Biochemistry 401G Lecture 30 Andres

Lecture Summary:

- Introduction to remainder of the course
  - Office hours
  - Web resources

- Discussion of the requirements of genetic material

- History of DNA

- Early experimental results: Protein or DNA as genetic material?

- The "central dogma"

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Office Hours: MWF 3-4 in MS627B

Appointments are outside of normal office hours are encouraged. However, prior notification is REQUIRED.

Three steps to success:

1) Come to class prepared. Read the text and download the web class notes.
2) Stay current- Do problem sets/old exams and seek help early and often.
3) Use class notes and old exams to fine-tune exam preparation.

The Requirements of Genetic Material

- **It must be able to replicate**

- **It must be able to control expression of traits**.

- **It must be able to change in a controlled way**

Hereditary Material is contained on Chromosomes

1. A variety of chromosome types, as defined by relative size and shape, were found to be present in the nucleus of each cell.

2. All of the cells of an organism, excluding sperm cells, egg cells, and red blood cells, and all organisms of the same species, were observed to have the same number of chromosomes.

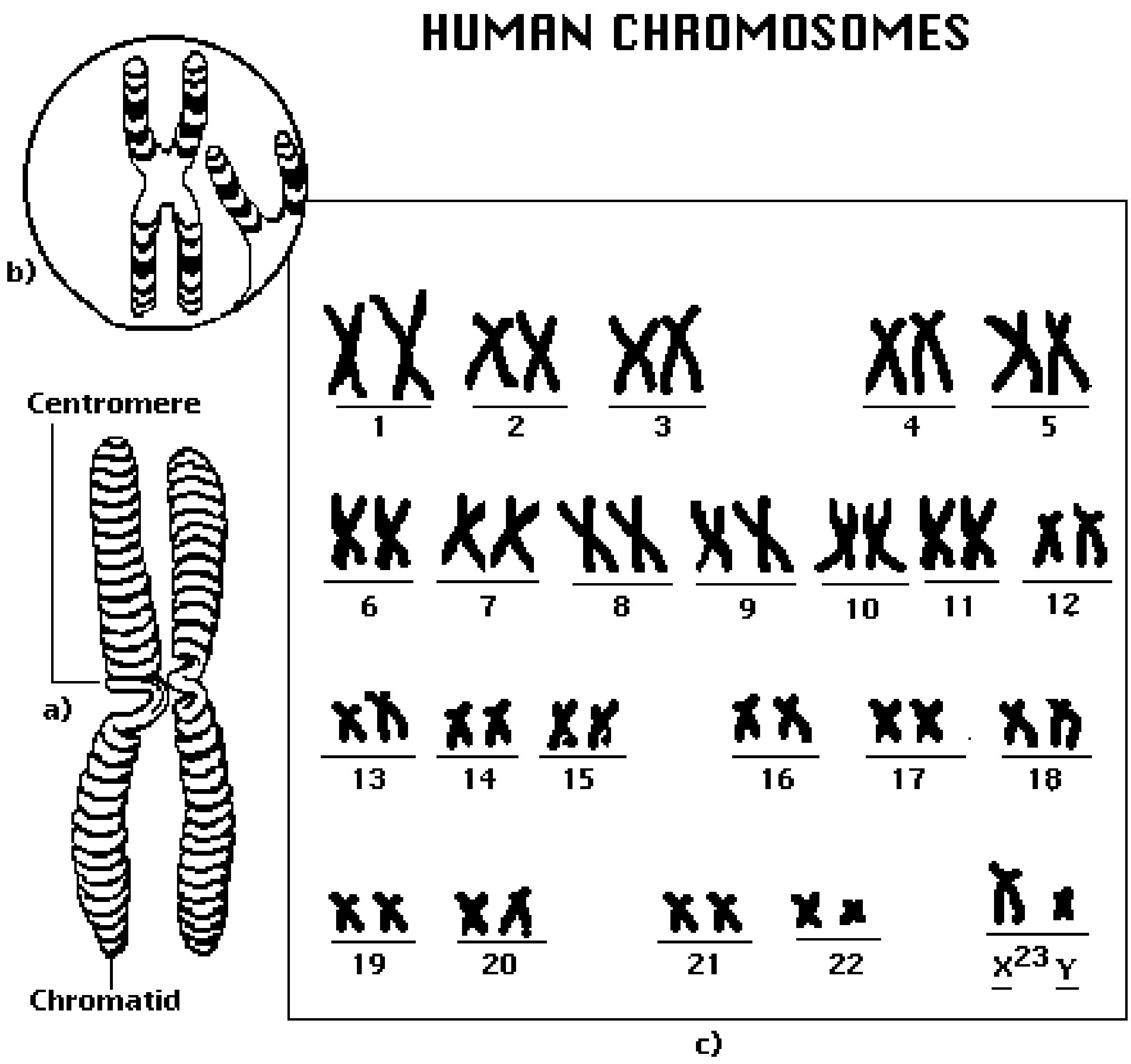
3. The number of chromosomes in any cell appeared to double immediately prior to the cell division processes of mitosis and cytokinesis.

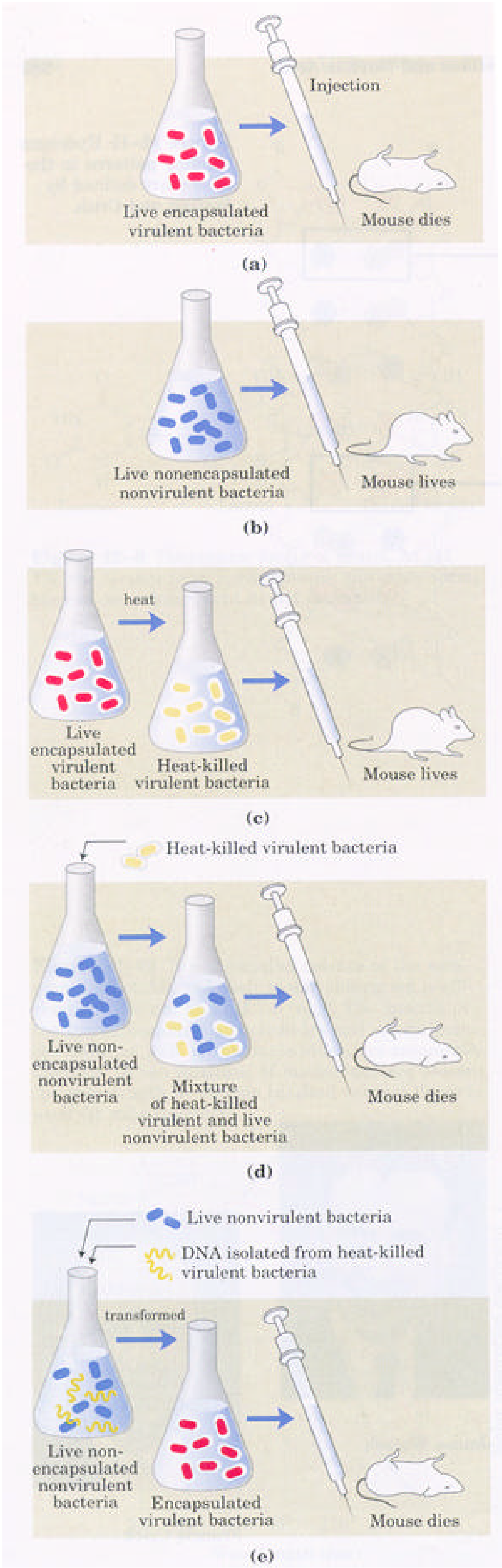
4. The sex or germ cells (i.e., sperm and egg) appeared to have exactly half of the number of chromosomes as were found in the non-germ or somatic cells of any organism. Furthermore, the germ cells were shown to have just one copy of each chromosome type. Such cells are called **haploid** cells.

5. The fertilization of an egg with a sperm cell produces a **diploid** cell called a zygote, which has the same number of chromosomes as the somatic cells of that organism.

These chromosomes behaved like the particles or factors that Mendel described. These hereditary factors were either located on the newly discovered chromosomes or were the chromosomes themselves.

Proof that the chromosomes were Mendel's hereditary factors did not come until 1905, when the first physical trait was shown to be the result of the presence of specific chromosomal material and, conversely, that the absence of that specific chromosome meant the absence of the particular physical trait.





Which Chromosomal Subunit Carries Hereditary Information?

Quantitative analysis of chromosomes shows a composition of about forty percent DNA and sixty percent protein.

<u>The Discovery of DNA</u>

Friedrich Miescher first identified DNA in 1868. He called the substance nuclein, noted the presence of phosphorous, and separated the substance into a basic part (which we now know is DNA) and an acidic part (a class of acidic proteins that bind to basic DNA-histones).

The Transforming Principle - DNA Might be the Genetic Material

In 1944, <u>Oswald Avery, Colin Macleod, and Maclyn McCarty,</u> discovered that different strains of the bacterium Strepotococcus pneumonae could have different effects on a mouse.

What transforming principle was the dead virulent strain giving to the avirulent strain to make it lethal?

<u>Background</u>:
The bacteria contain a capsular polysaccharide on its surface that protects it from host defenses. Occasionally, mutant

bacteria arise which have a defect in the production of the capsular polysaccharide.

These mutants have two characteristics:
1) They are **avirulent**, meaning that they are unable to mount an infection in the host.
2) Due to the lack of the capsular polysaccharide the surface of the mutant appears rough under the microscope and can be distinguished from the wild type bacteria (whose surface appears smooth).

The virulent smooth wild type pneumococcus can be heat treated and rendered avirulent (still appears smooth under the microscope however).

<u>Controls:</u>

w.t. (smooth) + mouse = DEAD mouse
mutant (rough) + mouse = live mouse
Heat-treated w.t. (smooth) + mouse = live mouse

<u>Combination:</u>

Heat-treated w.t. (smooth) + mutant (rough) + mouse = **DEAD mouse**

In this case when the bacteria were isolated from the dead mouse they were smooth virulent pneumococcus (i.e. indistinguishable from wild type).
The overall conclusion from these experiments was that there was a "transforming agent" in the heat treated bacteria which transformed the live rough (mutant) bacteria to be able to produce wild type capsular polysaccharide.

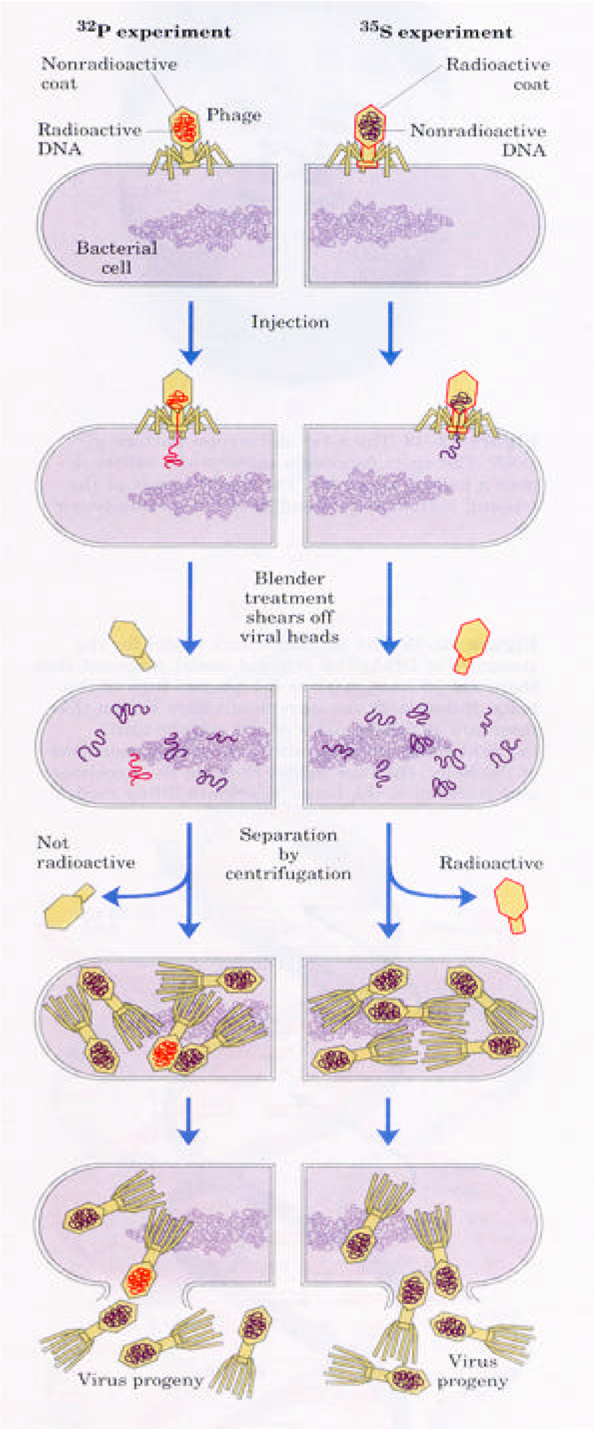
Question: Was the transforming factor DNA or protein?

<u>1952 Hershey and Chase Experiment:</u>

Background:
T2 is a virus that attacks the bacteria E. coli. The virus particles adsorb to the surface of the E. coli cells. It was known that some material then leaves the phage and enters the cell. The empty phage particles on the surface of infected cells can be physically removed by putting the cells in a blender and whipping them up. In any case, approximately 20 minutes after the phage adsorb to the surface of bacteria the bacteria burst open (lysis) and release a large number of progeny viruses.

If the bacteria are grown in the presence of ^{32}P labeled ATP, progeny phage could be recovered with this isotope incorporated into its DNA (normal proteins contain only hydrogen, nitrogen, carbon, oxygen, and sulfur atoms).

Likewise, if the media contained ^{35}S labeled methionine the resulting progeny phage could be recovered with this isotope present only in its protein components (normal DNA does not contain sulfur).



The experiment:

1) E. coli were infected with ^{35}S labeled phage. After infection, but prior to lysis, the bacteria were whipped up in a blender and the phage particles were separated from the bacterial cells. The isolated bacterial cells were cultured further until lysis occurred. The released progeny phages were isolated.

Where the ^{35}S label went:

- **Adsorbed phage shells 85%**
- **Progeny phage < 1%**

2) E. coli were infected with ^{32}P labeled phage. The same steps as in 1) above were performed.

Where the ^{32}P label went:

- **Adsorbed phage shells 30%**
- **Progeny phage 30%**

Conclusion:

The material being transferred from the phage to the bacteria during the infection appeared to be mainly DNA.

Chargaff- Nucleotide Content in DNA

In 1950, Erwin Chargaff discovered that no matter what tissue from an animal he looked at, the percentage of each of the four nucleotides was the same, though the percentages could vary from species to species. This insinuated that the structure of DNA was specific and conserved in each organism.

1. Base composition generally varied from species to species.

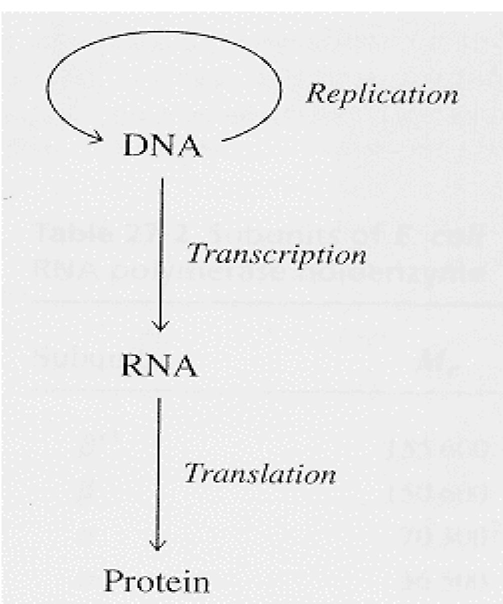
2. DNA specimens from different tissues of the same species have identical base composition.

3. The base composition of DNA in a given species DOES NOT change with the organism's age, nutritional state, or changing environment.

4. In all DNA's, regardless of species the number of A=T and G=C. The sum of purine residues is equal to the sum of pyrimidine residues.

$$\%G = \%C$$
$$\%A = \%T$$

The Central Dogma:



In this model he argued that DNA itself is not the direct template that orders amino acid sequences. Instead, the genetic information of DNA is transferred to another class of molecules, which then serve as the protein templates. These intermediate templates are molecules of ribonucleic acid (RNA). Their relation to DNA and to protein is summarized in the Central Dogma. The arrows indicate the direction of transfer of the genetic information. The arrow encircling DNA signifies that it is the template for its self-replication; the arrow

between DNA and RNA indicates that all cellular RNA molecules are made on DNA templates. Correspondingly, RNA templates determine all protein sequences.
Most importantly, both of these later arrows are unidirectional, that is, RNA sequences are never copied on protein templates; likewise, RNA never acts as a template for DNA.

The remaining lectures are arranged to follow this model through the logical progression from DNA to RNA to proteins. In the final lecture of the class we will return to the Central Dogma and discuss how a greater understanding of the complex processes which underlie this information transfer require that modifications be made to this simple linear relationship.