

## How was DNA shown to be the genetic material?

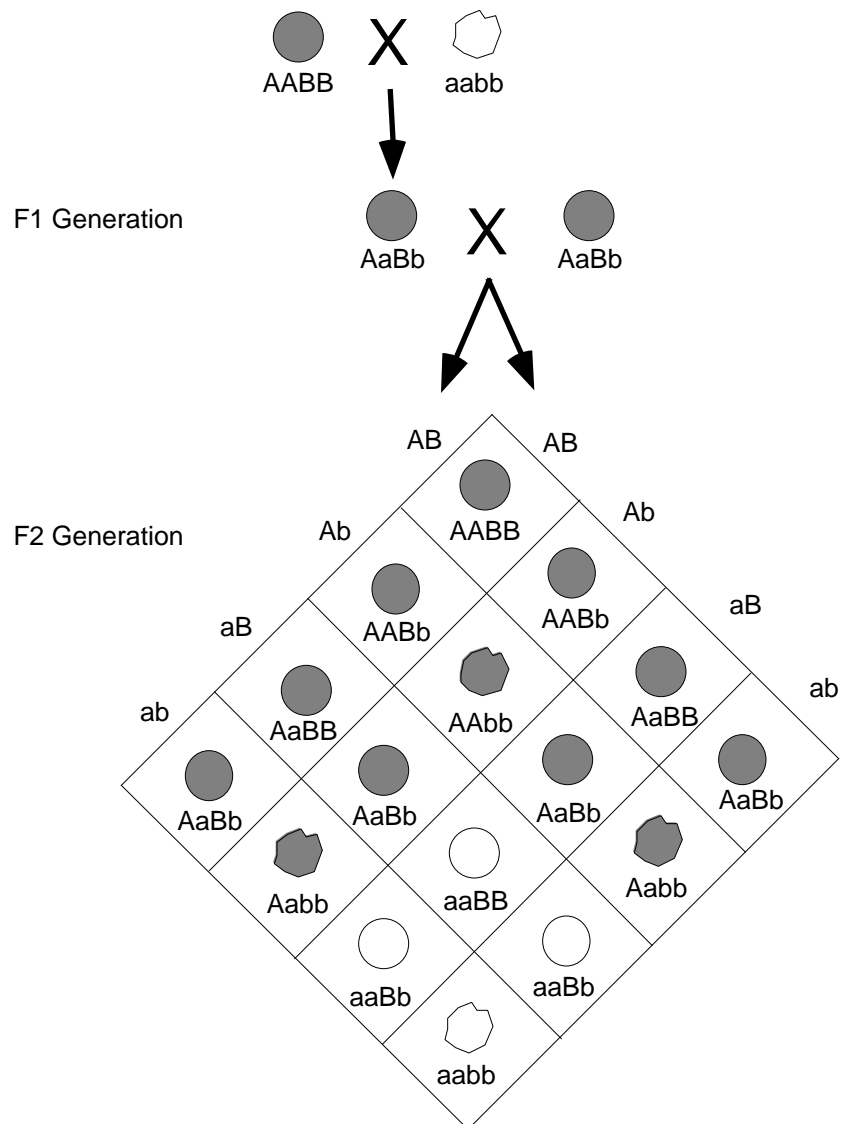
We need to discuss this in an historical context. During the 19th century most scientists thought that a bit of the essence of each and every body part was put into the sperm and egg and that at conception a blending essences occurred. This theory was called **Blending Inheritance**. It was based on a non-rigorous observation of nature. Complex characteristics were examined and careful counts of the number and type of progeny were not performed. *It is not correct.*

## How did the concept of the gene arise?

It was Gregor Mendel in 1865 who first introduced the concept of the gene. However, he did not use the word gene. Mendel was an Austrian Monk and he worked in Brno (Brunn) Czechoslovakia. Mendel's interest in botany began early because his family's chief occupation was the farming and the development of new apple varieties. At school, he was formally educated in mathematics, physics, botany and zoology.

In Czechoslovakia, Mendel studied the genetics of the pea, *Pisum sativum*. At the time his approach was unique. He first documented a number of simple traits and then established plant strains that bred true for these traits. That is, for a given trait (such as pea color or texture) all of the children were identical to the parents.

Mendel took these pure strains and crossed them to one another, collected large data sets that represented all of the progeny and searched for mathematical equations and relationships that described the results. Mendel would propose specific hypotheses and then experimentally test them. This alone set him apart from other geneticists of the time. This approach led him to propose his **particulate theory of inheritance**. This theory says that each characteristic is determined by discrete units inherited that are passed intact down through the generations. Mendel's *particles of heredity* are equivalent to the genetic units that we call the *gene*. Modern work indicates that the details of Mendel's Laws and concepts are not completely correct. But his overall concepts were sound.



### **An example of Mendel's work**

A simple example of one of Mendel's experiments. Here 'A' causes the pea to be dark in color and 'B' causes it to be smooth. 'a' causes it to be light in color and 'b' causes it to be wrinkled. 'A' and 'B' are dominant to 'a' and 'b' respectively. In the F1 generation all of the progeny are dark and smooth.

### **F2 punnet square**

Two of the double heterozygotes (AaBb) are crossed to one another. Analysis of the offspring indicated that the traits carried by the 'grandparents' have not been blended together. Mendel said that they had survived as particles of inheritance and been passed unchanged through the generations. It is very clear that blending has not occurred.

Mendel studied many different characters and was able to show that this was true for all of the ones he studied.

### **Mendel was ignored**

Mendel's work was published in 1865 but it was essentially ignored until 1900. Why was his work so unappreciated? It resulted from the lack of any known physical basis for the postulated genetic factors (genes).

### **Why was Mendel's work finally appreciated?**

A general understanding of Mendel's work had to wait until chromosomes were discovered. These structures provided a physical basis that could account for the behavior of the particles of inheritance.

In 1903 Sutton published a paper describing how chromosomes behaved during meiosis. Basically, they behaved in the same manner as Mendel's particular units. The figure on page 7.5 summarizes how chromosomes behave during meiosis. It shows how a diploid individual produces haploid gametes. Make sure that you understand this and see how it relates to the segregation of Mendel's particle units of inheritance.

# Insert Meiosis

### Comparison of Mendel's and the Modern view of a gene

Mendel's view	Modern view
Particle of inheritance	A gene is a chain of nucleic acids that carries the information that a cell requires to synthesize a particular polypeptide.
Each parent has 2 hereditary units for each trait.	Mendel's pea plants were <b>diploid</b> .
Each unit segregates into different gametes	The genes that he was studying were carried on different copies of the same chromosomes and segregated from one another at meiosis.
Responsible for producing a single characteristic in an organism.	The genes that Mendel studied encoded a single product.

## Discovery of the nature of the genetic material

By the 1930's, the scientific community had accepted the existence of discrete genetic elements and that these genetic elements were probably carried on or by chromosomes.

The burning question of the day was what type of molecule carried the genetic information. During the 1940's it was known that chromosomes contained both DNA and small basic proteins called histones.

It was also clear that the genetic material:

- 1) must be of sufficient complexity to encode tens of thousands of different proteins each of which are hundreds to thousands of amino acids long.
- 2) must be able to be replicated with high fidelity each and every cell division so that it could be passed down to future generations.
- 3) must be very stable, that is it must not be subject to a high rate of randomization. Some cells in your body survive for 80 years
- 4) must be able to be altered by mutations. We have not talked about these. Mutations are changes in the genetic material.

### Disbelief

Most people did not believe that it was possible for DNA to be the genetic material since it seemed much too simple a molecule for such a complex job. Remember that DNA is a polymer of only 4 different nucleotides. Proteins, however, are composed of 20 different amino acids and so had a very satisfying degree of complexity. In the 40's most scientists thought that the genetic information was encoded by strings of amino acids.

It was clear that the histones could not be the genetic material since they were not present in sperm. Sperm, however, do contain very small proteins called protamines.

### Fred Griffith

In 1928, Fred Griffith performed an experiment that provided a foundation for the discovery that DNA is the genetic material. He was studying the pathogenicity of *Streptococcus pneumoniae*. This bacteria causes a form of pneumonia in humans. He was not particularly interested in the genetic material debate.

He had two strains of Streptococcus. The S-strain was virulent and the R-strain was avirulent. He was interested in what enabled the S-strain to cause a diseased state. His virulence assay was to inject mice with the bacteria. A virulent strain would kill the mouse but an avirulent strain would not.

Two strains of Streptococcus

- one virulent AND • one avirulent.

These strains could also be distinguished from one another in the laboratory by biochemical tests.

### Characteristics of S and R-strains

#### S-strains

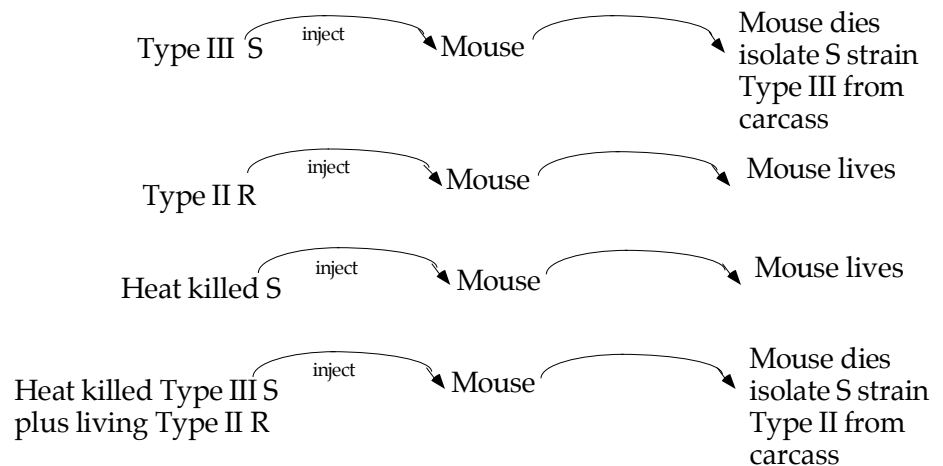
When grown in petri dishes, these pathogenic bacteria exuded a plump gelatinous polysaccharide outer coat causing them to produce smooth glistening colonies. Because of their colony morphology, these bacteria were said to have a Smooth phenotype and were called S-strains. It is the presence of this outer coat that protects them from the host's immune system and makes them more virulent than the R-strains.

#### R-strains

Avirulent 'cousins' to the S-strains They do not produce a gelatinous outer coat and therefore produce dull, well defined colonies in petri dishes. There are said to have a Rough phenotype and were referred to as R-strains.

The experiment:

Griffith performed an experiment that produced an unexpected result. ----->

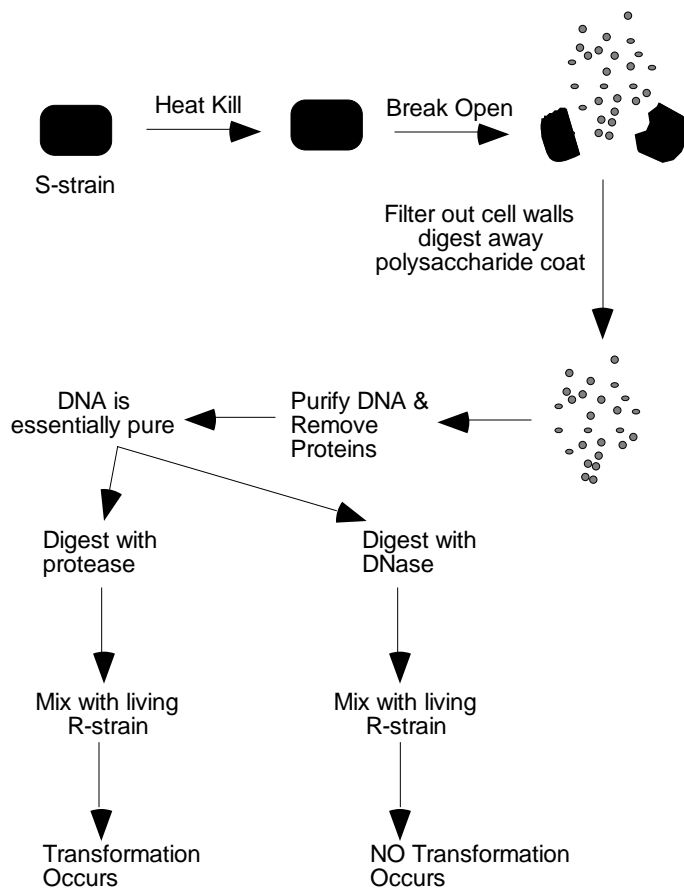


Look closely at the fourth experiment (above). The bacteria recovered from the dead mouse have the S-phenotype but they are also Type II. At the beginning of the experiment the Type II bacteria all had a Rough phenotype. Griffith found that this change in the bacteria was permanent. If the new Type II S strain was propagated on petri dishes, it remained Type II and S ! This is the only combination that produces this transformation.

Griffith said that the Type II R-strains had been transformed into Type II S-strains. Something from the cell debris of the S-strains had been picked up by the R-strains. He called this *something* the **transforming principle**. The term transformation is still in use today. Many scientists thought that the transforming principle was the polysaccharide coat itself. **NOT**.

**Oswald Avery, Colin MacLeod and MacLynn McCarty 1944**

No one had anything significant to say about what the transforming principle might be until 16 years later, in 1944 when Oswald Avery, Colin MacLeod and MacLynn McCarty demonstrated that the transforming principle was DNA. They did their transformations in a test tube. They showed that cell extracts prepared from S strains could cause the transformation. Furthermore, they showed that it was the DNA that was responsible for the transformation.



**Conclusion from this experiment:** *DNA is the transforming principle.*

Avery was as surprised by this as anyone else. When he began this series of experiments he fully expected that the transforming factor would be a component of the polysaccharide coat itself.

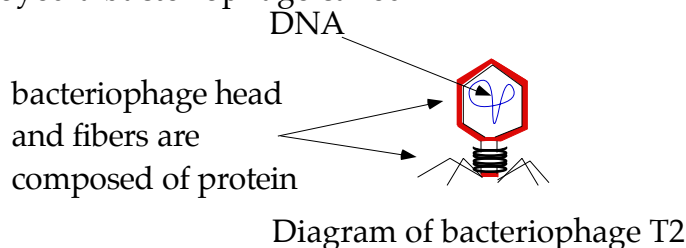
**Still there were dis-believers**

Still many in the scientific community chose to believe that Avery had missed something, and that a series of small proteins must be present in the DNA fraction. This was because DNA was thought to be a monotonous random repeat of four different nucleotides. It was a boring molecule. I am sure that you don't think this.

**Alfred Hershey and Martha Chase 1952**

In 1952, Alfred Hershey and Martha Chase stopped all of the arguing with the two experiments. This experiment employed a bacteriophage called T2.

The T2 bacteriophage is composed of only protein and DNA. It does not contain anything else. It cannot replicate on its own, it relies on its host cell for everything. It is an obligate parasite. T2 infects the bacterium *Escherichia coli*.

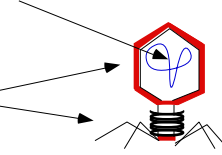


Genetic studies had shown that after entering a cell, the T2 genome directed the host to cell to make new phage. Here was the perfect opportunity to settle the debate. Hershey and Chase grew two cultures of T2. One culture grew in the presence of <sup>32</sup>P which primarily labels newly synthesized nucleic acids and the other culture grew in the presence of <sup>35</sup>S which primarily labels proteins.

$^{32}\text{P}$  is found primarily in the DNA

T2 raised in the presence of  $^{32}\text{P}$ .-->

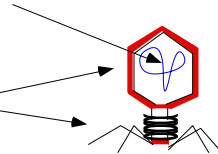
Little  $^{32}\text{P}$  is found in the protein components



Little  $^{35}\text{S}$  is found in the DNA

T2 raised in the presence of  $^{35}\text{S}$ .-->

$^{35}\text{S}$  is found primarily in the protein components



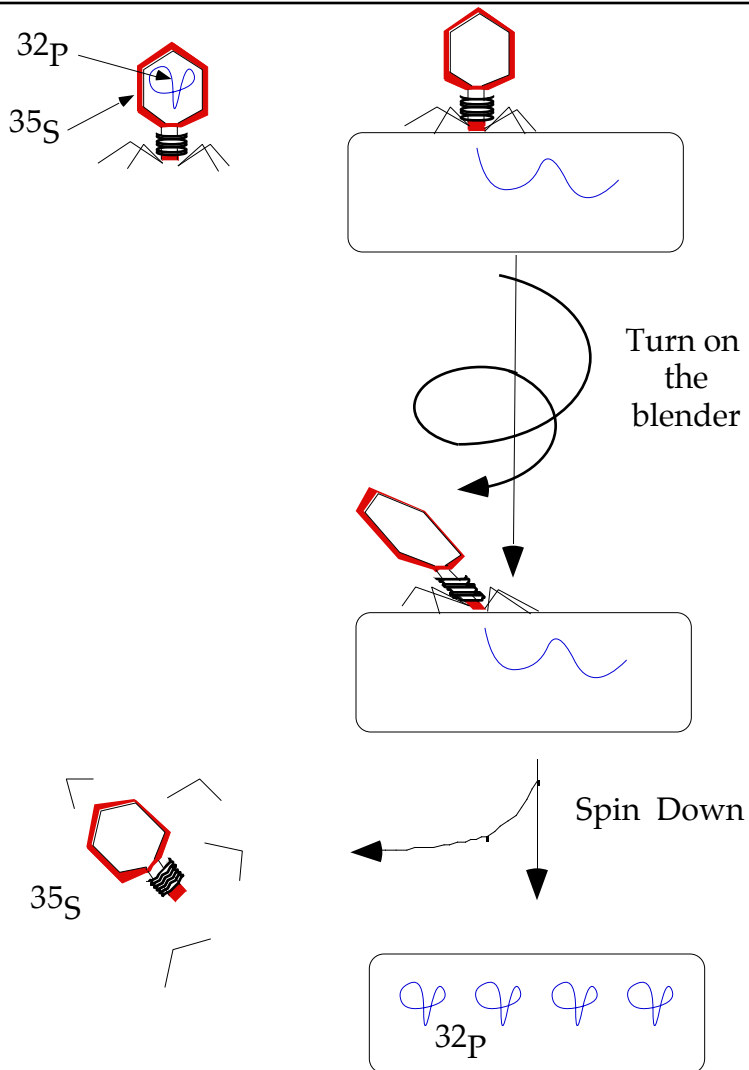
Each of the following two experiments was actually two experiments, one performed with the  $^{32}\text{P}$  grown phage and the other with the  $^{35}\text{S}$  grown phage. For simplicity's sake I will describe each experiment in a single diagram.

### Experiment 1.

The radiolabeled phage is allowed to infect a bacterial culture. The bacteria is growing in media without any radionuclides. The only source of radiolabel are the bacteriophage.

Following attachment of the phage, the culture is placed in a blender, which is then turned on. Blending removes the empty phage 'ghosts' from the bacteria. Next the mixture is then centrifuged. This separates the bacterial cells from everything else. Following centrifugation, the supernatant contains the  $^{35}\text{S}$  (bacteriophage protein coat) while the both the cells and the  $^{32}\text{P}$  (bacteriophage DNA) is found in the pellet.

This means that the only thing that has entered the cell is the DNA. Therefore, it must be the genetic material!

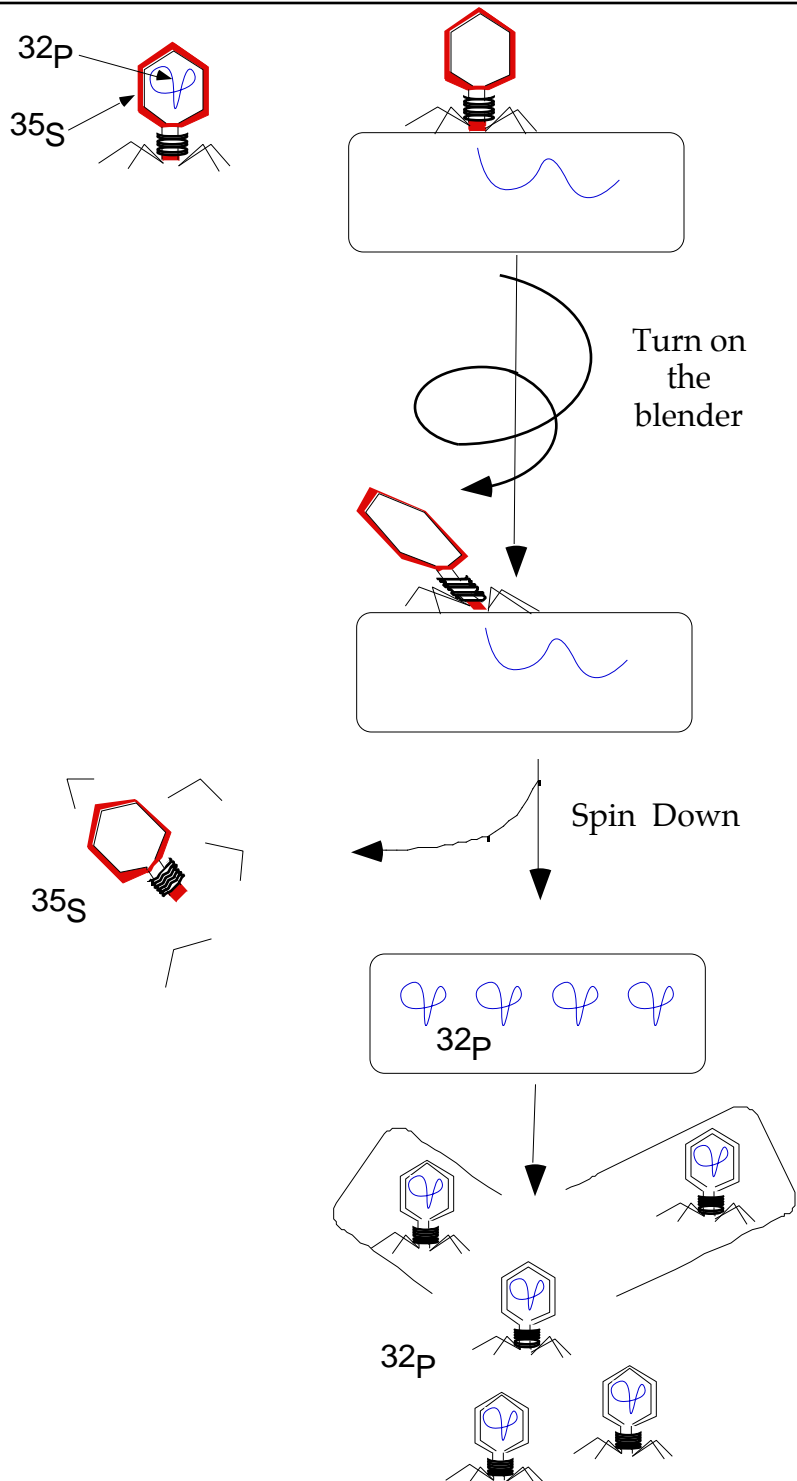


## Experiment 2.

Essentially the same as experiment 1 except that this time the phage is allowed to reproduce in the bacteria and the T2 offspring are harvested.

Now remember, the only source of radiolabel are the bacteriophage.

The phage replicate inside the bacteria and new offspring are produced. When examined, small amounts of  $^{32}\text{P}$  are found in the new phage but no  $^{35}\text{S}$  in found in them.



These experiments finally convinced the scientific world that DNA and not protein must be the genetic material. No other interpretation was reasonable. Now people had to face the fact that a repeating polymer of only 4 different nucleotides was able to encode every protein that a cell needed to function.



## Definitions

avirulent	Refers to a pathogen that is not very good at causing disease or death.
bacteriophage	A virus that infects bacteria. Bacteriophage T2, lambda ( $\lambda$ ) and M13 are examples. A bacteriophage is a virus that infects bacteria, takes over the cell and forces it to make new bacteriophage. They are obligate parasites. They are very simple entities, composed of only DNA and protein.
diploid	A diploid set of chromosomes contains two copies of each autosome and two sex chromosomes. We are diploids. Also referred to as 2N. Diploids usually produce gametes that are 1N.
DNase	An enzyme that specifically degrades DNA.
haploid	A haploid set of chromosomes contains one copy of each autosome and one sex chromosome. This is also referred to as 1N. Diploid (2N) organisms produce gametes that are 1N.
protease	An enzyme that specifically degrades proteins (polypeptides) to amino acids or short peptide fragments.
virulence	A measure of the ability of a pathogen to cause disease or death.
virulent	Refers to a pathogen that is good at causing disease or death.