycle: The Role of Protein Kinases

I. Cell fusion experiments revealed the existence of factors that regulated cell cycle, but provided no information about the biochemical properties of these factors
A. The nature of the agents that trigger replication & promote entry into mitosis/meiosis were first gained in studies of oocytes & early embryos of frogs in frog & invertebrates
B. Cell entry into M phase is initiated by a 2-subunit protein kinase (PK) called **maturation-promoting factor** (**MPF**)
    1. Catalytic subunit - moves PO_4^{-3} from ATP to certain serines/threonines of specific protein substrates
    2. Regulatory subunit – cyclin; since its concentration rises & falls predictably as cell cycle progresses; activated MPF kinase —> cell enters into M phase; only after [cyclin] reaches critical level
C. Cyclin levels determine MPF activity
    1. Low cyclin concentration —> kinase lacks cyclin subunit & thus is inactive so MPF activity is low
    2. When cyclin concentration rises —> the kinase is activated, causing cell to enter M phase

D. These results suggested that:
    1. Progression of cells into mitosis depends on an enzyme whose sole activity is to phosphorylate other proteins
    2. Activity of this enzyme is controlled by a subunit whose concentration varies from one cell cycle stage to another

II. MPF-like enzymes [**cyclin-dependent kinases** (**Cdks**)] in other cells (yeast, mammalian cells) have been studied over past decade; they are involved in M phase & are the key agents orchestrating cell cycle activities

A. Yeast cells very useful – temperature-sensitive mutants used to study cell cycle processes; grow fairly normally at lower, permissive temperature & restrictive at higher temperatures where they show defect

1. Temperature-sensitive mutants are excellent for studying roles of genes whose encoded proteins perform indispensable functions
2. Studies focus on 2 distantly related yeasts: *Saccharomyces cerevisiae* (reproduce through buds at 1 end of cell) & *Schizosaccharomyces pombe* (fission yeast; elongates, splits into 2 equal-sized cells)
3. Cell cycle regulation molecular basis has been remarkably conserved through eukaryote evolution
4. If gene involved in cell cycle control is found in one of the yeast species, homologues are sought & usually found in genomes of higher eukaryotes, including humans

B. Research on yeast cell cycle genetic control began in 1970s in 2 labs: Paul Nurse et al. (U. of Oxford) & Leland Hartwell et al. (U. of Washington) who worked on fission vs. budding yeast, respectively

1. Both found a gene that, when mutated, would cause growth of cells at elevated temperature to stop at certain points in cell cycle
2. Gene product was called *cdc2* in fission yeast & *CDC28* in budding yeast & was eventually found to be homologous to the catalytic subunit of MPF; it was a cyclin-dependent kinase
3. Concluded from research on yeast cells & many different mammalian cells that progression through cell cycle is regulated at distinct stages, one near the end of G_1 & one near the end of G_2
4. These are points in cell cycle where the cell is committed to starting a crucial event, either initiating replication or entering mitosis

III. Example in fission yeast: commitment process for S & mitosis (M phase) entry involves cyclic availability of different cyclins, but same Cdk (cdc2) – first transition point to S phase in yeast & mammals

A. First transition point is called START - just before G_1 end; passage through START requires cdc2 activation by $\geq$1 G_1 cyclins (their levels rise during late G_1)

1. Once cell passes START, it is irrevocably committed to replication & ultimately completing cell cycle
2. Activation of cdc2 by G_1 cyclins in fission yeast leads to replication initiation at sites where prereplication complexes had previously assembled

B. Mammalian cells pass through comparable point during G_1 (called the **restriction point**), at which time they become committed to DNA replication & ultimately to completing mitosis

1. Prior to restriction point, mammalian cells require presence of growth factors in their culture medium, if they are to progress in cell cycle
2. After they have passed the restriction point, these same cells will continue through the remainder of the cell cycle without external stimulation

IV. Commitment process for mitosis (M phase) entry involves cyclic availability of cyclins different from those needed to enter S, but same Cdk (cdc2) – 2nd transition point (just before G_2 end) & 3rd commitment

A. Requires cdc2 activation by mitotic cyclins (different from those at START); Cdks containing a mitotic cyclin (e.g., MPF) phosphorylate substrates needed for cell to enter mitosis:

B. G_2-activated, Cdk-phosphorylated cytoplasmic proteins start dynamic changes in organization of both chromosomes & cytoskeleton that characterize the shift from interphase to mitosis like those below:
   1. Nuclear proteins (histone H1) - phosphorylation may help compact chromosomes
   2. Nuclear lamins - phosphorylation leads to disassembly of nuclear envelope

C. Cells make a 3rd commitment during the middle of mitosis, which determines whether they will complete cell division & reenter G_1 of the next cycle
   1. Exit from mitosis & entry into G_1 depends on rapid decrease in Cdk activity that results from a plunge in mitotic cyclin concentration

V. Cyclin-dependent kinases, which are described as the engines that drive the cell through its various stages, are regulated by a number of factors that operate in combination to brake or accelerate a process, including:
   A. Cyclin concentration
   B. Cdk phosphorylation state
   C. Cdk inhibitors
   D. Controlled proteolysis
   E. Subcellular localization

Control of the Cell Cycle: Factors That Regulate Cyclin-Dependent Kinases (Cdks)

I. Cyclin concentration – Cdks are activated by association with a regulatory subunit or cyclin; the presence in the cell of a particular cyclin follows the activation of transcription of the gene encoding that cyclin
   A. Different cyclin genes are transcribed at different stages during the cell cycle
   B. When a cyclin is present in the cell, it binds to the Cdk catalytic subunit, causing a major change in that catalytic subunit's conformation
   C. X-ray crystallography - cyclin binding causes a flexible loop of the Cdk polypeptide chain to move away from the opening to enzyme's active site; allows Cdk to phosphorylate its protein substrates

II. Cdk phosphorylation state – cyclin binding is not sufficient by itself to turn on Cdk's kinase activity
   A. For activation, the Cdk subunit must also be phosphorylated at a critical threonine residue (Thr 161); done by another protein kinase (CAK [Cdk-activating kinase])
   B. On the other hand, phosphorylation of a key tyrosine residue (Tyr 15) inhibits the kinase's activity; this inhibitory phosphate is added by another kinase called wee1
      1. Phosphorylation by wee1 during G_2 inactivates the cdc2 enzyme; the enzyme remains deactivated until the end of G_2 when the inhibitory Tyr 15 phosphate is removed by a phosphatase (cdc25)
      2. The removal of the Tyr 15 phosphate activates the Cdk, driving the yeast cell into mitosis
   C. The balance between weeI kinase & cdc25 phosphatase activities, which determines whether the cell will remain in G_2 or progress into mitosis, is regulated, in turn, by other kinases & phosphatases
   D. The involvement of a series of enzymes in activation & inhibition of Cdks provides a number of targets at which information from inside & outside the cell can alter cell cycle progress

III. Cdk inhibitors – Cdk activity can be blocked by a variety of inhibitors; in budding yeast, a protein (Sic1) acts as Cdk inhibitor during G_1; if Sic1 degraded —> cyclin-Cdk present in cell initiates DNA replication

IV. Controlled proteolysis – cyclin concentrations oscillate during each cell cycle leading to changes in Cdk activity; cells regulate cyclin concentrations & other key cell cycle proteins
   A. They do this by adjusting both the synthesis rate & destruction rate of the molecule at different points in the cell cycle (degradation is accomplished via the ubiquitin-proteasome pathway)
   B. Cell cycle regulation requires 2 classes of multisubunit complexes (SCF & APC complexes) that function as **ubiquitin ligases**
      1. They recognize proteins that have been targeted for degradation & link them to polyubiquitin chain —> ensures their destruction in a proteasome
   C. SCF complexes are active from late G_1 through early mitosis & mediate the destruction of G_1 cyclins, Cdk inhibitors & other cell cycle proteins
      1. These proteins become targets for SCF after they are phosphorylated by the protein kinases (i.e., the Cdks) that regulate the cell cycle
      2. Mutations that inhibit SCFs from mediating proteolysis of key proteins, like G_1 cyclins or the Sic inhibitor, can prevent cells from replicating their DNA
   D. The APC complex acts in mitosis & degrades a number of key mitotic proteins, including the mitotic cyclins; destruction of these cyclins allows a cell to exit mitosis & enter a new cell cycle

V. Subcellular localization – cells contain a number of different compartments in which regulatory molecules can either be united with or separated from the proteins with which they interact
   A. Subcellular localization is a dynamic phenomenon characterized by movement of cell cycle regulators into different compartments at different stages
   B. Example: cyclin B1 (a major animal cell mitotic cyclin) shuttles between nucleus & cytoplasm until G_2, when it accumulates in the nucleus just prior to the onset of mitosis
      1. Nuclear accumulation of cyclin B1 is facilitated by phosphorylation of a cluster or serine residues that reside in its nuclear export signal (NES)
      2. Phosphorylation at this site presumably blocks subsequent export of cyclin back to cytoplasm
      3. If nuclear accumulation of cyclin is blocked, cells fail to initiate cell division

~~Control of the Cell Cycle: Control of the Cell Cycle in Mammalian Cells~~

~~I. As in yeast, successive waves of synthesis & degradation of different cyclins play key role in driving mammal cells from one stage to next, **however……..**~~
   ~~A. Unlike yeast cells (with only 1 Cdk), mammal cells make several different versions of this protein kinase~~
   ~~B. The pairing between individual cyclins & Cdks is highly specific; only certain combinations are found~~
      ~~1. Cyclin E-Cdk2 complex drives the cell into S phase~~
      ~~2. Cyclin B1-Cdk1 complex drives the cell into mitosis~~
   ~~C. Cdks do not always stimulate activities, but can also inhibit inappropriate events – cyclin B1-Cdk1 activity during G_2 prevents the inappropriate replication of DNA~~

~~II. Studies aimed at identifying the roles of various cyclins & Cdks in mammalian cells have used genetically engineered (knockout) mice that lack a functional gene for that particular protein~~
   ~~A. The phenotypes of these mice depend on the gene that has been eliminated~~
      ~~1. Mice lacking Cdk1 or cyclin B1 do not survive; thus these genes are essential for cell proliferation~~

2. Mice lacking a gene encoding several other Cdks or cyclin subunits develop surprisingly well; suggests that other members of gene family can take over the functions of those that are lacking

B. Despite this genetic redundancy, the absence of any of these cell cycle regulatory proteins produces distinct abnormalities

1. Cdk4-deficient mice are much smaller than controls; stems from drop in level of whole body's cell division; also diabetic due to specific failure in differentiation of pancreatic insulin-secreting cells
2. Mice lacking a gene for cyclin D1 also exhibit growth retardation, but display a particular lack of cell proliferation during development of the retina

Control of the Cell Cycle: Checkpoints, Kinase Inhibitors and Cellular Responses

I. Ataxia-telangiectasia (AT) – rare, recessive genetic disorder; irradiated cells go into mitosis despite damage

A. Characterized by a host of diverse symptoms, including a greatly increased risk of certain types of cancer; the basis of the first 2 symptoms below have yet to be determined

1. Unsteady posture (ataxia) resulting from degeneration of nerve cells in cerebellum
2. Permanently dilated blood vessels (telangiectasia) in face & elsewhere
3. Susceptibility to infection
4. Cells with an abnormally high number of chromosome aberrations

B. Late 1960s - AT patients died during radiation therapy; they were extremely sensitive to ionizing radiation; patients' cells are sensitive as well, lacking crucial protective response seen in normal cells

1. When normal cells are subjected to treatments that damage DNA (ionizing radiation or DNA-altering drugs), their progress through cell cycle stops while the damage is repaired
2. If normal cell is irradiated during cell cycle G_1 phase, it delays progression into S phase; if cells are irradiated in S phase, further DNA synthesis is delayed; cells irradiated in G_2 delay entry into mitosis

II. Leland Hartwell & Ted Weinert (1988) – worked in yeast & proposed that cells possess checkpoints as part of their cell cycle

A. Checkpoints are surveillance mechanisms that halt progress of cell cycle if:

1. Any of the chromosomal DNA is damaged **or**
2. Certain critical processes, such as DNA replication during S phase or chromosome alignment during M phase, have not been properly completed

B. Checkpoints ensure that each of the various events that make up the cell cycle occurs accurately & in the proper order

1. Mostly, the proteins of checkpoint machinery have no role in normal cell cycle events & are only called into action when an abnormality appears
2. Genes encoding checkpoint proteins were first found in screens for mutant yeast cells that kept going through cell cycle, despite suffering DNA damage or other abnormalities that caused serious defects

III. Checkpoints are activated throughout the cell cycle by a poorly understood system of sensors that recognize DNA damage or cellular abnormalities

A. If a sensor detects the presence of a defect, it triggers a response that temporarily arrests further cell cycle process

B. The cell can then use the delay to repair the damage or correct the defect rather than continuing on to the next stage
   1. This is especially important because mammalian cells that undergo division with genetic damage run the risk of becoming transformed into a cancer cell
   2. If the DNA is damaged beyond repair, the checkpoint mechanisms can transmit a signal that leads to the death of the potentially hazardous cell

C. The presence of a single break in one of cell's DNA molecules is sufficient to cause cell cycle arrest
   1. The gene responsible for AT (*ATM*) encodes a protein kinase that is activated by certain DNA lesions, particularly double-stranded breaks
   2. A related protein kinase (ATR) is activated by other types of lesions, including those resulting from incompletely replicated DNA or UV irradiation
   3. Both ATM & ATR are part of multiprotein complexes that can bind to damaged DNA; once bound, ATM & ATR can phosphorylate a large variety of proteins that participate in cell cycle checkpoints

IV. How does a cell stop its progress through cell cycle?
A. 2 pathways are available to mammalian cells to arrest cell cycle; both pathways involve the sequential action of a number of proteins
B. Mutant versions of these proteins lead to checkpoint failure & are associated with inherited disorders characterized by an elevated risk of developing cancer
   1. There is no known human disease characterized by mutations in the *ATR* or *CHK1* genes
   2. When either of these two genes are knocked out in mice, the animals die as early embryos whose cells exhibit extensive chromosome abnormalities
   3. It is presumed that human embryos carrying homozygous *ATR* or *CHK1* mutations are not viable

~~V. Arrest pathway #1 - the entry of a cell into mitosis is triggered by the removal of inhibitory phosphates from the Cdk by the phosphatase Cdc25~~
~~A. When a G_2 cell detects the presence of DNA damage caused by UV irradiation, ATR kinase is activated & phosphorylates a checkpoint kinase, called Chk1~~
~~B. Once activated, Chk1 phosphorylates Cdc25 on a particular serine residue (Ser 216 in humans)~~
~~C. Once this serine is phosphorylated, the Cdc25 molecule becomes a target for a special adaptor protein that binds to the Cdc25 phosphatase in the cytoplasm~~
~~D. This interaction inhibits Cdc25's phosphatase activity & prevents its reimportation into nucleus~~
~~E. The absence of Cdc25 from the nucleus leaves the Cdk in an inactive state & the cell is stuck in G_2~~

VI. Arrest pathway #2 - damage to DNA also leads to synthesis of proteins that directly inhibit the cyclin-Cdk complex that drives the cell cycle; ATM is involved in this checkpoint mechanism
A. Cells exposed to ionizing radiation in G_1 synthesize a protein called p21 (molecular mass of 21 kD) that inhibits the kinase activity of the G_1 Cdk & thus prevents the cells from entering S phase
B. ATM phosphorylates & activates another checkpoint kinase (Chk2) that phosphorylates transcription factor (p53), which leads to transcription & translation of *p21* gene & subsequent inhibition of Cdk
   1. ~50% of all human tumors show evidence of mutations in the *p53* gene, which reflects its importance in the control of cell growth

C. p21 (see above) is 1 of at least 7 known Cdk inhibitors - a different Cdk inhibitor (p27) & another cyclin-Cdk complex interact as well
1. The p27 molecule drapes itself across both subunits of the cyclin A-Cdk2 complex
2. This changes the conformation of the catalytic subunit & inhibits its kinase activity
3. In many cells, p27 must be phosphorylated & then degraded before progression to S phase can occur

VII. Cdk inhibitors, like p21 & p27, are also active in cell differentiation; just before they differentiate, cells of all types (muscle, liver, blood, etc) typically withdraw from the cell cycle & stop dividing
A. Cdk inhibitors are thought to either allow or directly induce cell cycle withdrawal
B. Study Cdk inhibitors with genetically engineered knockout mice unable to make one of them
1. *p27* gene knockout mice – distinctive phenotype; larger than normal mice; certain organs (thymus gland, spleen) contain a significantly greater number of cells than those of a normal animal
2. In normal mice, thymus & spleen cells make relatively high levels of p27; it is thought that absence of p27 in p27-deficient animals allows cells to divide several more times before differentiation

~~C. Certain Cdk inhibitors seem to prevent uncontrolled growth that can lead to cancer development - most apparent when Cdk inhibitor is not made so that checkpoint control is disturbed - ex.: Cdk inhibitor p16~~
~~1. Gene encoding Cdk inhibitor p16 is often deleted in a variety of human tumors~~
~~2. Knockout mice lacking p16 Cdk inhibitor gene exhibit greatly increased incidence of cancer~~
~~3. Mimicking effects of such Cdk inhibitory proteins could lead to synthesis of new anticancer drugs that block uncontrolled cell growth~~

M Phase: Mitosis and Cytokinesis

I. Mitosis - from Greek *mitos*, for thread; named in 1882 by German biologist Walther Flemming to describe threadlike chromosomes that mysteriously appeared just before a cell divided in two
A. What we know about M phase is largely based on years of animal & plant observations & research
B. Mitosis is process of nuclear division in which replicated DNA molecules of each chromosome are faithfully partitioned into 2 nuclei
1. Mitosis is usually accompanied by cytokinesis – dividing cell splits in two, partitioning cytoplasm into 2 cellular packages
2. The 2 daughter cells are genetically identical to each other & mother cell after mitosis & cytokinesis
C. It maintains chromosome number & generates new cells for organism growth, maintenance & repair
D. Happens in diploid or haploid (fungi, plant gametophytes, a few animals like male bees [drones]) cells
E. Most metabolic activities (transcription, translation) are curtailed; cell is unresponsive to external stimuli; virtually all cell energy is devoted to one activity - chromosome segregation

~~II. Our understanding of events that occur during mitosis has been greatly aided by the use of extracts prepared from frog eggs; extracts contain all materials needed to support mitosis (histones, tubulins, etc.)~~

A. May use original frog egg nucleus present in egg or a foreign nucleus added to egg extract
   1. Add foreign chromatin (or DNA) to egg extract —> chromatin converted into mitotic chromosomes, which are divided by a mitotic spindle that assembles spontaneously in cell-free mixture
B. Can study role of particular protein by removing that protein from egg extract by immunodepletion (addition of antibody against it) & determining whether the process can continue in its absence

III. Usually divided into 5 distinct stages - prophase, prometaphase, metaphase, anaphase, telophase

A. Each is characterized by a particular series of events
B. Each stage represents a segment of a continuous process - division into arbitrary phases is done only for the sake of discussion & experimentation

The Stages of Mitosis: Prophase

I. During prophase, duplicated chromosomes are prepared for segregation & mitotic machinery is assembled
   A. Formation of mitotic chromosomes
   B. Formation of mitotic spindle
   C. Dissolution of nuclear envelope and fragmentation of cytoplasmic organelles

II. Mitotic chromosome formation
   A. Interphase cell nucleus contains tremendous lengths of chromatin fibers; this extended state is ideal for transcription & replication, but not for segregation into daughter cells
      1. Chromosomes are converted into much shorter, thicker structures by chromosome compaction or condensation that occurs during early prophase
      2. Interphase chromatin is organized into fibers ~30 nm in diameter; mitotic chromosomes are made of similar types of fibers as seen in EM of whole chromosomes isolated from mitotic cells
   B. Thus, chromosome compaction does not alter the nature of a chromatin fiber, but rather its packaging
      1. Treat mitotic chromosomes with solutions to solubilize histones & most nonhistone proteins
      2. After treatment, EMs of the treated chromosomes reveal a structural framework or scaffold that retains the basic shape of the mitotic chromosome
      3. DNA loops attach at their base to the nonhistone proteins that form chromosome scaffolding
      4. During interphase, scaffold proteins are dispersed within nucleus, probably as part of nuclear matrix

C. In recent years, research on chromosome compaction has focused on an abundant multiprotein complex called **condensin**
   1. Its proteins were found by incubating nuclei in frog egg extracts & identifying the proteins that associated with chromosomes as they underwent compaction
   2. Removal of any of the condensin proteins from the extracts prevented chromosome compaction

D. How does condensin induce compaction? – supercoiled DNA fills a much smaller volume than relaxed DNA; supercoiling may play role in compacting chromatin into tiny volume of mitotic chromosome
   1. In presence of topoisomerase & ATP, condensin can bind to DNA *in vitro* & curl it into positively supercoiled loops
   2. This fits with the observation that chromosome compaction at prophase *in vivo* requires topoisomerase II, one of the major nonhistone proteins of the mitotic chromosome
   3. Condensin is activated at mitosis onset by phosphorylation of several of its subunits by the Cdk-cyclin responsible for driving cells from G_2 into mitosis
   4. Presumably, condensin is one of targets through which Cdks trigger cell cycle activities

E. Due to compaction, mitotic chromosomes appear as distinct, rodlike structures; each one is made of 2 mirror-image sister **chromatids** formed during replication in previous interphase
   1. Before replication, DNA of each interphase chromosome becomes associated along its length with a multiprotein complex called **cohesin**
   2. After replication, cohesin functions as a physical bridge that holds the 2 sister chromatids together through G_2 & into mitosis, when they are ultimately separated
   3. Condensin & cohesin have a similar structural organization – EMs suggest that cohesin adopts a circular configuration with a latch at one end that can open & close the ring

F. In vertebrates, cohesin is released from chromosomes in 2 distinct stages
   1. Most of the cohesin dissociates from the chromosome arms as they become compacted during prophase; dissociation is induced by phosphorylation of cohesin subunits
   2. Release of remaining cohesin from centromeres is delayed until anaphase

G. After phosphorylation, the chromatids of each mitotic chromosome are held relatively loosely along their extended arms, but much more tightly at their centromeres, where cohesin stays bound
   1. Mutations in any of the genes encoding cohesin subunits can cause sister chromatids to separate from one another prematurely during mitosis
   2. Sister chromatids are also linked by their DNA molecules, which fail to completely separate from one another during replication
   3. Due to these 2 types of connections (cohesin & DNA), sister chromatid segregation at anaphase requires a protease to cleave cohesin complex & topoisomerase II to unlink the DNA molecules

III. Centromeres and kinetochores – the most notable mitotic chromosome landmark is **primary constriction**, an indentation that marks the position of the centromere
   A. Centromere is residence of highly repeated DNA sequences that are binding sites for specific proteins
   B. At outer surface of centromere of each chromatid is proteinaceous, buttonlike structure (**kinetochore**) – seen in sections through a mitotic chromosome
      1. Kinetochore assembles on centromere during prophase
      2. It serves as chromosome attachment site for dynamic MTs of mitotic spindle & as the residence of several MT-based motor proteins & as a key component of an important mitotic checkpoint

IV. Mitotic spindle formation – as cells move from G_2 to mitosis, G_2 MTs undergo sweeping disassembly, then reassemble forming mitotic spindle with focus at **centrosome**, a special animal cell MT-organizing structure
   A. Rapid disassembly of interphase cytoskeleton is thought to be accomplished by inactivation of proteins that stabilize MTs (MT-associated proteins or MAPs) & activation of proteins that destabilize MTs

B. Centrosome cycle as it progresses in concert with cell cycle - animal cells after mitosis have 1 centrosome with 2 centrioles situated at right angles to one another
   1. Each centriole of centrosome starts its replication in cytoplasm at the onset of S phase as DNA replication begins in nucleus; process starts as centrioles move apart within centrosome
   2. Soon, a small daughter centriole appears next to each preexisting (maternal) centriole oriented at right angles to it; later, MTs of new centriole elongate, bringing the centriole to full length
   3. At the start of mitosis, the centrosome splits into 2 adjacent centrosomes, each containing a pair of mother-daughter centrioles
   4. The start of centrosome duplication at G_1-S transition is triggered by phosphorylation of a centrosomal protein by cyclin E-Cdk2, the same agent that triggers the onset of DNA replication
   5. Centrosome duplication errors can lead to abnormal cell division & may help cancer develop

C. First stage of spindle formation in typical animal cell – MTs appear in sunburst arrangement (aster) around each centrosome during early prophase
   1. Phosphorylation of key proteins by newly active mitotic Cdk increases centrosome MT-nucleating activity at mitosis; the mitotic Cdk controls G_2 to M progression
   2. Aster formation is followed by centrosomes separating from each other & their subsequent movement around the nucleus to the opposite ends of the cell
   3. Centrosome separation is driven by motor proteins associated with the adjacent MTs
   4. As centrosomes separate, MTs stretching between them increase in number & elongate
   5. Eventually, they reach points opposite one another & establish 2 poles of bipolar mitotic spindle
   6. After mitosis, one centrosome is distributed to each daughter cell

D. Centrosomes are not essential components in formation of bipolar mitotic spindle in all cells
   1. Some animal cells (early mouse embryo) & most higher plant cells lack centrosomes, yet all of these cells undergo relatively typical mitosis
   2. Mitotic spindles even form in mutant *Drosophila* cells lacking centrosomes or in mammalian cells in which centrosome was experimentally removed
   3. Although these mutant or surgically altered cells form a mitotic spindle & segregate their chromosomes normally, they do not continue to proliferate
   4. Many of these cells fail to undergo cytokinesis & those that divide do not continue into S phase of next cell cycle
   5. It appears that centrosomes play an essential role in cell cycle progression even if it doesn't involve spindle formation

E. In cells without centrosomes, spindle forms by very different pathway than in cell having a centrosome – rather than being nucleated at poles, the MTs of spindle are nucleated near the chromosomes
   1. Once polymerized, the minus ends of MTs are brought together (focused) at each spindle pole through the activity of molecular motor proteins
   2. Cells have fundamentally different mechanisms to achieve similar end results; if one is rendered inactive, the alternate mechanism can finish the job (redundancy)

V. Nuclear envelope dissolution & cytoplasmic organelle partitioning
   A. Nuclear envelope breakdown occurs at prophase end; it allows spindle-chromosome interaction since spindle forms in the cytoplasm & chromosomes compact in the nucleus
      1. Classical view of nuclear envelope breakdown – nuclear envelope is fragmented into a population of small vesicles that disperse throughout mitotic cell

B. This view has been challenged in recent years by mammalian cell studies that suggest that the nuclear envelope is torn apart mechanically by forces exerted through MTs & molecular motors
   1. Events begin with the association of cytoplasmic dynein with the outer surface of nuclear envelope
   2. Dynein molecules then move along MTs toward their minus ends (toward centrosome) pulling on attached nuclear envelope, which forms deep invaginations in the vicinity of centrosomes
   3. These invaginations containing a nearby centrosome have been seen for years in mitotic cell EMs
   4. Nuclear envelope invagination on one side of nucleus is proposed to produce a stretching force (tension) at the opposite side of the nucleus, which tears open nucleus
   5. The opening in the membrane spreads & eventually produces fragments that are transported along MTs away from chromosomes & toward centrosomes
   6. The disintegration of nuclear envelope provides access to the chromosomes by MTs of the spindle

C. Some membranous cytoplasmic organelles remain relatively intact through mitosis - mitochondria, lysosomes, peroxisomes, plant chloroplasts

D. Golgi complex partitioning mechanism during mitosis has been controversial in recent years - 3 views of what happens; ultimately, we may learn that different cell & organism types use different mechanisms
   1. Forward (anterograde) membrane/material movement from ER to Golgi stops during prophase, but vesicle movement in opposite (retrograde) direction continues
      a. Thus Golgi complex contents are incorporated into ER & Golgi ceases to exist briefly as distinct organelle
   2. In alternate view, Golgi membranes are fragmented to form a distinct population of small vesicles that are partitioned between daughter cells
   3. Third view based primarily on algae & protist studies – entire Golgi complex splits in two, with each daughter cell receiving half of original structure

E. Ideas about fate of ER have changed & are somewhat controversial as well – recent studies on living, cultured mammalian cells suggest that the ER network remains relatively intact during mitosis
   1. This view challenges earlier studies performed largely on eggs & embryos that suggested that ER undergoes fragmentation during prophase

The Stages of Mitosis: Prometaphase

I. Prometaphase - starts with dissolution of the nuclear envelope
   A. During this stage, mitotic spindle assembly is completed
   B. Chromosomes are moved into position at center of cell

II. At prometaphase start, compacted chromosomes are scattered throughout space that was nuclear region
   A. As spindle MTs penetrate central cell region, MT free ends grow & shrink in highly dynamic fashion as if they are searching for chromosome; those that contact a kinetochore are captured & stabilized
      1. Lateral surface (side-wall) of MT makes initial contact with kinetochore, not its free end
      2. Once initial contact is made, some chromosomes then move actively along MT wall powered by motor proteins located in the kinetochore

3. Soon, kinetochore tends to become stably associated with plus end of ≥1 spindle MTs from one of poles; then unattached sister chromatid kinetochore captures its own MTs from opposite spindle pole
4. The 2 sister chromatids become connected by kinetochores to MTs extending from opposite poles

B. Chromosomes not moved directly to spindle center; they oscillate back & forth in both a poleward & anti-poleward direction; ultimately moved by process (**congression**) to spindle center midway between poles
1. Forces needed are generated by motor proteins associated with both kinetochores & chromosome arms
2. As chromosomes move to spindle center, longer MTs attached to one kinetochore are shortened, while shorter ones attached to sister kinetochore are elongated
3. MT elongation & shortening is result of gain or loss of subunits at MT (+) end; remarkably, this dynamic activity occurs while each MT (+) end remains attached to kinetochore
4. Motor proteins at kinetochores may play a key role in tethering MT to chromosome, while it loses & gains subunits
5. Eventually, each chromosome moves into position along a plane at spindle center so that MTs from each pole are equivalent in length

The Stages of Mitosis: Metaphase

I. Starts with chromosomes aligned at spindle equator in a plane (**metaphase plate**); one chromatid attached by its kinetochore to spindle fiber from one pole, other chromatid attached to fiber from opposite pole

II. Metaphase MTs are highly organized array that is ideally suited for the task of separating duplicated chromatids & the MTs of animal cell are divided into 3 groups of fibers, all of which have same polarity
A. Astral MTs - radiate outward from centrosome into region outside the body of the spindle; they help position the spindle apparatus in the cell & determine the plane of cytokinesis
B. Chromosomal (kinetochore) MTs - extend from centrosome to chromosome kinetochores; exert a pulling force on kinetochores during metaphase
1. In mammalian cells, each kinetochore is attached to a 20 – 30 MT bundle, forming a spindle fiber
2. Maintain chromosomes in equatorial plane by tug-of-war between balanced pulling forces exerted by chromosomal spindle fibers from opposite poles
3. During anaphase, chromosomal MTs are needed for movement of chromosomes toward the poles

C. Polar (interpolar) MTs - extend from centrosome past chromosomes; they form a structural basket that maintains spindle mechanical integrity
1. Polar MTs from one centrosome overlap with their counterparts from the opposite centrosome

III. Metaphase appears to be a stage during which the cell pauses for a brief period as if all the mitotic activities suddenly come to a halt, however, analysis shows that important events occur during this time

IV. Microtubule flux in the metaphase spindle - even though chromosomal MTs do not appear to change in length while chromosomes are aligned at metaphase plate, studies suggest they are in highly dynamic state

A. When they use fluorescently labeled tubulin to follow MT's state, subunits are rapidly lost & added from the plus ends of chromosomal MTs, even though these ends are presumably attached to kinetochore
   1. Thus, kinetochore does not act like cap at end of MT, blocking entry or exit of terminal subunits
   2. Instead, it is the site of dynamic activity
B. Since more subunits are added than lost at plus end, there is a net addition of subunits at the kinetochore; meanwhile, the minus end of the MTs experiences a net loss
   1. Thus, subunits are thought to move (treadmill) along chromosomal MTs from kinetochore to pole
   2. Neither the molecular basis nor function of the tubulin subunit poleward flux in a mitotic spindle is well understood

The Stages of Mitosis: Anaphase

I. Starts as sister chromatids of each chromosome split apart & begin movement toward opposite poles – the control of anaphase & its initiation mechanism have been revealed by genetic & biochemical approaches
   A. 2 distinct multiprotein complexes (SCF & APC) act at different stages of the cell cycle to ubiquinate proteins & target them for destruction by a proteasome
      1. SCF acts primarily during interphase
      2. **A**naphase **p**romoting **c**omplex (APC) could be described as the mitotic terminator; it plays a key role in regulating the events that occur toward the end of mitosis
   B. APC contains ~12 subunits, one of which plays a key role in determining which proteins will serve as APC substrate; this "substrate-targeting" subunit is represented by members of a family of proteins
      1. 2 members of the family in budding yeast cells (Cdc20 & Cdh1) play an important role in substrate selection during mitosis
      2. APC complexes having one or the other of these subunits are called APC^{Cdc20} or APC^{Cdh1}
   C. APC^{Cdc20} is activated at the metaphase/anaphase transition
      1. When present as an APC subunit, Cdc20 directs the enzyme complex to ubiquinate a major anaphase inhibitor (**securin**) since it secures the attachment between sister chromatids
      2. Destruction of securin by proteasomes at the end of metaphase releases an active protease (**separase**) that had been bound by the inhibitor
      3. Separase then cleaves a key subunit of the cohesin molecules that hold sister chromatids together
      4. Cleavage of cohesin triggers the separation of the sister chromatids to mark the onset of anaphase
   D. Near the end of mitosis in budding yeast cells, the Cdc20 subunit of APC is replaced by the other substrate-targeting subunit, Cdh1
      1. When Cdh1 is attached to APC, the enzyme completes the ubiquitination of mitotic cyclins
      2. Destruction of these mitotic cyclins leads to a precipitous drop in activity of the mitotic Cdk & the progression of the cell out of mitosis & into the G_1 phase of the next cell cycle

II. Events of anaphase – all metaphase plate chromosomes split synchronously at anaphase onset; the chromatids are now referred to as chromosomes, since they are no longer attached to their duplicates
   A. The chromosomes then begin to migrate poleward

1. Chromosome movement toward pole is accompanied by shortening of MTs attached to its kinetochore
2. As chromosome moves during anaphase, its centromere is seen at its leading edge with the arms of the chromosome trailing behind

B. Chromosomes move at ~1 μm/min (very slow compared to other types of cell movements); completed in anywhere from 2 to 60 min; equivalent to a trip from North Carolina to Italy in ~14 million years
1. Slow rate of movement may ensure that chromosomes segregate accurately without entanglement

III. Anaphase chromosomes exhibit 2 types of movement – anaphase A & anaphase B
A. Anaphase A – movement of chromosomes toward poles
B. Anaphase B – a separate but simultaneous movement; the 2 spindle poles move farther apart
1. Polar MTs from opposite poles overlap in the region of the spindle equator
2. Separation of poles during anaphase B is accomplished by the sliding of overlapping MTs from opposite poles over one another in opposite directions
3. The elongation of the mitotic spindle during anaphase B is accompanied by the net addition of tubulin subunits to the plus ends of the polar MTs
4. Thus, subunits can be preferentially added to polar MTs & removed from chromosomal MTs at the same time in different regions of the same mitotic spindle

IV. Forces required for chromosome movements at anaphase
A. Over the past two or three decades, two broad proposals to explain the forces required for chromosome segregation during anaphase have been debated – both factors likely contribute to force generation
1. Some think that motor proteins provide the necessary forces
2. Others argue that microtubule dynamics is responsible
B. Molecular motors – cytoplasmic dynein & at least 2 kinesin-related proteins have been identified at the kinetochores of mitotic chromosomes
1. Thus, the chromosomes are endowed with all of the motor equipment needed to move themselves from one place to another along a MT
2. Fluorescently labeled antibodies against dynein show that during interphase, the protein is localized in cytoplasm; as cell enters mitosis, the motor protein is found at both spindle poles & kinetochores
3. Cytoplasmic dynein moves along MT surface toward its minus end & thus would tend to move an attached chromosome toward the poles
4. Inhibition of dynein function at anaphase greatly slows chromosome movement, suggesting that this motor protein at least contributes to poleward chromosome migration
C. Poleward movement of chromosomes is accompanied by the obvious shortening of chromosomal MTs – several processes can contribute to this phenomenon
1. It has long been appreciated that tubulin subunits are lost from the plus (kinetochore-based) end of chromosomal MTs during anaphase A
2. The loss of tubulin subunits from the plus end may be aided by the presence at the kinetochore of a type of kinesin that promotes MT depolymerization rather than movement
3. Subunits are also lost from the minus end of these MTs as a result of the continued poleward flux of tubulin subunits that occurs during prometaphase & metaphase
4. The primary difference in MT dynamics between metaphase & anaphase is that subunits are added to MT plus ends during metaphase, whereas subunits are lost from the plus ends during anaphase

5. Addition of subunits to plus ends at metaphase keeps the length of chromosomal fibers constant, whereas the loss of subunits at plus ends during anaphase results in chromosomal fiber shortening
6. The basis for this change in MT dynamics at the kinetochore remains unclear

D. Shinya Inoué (early 1960s; Marine Biological Lab, Woods Hole) said that chromosomal MT disassembly during anaphase was not simply a consequence of chromosome movement, but the cause of it
1. He suggested that disassembly of MTs that compose a spindle fiber could generate sufficient mechanical force to pull a chromosome forward
2. One would not expect dissolution of fiber at one or both ends to provide much pulling force
3. But, calculations show that very little force is needed to move an object as small as a chromosome such a short distance (as little as 10^{-8} dyne; equals the hydrolysis of 20 – 30 ATP molecules)

E. Experimental support for the disassembly model has come from studies in which chromosomes attached to MTs undergo movement as the result of the depolymerization of the MTs
1. Movement of a MT-bound chromosome occurs *in vitro* after dilution of the medium
2. Dilution reduces the concentration of soluble tubulin, which, in turn, promotes MT depolymerization
3. Chromosome movement occurs in the absence of ATP, which would be required if the force were generated by a motor protein
4. This & other experiments suggest that MT disassembly alone can generate enough force to pull chromosomes considerable distances, but whether or not cells use this mechanism is open to debate

F. Even if MT disassembly does not generate forces that move chromosomes, chromosomal fiber shortening is thought to be the slowest step in overall process & thus govern chromosome movement rate
1. Conversely, even if MT motors do not serve as force-generating agents, they would still play a critical role in anchoring kinetochores to chromosomal MT plus ends as they lose subunits

V. The spindle checkpoint – operates at transition between metaphase & anaphase; best revealed when one or more chromosomes fails to align properly at metaphase plate
A. When this happens, the checkpoint mechanism delays onset of anaphase until misplaced chromosome has assumed its proper position along the spindle equator
B. If chromosome segregation were not delayed, the risk of an abnormal number of chromosomes in daughter cells would be greatly elevated
1. In fact, the spindle checkpoint is lacking in many tumor cells
2. They keep dividing while producing cells with an increasing number of chromosome abnormalities

VI. How do cells know if chromosomes have aligned themselves properly at metaphase plate? – cells monitor at least 2 distinct properties of mitotic spindle
A. If metaphase cell has chromosome connected by MTs to only one spindle pole, cell delays anaphase onset until chromosome becomes attached to spindles from both poles & aligned at the equator
1. Unattached kinetochores contain a complex of proteins (best-studied one is Mad2) that mediate the spindle checkpoint
2. The presence of these proteins at an unattached kinetochore sends a "wait" signal to the cell-cycle machinery that prevents the cell from continuing into anaphase

3. Once the wayward chromosome is attached to spindle fibers from both spindle poles & becomes properly aligned at the metaphase plate, the signaling complex leaves the kinetochore
4. This turns off the "wait" signal & allows the cell to progress into anaphase

B. Evidence for above – in mitotic spindle of cell arrested prior to metaphase due to a single unaligned chromosome, the unaligned chromosome kinetochore is the only one that still contains Mad2 protein
1. As long as cell contains unaligned chromosomes, Mad2 molecules can inhibit cell cycle progress
2. Inhibition is achieved through direct interaction between Mad2 & the APC activator Cdc20
3. While Cdc20 is bound to Mad2, APC complexes cannot ubiquinate securin (the anaphase inhibitor), keeping all of the sister chromatids attached to one another by their cohesin "glue"

C. What trait of an unattached kinetochore makes it a binding site for checkpoint proteins like Mad2? – 2 properties distinguish an unattached kinetochore; lack of either one can arrest a cell in metaphase
1. A lack of physical interaction with microtubules **and**
2. A lack of tension that is normally exerted at the kinetochore by attached microtubules

D. Another abnormality that can delay anaphase is the occasional attachment of both chromatids of a chromosome to MTs from the same spindle pole; this leaves the chromosome lacking bipolar tension
1. Kinetochores possess a corrective mechanism that is mediated by an enzyme called Aurora B kinase & thought to respond to a lack of tension
2. The kinase molecules of the incorrectly oriented chromosome phosphorylate an unidentified substrate, which destabilizes MT attachment to both kinetochores
3. Once freed of their bonds, the kinetochores of each sister chromatid have a fresh opportunity to become attached to MTs from opposite spindle poles
4. Aurora B kinase inhibition in cells or extracts leads to chromosome misalignment & missegregation

The Stages of Mitosis: Telophase

I. Telophase is marked by chromosomes collecting in mass as they near their respective poles

II. During telophase, daughter cells return to their interphase condition

A. Nuclear envelope reforms – membranous vesicles attach to chromosome surfaces & fuse laterally with one another forming increasingly larger double membrane envelope

B. Chromosomes disperse until they disappear from view under microscope

C. Vesicles that were once part of ER fuse & begin to reform cell's membranous cytoplasmic network

D. Partitioning of the cytoplasm into 2 daughter cells occurs by cytokinesis

Forces Required for Mitotic Movements

I. Mitosis is characterized by extensive movements of cellular structures

A. Prophase – spindle poles move to opposite ends of cells

B. Prometaphase – chromosomes move to the spindle equator

C. Anaphase A – chromosomes move from spindle equator to its poles

D. Anaphase B – elongation of the spindle

II. Molecular motors are responsible for movements during mitosis
   A. A number of different molecular motors have been identified in different locations in mitotic cells of widely diverse species; all of those thought to be involved in mitotic movements are MT motors
      1. A number of different kinesin-related proteins & cytoplasmic dynein have been shown to be involved in mitosis
      2. Some motors move toward microtubule plus ends, some toward minus ends; one kinesin doesn't move anywhere, but promotes microtubule depolymerization
   B. Motor proteins found at spindle poles, along spindle fibers & within kinetochores & chromosome arms

III. Studies that reveal roles of specific motor proteins
   A. Analysis of phenotypes of cells lacking motor because of mutation in gene coding for part of motor
   B. Injection of antibodies against motor or inhibitors of motor into cells at various stages of mitosis
   C. Depletion of the motor from cell extracts in which mitotic spindles are formed

IV. Tentative interpretations & conclusions about functions of various motor proteins:
   A. Motor proteins located along polar MTs probably contribute by keeping the poles apart
   B. Motors on chromosomes are probably important in chromosome movements during prometaphase, in maintaining chromosomes at metaphase plate & in separating them during anaphase
   C. Motor proteins situated along overlapping polar MTs in the region of the spindle equator are probably responsible for sliding MTs over one another, thus elongating the spindle during anaphase B

Cytokinesis

I. Cytokinesis – process during which cell is divided into 2 daughter cells; usually coordinated with mitosis

II. First hint of cytokinesis – occurs in late anaphase with cell surface indentation in narrow band around cell
   A. As time progresses, indentation deepens & forms furrow completely encircling cell
      1. Furrow plane lies in same plane previously occupied by metaphase plate chromosomes (perpendicular to spindle long axis); ensures that chromosome sets are partitioned into 2 different cells
      2. As one cell becomes two, additional plasma membrane is delivered to cell surface via cytoplasmic vesicles that fuse with the advancing cleavage furrow
      3. Furrow continues to deepen until opposing surfaces meet in cell center, fuse & split cells in two
      4. In some cells like the ctenophore egg, the furrow initiates from only one side
   B. Contractile ring theory (Douglas Marsland, late 1950s) – the force required to cleave cell is generated in a thin band of contractile cytoplasm found in cortex just under plasma membrane of furrow
      1. Large numbers of actin filaments are found in the cortex under the furrow of a cleaving cell aligned in an array parallel to cleavage furrow, seen in microscopic examination
      2. Smaller number of short, bipolar myosin II filaments are interspersed among the actin filaments (identified by their ability to bind anti-myosin II antibodies)

C. Importance of myosin II in cytokinesis is evident from following experiments:
1. Inject anti-myosin II antibodies into dividing cell —> rapid cessation of cytokinesis
2. If cells lack functional myosin II gene —> cannot divide normally into daughter cells, but they do carry out nuclear division by mitosis

D. Force-generating mechanism operating during cytokinesis is thought to be similar to actinomyosin-based contraction of muscle cells
1. Sliding of actin filaments in muscle shortens muscle fiber; in contractile ring, sliding filaments pull cortex & attached plasma membrane into center of cell
2. Contractile ring constricts equatorial region of cell much like a purse string narrows a purse opening

E. Contractile ring assembles rapidly just before cytokinesis & is then dismantled when it ends
1. Actin filaments of contractile ring are made of the same subunits that were previously part of the cytoskeleton in interphase cell
2. Early sea urchin egg studies show that the contractile ring forms in plane midway between the spindle poles, even if one of the poles is displaced by microneedle inserted in the cell
3. Suggests that actin-filament assembly site & thus the cytokinesis plane is determined by signal emanating from spindle poles (thought to travel from spindle poles to cell cortex along astral MTs)
4. When one modifies the distance between the poles & the cortex experimentally, the timing of cytokinesis can be dramatically altered
5. Cultured mammalian cell studies support idea of cytokinesis signal, but suggest that the source of the stimulus in mammalian cells emanates from central part of mitotic spindle, rather than its poles

F. Observations of dividing mammalian cells has revealed a remarkable relationship between the centrosome & cytokinesis
1. The final constriction of a cell by an advancing cleavage furrow is usually delayed for a brief time due to the presence in the cell center of the remnants of the mitotic spindle, the midbody
2. The midbody must be severed before a cell can be split in two
3. During this time, one of the 2 centrioles within each centrosome leaves its position at the spindle pole & migrates across the cell to the midbody where it stays for about 15 minutes
4. It is only after the centriole moves out of the midbody & back toward the centrosome that cytokinesis is completed
5. These findings have led to a model in which the migrating centriole is part of a cytokinesis checkpoint that monitors events at the very end of mitosis
6. If the centriole is missing, or cannot reposition itself as described, the cell is generally unable to complete cytokinesis & reenter interphase

~~III. Cytokinesis in plant cells: formation of the cell plate – plant cells do cytokinesis by a very different mechanism because they are enclosed by a relatively inextensible cell wall~~

~~A. Plant cells must construct an extracellular wall inside a living cell~~

~~1. Wall formation starts in plane at the center of cell & grows outward to meet the existing lateral walls~~

~~2. The formation of new cell wall starts with the construction of a simpler precursor, the **cell plate**~~

~~B. The plane in which the cell plate forms is perpendicular to the mitotic spindle axis, but unlike animals, the plane is not determined by the spindle position~~

~~1. The orientation of the mitotic spindle & cell plate are determined by a belt of cortical MTs, the **preprophase band**; it forms in late G_2~~

2. Even though the preprophase band has disassembled by prophase, it leaves an invisible imprint that determines the future division site

C. The first sign of cell plate formation is seen in late anaphase with appearance of **phragmoplast** in the center of the dividing cell - steps in process below
1. Phragmoplast is made of clusters of interdigitating MTs oriented perpendicular to future plate along with membranous vesicles & electron-dense material; its MTs arise mainly from spindle remnants
2. After phragmoplast forms, small Golgi-derived secretory vesicles move into region, probably transported along MTs, & become aligned along a plane between the daughter nuclei

D. How do Golgi-derived vesicles become reorganized into cell plate? - EMs of rapidly frozen tobacco cells; steps in the process below:
1. Vesicles send out fingerlike tubules that contact & fuse with neighboring vesicles to form an interwoven tubular network in center of cell
2. Additional vesicles are then directed along MTs to lateral edges of network; these newly arrived vesicles continue tubule formation & fusion process, which extends network in an outward direction
3. Eventually, the leading edge of growing network contacts parent plasma membrane at cell boundary
4. Ultimately, tubular network loses its cytoplasmic gaps & matures into a continuous, flattened partition; tubular network membranes become plasma membranes of the 2 adjacent daughter cells
5. Secretory products that had been carried within vesicles contribute to the intervening cell plate
6. Once cell plate is finished, cellulose & other materials are added over time to make mature cell wall

Meiosis: Background and Overview

I. Meiosis - process during which chromosome number is reduced; the cells made only have 1 member of each homologous pair; sexual reproduction includes union of 2 cells each with full haploid chromosome set

A. Why reduction via meiosis needed? - without it, chromosome number would double with each generation, and sexual reproduction would be impossible
1. Doubling of chromosome number at fertilization is compensated by an equivalent reduction in chromosome number at a stage prior to gamete formation
2. The reduction is accomplished by meiosis (coined in 1905 from Greek word meaning "reduction")

B. Meiosis ensures production of haploid phase in the life cycle; fertilization ensures a diploid phase

II. Prior to both mitosis & meiosis, diploid G_2 cells contain pairs of homologous chromosomes, with each chromosome consisting of 2 chromatids

A. During mitosis, each chromosome's chromatids are split apart & separated into 2 daughter nuclei in a **single** division --> cells have homologous chromosome pairs & are genetically identical to their parents
1. But G_1 cell chromosomes no longer contain 2 chromatids

B. Mitosis events contrast with those of meiosis, in which the 4 chromatids of a pair of replicated homologous chromosomes are distributed among 4 daughter nuclei
1. This is accomplished with 2 sequential divisions without an intervening round of DNA replication

2. In first meiotic division, each chromosome (with its 2 chromatids) is separated from its homologue; each cell now contains one member of each pair of homologous chromosomes
3. To ensure that each daughter nucleus formed by meiosis has one member of each homologous pair, chromosomes are paired during prophase I by an elaborate process with no mitotic counterpart
4. During pairing, homologous chromosomes engage in genetic recombination that produces chromosomes with new combinations of maternal & paternal alleles; increases variability
5. Variability important for evolution; species better responds to adverse environmental changes
6. In second meiotic division, the 2 chromatids of each chromosome are separated from one another

III. Eukaryotic life cycle stage at which meiosis occurs varies as does duration of haploid phase - 3 groups

A. Gametic or terminal meiosis – includes all multicellular animals & many protists
1. Meiotic divisions are closely linked to gamete formation; for example, meiosis occurs just prior to the differentiation of the spermatozoa
2. Spermatogonia (mitosis) —> primary spermatocytes (undergo 2 meiotic divisions) —> 4 spermatids differentiate into 4 spermatozoa; male vertebrates - meiosis occurs just prior to sperm differentiation
3. Oogonia (mitosis) —> primary oocytes (undergo greatly extended meiotic prophase I; oocyte grows, fills with yolk/other materials)
4. When differentiation is complete (oocyte has reached essentially the same state as when it is fertilized) —> meiotic divisions then finish
5. Vertebrate eggs are typically fertilized at a stage before meiosis completion (usually at metaphase II); meiosis is completed after fertilization while the sperm still resides in the egg cytoplasm

B. Zygotic or initial meiosis – includes only protists & fungi
1. Meiotic divisions occur just after fertilization, so all cells haploid; process produces haploid spores
2. Spores divide by mitosis to produce haploid adult generation
3. Life cycle diploid stage is restricted to brief period after fertilization when individual is still a zygote

C. Sporic or intermediate meiosis – includes all plants & some algae
1. Meiosis occurs at a stage unrelated to either gamete formation or fertilization
2. Life cycle begins with union of male gamete (pollen grain) & female gamete (the egg)
3. Diploid zygote thus formed undergoes mitosis & develops into a **diploid sporophyte**
4. Sometime during sporophyte development, **sporogenesis** (including meiosis) occurs
5. Spores produced by sporogenesis germinate directly into haploid gametophyte (either an independent stage or a tiny structure kept in ovules, as in seed plants)
6. In either case, the gametes are produced from haploid gametophyte by mitosis

IV. The prelude to meiosis includes DNA replication - premeiotic S takes several times longer than premitotic S

The Stages of Meiosis: Prophase I

I. Prophase I - typically lengthened extraordinarily compared to prophase of mitosis; in human female - oocytes initiate prophase I prior to birth & then enters a period of prolonged arrest

A. Oocytes resume meiosis just prior to the time they are ovulated, which occurs every 28 days or so after an individual reaches puberty
B. Thus, many human oocytes remain arrested in same approximate stage of prophase for several decades
C. The first meiotic prophase is also very complex & is customarily divided into several (5) stages that are similar in all sexually reproducing eukaryotes

II. Leptotene - first prophase I stage; chromosomes become gradually visible in light scope; lasts a few hours
A. There is no indication that there are 2 chromatids in light microscope (but there are since the chromosomes replicated at an earlier stage), can be seen in EM
B. Compaction of chromosomes continues through leptotene until homologues can be seen to associate with one another; this process of chromosome pairing is called **synapsis** & is first event in next stage

III. Zygotene – the second prophase I stage; synapsis (process by which homologues joined) occurs; lasts a few hours
A. A number of questions about synapsis remain unanswered
1. On what basis do the homologues recognize one another?
2. How does the pair become so perfectly aligned?
3. When does recognition between homologues first occur?
B. Homologous DNA regions of homologous chromosomes make contact during yeast leptotene - Nancy Kleckner et al. (Harvard); originally thought this began in zygotene at synapsis start, not leptotene
1. Chromosome compaction & synapsis during zygotene simply make this arrangement visible under scope
2. First step in genetic recombination is incidence of double-stranded breaks in aligned DNA molecules
3. Yeast & mouse studies suggest DNA breaks occur in leptotene, well before chromosomes are visibly paired

C. The above findings are supported by studies aimed at locating particular DNA sequences within the nuclei of premeiotic & meiotic cells
1. Individual chromosomes are thought to occupy discrete regions within nuclei rather than being randomly dispersed throughout the nuclear space
2. Recent studies in yeast show that cells just entering meiotic prophase have each pair of homologues sharing a joint territory, distinct from those shared by other homologous pairs
3. Suggests that homologous chromosomes are paired to some extent before meiotic prophase begins
D. In maize, homologues are paired very early in meiotic prophase, but not as early as in yeast - maize leptotene chromosome telomeres (terminal segments) are distributed throughout nucleus
1. Near leptotene end, telomeres are localized at nuclear envelope inner surface at one side of nucleus
2. This telomere clustering occurs in wide variety of eukaryotic cells & causes chromosomes to look like flower bouquet (bouquet stage); may facilitate chromosome alignment in preparation for synapsis
3. In most organisms, synapsis begins at one end of each homologous pair & progresses along the length of the chromosomes during zygotene

E. Synapsis accompanied by synaptonemal complex (SC) formation, a complex, ladderlike structure made of 3 parallel bars with transverse protein filaments connecting central element with 2 lateral elements
1. Chromatin of each homologue is organized into loops extending from one of the SC lateral elements, which are made primarily of cohesin (presumably binds together the chromatin of sister chromatids)
2. For years, SC was thought to hold each homologous pair in proper position to start genetic recombination between homologous DNA strands, but it is now evident that it is not required
3. SC forms after genetic recombination has been initiated; mutant yeast cells unable to form SC still engage in genetic recombination between homologues
4. It is currently thought that the SC functions primarily as scaffold to allow interacting chromatids to complete crossover activities
5. Complex formed by a pair of synapsed homologous chromosomes is called **bivalent** (referring to 2 homologues) or **tetrad** (referring to 4 chromatids)

F. The end of zygotene is marked by the end of synapsis

IV. Pachytene - starts when synapsis ends; often inordinately long (extended for a period of days or weeks unlike leptotene & zygotene which generally last a few hours)

A. Homologues are held closely together along their length by SC; characterized by a fully formed SC - DNA of sister chromatids is extended into parallel loops

B. Within SC center, a number of electron-dense bodies (~100 nm dia) are seen in EM at irregular intervals
1. These bodies called **recombination nodules**; they contain some of the enzymatic machinery that facilitates genetic recombination, which is completed by the end of pachytene
2. They correspond to the sites where crossing over is taking place as evidenced by the associated synthesis of DNA that occurs during intermediate steps of recombination

V. Diplotene – its beginning is usually recognized by SC dissolution & the tendency of homologous chromosomes of the bivalents to pull away somewhat from each other

A. As they separate, homologous chromosomes are seen to remain attached to each other at specific points by X-shaped structures (**chiasmata; chiasma** is the singular) at crossover sites
1. They are located at sites on chromosomes where crossing-over between DNA molecules from the two chromosomes had previously occurred
2. Chiasmata are formed by covalent junctions between a chromatid from one homologue & a non-sister chromatid from the other homologue; shows extent of genetic recombination

B. Diplotene is an extremely extended phase of oogenesis, during which the bulk of oocyte growth occurs
1. Many spermatocytes & oocytes – diplotene chromosomes become dispersed into a particular configuration not seen at any other time during the organism's life cycle, **lampbrush chromosomes**
2. Lampbrush chromosomes have an axial backbone from which pairs of loops extend out in opposite directions; they arise in pairs since each chromosome consists of a pair of duplicated chromatids
3. Each loop is a projection from a single chromatid, and the two homologues still remain attached to each other at the chiasmata
4. DNA situated between loops is tightly compacted & transcriptionally inactive

5. In contrast, loop DNA is highly extended & the site of intense transcriptional activity; RNAs made are used in protein synthesis during both oogenesis & early embryo development after fertilization

VI. Diakinesis - meiotic spindle assembled & chromosomes are prepared for separation; final stage of meiotic prophase I
   A. In those species in which chromosomes become highly dispersed during diplotene, the chromosomes become recompacted during diakinesis
   B. Diakinesis ends with the disappearance of the nucleolus, nuclear envelope breakdown & tetrad movement to the metaphase plate
      1. In vertebrate oocytes, these events are triggered by an increase in the protein kinase activity of MPF (maturation-promoting factor)
      2. MPF was first identified by its ability to initiate these events, which represent oocyte maturation

The Stages of Meiosis: Metaphase I

I. In most eukaryotes, homologous chromosomes at meiosis I metaphase plate often contain visible chiasmata
   A. In fact, chiasmata hold homologues together as a bivalent during this stage
      1. In humans & other vertebrates, every homologous pair typically contains ≥1 chiasma; longer chromosomes tend to have 2 or 3 of them
      2. It is thought that some mechanism exists to ensure that even smallest chromosomes have at least 1
      3. If there is no chiasma between homologues, they tend to separate after SC dissolution
      4. This premature homologue separation can result in nondisjunction & an abnormal chromosome number in nucleus; chiasmata are probably a way of preventing abnormal chromosome segregation
   B. A mechanism also exists to prevent formation of >2 or 3 chiasmata regardless of chromosome length; inhibition of excess chiasmata is called crossover interference & is thought to be mediated by SC
   C. At metaphase I, the 2 homologous chromosomes of each bivalent are connected to the spindle fibers from opposite poles
      1. In contrast, kinetochores of sister chromatids are connected as a unit to MTs from the same spindle pole
      2. The orientation of maternal & paternal chromosomes of each bivalent on metaphase I plate is random; the maternal member of a particular bivalent has an equal likelihood of facing either pole
      3. Thus, when homologous chromosomes separate during anaphase I, each pole receives a random assortment of maternal & paternal chromosomes
      4. Thus, anaphase I is the cytological event corresponding to Mendel's law of independent assortment; due to independent assortment, organisms can generate a nearly unlimited variety of gametes
   D. Abnormalities in formation of metaphase I spindle trigger the arrest of meiosis by a checkpoint mechanism similar to that in mitosis

The Stages of Meiosis: Anaphase I, Telophase I and Interkinesis

I. Anaphase I – cohesion between chromosome arms is lost; homologous chromosomes of each bivalent separate, while cohesion of joined centromeres of sister chromatids stays strong & they stay together

A. Separation of homologous chromosomes at anaphase I requires the dissolution of the chiasmata that hold the bivalent together

1. Chiasmata are maintained by cohesion between sister chromatids in regions that flank these sites of recombination
2. Chiasmata disappear at the metaphase I – anaphase I transition, as the arms of each bivalent's chromatids lose cohesion

B. Loss of cohesion between the arms is accomplished by proteolytic cleavage of the cohesin molecules in those regions of the chromosome

1. Cohesion between the joined centromeres of sister chromatids remains strong, because the cohesin situated there is protected from proteolytic attack
2. Thus, sister chromatids stay firmly attached to each other as they move toward a spindle pole during anaphase I; each chromosome made of 2 chromatids attached at centromere throughout stage

II. Telophase I – less dramatic changes than mitotic telophase; chromosomes usually disperse a bit, but they do not reach the extremely extended state of interphase nucleus; nuclear envelope may or may not reform

III. Interkinesis - stage between the 2 meiotic divisions; generally short-lived

A. Animal cells in this fleeting stage are called **secondary spermatocytes** or **secondary oocytes**

B. These cells have haploid number of chromosomes (one member of each homologous pair), but they have diploid amount of nuclear DNA, since each chromosome still consists of 2 chromatids

1. Secondary spermatocytes are said to have a 2C amount of DNA, half as much as a primary spermatocyte with a 4 C amount of DNA & twice as much as a sperm cell with a 1C DNA content

The Stages of Meiosis: Meiosis II

I. Meiosis II - very similar to mitotic division, except haploid number of chromosomes involved

II. Prophase II – much simpler than prophase I; chromosomes become recompacted & line up at metaphase plate; if nuclear envelope had reformed in telophase I, it is broken down again

III. Metaphase II – unlike metaphase I, kinetochores of sister chromatids of metaphase II face opposite poles & become attached to opposing sets of chromosomal spindle fibers

A. Oocytes of vertebrates halt here & await fertilization; progression through meiosis stops here

B. Metaphase II arrest is brought about by factors that inhibit cyclin B degradation

1. As long as cyclin B levels remain high within oocyte, Cdk activity is maintained & the cells cannot progress to the next meiotic stage
2. Metaphase II arrest is only released when the oocyte (now called egg) is fertilized
3. Fertilization leads to rapid influx of Ca^{2+} ions, the activation of APC^{Cdc20} & cyclin B destruction
4. Fertilized egg responds to these changes by completing meiosis II; only happens if egg is fertilized

IV. Anaphase II - begins with the synchronous splitting of the centromeres, which hold the sister chromatids together; allows chromosomes to move toward opposite poles of the cell

V. Telophase II - ends meiosis II; chromosomes are once again enclosed by a nuclear envelope; cells have haploid amount (1C) of nuclear DNA (half that in normal G_1 cell) & haploid chromosome number

Genetic Recombination During Meiosis

I. Meiosis reduces chromosome number as required by sexual reproduction; it also raises genetic variability in a population of organisms from one generation to the next - how is this genetic variability introduced?

A. Independent assortment allows maternal & paternal chromosomes to be shuffled as gametes form

B. Genetic recombination (crossing over) allows maternal & paternal alleles on a given chromosome to be shuffled as well

1. Without genetic recombination, alleles along a particular chromosome would remain tied together from generation to generation
2. By mixing maternal & paternal alleles between homologous chromosomes, meiosis generates organisms with novel genotypes & phenotypes upon which natural selection can act

II. Recombination frequency between 2 alleles on a chromosome is proportional to the distance between them

A. This has allowed the mapping of the relative positions of genes along chromosomes in organisms ranging from bacteria to humans

1. Actually, recombination does not occur uniformly across a chromosome; rather each chromosome has "hot spots" where it is most likely to occur & "cold spots" where it is less likely to occur
2. Recombination hot spots are probably sites that are relatively devoid of nucleosomes, which may inhibit access to the recombination machinery

B. T. H. Morgan's early view & that of others (first decade of 1900s) - tension generated by chromosomes twisting around each other during meiotic prophase caused breakage

1. Thought this breakage led to exchange of pieces of chromatids between homologous chromosomes

2. Crossing-over thus would involve actual physical **breakage & reunion** of chromosomal material

C. J. Herbert Taylor (Columbia U.) used grasshopper meiotic chromosomes labeled with ^{3}H-T; eukaryotic crossover exchange via a breakage & reunion mechanism was confirmed using autoradiography

1. Germ cells were exposed to hot DNA precursor during S phase of the previous mitotic division --> at meiotic prophase I, only 1 chromatid of each chromosome was labeled, while the other was not
2. After crossing over had occurred, Taylor did autoradiography --> certain chromatids had exposed silver grains over only part of their length
3. The labeled & unlabeled segments of one chromatid showed a reciprocal pattern to that of another chromatid of the same tetrad
4. This indicated that physical exchange had occurred between labeled & unlabeled chromatids

III. Mechanism of recombination

A. Original view of breakage & reunion was that each homologous chromosome split entirely across into 2 pieces —> a piece from one chromosome was exchanged with corresponding piece from the homologue
1. But meiotic chromosomes contain large numbers of DNA fibers lying side-by-side
2. Thus, breakage of an entire compacted chromatid would sever DNA of each chromatid many times, splitting 100s of genes that could in no way be reunited with counterparts on other chromosome

B. Now know it involves physical breakage of individual DNA molecules & ligation of split ends from one DNA duplex with split ends of the homologous chromosome duplex
1. Mechanism is remarkably precise; occurs between corresponding sites on homologous DNA molecules without loss or addition of a single base pair
2. DNA repair enzymes involved ensure precision by filling in gaps created during exchange process

IV. Simple model of recombination – major steps during recombination in eukaryotic cells

A. 2 DNA duplexes that are about to recombine align next to each other as result of an homology search in which homologous DNA molecules associate with one another in preparation for recombination

B. Once aligned, double-stranded break is introduced into one duplex by an endonuclease (Spo11)

C. Then the gap is subsequently widened by the action of a 5' —> 3' exonuclease
1. In *E. coli*, this step is carried out by a remarkable complex of 3 cooperating enzymes (RecBCD), 2 helicases that unwind the DNA & a nuclease that degrades it
2. As a result of exonucleolytic digestion, the broken strands possess exposed single-stranded tails, each bearing a 3'-OH terminus
3. One of the single-stranded tails leaves its own duplex & invades the DNA molecule of a non-sister chromatid, H-bonding with the complementary strand in the neighboring duplex
4. In *E. coli*, this process (in which a single strand invades an homologous duplex & displaces the corresponding strand in that duplex) is catalyzed by the multifunctional RecA protein
5. RecA protein polymerizes to form a filament that binds along a length of single-stranded DNA & facilitates that strand's invasion of an uncut, homologous double helix
6. Eukaryotic cells have RecA homologues (e.g., Rad51) that are thought to catalyze strand invasion, which activates a DNA repair activity that fills the gaps in the duplex

D. Due to reciprocal exchange of DNA strands, the 2 duplexes are covalently linked to one another to form a joint molecule (**heteroduplex**) that contains a pair of DNA crossovers (**Holliday junctions**)
1. The Holliday junctions flank the region of strand exchange & are named after Robin Holliday, the researcher who proposed their existence in 1964
2. This type of recombination intermediate need not be static structure because the linkage point may move in one direction or another, an event known as **branch migration**
3. This is done by breaking H bonds holding the original pairs of strands together & reforming H bonds between strands of newly joined duplexes (may require recombination enzymes); see it in EM

E. To resolve the interconnected Holliday junctions & restore DNA to 2 discrete duplexes, another round of DNA cleavage must occur
1. Depending upon particular DNA strands cleaved & ligated, 2 alternate products can be generated
2. In one case, the 2 duplexes contain only short stretches of genetic exchange, which represents a noncrossover

3. In the alternate pathway, the duplex of one DNA molecule is covalently joined to the duplex of the homologous molecule, creating a site of genetic recombination, a crossover

F. In addition, the fusion of a maternal & paternal chromosome leads to the formation of a chiasma, which is required to hold the homologues together during meiosis

The Human Perspective: Meiotic Nondisjunction and Its Consequences

I. Meiosis is a complex process & meiotic mistakes in humans appear to be surprisingly common

A. What kinds of mistakes might occur?

1. Homologous chromosomes may fail to separate from each other during meiosis I
2. Sister chromatids may fail to come apart during meiosis II

B. When either of these situations occurs, gametes are formed that contain an abnormal number of chromosomes — either an extra chromosome or a missing chromosome

1. If one of these gametes happens to fuse with a normal gamete, a zygote with an abnormal number of chromosomes forms & serious consequences arise
2. In most cases, the zygote develops into an abnormal embryo that dies at some stage between conception & birth
3. Sometimes, zygote develops into infant whose cells have an abnormal chromosome number (**aneuploidy**); its effect depends on which chromosome or chromosomes are affected

C. The normal human chromosome complement is 46: 22 pairs of autosomes & 1 pair of sex chromosomes

1. An extra chromosome (producing a total of 47 chromosomes) creates a condition called **trisomy**, e.g., a person whose cells contain an extra chromosome 21 has trisomy 21
2. A missing chromosome (producing a total of 45 chromosomes) produces a **monosomy**

II. The absence of one autosomal chromosome, regardless of which chromosome is affected, invariably proves to be lethal at some stage during embryonic or fetal development

A. A zygote containing an autosomal monosomy does not give rise to a fetus that is carried to term

B. One might not expect that possession of an extra chromosome would be life-threatening, but trisomies do not fare much better than monosomic zygotes

1. Of the 22 different autosomes in the human chromosome complement, only people with trisomy 21 survive beyond the first few weeks or months of life
2. Most of the other possible trisomies are lethal during development, whereas trisomies of chromosomes 13 & 18 are often born alive but have such severe abnormalities that they die soon after birth
3. >25% of spontaneously aborted fetuses have a chromosomal trisomy
4. It is thought that many more zygotes carrying abnormal chromosomal numbers produce embryos that die early in development before pregnancy is recognized
5. For every trisomic zygote formed at fertilization, there is presumably an equal number of monosomic zygotes that fare even less well

C. It is estimated that as many as 20% of human oocytes are aneuploid, which is much higher than any other species that has been studied

1. Mouse eggs typically exhibit an aneuploidy level of 1 – 2%
2. It was recently found that exposure of mice to an estrogen-like compound (bisphenol A) used to manufacture polycarbonate plastics greatly raises the level of nondisjunction during mouse meiosis
3. This is the first clear demonstration of a relationship between synthetic compounds present in the environment & meiotic aneuploidy

4. Whether this or other environmental agents contribute to the high aneuploidy rate in human oocytes remains unclear
5. Whatever the reason, meiosis in males occurs with a much lower level of chromosomal abnormalities than in females

III. Even though chromosome 21 is practically the smallest human chromosome, the presence of an extra copy of this chromosome has serious consequences, producing a condition called **Down syndrome**
   A. Symptoms of Down syndrome
      1. Varying degrees of mental impairment
      2. Alteration in certain body features
      3. Circulatory problems
      4. Increased susceptibility to infectious diseases
      5. A greatly increased risk of developing leukemia
      6. Early onset of Alzheimer's disease
   B. All of these medical problems are thought to result from an abnormal level of expression of genes located on chromosome 21

IV. The presence of an abnormal number of sex chromosomes is much less disruptive to human development
   A. Turner syndrome - a zygote with only 1 X chromosome & no second sex chromosome (denoted as XO)
      1. The individual develops into a female, in which general development is arrested in the juvenile stage
      2. The ovaries fail to develop & body structure is slightly abnormal
   B. Since a Y chromosome is male determining, persons with at least one Y chromosome develop as males
      1. Male with extra X chromosome (XXY) develops Klinefelter syndrome, characterized by mental retardation, genitalia underdevelopment, some feminine physical traits (like breast enlargement)
      2. Extra Y (XYY) - physically normal male; often taller than average; controversial claims that XYYs tend to show more aggressive, antisocial & criminal behavior than XY males were never substantiated

V. Likelihood of having a child with Down syndrome rises dramatically with the age of the mother – from 0.05% for mothers 19 years of age to nearly 3% for women over the age of 45
   A. Most studies show no such correlation between the age of the father & the likelihood of having a child with trisomy 21
   B. Estimates based on comparisons of DNA sequences between offspring & parents indicate that ~95% of trisomies 21 can be traced to nondisjunction having occurred in the mother

VI. Abnormal chromosome number can result from nondisjunction at either of the two meiotic divisions
   A. Although these different nondisjunction events produce the same effect in terms of chromosome numbers in zygote, they can be distinguished by genetic analysis
      1. Primary nondisjunction transmits 2 homologous chromosomes to zygote
      2. Secondary nondisjunction transmits 2 sister chromatids (most likely altered by crossing over) to zygote
   B. Studies indicate that most of the mistakes occur during meiosis I

1. In one study of 433 trisomy 21 cases that resulted from maternal nondisjunction, 373 resulted from errors that had occurred during meiosis I & 60 resulted from errors during meiosis II

C. Why is meiosis I more susceptible to nondisjunction than meiosis II? – do not know answer precisely

1. It almost certainly reflects the fact that oocytes of older women have remained arrested in meiosis I for longer periods within the ovary
2. Chiasmata, the visual indicators of genetic recombination, play an important role in holding a bivalent together during metaphase I
3. One hypothesis - meiotic spindles of older oocytes are less able to hold together weakly constructed bivalents than those of younger oocytes
4. Weakly constructed bivalents are those with only one chiasma located near the tip of the chromosome)
5. This increases the likelihood that homologous chromosomes will missegregate at anaphase I

LECTURE HINTS

The Cell Cycle: Introduction

Introduce your students to the cell cycle and its stages. Define for them what happens in each one and about how long each one usually lasts. Describe the experiments that allowed the lengths of each stage to be determined. Outline for students the various types of cell cycles exhibited by different cell types *in vivo*: cells that have lost the ability to divide, cells that usually do not divide, but can if properly stimulated and cells that are normally highly mitotic.

Control of the Cell Cycle

Discuss with the students the evidence for control points in the cell cycle. Two major control points are presently known: the initiation of DNA replication (at the transition between G_1 & S) and the initiation of mitosis (at the transition between G_2 & M). Describe the experiments covered in the text that outline the evidence for the effect of cytoplasmic regulatory factors on nuclear activity. Emphasize the role of protein kinases in this regulation. If time permits, a discussion of maturation-promoting factor (MPF) and the cyclins that alternatively turn it on and off is valuable. I don't recommend going into this story too deeply. The dizzying abundance of kinases, cyclin-dependent kinases, phosphatases, catalytic subunits and regulatory subunits can rapidly bring a glaze to the eyes of your students. The layers of regulation of cell activities is staggering and, thus far, we have only scratched the surface. It is worthwhile, however, to emphasize the importance of these molecules in regulating cell cycle activities.

Emphasize the cell cycle checkpoints and the functional reasons for them. It would not be wise for a cell to divide if sufficient nutrients were not present to support growth. Furthermore, failure to repair DNA damage prior to cell division would be fatal for the cell. It, therefore, makes sense for the cell to delay cell division until sufficient nutrients are present or until DNA damage is repaired. In addition, stress the connection between defects in the mechanisms controlling the cell cycle and the development of cancer. Especially important are proteins that inhibit the cell cycle. Mutants of these proteins can remove any control on the cycle and allow it to continue, when normally it would be dormant.

Mitosis and Cytokinesis

For reasons that I have not yet been able to determine, students are tremendously confused by mitosis and meiosis. This is true no matter how many times these two processes have been

described to them. The most comforting thing I can say is that each time they hear it, the group of students that understands gets larger. Before describing the stages of mitosis, give the class an overview of the purpose of the process - to turn one cell into two exact copies of itself. Differentiate between mitosis (nuclear division) and cytokinesis (the equal parceling out of the cell cytoplasm to daughter cells). Emphasize that while mitosis and cytokinesis are usually tightly linked, there are times when they are separated (embryonic development in *Drosophila*). I have also found it useful to review the concepts of genes and alleles and their relationship to chromosomes before talking about stages of mitosis.

Analogy

The Bicycle Instructions Analogy

I liken a cell's genetic information to the instructions for assembling a bicycle from scratch - with a few twists. Each chromosome can be considered to be a page of instructions for assembling a bicycle. To assemble a complete and functional bicycle, you would need the full assembly instruction manual. Assume that the bicycle in question requires an instruction manual of 23 pages. Assume further that the manufacturer sends two copies of the instructions so that there are two copies of every page. The instructions on each page are not exactly the same. If you were to follow either of the single sets of instructions, you would assemble a working bicycle. You could also correctly assemble the bicycle by following randomly a particular instruction from either one manual or the other, that is by using both manuals but deciding at random which manual to use for each step. For example, you may get to a step that tells you to paint the bike red. The corresponding instruction in the other manual may tell you to paint it blue. It is possible that the instruction for a particular step in one of the manuals is illegible or nonsense or in some other way faulty. You could then go to the corresponding instruction in the other manual and follow it. Only if the same instruction in both manuals were faulty would you have a problem assembling your bicycle. Of course, each page in the instruction manual represents a chromosome, each manual the maternal or paternal set of chromosomes, the two sets of instructions together a diploid organism and a cell with only one set of instructions a haploid cell. Each instruction would represent the allele of a particular gene. If a particular instruction differs in the two sets, the gene would be represented by different alleles on the two chromosomes. Such an individual would be heterozygous for this gene. If both instructions were identical, the individual would be homozygous. This analogy can be pushed pretty far and it can be extended to cover both mitosis and meiosis.

Once you have given the students an overview of mitosis, discuss each stage with them. While you are doing this, show them slides or overheads of the process. Emphasize that mitosis (and meiosis) is a continuum. The stages are to some degree arbitrary divisions. I stress that the dividing cell does not "click" from one stage to another, but instead moves smoothly through the stages (it is analog vs. digital). This makes identification of the stages somewhat difficult. Thus, I give the students traits to look for that will allow them to distinguish between the different stages. For example, prophase is marked by the condensation of chromosomes, the beginning of the dissolution of the nuclear membrane and the disappearance of the nucleoli. Prometaphase is marked by the movement of chromosomes into the center of the cell and the completion of membrane dissolution. Metaphase is easily identifiable because of the alignment of the chromosomes at the metaphase plate. Anaphase begins when the chromatids separate. An

earmark of telophase is the reformation of the nuclear membrane and the dispersion of the mitotic chromosomes.
In addition to describing the mitotic stages, discuss the attachment of chromosomes to the spindle at the centromere/kinetochore, the breakdown and distribution of organelles and the way in which the force that moves chromosomes is generated.

Spend some time discussing cytokinesis and the mechanisms involved. Differentiate between cytokinesis in plants and animals. Talk about the contractile ring of animal cells and the involvement of actin and myosin in the process. If time permits, cover the formation of the cell plate in plants in some detail.

Meiosis

Before describing the actual process of meiosis, distinguish between the purposes of mitosis and meiosis. The purpose of mitosis is to create exact copies of cells. Meiosis is responsible for converting diploid cells into haploid cells. While most students have been told before where meiosis fits into the life cycles of different organisms, you may wish to refresh your students' memories about the stages in the life cycles of organisms during which meiosis occurs and the patterns of the corresponding life cycles (gametic, zygotic, sporic). Ask the class why it is necessary to reduce the number of chromosomes during the production of gametes. Ask them what would happen if there were no reduction division. I often point out to my students that without the reduction division of meiosis, an organism that started with two chromosomes would have 1,048,576 chromosomes after 20 generations. One description that grabs their attention is the image that without reduction divisions, it would not take all that many generations to get a nucleus larger than the planet itself. This is a decidedly impractical situation, even more so in an organism that has more than two chromosomes. Remind them that DNA is replicated before both processes begin. However, meiosis involves two separate rounds of division with no further replication in between, while mitosis consists of only one round.

Describe the stages of meiosis. Emphasize the difference between the two separate rounds of division: meiosis I is the reduction division and meiosis II is similar to mitosis except that a haploid number of chromosomes is distributed to daughter cells. As I do with mitosis, I suggest that you emphasize the features of each stage that allow their identification. Also, focus on the number of chromosomes (haploid vs. diploid chromosome number) and whether they have two chromatids or one. I expect my students to be able to identify the stage of mitosis or meiosis in which a cell finds itself, either from a picture or a description. You may or may not decide to cover the stages of prophase of meiosis I in detail. Because of time constraints, I generally just lump it all together into prophase I after telling my students that it is divided into stages.

Once you have described meiosis, show your class where the process fits into the development of sperm and egg. Emphasize the differences in the two processes: four functional sperm vs. one functional egg and equal cytoplasmic division in sperm vs. unequal division in egg development, for example. Stress the length of time spent in meiosis by the eggs of some species compared to the constant production of sperm, in most cases.

During your coverage of these two processes, drill your students on the number of chromosomes expected in a particular organism at different times or in different cells (somatic vs. germ cells) after telling them what the diploid or haploid number of chromosomes in the organism is.

Genetic Recombination During Meiosis

Emphasize the evolutionary importance of recombination (crossing over). Describe what is known about the mechanism of recombination in prokaryotes. Define what is meant by breakage and reunion and emphasize the precision of the process. Ask the class what might happen if recombination is off by as little as one nucleotide. Describe Holliday junctions and how they result in recombination. Cover the hypothesized variations that may occur in eukaryotes.