

C2005/F2401 '10 -- Lecture 19 -- Last Edited: 11/17/10 02:14 PM

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Handouts for today & next time: 19A = Meiosis/Mitosis

19B = Chromosome squashes, karyotypes & chromosome structure

20A = Meiotic Chromosome Cycle

20B = Super Cycle & Nondisjunction

I. Splicing -- wrap up of Introns & Exons. See notes of last lecture, Topic V, and [handout 18 C](#), bottom.

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

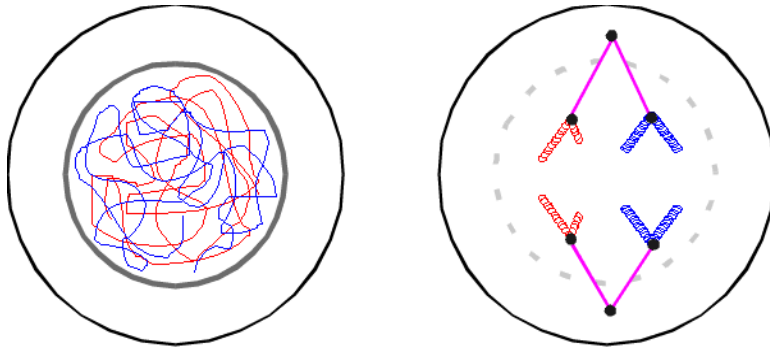
1. Structure

- Many chromosomes (multivolume encyclopedia as vs one vol.) = genetic material is divided into several pieces.
- Chromosomes are linear and not attached to anything.
- Much more DNA per cell and more per piece. $2-5 \times 10^7$ 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).
- 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	3×10^6 base pairs for <i>E. coli</i>	$2-5 \times 10^7$ 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 'two state' solution -- Have 2 different states of nucleus & DNA (see diagram below or Sadava fig. 11.8 (9th ed.)). Also have separate times for synthesis of DNA and for distribution of DNA. Always have DNA in double helix and super coiled with proteins. The difference between the two states is the extent of super-super coiling. (Details next term.)



< Cell During Interphase >

< Cell During Division >

A. State one -- between divisions (during interphase) -- DNA accessible to polymerases

1. *Chromatin*. DNA + associated proteins (mostly histones) form tangled mass called chromatin .

- Relatively loose coiling of DNA.
- DNA accessible to polymerases for transcription and replication.
- DNA not ready to distribute.
- No distinct structures visible in microscope.

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. *DNA can act as template* -- All transcription and replication occurs in this stage

B. State two -- for distribution 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.
- DNA is condensed > 10,000 X.
- State 1 vs state 2: Like 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. *DNA can not serve as template* -- No transcription or replication in this stage.

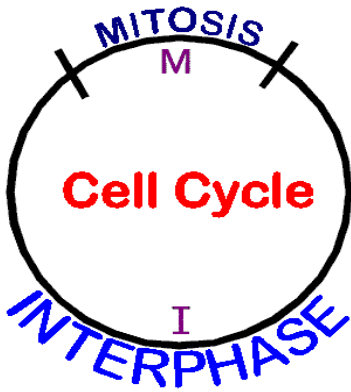
C. Reminder: all eukaryotic DNA is in double helix, supercoiled, AND associated with special proteins called histones at all times -- it's super-supercoiling and association with additional proteins that changes. (More details next term.)

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 or Sadava fig. 11.3 (9.3).

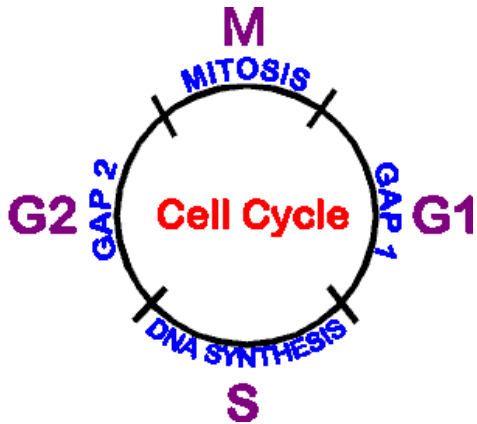
You can divide the cycle into 2 major stages described above: I (interphase) and M (mitosis or division). The picture below puts the element of time in -- cell goes around and around. The two stages of I and M corresponds to the two states of DNA described above.



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

1. DNA made in I, prior to M. (How we know is below.)
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:



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.

Q: If you supply labeled/radioactive U or amino acids, will these precursors be made into macromolecules throughout interphase? During M?

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 bottom panel, and box on 19A. Also see Sadava, fig. 9.7 (8th ed).

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 Sadava 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 fig. 11.12 (9.11) The centromere can be at the end of the chromosome or anywhere 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. 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 bottom panel of 19B.
- **Double chromosomes may not look doubled:** When chromosomes are observed, in the beginning of M, the two chromatids of a double chromosome can stick together and look like a single structure.

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 -- How DNA is distributed (see handout 19A) First let's go through stages as shown. See Sadava fig. 11.11 (9.10) or Becker 19-20 & 21. When we get to meiosis we'll compare and contrast the two processes (mitosis & meiosis) as listed in table on handout (& see Sadava fig. 11.20 (9.19)). 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 or 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 the 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 an attachment to a structure at its centromere. Chromosomes 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 fig. 11.10 (9.9) or Becker 19-22, 24 & 25. 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 fig. 11.13 (9.12) or Becker fig. 19-28 & 29 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

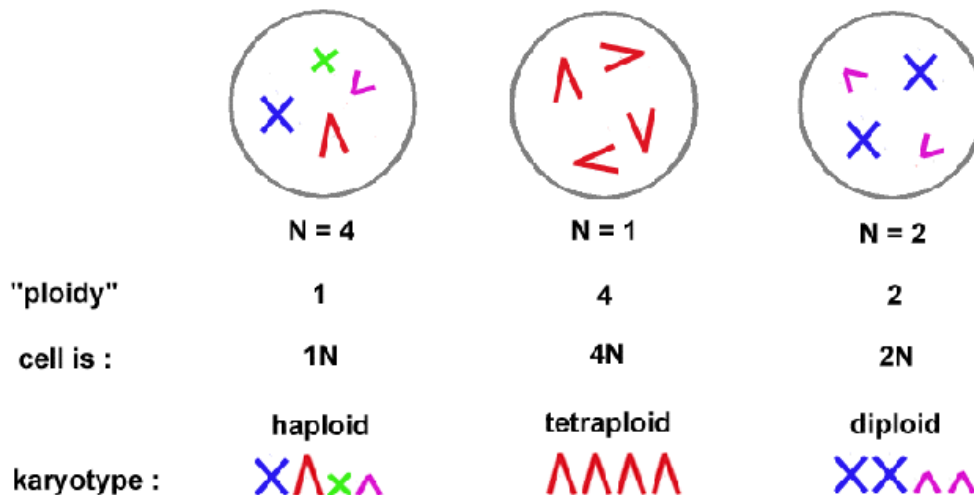
A. What are Karyotypes and how do you get them?

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 fig. 11.16 (9.15), 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 or Sadava fig. 11.16 (9.15).

Chromosome Squashes and Karyotypes



B. What do you see in a normal squash or karyotype? (Any of the topics below that are not covered in this lecture 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 (8th ed). 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 of each gene. Each alternative version of a gene is called an allele. Examples:

(1). Gene for Blue vs Brown Eye Color. The "eye color gene" determines blue vs brown eye color (if everything else is held constant). This gene is in the same place (the eye color locus) on both homologs, but the eye color gene on a particular chromosome could be the blue-determining version or the brown-determining version. Each homolog carries one allele of the eye color gene. Homologs carry the same genes, but not necessarily the same alleles.

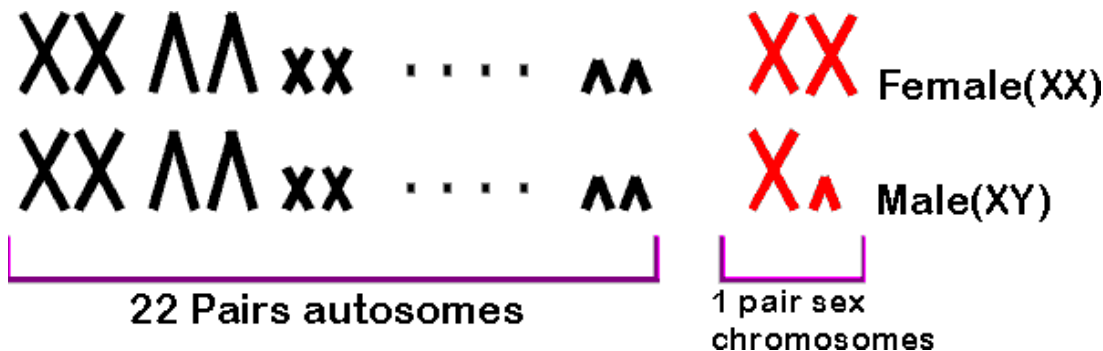
(2). Gene for Beta Chain of Hemoglobin. The beta chain gene is always in the same position, the beta chain locus. However an individual chromosome could carry the Hb A or Hb S allele (version) at the beta chain locus.

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.

See problem 8-8, part A.

5. Human karyotypes -- sex chromosomes & autosomes. See Sadava fig. 11.16 (9.15) 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.

To review mitosis and normal karyotypes, try problem **8-8 parts A-D, & G**.

VIII. Overview of Meiosis -- See handout 20A -- if time is short, this will be covered in lecture 20.

A. What is meiosis for?

1. *Need for meiosis/reduction division -- to keep karyotype & ploidy constant from generation to generation.*

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 figs. 11.18 & 11.19 (9.17 & 9.18) or Becker fig 20-16 (20-17). 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.

B. How many chromosomes, chromatids & cells during meiosis? What happens if there is one pair of homologs?

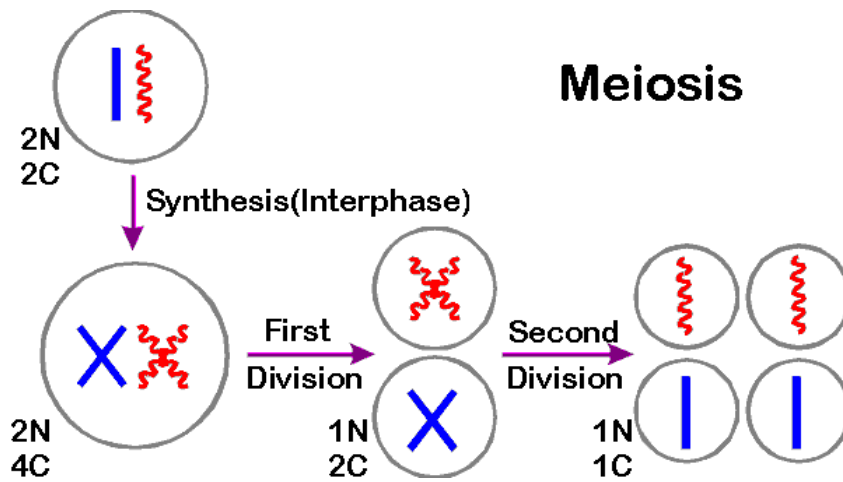
Picture below or on bottom of Handout 20A shows what happens to **one pair** of chromosomes. It shows **all cells** at each stage -- before DNA synthesis, after S, after 1st div, and after 2nd div. See Becker fig. 20-3 for a similar diagram of meiosis in a cell with **2 pairs** of chromosomes.

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:



C. What happens to chromosomes per cell during meiosis?

Handout 20A (top part) shows a different view of the stages shown above. It emphasizes the 'chromosome cycle' -- the number of chromosomes, the number of chromatids, and the DNA content **per cell** at each stage. It summarizes the chromosome cycle for cells with one chromosome pair ($N = 1$), for 3 pairs ($N = 3$), and for any general value of N .

D. 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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