C2005/F2401 '07 -- Lecture 19 -- Last Edited:

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Handouts for today: 19A = Meiosis/Mitosis ; 19 B = Meiotic Chromosome Cycle (You'll also need handouts 18B & 18C.)

I. Wrap up splicing and RNA processing in eukaryotes. See previous lecture, topic VI, & handouts 18B & 18C.

II. Problems/issues of Cell Division

A. So we know how prokaryotes divide. How do eukaryotes do it? First let's consider how individual cells (or unicellular eukaryotes) → 2 and then how a multicellular organism → 2.

B. How do individual eukaryotic cells do it? Eukaryotic cell structure and implications

1. Structure

a. Many chromosomes (multivolume encyclopedia as vs one vol.) = genetic material is divided into several pieces.

b. Chromosomes are linear and not attached to anything.

c. Much more DNA per cell and more per piece. 2-5 X 10^7 BP per euk. chromosome (or more); clearly > million; *E. coli* only 3 million BP total and all in one chromosome/piece. Also replication forks move more slowly (so replication takes longer even for same # nucleotides).

d. Chromosome and cell structure is more complex. Entire nuclear & chromosome structure as well as DNA must duplicate.

- Chromosomes are in a separate compartment = the nucleus. In prokaryote, entire cell is one compartment.
- More complex chromosome structure in eukaryotes; DNA is complexed with proteins called histones. Tune in next term for details.

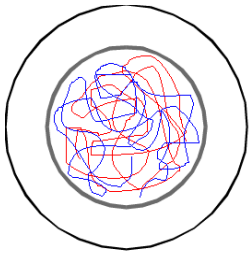
Comparison of Organization of Genetic Information in Eukaryotes & Prokaryotes		
Property of Chromosomes	Prokaryotes	Eukaryotes
Number	one	many
Shape	circular	linear
Attached to something?	yes	no
Size	3 X 10^6 base pairs for *E. coli*	2-5 X 10^7 base pairs/ av. chromosome
In separate compartment?	no (no nucleus)	yes (in nucleus)
DNA associated with histones?	no	yes

2. Resulting Problems -- how will cell get DNA (& histones) doubled in time and distributed properly?

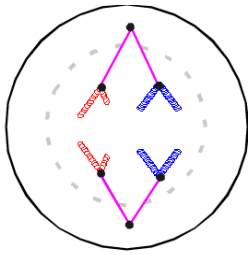
III. Basic Eukaryotic solution -- Have 2 different states of nucleus & DNA, and separate times (& states) for synthesis and distribution. Both states have DNA in double helix and super coiled with proteins. The issue here is super-super coiling.

A. State one -- interphase (between divisions)

1. Chromatin. DNA + associated proteins (mostly histones) form tangled mass called chromatin -- relatively loose coiling of DNA, accessible to polymerases for transcription and replication, not ready to distribute. No distinct structures visible in microscope.



< Cell During Interphase >



< Cell During Division >

2. Nuclear membrane (and nuclear structure) intact -- nucleus organized for RNA and DNA synthesis, processing, transport, etc. = chromatin in separate compartment

3. No spindle -- DNA not attached to anything.

4. All transcription and replication occurs in this stage

B. State two -- during divisions

1. Chromosomes. DNA (+ associated proteins) visible in microscope as individual structures called chromosomes. DNA tightly coiled, easy to distribute but not accessible to enzymes of replic. and transc. (condensed > 10,000 X). Individual balls of string (in this state) vs unwound, tangled mess (between divisions). Chromosomes can look like J's, V's, rods or X's, depending on how the parts are connected and what stage of division you are looking at; see below.

2. Nuclear membrane (compartmental separators) disassembled . Disassembly is temporary -- membrane components not lost, just taken apart into subunits. (Lego castle disassembled -- will be reassembled into two smaller castles after division).

3. Spindle -- have set of fibers attached to chromosomes (and to structures at poles). Assembly of spindle is temporary -- fiber components are not new, but were rearranged to form a new structure. (Building blocks rearranged -- take apart one structure and build another using the same pieces.)

4. No transcription or replication in this stage.

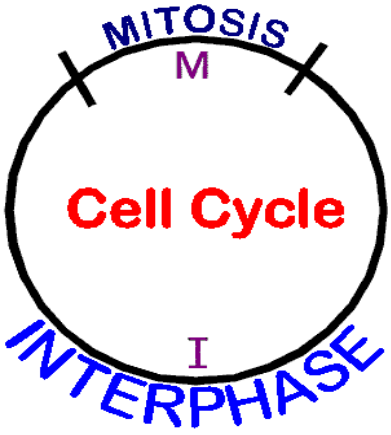
C. Reminder: all eukaryotic DNA is in double helix, supercoiled, AND associated with special proteins called histones at all times -- it's super-supercoiling and association with additional proteins that changes.

IV. Cell Cycle

A. What is it? Look at what goes on during the various stages as a single cell goes from newborn (small; just made by cell division) to double-size = ready to divide again → division → start over. This called the cell cycle. See Becker fig. 19-1 (17-1) or Sadava 9.3.

You can divide the cycle into 2 major stages described above: I (interphase) and M (mitosis or division). The picture below puts the element of time in -- cell goes around and around. The two stages of I and M corresponds to the two states of DNA described above.

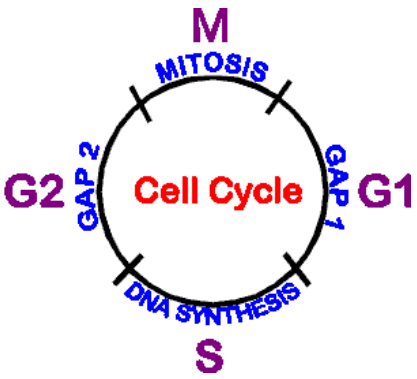
B. When is DNA made? Can I (interphase) be subdivided?



1. DNA made in I, prior to M.

- Pictures: Doubling of DNA prior to M is often implied by pictures of doubled chromosomes at start of M. This was not obvious when M first discovered, because two parts of a doubled chromosome can stick together and look like one structure.
- Terminology: Whether chromosomes are single or double refers to whether the DNA has been replicated yet or not. A 'single' or 'undoubled' chromosome does NOT contain single stranded DNA. It contains double stranded DNA that has not replicated yet. See chromatids and chromosomes below and/or see box on 19A or middle panel of 19B.

2. Stages -- Can I be subdivided? Does DNA replication take all of I?



a. G-1. There is a period in interphase, after division but before DNA made; this is called G-1 or gap 1.

b. G-2. There is a period after DNA is made but before division which is called G-2 or gap 2.

c. S. DNA is made in the middle of interphase -- the period when DNA is made is called S, for Synthesis (of DNA)

Therefore interphase can be divided into G-1, S (period of DNA synthesis) and G-2 as follows (above):

3. How were stages discovered?

If you supply radioactive T (T*) when cell isn't in S, the T* doesn't get into DNA. In other words, cells don't make radioactive macromolecules (from T*) when they are in G-1, G-2 or M. (If supply labeled/radioactive U or aa, it's different. These precursors are made into macromolecules throughout I.)

4. Lengths of stages. FYI only -- Typical lengths of stages for mammalian cells:

S about 8 hrs; M = 1, G2 = 3 and G1 = 3 to 12 (in culture); >12 in adult tissues. G-1 varies most; more details next term.

5. Replication of Eukaryotic DNA -- The replication fork in eukaryotes moves more slowly than the fork in prokaryotes. Therefore getting the DNA replicated in time (since S is so short) is a complex process requiring multiple origins of DNA replication and other details which we will skip for the time being. Next term we will discuss some of the complications and regulation of the stages of the cell cycle. For now we will assume DNA can be replicated properly and packaged into chromosomes (with histones) and we will concentrate on how the DNA is distributed to the daughter cells.

V. Chromosomes, Chromatids and Centromeres.

See handout 19B middle panel, and box on 19A.

Chromosomes at start of cell cycle (before S) contain one double-stranded DNA molecule. Chromosomes after S contain two double-stranded DNA molecules. How are these DNA molecules related and/or connected? (Remember you can't see the chromosomes during interphase.)

- *Terminology:* By the end of the cell cycle, chromosomes are doubled -- each chromosome has two (identical) parts called **chromatids** (sister chromatids) which are connected (by proteins) at a section of the chromosome called a **centromere**. see Sadave 9.7 (9.5)
- *How much DNA per chromatid?* Each chromatid contains one double-stranded DNA molecule.

- *Sister/Sibling Chromatids*: The DNA molecules in sister chromatids are identical because they are the two products of a single semi-conservative DNA replication.
- *How many chromatids per chromosome?* Can be 1 or 2; depends on where cell is in the cell cycle.
  Before S, each chromosome has one chromatid (containing one double-stranded DNA molecule).
  After S, each chromosome has 2 chromatids (each containing one double-stranded DNA molecule).
- *Centromere position*. The centromere = the region where the two sister chromatids are connected. The connection forms at a specific region of the DNA (with a specific sequence). The connecting material itself is protein. See Sadava 9.11 (9.9) The centromere can be at the end of the chromosome or in the middle, so a doubled chromosome containing two chromatids can look like a V or an X.
  The term 'centromere' can refer to the region of the DNA where the connection forms (the centromeric DNA), or to the structure connecting the two chromatids. See the key to 8-2 in the problem book.
- *Double vs single chromosomes.* Whether a chromosome is said to be single or double refers to the number of chromatids per chromosome. Not to whether the DNA in the chromosome is double or single stranded. The DNA is always double stranded.

To review the terminology, try problem 8-0, 8-1, parts A-B, and 8-2 Parts A-C. If you are feeling very confident, try 8-6.

VI. Mitosis

(see handout 19A) First let's go through stages as shown. See Sadava 9.10 (9.8) or Becker 19-20 & 21 (17-19 & 20). When we get to meiosis we'll compare and contrast the two processes (mitosis & meiosis) as listed in table on handout (& see Sadava 9.19 (9.17)). Important points to notice about each stage of mitosis.

- *Interphase:* All DNA is doubled (in S prior to division) **before** M.
- *Prophase:* this stage is reached when you can see chromosomes (as opposed to just chromatin) and nuclear membrane starts to break down. Chromosomes are doubled (2 chromatids/chromosome) but the two sister chromatids can stick together and appear as a single unit. So chromosomes may not *look* doubled (in microscope) even though they are. When they don't look doubled, the centromere is often visible as a constricted region of the chromosome.
- *Metaphase:* Chromosomes achieve the maximum degree of condensation; all chromosomes are lined up in the same plane (metaphase plate) = slice through equator on handout. Idea of mitosis is to separate or segregate sister chromatids, so the chromatids line up in pairs. (In meiosis, chromosomes, not chromatids, will line up in pairs.)
- *Anaphase:* Separate sister chromatids; each chromatid now becomes a full fledged chromosome and is pulled to pole by its centromere. Can appear V or J or rod shaped, depending on position of centromere. (Pulling done by spindle fibers; not shown on handout. For pictures see Sadava 9.9 (9.7) or Becker 19-22, 24 & 25 (17-21, 23 & 24). See Becker for details of spindle and mechanism of chromosome movement if you are interested. More details will be discussed next term. For now, emphasis is on where the genetic material ends up.)
- *Telophase:* Start putting cells back to normal. Start reassembling nuclear membrane, decondensing chromosomes, and starting to divide cytoplasm. (See Sadava 9.12 (9.10) or Becker fig. 19-28 & 29 (17-27 & 28) for how cytoplasm is divided.)
- *Daughter cell stage:* End product of mitosis = two cells with genetic information identical to that of original.

To review mitosis and the cell cycle, do problem 8-13.

VII. Karyotypes

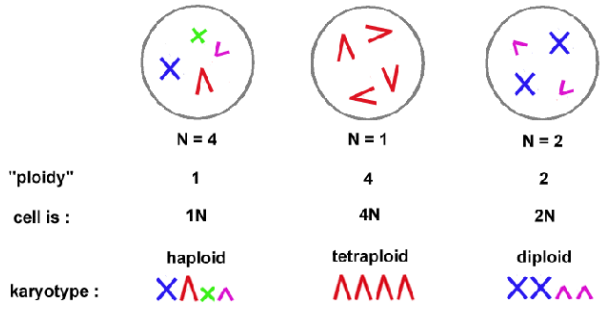
A. What are Karyotypes and how do you get them?

There are drugs that stop cells at metaphase (the drugs interfere with spindle fiber function). So what? This allows you to conveniently collect lots of cells at metaphase and look at the chromosomes.

1. Chromosome squash. Can squash cells in plane of metaphase plate and see all chromosomes spread out. Picture of this = chromosome squash. (See picture below or handout 19B or Sadava 9.15 (9.13), left.)

2. Karyotype. If make squash, cut out each chromosome and line them up in order of size, this = karyotype. Gives standard pattern for each species. (Squash is harder to analyze, if there are a lot of chromosomes, since chromosomes are in random order.) See picture below or Becker fig. 19-23 (17-22) or Sadava 9.15 (9.13).

Chromosome Squashes and Karyotypes



B. What do you see in a normal squash or karyotype? (Banding, and related topics, may not be covered in this lecture; if we don't get to them they will be discussed next time.)

1. Can see number of chromosomes, size and shape (determined by position of centromere) for each chromosome and can identify each individual chromosome by banding techniques. (Banding = procedure to stain chromosomes with standard dyes; different dyes give different patterns of dark and light regions. Each band = block of 100's of genes, not a single gene.)

2. Each species has a standard karyotype with a fixed number of chromosomes. You can use similarities and differences to evaluate relationships between species and to detect certain abnormalities which we will discuss later. Same number in all body (somatic) cells and in each generation.

3. Important general features of a (normal) karyotype

a. "N" -- Number of different types or kinds of chromosomes is called N. For humans, N = 23. See Sadava table 9.1 for typical values of N.

b. Ploidy = number of chromosomes of each type. Cell can be

- haploid -- 1N -- have one of each type of chromosome (for humans, this occurs in gametes -- eggs and sperm)
- diploid -- 2N -- have two of each type of chromosome (for most multicellular organisms, this is the state in most body cells).
- triploid (3N) or tetraploid (4N) -- has 3 or 4 of each type of chromosome. (Higher multiples of N are possible too in plants.)

4. Homologs

a. Definition: Homologs = all the chromosomes of each type. Except for sex chromosomes, homologs = all chromosomes of same size, banding pattern, & position of centromere (shape).

b. Number: There are 2 homologs = 2 of each type of chromosome in diploid cells. One from mom, one from dad.

c. Relationship of genes on homologs; alleles. Homologs (except for sex chromosomes) carry homologous DNA. They carry the same genes, in the same order, in corresponding places (loci), but they do not necessarily carry the same version (allele) of each gene.

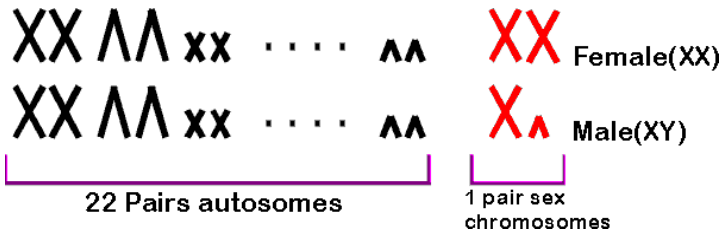
For example, the gene for eye color is in the same place on both homologs, but the "eye color gene" on a particular chromosome could be the blue-determining version or the brown-determining version. Each alternative version of a gene is called an allele. Each homolog carries one allele of the eye color gene. Homologs carry the same genes, but not necessarily the same alleles.

Another example: Consider the gene for the beta chain of Hemoglobin -- the beta chain gene is always in the same position, but the chromosome could carry the Hb A or Hb S allele (version) in the Hb position (locus).

d. Sister chromatids vs homologs: Sister chromatids = 2 halves of a doubled chromosome. Why are they identical? Because they contain the two products of a semi-conservative DNA replication. Homologs **need not be** identical -- each came from a different source (a different parent). Important: be sure you know the difference between homologs (homologous chromosomes) and sister chromatids.

5. Human karyotypes -- *sex chromosomes & autosomes.* See Sadava 9.15 (9.13) for a real human karyotype. Many more examples can be found on the web. (Try the images on Google for a large assortment.) If you want to try making a karyotype for yourself, go to http://bluehawk.monmouth.edu/~bio/karyotypes.htm. For another simulation try http://www.biology.arizona.edu/human_bio/activities/karyotyping/karyotyping.html.

If you do karyotypes on human cells, you will discover that the pattern is different from males and female, as follows:



Both sexes have 22 pairs of chromosomes that look the same regardless of sex, but the 23rd pair is not the same in both sexes. In females, the 23rd pair consists of 2 large chromosomes that look alike. In males the 23rd pair consists of a large and a small chromosome that do not look alike but act as a pair during meiosis. The 22 pairs of chromosomes that are the same in both sexes are called autosomes. The remaining pair are called sex chromosomes, and the big one is called the X chromosome and the little one the Y chromosome. So females are XX and males are XY.

C. What can you see in an abnormal karyotype?

1. Types of visible mutations. Since you can do banding, you can tell all the chromosomes and chromosome regions apart. Therefore you can detect large abnormalities affecting whole chromosomes and/or large blocks of genes (so called "chromosomal" mutations) from looking at karyotypes. Many of these abnormalities are associated with known genetic conditions -- diseases and/or tendencies thereto. What can you see?

a. Rearrangements. You can pick up extra, missing and rearranged pieces. Note these are large changes. You ***can not*** see base changes or even changes of whole genes -- only changes in large sections containing many genes (kilobases not bases) are visible in karyotypes. (If large enough, loss, additions, inversions, or translocations are visible.) Smaller changes must be detected using other methods.

b. Aneuploidy. Since you can tell all the individual chromosomes apart, you can see cases of missing or extra chromosomes. Cells are normally haploid (N), diploid (2N) etc. Cells with extra or missing chromosomes (2N + 1, or N -1, etc.) are called aneuploid.

2. Details of Aneuploidy

a. Terminology. The terms "monosomic" and "trisomic" apply to diploid cells as follows:

(1). Monosomic or monosomy = one chromosome missing = one chromosome has no partner (no homolog), but all other chromosomes still occur in pairs.

(2). Trisomic or trisomy = one chromosome has 3 copies (3 homologs) but all other chromosomes still occur in pairs. Note trisomic is different from triploid: trisomic means 3 copies of one type of chromosome, say #2; triploid means three copies of all the chromosomes.



b. What types of aneuploidy are common?

(1). Trisomy 21. Most aneuploid fetuses abort spontaneously but a few survive to birth. The only autosomal aneuploidy that is not regularly lethal early in life is trisomy 21 or Down's syndrome. (Chromosome 22 may look smaller, but 21 is the autosome with the smallest amount of genetic information.) Individuals who are trisomic for chromosome 21 have multiple developmental problems which usually result in significant mental retardation, distinctive facial features and a tendency to develop Alzheimers at a relatively early age. (The gene coding for the protein that clogs the brain in cases of Alzheimers is on chromosome 21.) All these abnormalities are thought to be due to a "gene dosage" effect. All the gene copies are normal, but trisomics have 3 copies of the genes on chromosome 21 instead of 2. The extra copies of the genes produce extra protein (for a total of 3 doses instead of 2). The extra amount of protein is what messes up development.

(2). Aneuploidy of the sex chromosomes. This is usually not lethal as long as there is at least one X.

a. Examples. Individuals are known who are XXY, XO (O stands for no 2nd sex chromosome), XYY, XXX etc. Humans who are XO are female, but have certain abnormalities called Turner's syndrome. Humans who are XXY are male, and have Klinefelter's syndrome.

b. What determines maleness? The Y or the single X? The sex of the aneuploid individuals described above indicates that it is the presence of the Y that is the male-determining factor in humans, not the absence of the second X. The human Y chromosome has very few genes, but it has one critical gene that triggers a sequence of events leading to male development; the default is female. (The case in fruit flies is different: XO flies are male, and XXY flies are female. In flies it is the ratio of X's to autosomes that determines sex.)

c. Why do XO and XXY survive? Why is an extra and/or missing X compatible with a more or less normal existence while a missing or extra autosome is almost always deadly? Because variation in the number of X's is "normal" -- females have twice as many as males, yet both males and females are "normal." So there must be a mechanism to cope with "extra" X's (or missing X's, depending on your point of view). This will be discussed next time.

d. Secondary Sex Characteristics. Most genes on X and Y have nothing to do with secondary sex characteristics (beard growth, breast development); most genes for secondary sex characteristics are autosomal (although some are on the X). Presence of Y determines which hormones are made and therefore which autosomal (and X linked) genes are turned on. If you add hormones externally, either sex can develop secondary sex characteristics of the other. Also note there is ***no*** correlation between unusual combinations of sex chromosomes and sexual preferences.

e. The birds & the bees. The mechanism of sex determination is similar in many other organisms, in that one sex has a matching pair of chromosomes (the homogametic sex) and the other has a non-matching pair (the heterogametic sex). Which is which, and the fine points of how the balance determines sex, varies. The sex ratio (males/females) is about 1:1 in all these cases because the heterogametic sex produces male-determining and female-determining gametes in equal proportions. (In birds, the female, not the male, is the heterogametic sex. In bees, one sex is diploid and one is haploid -- an extension of the sex-is-determined-by-chromosome-balance principle to the whole set of chromosomes. So when they say they are going to tell you about the birds and the bees, it's not a good way to explain human sex!)

How do aneuploidies occur? Next time. To review mitosis and karyotypes, try problem **8-8 parts A-D, & G.**

VIII. Ploidy and the need for meiosis

A. Karyotype of a species is constant, so N and ploidy stays constant from generation to generation.

B. How is ploidy kept constant?

1. Need for meiosis/reduction division

Most of the cells of most higher organisms are diploid. Humans, for example, have 46 chromosomes, or 23 pairs, in virtually all of their cells. If eggs and sperm also have 46 chromosomes, the next generation, formed from the fusion of an egg and a sperm, would have 92 chromosomes. But clearly the chromosome # does not double each generation. So the eggs and sperm, unlike all other cells, must have only 23 chromosomes and be haploid. So there must be a way to make haploid cells from diploid cells. There is, and the process is called meiosis. During meiosis, one chromosome from each *pair* is picked at random so that the resulting haploid has 23 chromosomes instead of 23 pairs. Then 2 such haploids fuse, during fertilization, to give you back a diploid with 23 pairs.

2. Why bother with all this? Why sex?

After all, you could start the next generation with one complete diploid cell from either parent and save yourself a lot of trouble! Some organisms do reproduce this way, at least some of the time, but most organisms engage in sexual reproduction. They probably do so because each cycle of meiosis, followed by fusion, allows for a new combination of chromosomes. (Crossing over, which occurs at meiosis, also allows for new combinations of genes within chromosomes as well.) So it looks like sexual reproduction is useful because it allows reshuffling of the genetic material (same argument as for bacteria). Reshuffling is needed to give new variety (for selection to act on) and/or for repair (& replacement) of damaged copies.

3. How reshuffling works

a. Reshuffling Chromosomes.

Suppose one person has 2 identical copies of chromosome #1 and 2 identical copies of chromosome #2. (Draw these chromosomes in one color, say pink.) Another person has 2 copies of chromosome #1 that are the same as each other but different from the copies in the first person, and similarly for chromosome #2. (Draw these chromosomes in another color, say white.) The offspring of these two people will have a mixture of "pink" and "white chromosomes. After several generations, it will be possible to get all conceivable combinations of "pink" and "white" chromosomes. **(See problem 8-4 parts A & B.)**

b. Reshuffling genes:

In addition to reshuffling whole chromosomes, equivalent parts of chromosomes can be reshuffled or exchanged. Homologous chromosomes pair and can exchange equivalent sections during meiosis by crossing over. (This is equivalent to what happens to bacteria during transformation, transduction, etc., but in eukaryotes the process is restricted to prophase I of meiosis.) See Sadava 9.17 & 9.18 (9.15 and 9.16) or Becker fig 20-17 (18-17). Note: the term "genetic recombination" usually refers to reshuffling of genes by crossing over. It is sometimes used in a more inclusive sense to mean all kinds of reshuffling (of genes and/or chromosomes) whether crossing over is involved or not.

IX. Meiosis -- Overview of the Chromosome Cycle -- Probably next time.

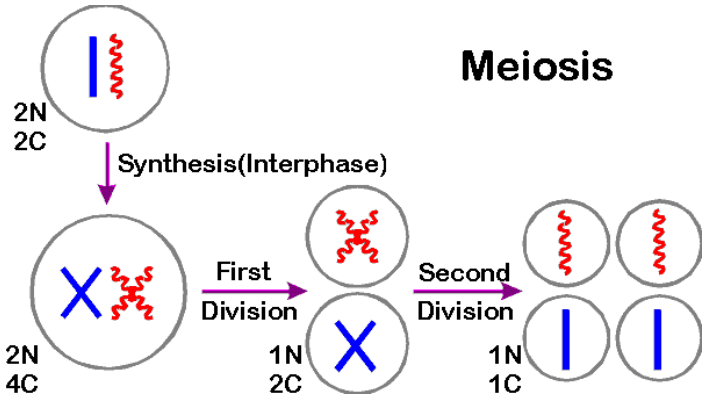
A. What happens to chromosomes during meiosis? (See handout 19B.)

1. DNA synthesis occurs first -- before division. Meiosis is preceded by DNA duplication just as mitosis is. During the S before meiosis (or mitosis) the cell doubles the DNA content and # of chromatids per chromosome. So cell starts with pairs of doubled chromosomes = 4 copies of each chromosome.

2. Products: There are 4 products, each haploid (from meiosis), instead of 2 products, each diploid (from mitosis). To cut the number of copies of each chromosome from 4 to one requires 2 division, not one.

3. Two divisions of meiosis: The first division of meiosis separates homologs; the second division of meiosis separates sister chromatids.

4. What happens to N, c and # of chromatids/chromosome? The first division cuts the chromosome number per cell in half from 2N to N and cuts the DNA content per cell in half from 4c to 2c ("c" is defined below). The second division halves the DNA content per cell (from 2c to c), halves the number of chromatids/chromosome (from 2 to 1) and halves the total chromatid # per cell (from 2N to N). What happens in a cell with one pair of chromosomes is as follows:



Handout 19B summarizes c, N etc. for cells with one chromosome pair (N = 1) and for 3 pairs (N = 3). Handout shows chromosomes per cell at each stage (before S, after S, after 1st div. of meiosis and after 2nd div.) See Becker fig. 20-3 (18-3) for a similar diagram of meiosis in a cell with 2 pairs of chromosomes.

B. Definition of c

"c" is a measure of DNA content per cell, not the number of chromosomes or chromatids.

c = minimum DNA content per haploid cell of an organism = DNA content of haploid cell before S (with unreplicated chromosomes) = DNA content of one set of chromatids. C is NOT equal to N; c is the DNA content of N chromosomes (with one chromatid/chromosome).

To review Meiosis (so far), and compare to Mitosis, do or finish problems 8-1, 8-2 (parts A to E), 8-3, & 8-8 (parts A-D & G). *Details of meiosis next time or see handout 19A.*

Next Time: Whatever parts of meiosis/mitosis we don't finish and then life cycles and nondisjunction (how aneuploidy occurs).

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