Lecture 7:

The identity
of the genetic material

Course 371

Aims

- Introduce the experiments involved in the search of the identity of the genetic code.
- Explain old experiments using our current understanding.
- Explain biological concepts related to the experiments.
- Highlight the importance of experimental design.

Griffith's transformation experiment

VOLUME XXVII JANUARY, 1928 No. 2

THE SIGNIFICANCE OF PNEUMOCOCCAL TYPES.

BY FRED. GRIFFITH, M.B.

(*A Medical Officer of the Ministry of Health.*)

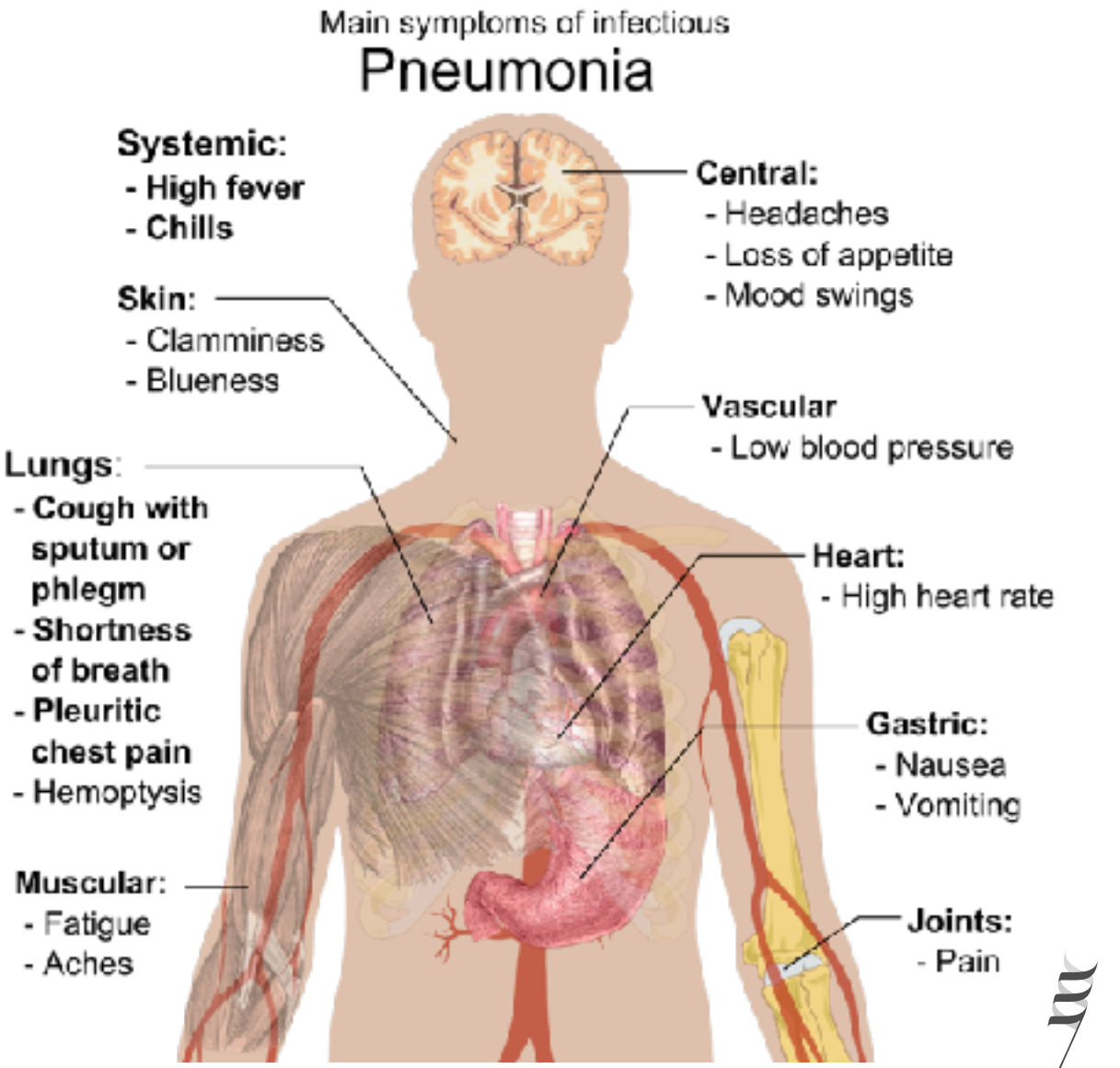
(*From the Ministry's Pathological Laboratory.*)

CONTENTS.

Griffith, F. (1928). The Significance of Pneumococcal Types. The Journal of Hygiene, 27(2), 113–159.

Griffith's Model organism

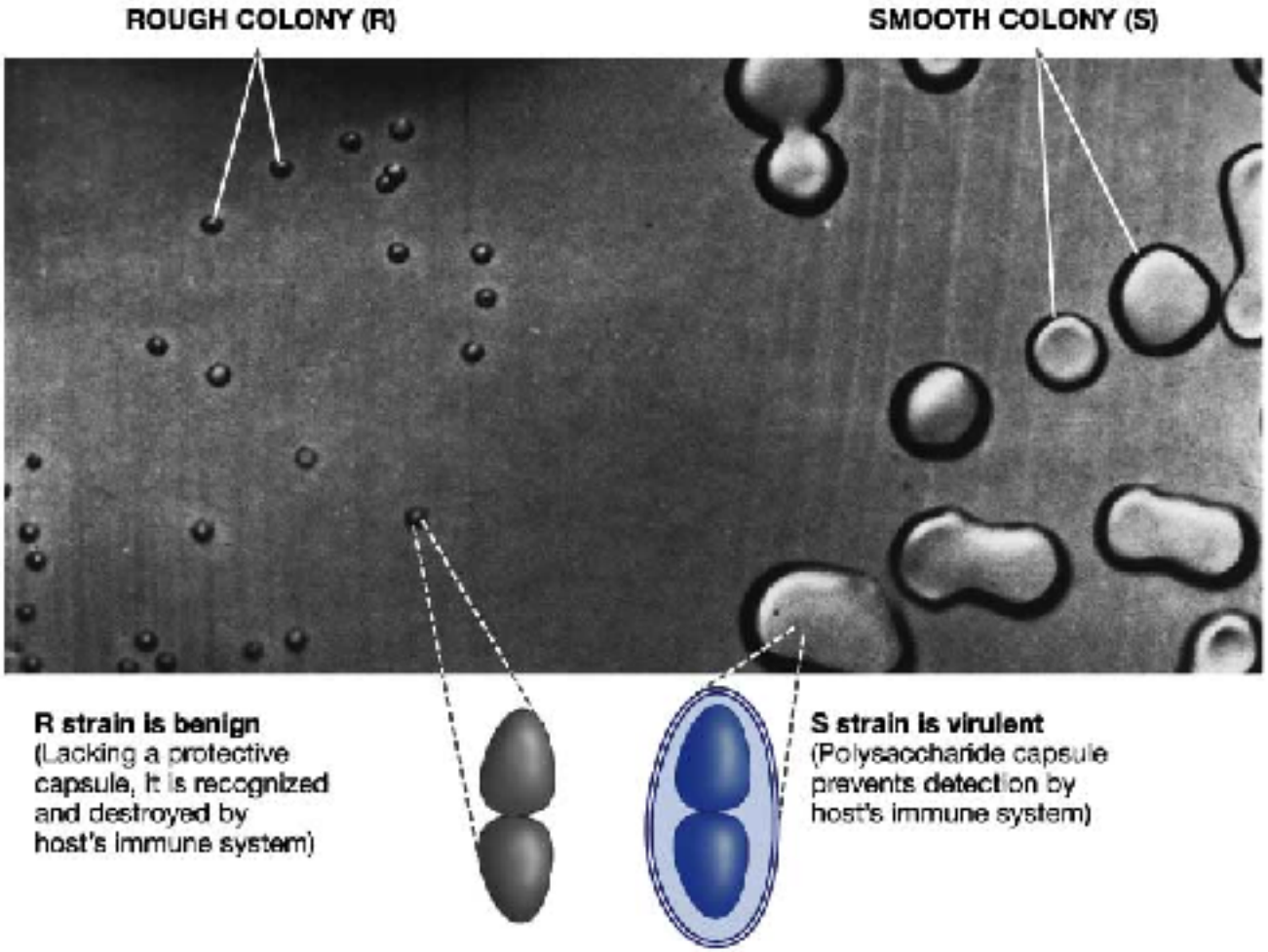
Streptococcus pneumoniae (causes lung disease – pneumonia).



Griffith's Model organism

Streptococcus pneumoniae

There are two strains of *Streptococcus pneumoniae*.



S. pneumoniae strains and mice survival

- Infection with R strain results in the survival of mice.
- Infection with S strain results in the death of mice.

Griffith's Model organism

What makes R strain's colonies rough?

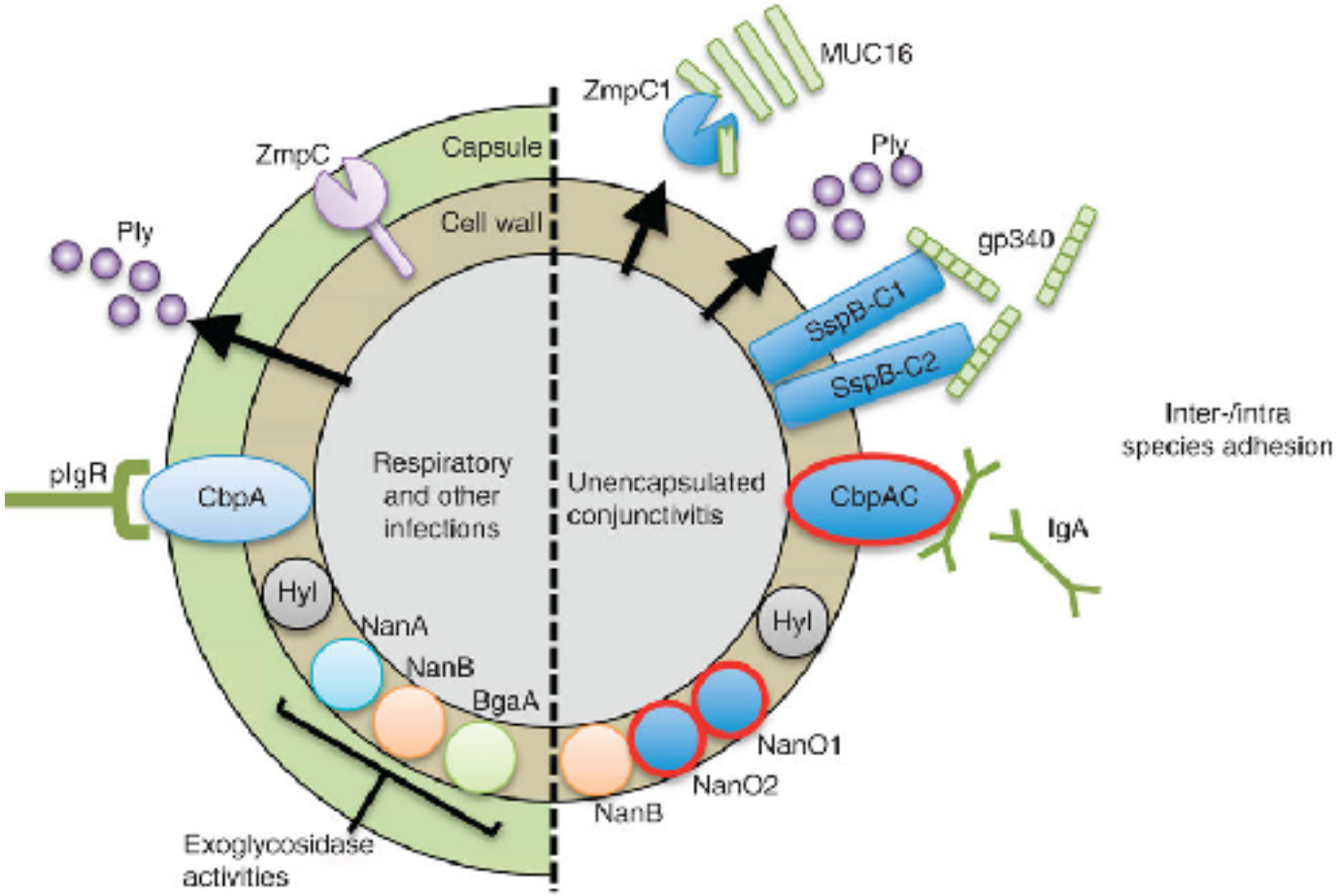
What makes the S strain colonies smooth?

What makes R strain tolerable by the immune system of the host (not pathogenic)?

What makes S strain intolerable by the immune system of the host (pathogenic)?

Griffith's Model organism

The capsule of the S strain gives it is smooth colony phenotype and it is reason for virulence



Griffith's Model organism

Where does the capsule of the S strain come from?

Streptococcus pneumoniae genomics

R strain genome sequence.

Genome size 2Mb.

JOURNAL OF BACTERIOLOGY, Oct. 2001, p. 5709–5717 Vol. 183, No. 19
0021-9193/01/$04.00+0 DOI: 10.1128/JB.183.19.5709–5717.2001

Genome of the Bacterium *Streptococcus pneumoniae* Strain R6

JOANN HOSKINS,[1] WILLIAM E. ALBORN, JR.,[1] JEFFREY ARNOLD,[2] LARRY C. BLASZCZAK,[1] STANLEY BURGETT,[2] BRADLEY S. DEHOFF,[2] SHAWN T. ESTREM,[1] LORI FRITZ,[2] DONG-JING FU,[2] WENDY FULLER,[2] CHAD GERINGER,[2] RAYMOND GILMOUR,[1] JENNIFER S. GLASS,[1] HAMID KHOJA,[1] ANGELIKA R. KRAFT,[1] ROBERT E. LAGACE,[3] DONALD J. LEBLANC,[1] LINDA N. LEE,[1] ELLIOT J. LEFKOWITZ,[4] JIN LU,[1] PATTI MATSUSHIMA,[1] SCOTT M. MCAHREN,[2] MARGARET MCHENNEY,[2] KEVIN MCLEASTER,[2] CHRISTOPHER W. MUNDY,[2] THALIA I. NICAS,[1] FRANKLIN H. NORRIS,[2] MARYJEANNE O'GARA,[1] ROBERT B. PEERY,[1] GREGORY T. ROBERTSON,[1] PAMELA ROCKEY,[2] PEI-MING SUN,[1] MALCOLM E. WINKLER,[1] YONG YANG,[1] MICHELLE YOUNG-BELLIDO,[1] GENSHI ZHAO,[1] CHRISTOPHER A. ZOOK,[1] RICHARD H. BALTZ,[1] S. RICHARD JASKUNAS,[1] PAUL R. ROSTECK, JR.,[2] PAUL L. SKATRUD,[1] AND JOHN I. GLASS[1]*

Infectious Diseases Research and Clinical Investigation[1] and Biological Research Technologies and Proteins,[2] Eli Lilly and Company, Indianapolis, Indiana 46285; Incyte Genomics, Palo Alto, California, 94304[3]; and Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294[4]

Received 20 June 2001/Accepted 13 July 2001

***Streptococcus pneumoniae* is among the most significant causes of bacterial disease in humans. Here we report the 2,038,615-bp genomic sequence of the gram-positive bacterium *S. pneumoniae* R6. Because the R6 strain is avirulent and, more importantly, because it is readily transformed with DNA from homologous species and many heterologous species, it is the principal platform for investigation of the biology of this important pathogen. It is also used as a primary vehicle for genomics-based development of antibiotics for gram-positive bacteria. In our analysis of the genome, we identified a large number of new uncharacterized genes predicted to encode proteins that either reside on the surface of the cell or are secreted. Among those proteins there may be new targets for vaccine and antibiotic development.**

S strain genome sequence comparison to that of R strain genome.

JOURNAL OF BACTERIOLOGY, Jan. 2007, p. 38–51 Vol. 189, No. 1
0021-9193/07/$08.00+0 doi:10.1128/JB.01148-06

Genome Sequence of Avery's Virulent Serotype 2 Strain D39 of *Streptococcus pneumoniae* and Comparison with That of Unencapsulated Laboratory Strain R6[▽]‡

Joel A. Lanie,[1]† Wai-Leung Ng,[1]† Krystyna M. Kazmierczak,[1]† Tiffany M. Andrzejewski,[1] Tanja M. Davidsen,[2] Kyle J. Wayne,[1] Hervé Tettelin,[2] John I. Glass,[3] and Malcolm E. Winkler[1]*

Department of Biology, Indiana University, Bloomington, Indiana 47405[1]; The Institute for Genomic Research, Rockville, Maryland 20850[2]; and J. Craig Venter Institute, Rockville, Maryland 20850[3]

Received 28 July 2006/Accepted 6 October 2006

Streptococcus pneumoniae **(pneumococcus) is a leading human respiratory pathogen that causes a variety of serious mucosal and invasive diseases. D39 is an historically important serotype 2 strain that was used in experiments by Avery and coworkers to demonstrate that DNA is the genetic material. Although isolated nearly a century ago, D39 remains extremely virulent in murine infection models and is perhaps the strain used most frequently in current studies of pneumococcal pathogenesis. To date, the complete genome sequences have been reported for only two *S. pneumoniae* strains: TIGR4, a recent serotype 4 clinical isolate, and laboratory strain R6, an avirulent, unencapsulated derivative of strain D39. We report here the genome sequences and new annotation of two different isolates of strain D39 and the corrected sequence of strain R6. Comparisons of these three related sequences allowed deduction of the likely sequence of the D39 progenitor and mutations that arose in each isolate. Despite its numerous repeated sequences and IS elements, the serotype 2 genome has remained remarkably stable during cultivation, and one of the D39 isolates contains only five relatively minor mutations compared to the deduced D39 progenitor. In contrast, laboratory strain R6 contains 71 single-base-pair changes, six deletions, and four insertions and has lost the cryptic pDP1 plasmid compared to the D39 progenitor strain. Many of these mutations are in or affect the expression of genes that play important roles in regulation, metabolism, and virulence. The nature of the mutations that arose spontaneously in these three strains, the relative global transcription patterns determined by microarray analyses, and the implications of the D39 genome sequences to studies of pneumococcal physiology and pathogenesis are presented and discussed.**

Streptococcus pneumoniae genomics

Comparing the two genomes allows the identification of key genes associated with pathogenicity.

TABLE 3. Virulence and physiologically important genes with altered sequences in strain D39 compared to strain R6[a]

D39 locus tag	Gene	Function	Source or reference[b]
SPD_0063	*strH*	β-*N*-Acetylhexosaminidase	STM (43)
SPD_0065	*bgaC*	β-Galactosidase	52
SPD_0080		SSURE fibronectin binding	21
SPD_0081	*rr08*	Response regulator	63, 111
SPD_0168	*ribE*	Riboflavin synthesis	71
SPD_0315-SPD_0323	Δ*cps*	Capsule biosynthesis	3, 69
SPD_0336	*pbp1A*	Penicillin-binding protein transglycosylase/transpeptidase	49, 64, 90
SPD_0479	*nusA*	Transcription termination/antitermination	18
SPD_0636	*spxB*	Pyruvate oxidase	STM/experimental (12, 65, 87, 91, 92, 106)
SPD_0660	*ftsX*	ABC transporter/cell division	102
SPD_0665	*pyrDA*	Dihydro-orotate dehydrogenase (pyrimidine biosynthesis)	5
SPD_0773	*fruA*	Fructose PTS	STM (43)
SPD_0854	*flpA*(*pavA*)	Fibronectin binding	STM/experimental (47, 64, 95)
SPD_0967	*murA1*	Homolog of MurA (first step in murein biosynthesis)	19, 70
SPD_1131	*carB*	Carbamoylphosphate synthase, heavy subunit (pyrimidine biosynthesis)	73
SPD_1337	*atpA*	Proton-translocating ATPase, F1 α subunit	32
SPD_1346		Hypothetical	STM (43)
SPD_1461	*psaB*	Manganese transport	Experimental (24, 27, 72, 75, 85)
SPD_1462	*psaC*	Manganese transport	Experimental (24, 27, 72, 75, 85)
SPD_1512	*secA*	Preprotein translocase subunit	93
SPD_1671	*amiA*	Oligopeptide transport	2, 57
SPD_1740	*cinA*	Competence induced	STM (43)
SPD_1758	*rpoC*	RNA polymerase β′ subunit	116
SPD_1797	*ccpA*	Catabolite control	STM/experimental (37, 53)
SPD_1961		Hypothetical (putative transcription regulator)	STM (43)
SPD_1965	*pcpA*	Choline-binding protein	STM/experimental (43, 101)
SPD_1974		Conserved hypothetical	STM (43)
SPD_1987		Hypothetical (fucolectin-related protein)	STM (43)
SPD_2005	*dltA*	D-Ala ligase	STM (43)
SPD_2012	*glpO*	α-Glycerophosphate oxidase	98
SPD_2022	*clpC*	Stress-related ATPase	Experimental (22, 23, 51, 96, 97)
SPD_2028	*cbpD*	Murein hydrolase	STM/experimental (, 40, 43, 46, 56)

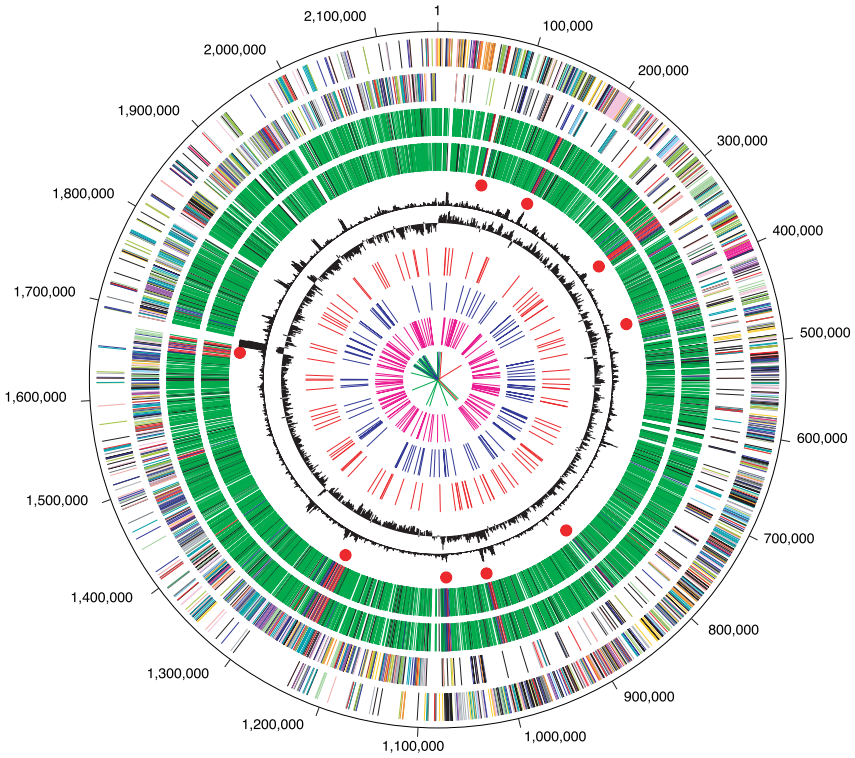
[a] Specific mutations are listed in Tables S1 and S3 in the supplemental material.
[b] Evidence for a role in virulence exists where indicated. STM, signature-tagged mutagenesis.

Streptococcus pneumoniae genomics

Complete Genome Sequence of a Virulent Isolate of *Streptococcus pneumoniae*

Hervé Tettelin,[1] Karen E. Nelson,[1] Ian T. Paulsen,[1,2] Jonathan A. Eisen,[1,2] Timothy D. Read,[1] Scott Peterson,[1,3] John Heidelberg,[1] Robert T. DeBoy,[1] Daniel H. Haft,[1] Robert J. Dodson,[1] A. Scott Durkin,[1] Michelle Gwinn,[1] James F. Kolonay,[1] William C. Nelson,[1] Jeremy D. Peterson,[1] Lowell A. Umayam,[1] Owen White,[1] Steven L. Salzberg,[1,4] Matthew R. Lewis,[1] Diana Radune,[1] Erik Holtzapple,[1] Hoda Khouri,[1] Alex M. Wolf,[1] Terry R. Utterback,[1] Cheryl L. Hansen,[1] Lisa A. McDonald,[1] Tamara V. Feldblyum,[1] Samuel Angiuoli,[1] Tanja Dickinson,[1] Erin K. Hickey,[1] Ingeborg E. Holt,[1] Brendan J. Loftus,[1] Fan Yang,[1] Hamilton O. Smith,[1*] J. Craig Venter,[1*] Brian A. Dougherty,[5] Donald A. Morrison,[6] Susan K. Hollingshead,[7] Claire M. Fraser[1,3†]

The 2,160,837–base pair genome sequence of an isolate of *Streptococcus pneumoniae*, a Gram-positive pathogen that causes pneumonia, bacteremia, meningitis, and otitis media, contains 2236 predicted coding regions; of these, 1440 (64%) were assigned a biological role. Approximately 5% of the genome is composed of insertion sequences that may contribute to genome rearrangements through uptake of foreign DNA. Extracellular enzyme systems for the metabolism of polysaccharides and hexosamines provide a substantial source of carbon and nitrogen for *S. pneumoniae* and also damage host tissues and facilitate colonization. A motif identified within the signal peptide of proteins is potentially involved in targeting these proteins to the cell surface of low–guanine/cytosine (GC) Gram-positive species. Several surface-exposed proteins that may serve as potential vaccine candidates were identified. Comparative genome hybridization with DNA arrays revealed strain differences in *S. pneumoniae* that could contribute to differences in virulence and antigenicity.



Red circles: 9 gene clusters differ between the two strains.

Tettelin et al. (2001) Complete genome sequence of a virulent isolate of Streptococcus pneumoniae. Science. 20;293(5529):498-506.

Streptococcus pneumoniae genomics

Who cares?

Identifying key genes associated with pathogenicity allows the development of specific antibiotics and vaccines.

Keeping these facts in mind, let's go over Griffith's transformation experiment.

Griffith's Model organism

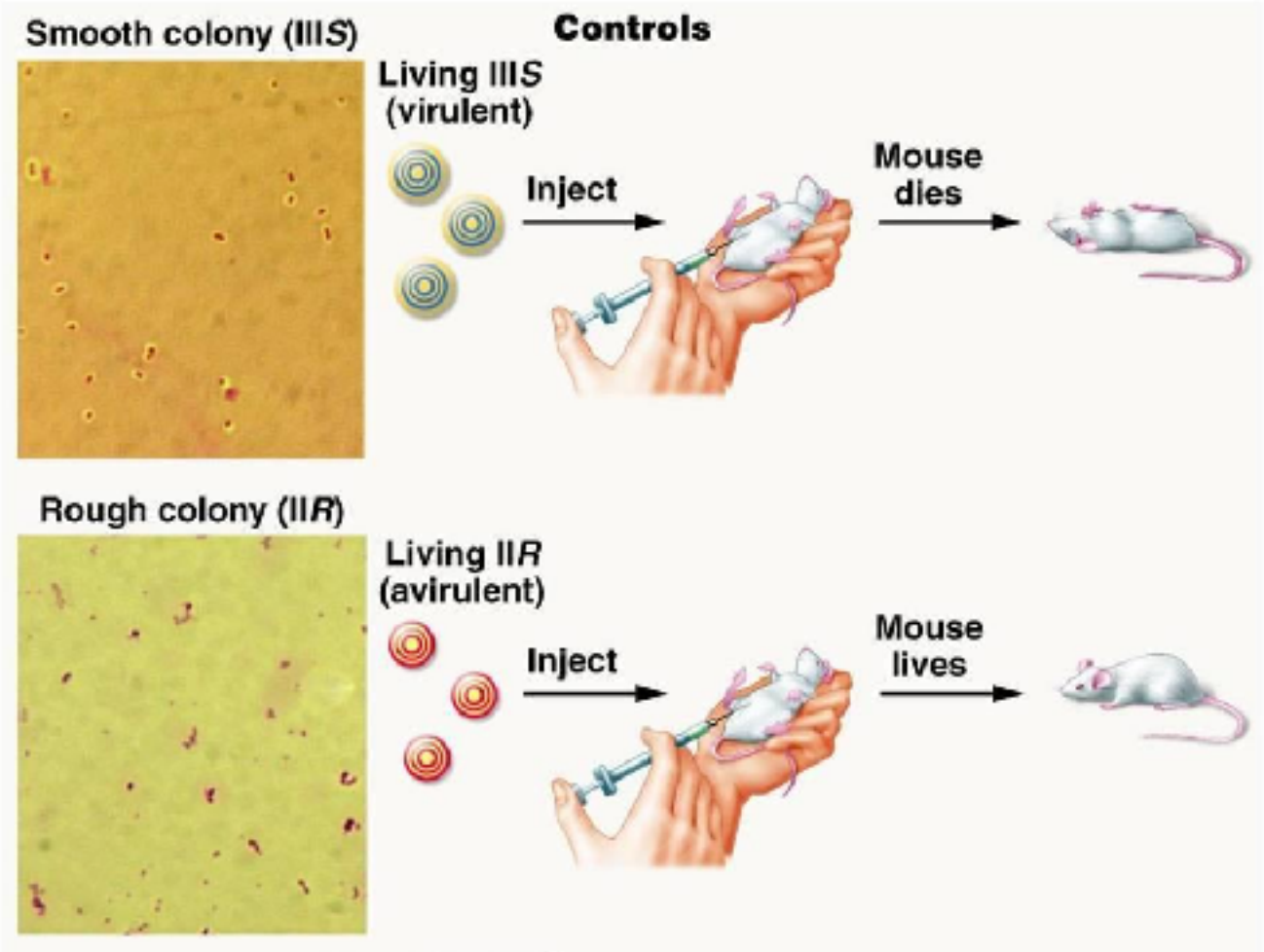
What phenotypes Griffith observed and designed his experiments based on?

Colony appearance?

strain virulence/pathogenicity?

Griffith's phenotypes

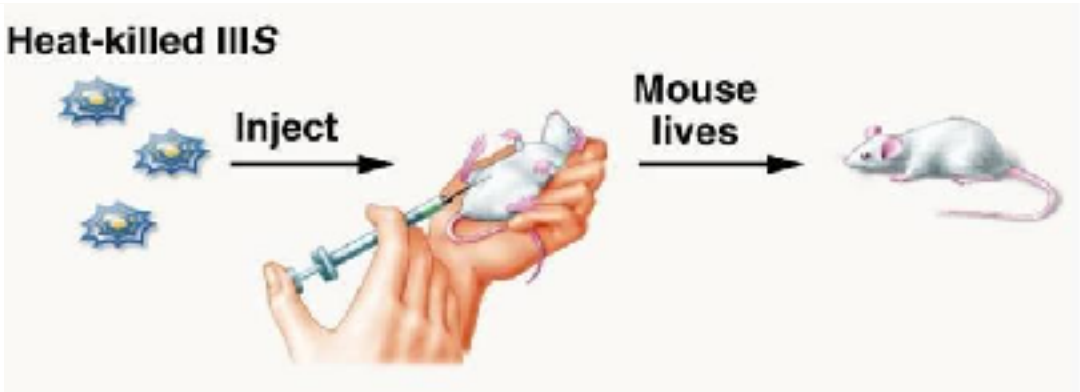
Griffith used the effect of each strain on the survival of mice as a phenotype.



Griffith's phenotypes

Griffith observed that heat-killed S strain sample loses its pathogenicity.

Mouse injected with heat-killed virulant S strain lives.



What happens when the S strain sample is heated?

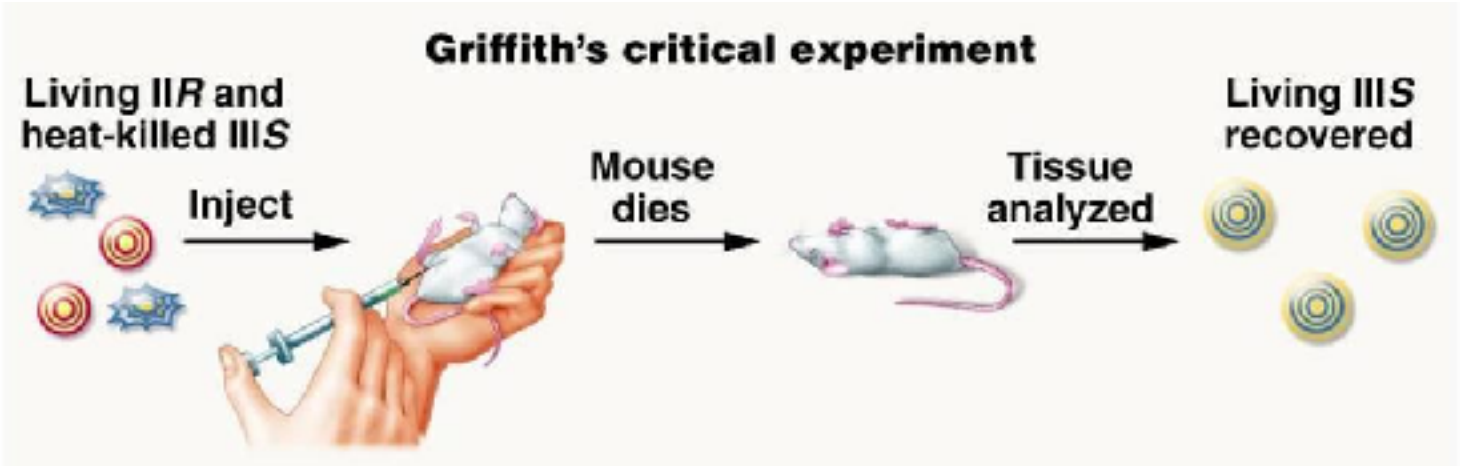
Griffith's transformation experiment

Fredrick Griffith question:

What happens when the heat-killed S strain is combined with the living R strain?

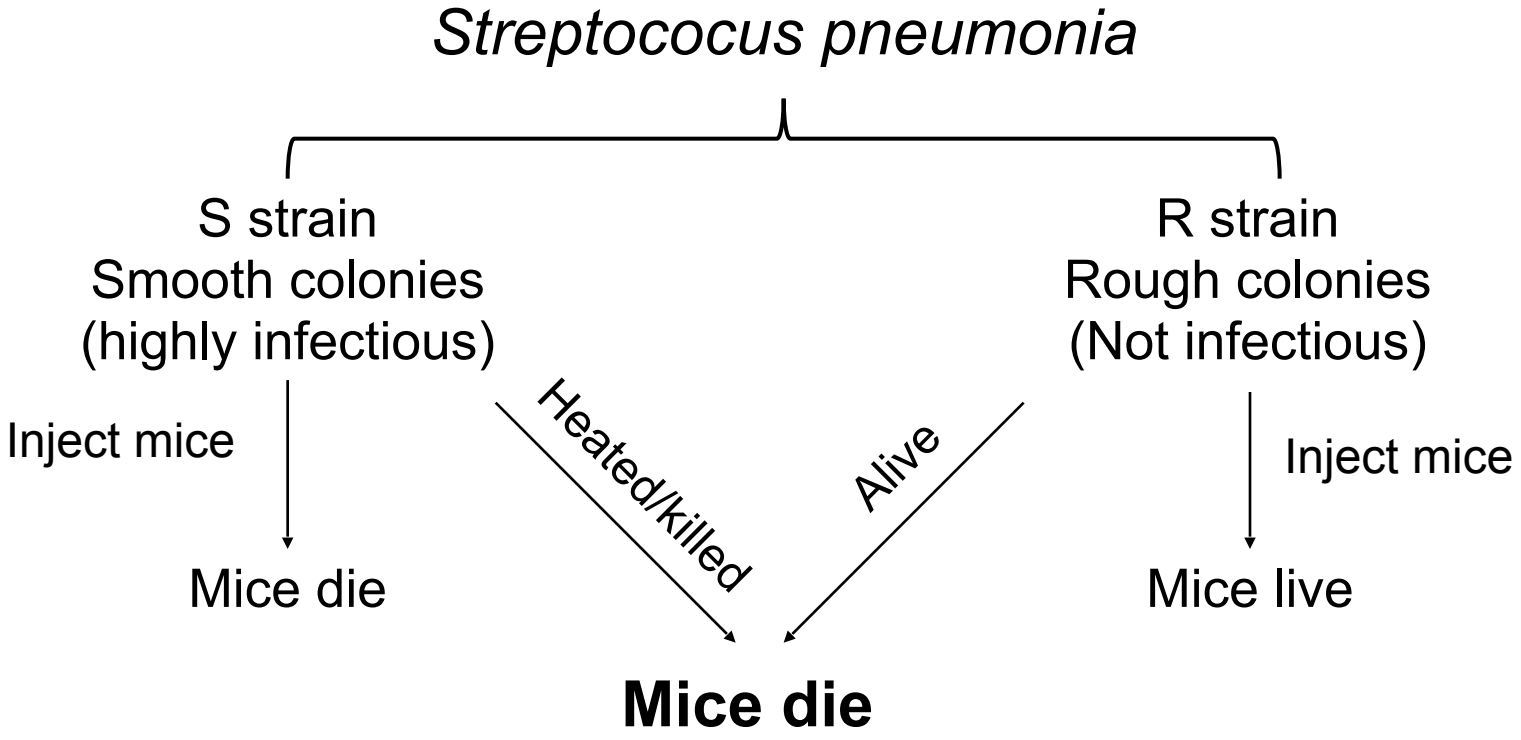
Griffith's transformation experiment

Griffith observed that wen heat-killed S strain sample is combined with living R strain, the R strain **transforms** into a deadly S strains.



Mouse injected with both heat-killed S strain and live non-virulant R strain dies.

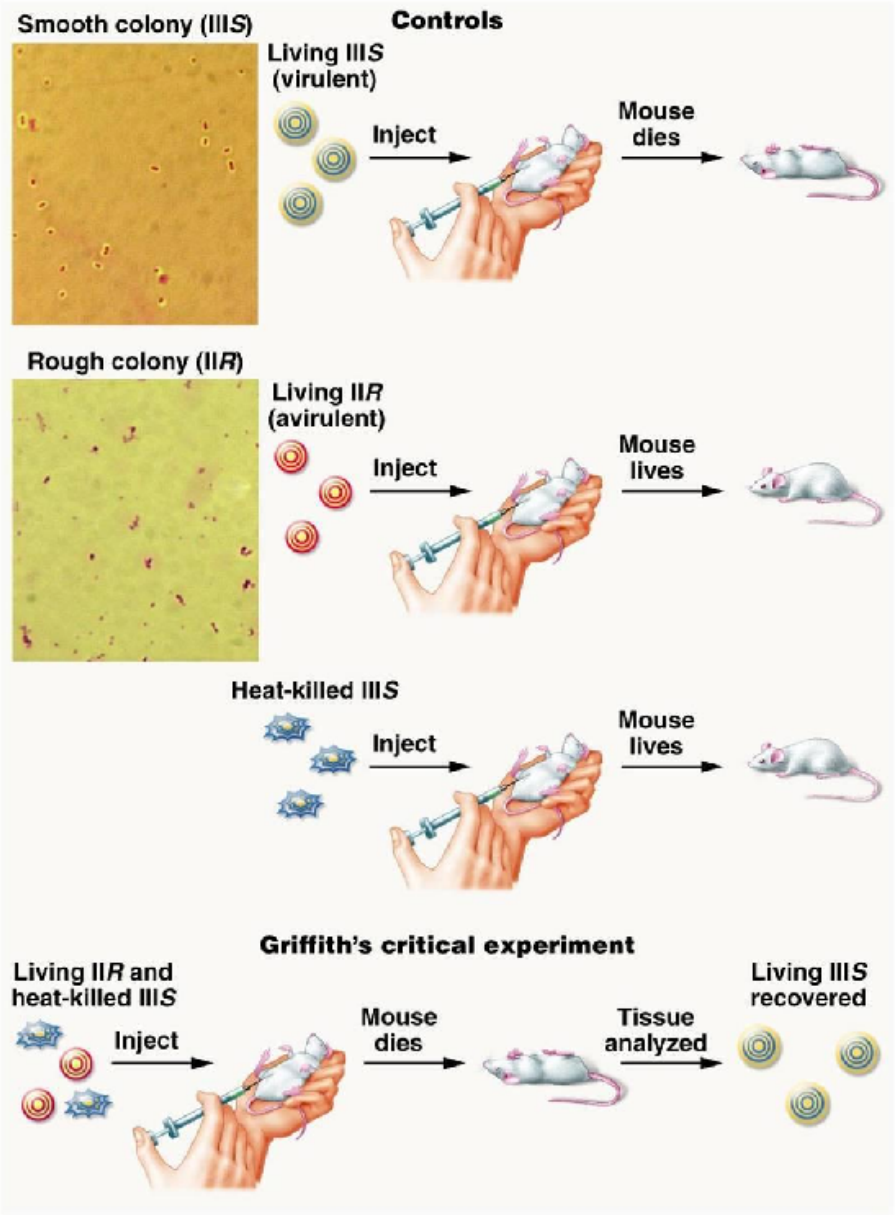
Griffith's transformation experiment



So R living cells were **transformed** by interacting with molecules of dead S starin

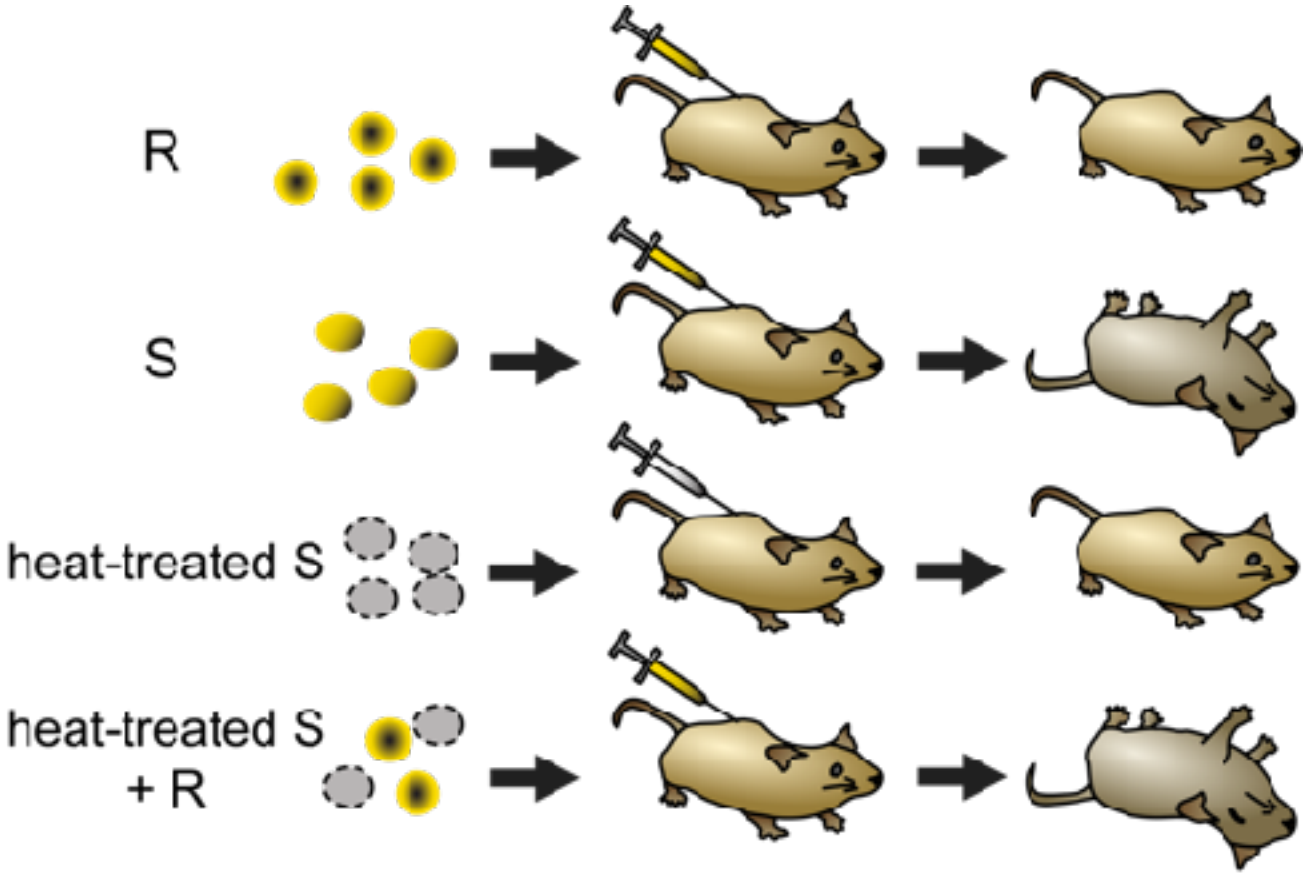
Griffith's transformation experiment

What is unique in the design of Griffith's transformation experiment?



Griffith's transformation experiment

- Griffith's conclusion: R changes into S by acquiring a molecule.
- He called the molecule (**transforming principle**).



Griffith's transformation experiment

How did the R strain become deadly S by the interaction with S strain soup?

The molecule that makes S strain pathogenic entered the R strain and changed it.

Genetic exchange

Bacteria transmit genetic material vertically and horizontally.

Vertical transmission involves passing genes to the next generation.

Horizontal transmission is the exchange of genetic material within the same generation

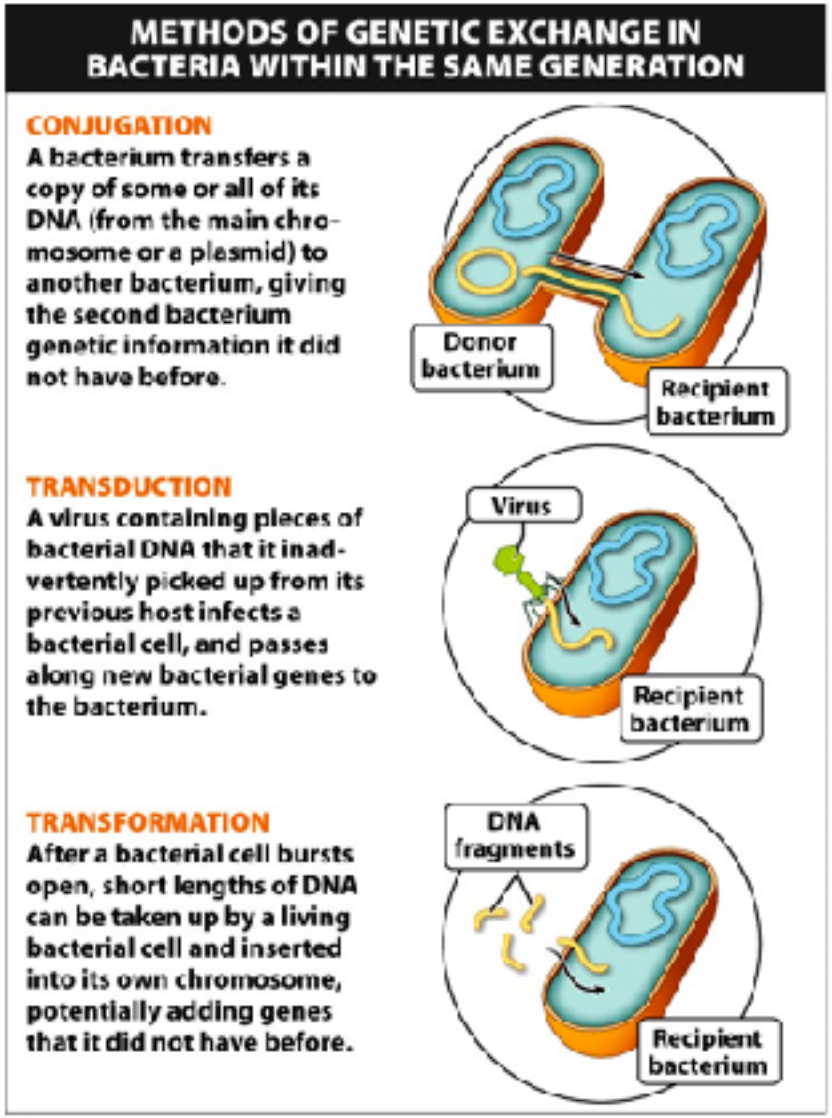
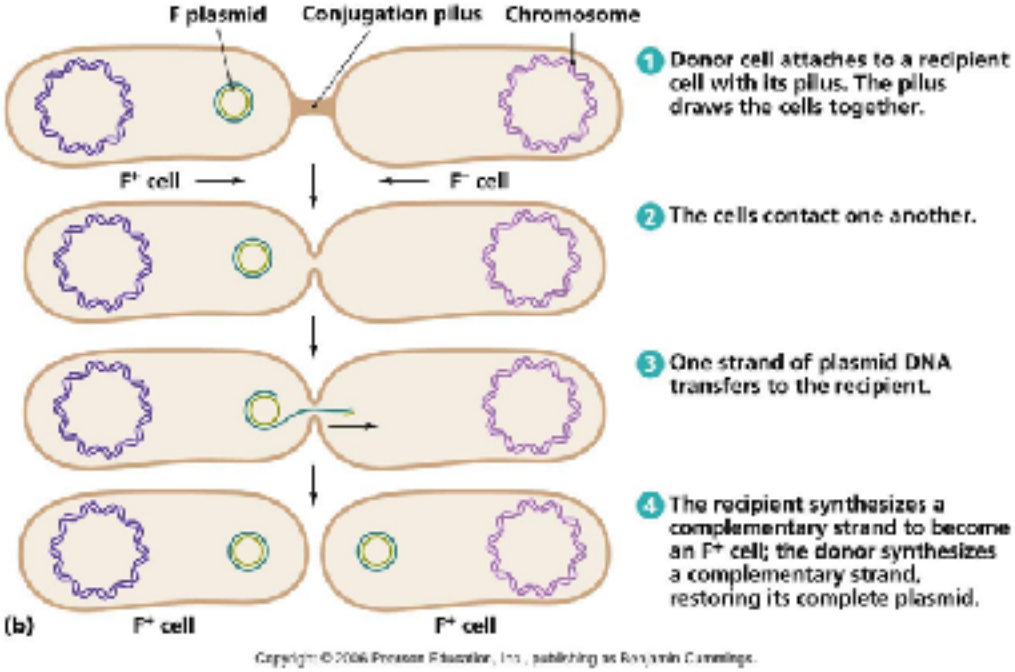
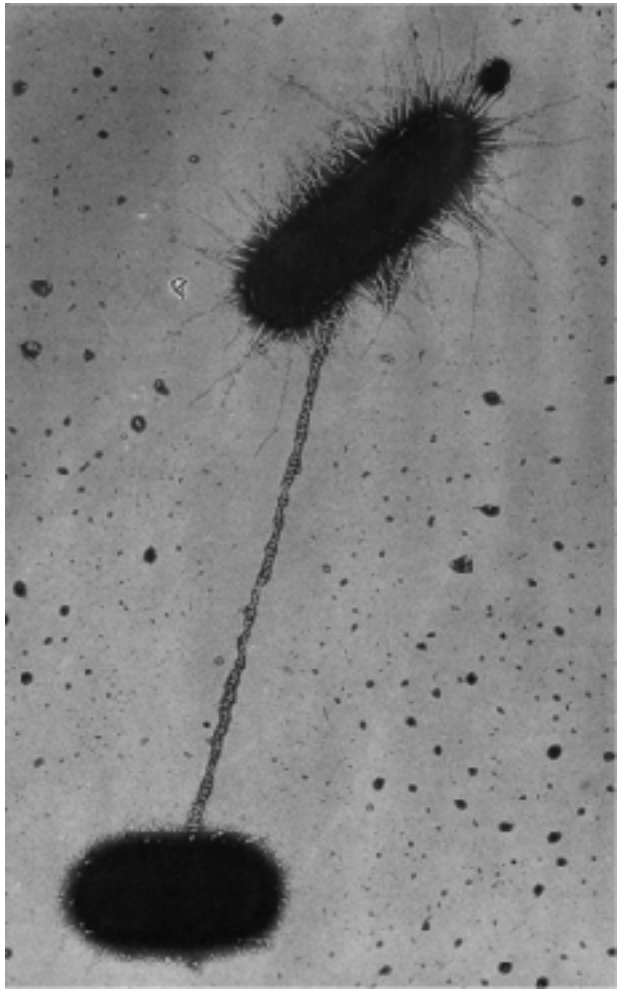


Figure 13-8
What is Life? A Guide To Biology

Conjugation

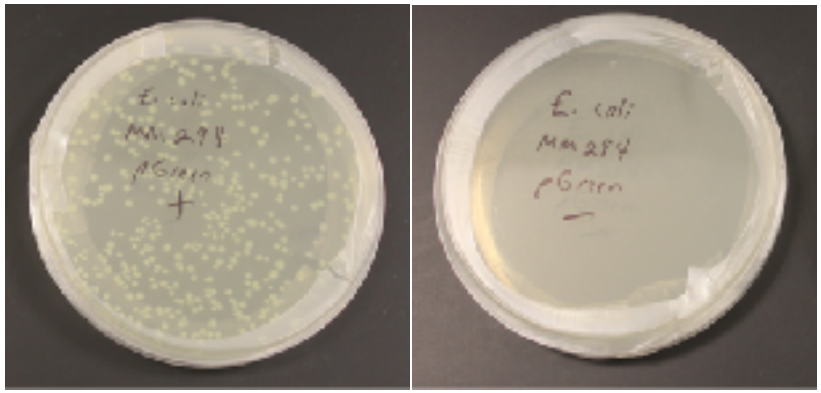
- Exchange of genetic material between two bacteria (bacterial sexual cycle?).
- Mostly transfer of plasmids.
- What is a plasmid?

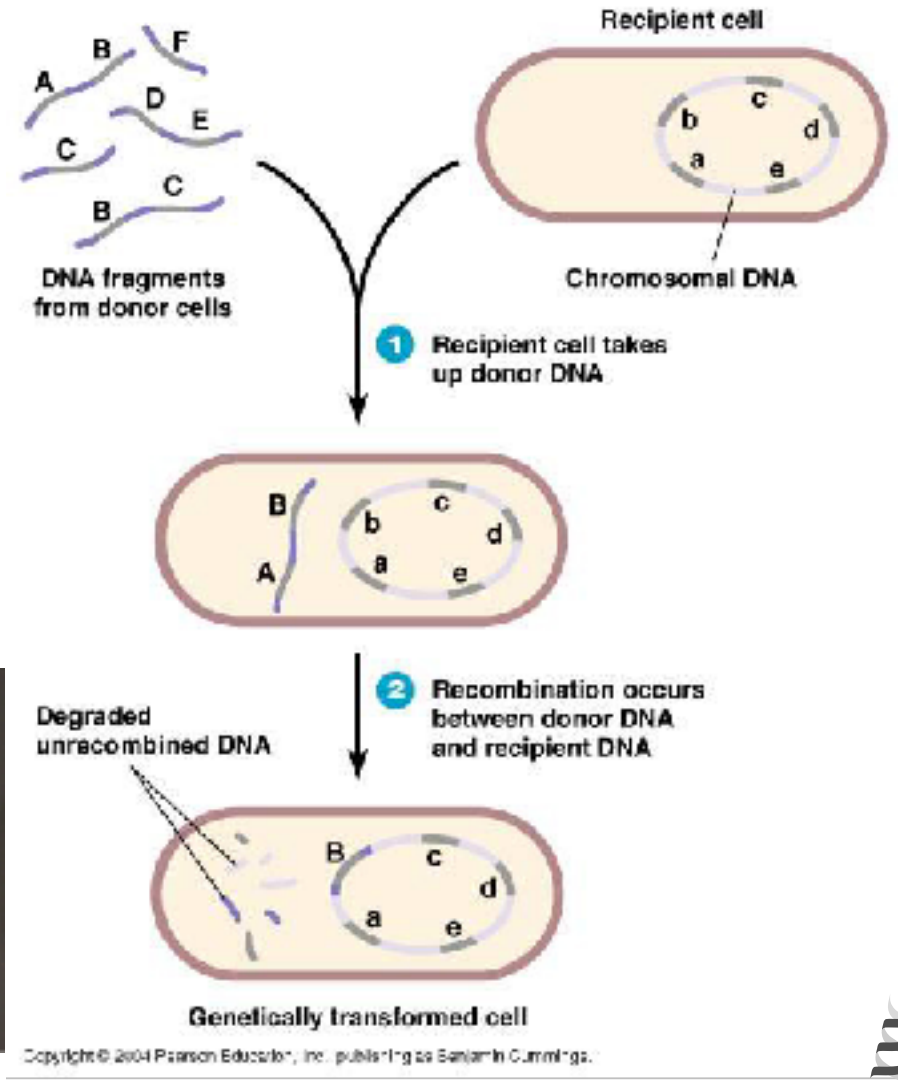




Transformation

- Bacteria acquires genetic material by recombination of similar genomic parts.
- Very important technological advancement.



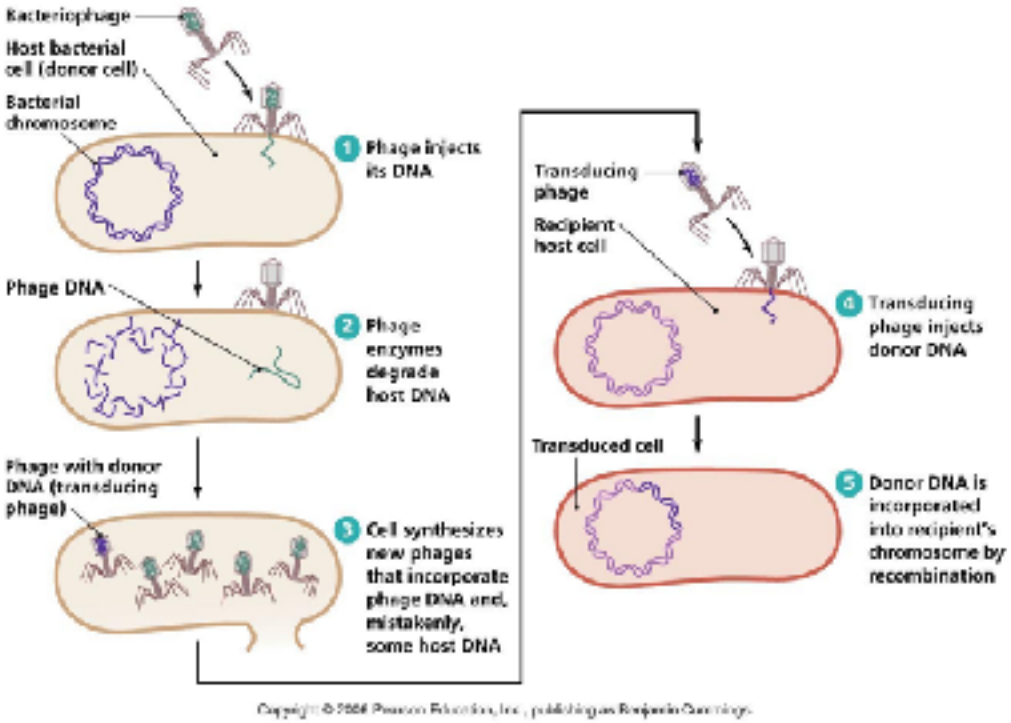


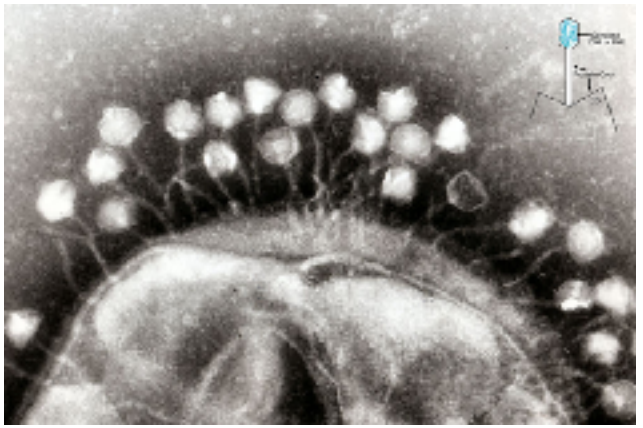
Transformation

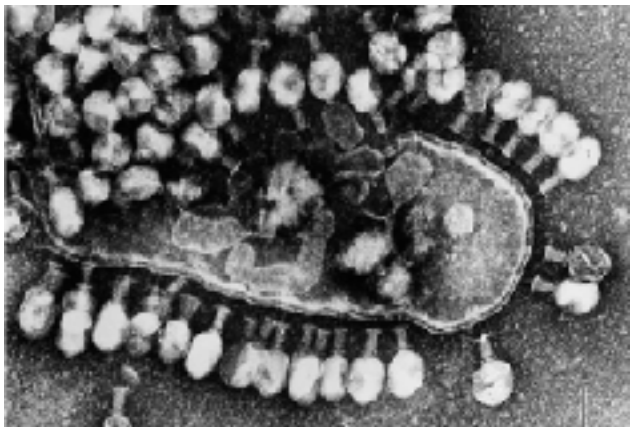
What is the requirement for recombination to occur?

Transduction

- Bacteria acquires genetic material transferred (un-intentionally?) by bacteriophages.
- Recombination?







Transduction

Does transduction require sequence similarity to occur?

Griffith's transformation experiment

Back to Griffith's transforming principle

Did Griffith prove which molecule is the transforming principle?

Avery's transformation experiment

STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III

BY OSWALD T. AVERY, M.D., COLIN M. MACLEOD, M.D., AND MACLYN MCCARTY,* M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research*)

PLATE 1

(Received for publication, November 1, 1943)

Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters. Among microörganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of Pneumococcus. This phenomenon was first described by Griffith (1) who succeeded in transforming an attenuated and non-encapsulated (R) variant derived from one specific type into fully encapsulated and virulent (S) cells of a heterologous specific type. A typical instance will suffice to illustrate the techniques originally used and serve to indicate the wide variety of transformations that are possible within the limits of this bacterial species.

Griffith found that mice injected subcutaneously with a small amount of a living R culture derived from Pneumococcus Type II together with a large inoculum of heat-killed Type III (S) cells frequently succumbed to infection, and that the heart's blood of these animals yielded Type III pneumococci in pure culture. The fact that the R strain was avirulent and incapable by itself of causing fatal bacteremia and the additional fact that the heated suspension of Type III cells contained no viable organisms brought convincing evidence that the R forms growing under these conditions had newly acquired the capsular structure and biological specificity of Type III pneumococci.

The original observations of Griffith were later confirmed by Neufeld and Levinthal (2), and by Baurhenn (3) abroad, and by Dawson (4) in this laboratory. Subsequently Dawson and Sia (5) succeeded in inducing transformation *in vitro*. This they accomplished by growing R cells in a fluid medium containing anti-R serum and heat-killed encapsulated S cells. They showed that in the test tube as in the animal body transformation can be selectively induced, depending on the type specificity of the S cells used in the reaction system. Later, Alloway (6) was able to cause

* Work done in part as Fellow in the Medical Sciences of the National Research Council.

Avery, O. T., MacLeod, C. M., & McCarty, M. (1944). STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES : INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III. The Journal of Experimental Medicine, 79(2), 137–158.

Avery's transformation experiment

Avery is asking the question:

Which component of the S cells is the transforming principle (genetic code)?

The idea is to rerun Griffith experiment with some modifications.

Avery's transformation experiment

Lysed cells (heat-killed S strain) contain:

- Proteins
- Polysaccharides
- RNA
- DNA

One of these molecules must be the genetic material

Avery's transformation experiment

How can we destroy each of the molecules only one at a time?

Proteins

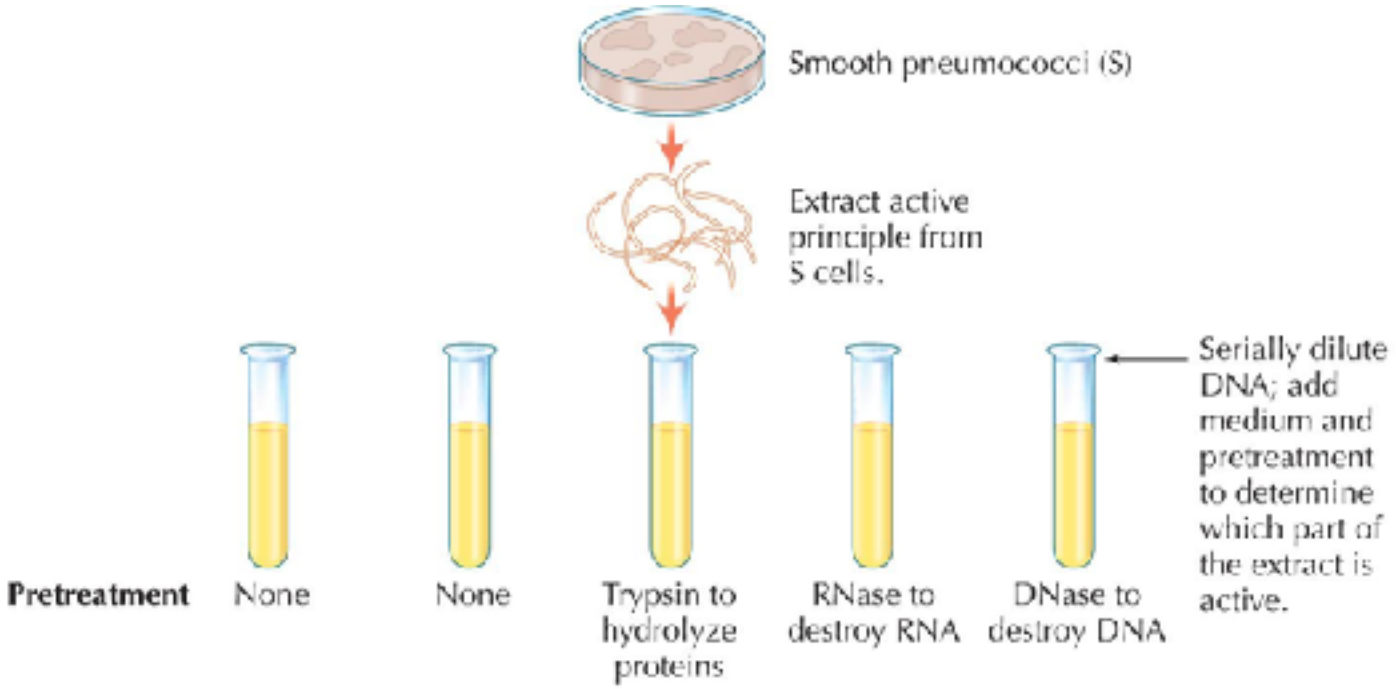
RNA

DNA

Avery's transformation experiment

Avery's Experimental design:

Treat S strain soup with different classes of enzymes and observe resulting transformation when soup is added to R strain



Avery's transformation experiment

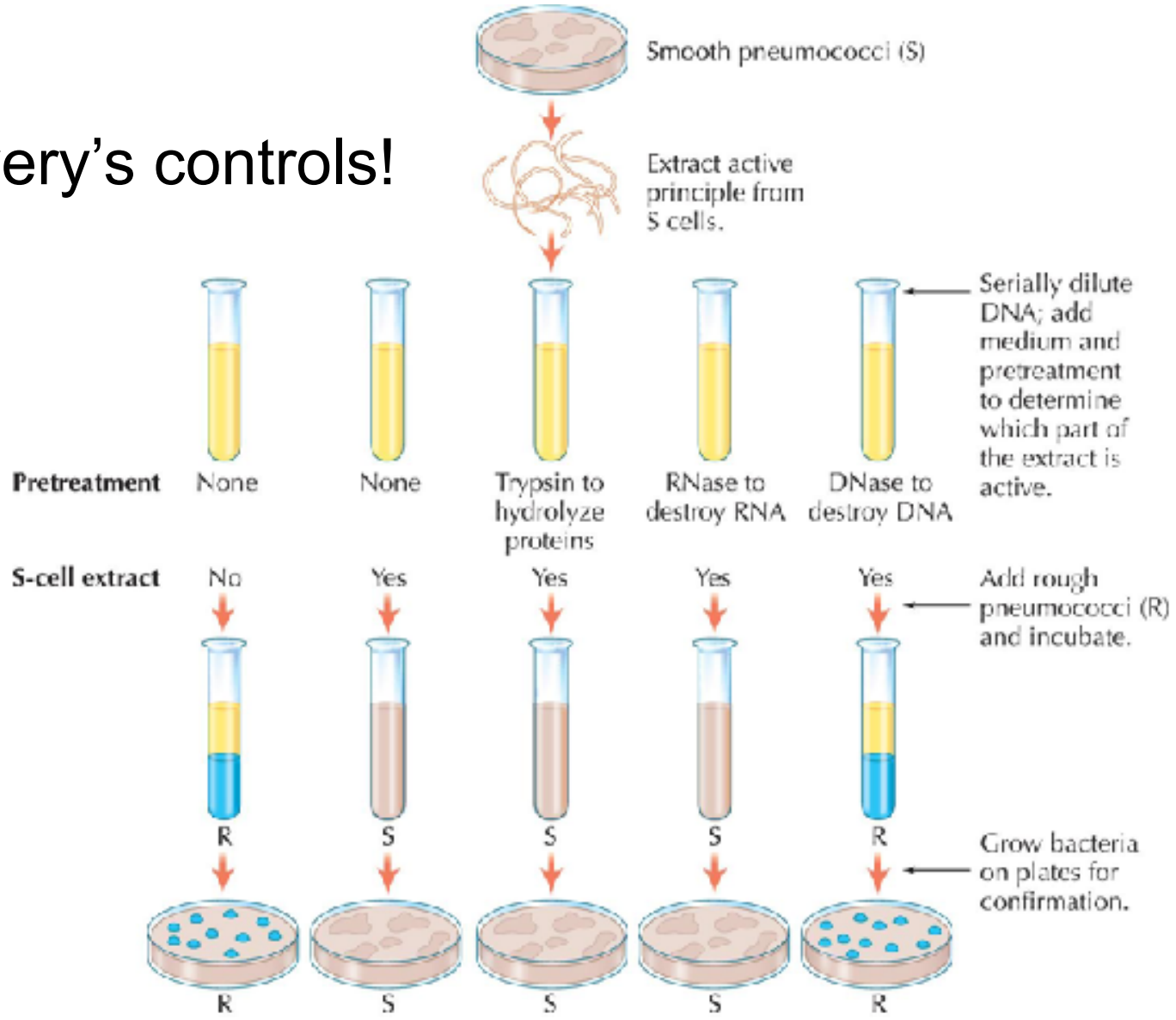
What phenotypes Avery's observed and designed his experiments based on?

Colony appearance?

strain virulence/pathogenicity?

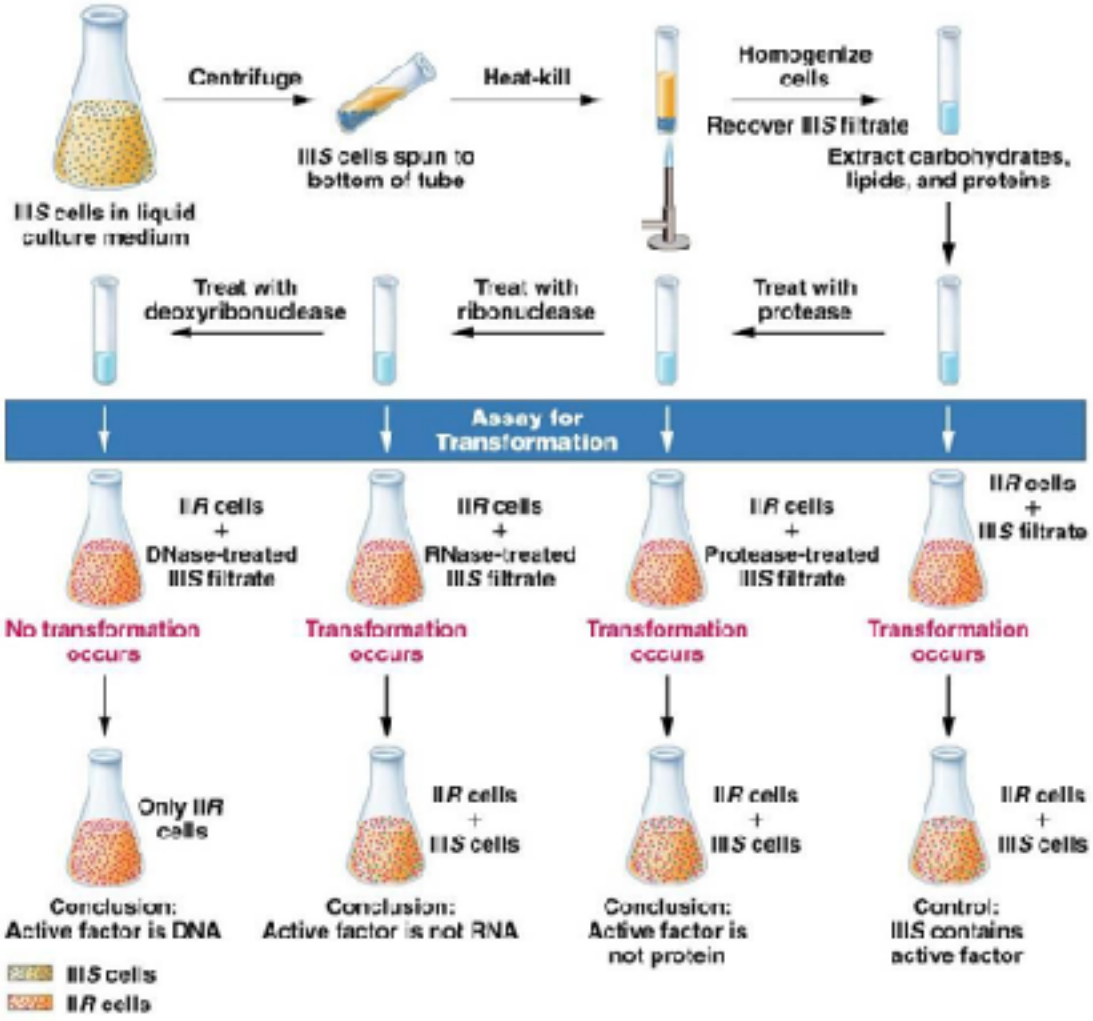
Avery's transformation experiment

Avery's controls!



Avery's transformation experiment

The molecule when degraded R cells do not change to S is the transforming principle



Avery's conclusion

SUMMARY

1. From Type III pneumococci a biologically active fraction has been isolated in highly purified form which in exceedingly minute amounts is capable under appropriate cultural conditions of inducing the transformation of unencapsulated R variants of Pneumococcus Type II into fully encapsulated cells of the same specific type as that of the heat-killed microorganisms from which the inducing material was recovered.

2. Methods for the isolation and purification of the active transforming material are described.

3. The data obtained by chemical, enzymatic, and serological analyses together with the results of preliminary studies by electrophoresis, ultracentrifugation, and ultraviolet spectroscopy indicate that, within the limits of the methods, the active fraction contains no demonstrable protein, unbound lipid, or serologically reactive polysaccharide and consists principally, if not solely, of a highly polymerized, viscous form of desoxyribonucleic acid.

4. Evidence is presented that the chemically induced alterations in cellular structure and function are predictable, type-specific, and transmissible in series. The various hypotheses that have been advanced concerning the nature of these changes are reviewed.

CONCLUSION

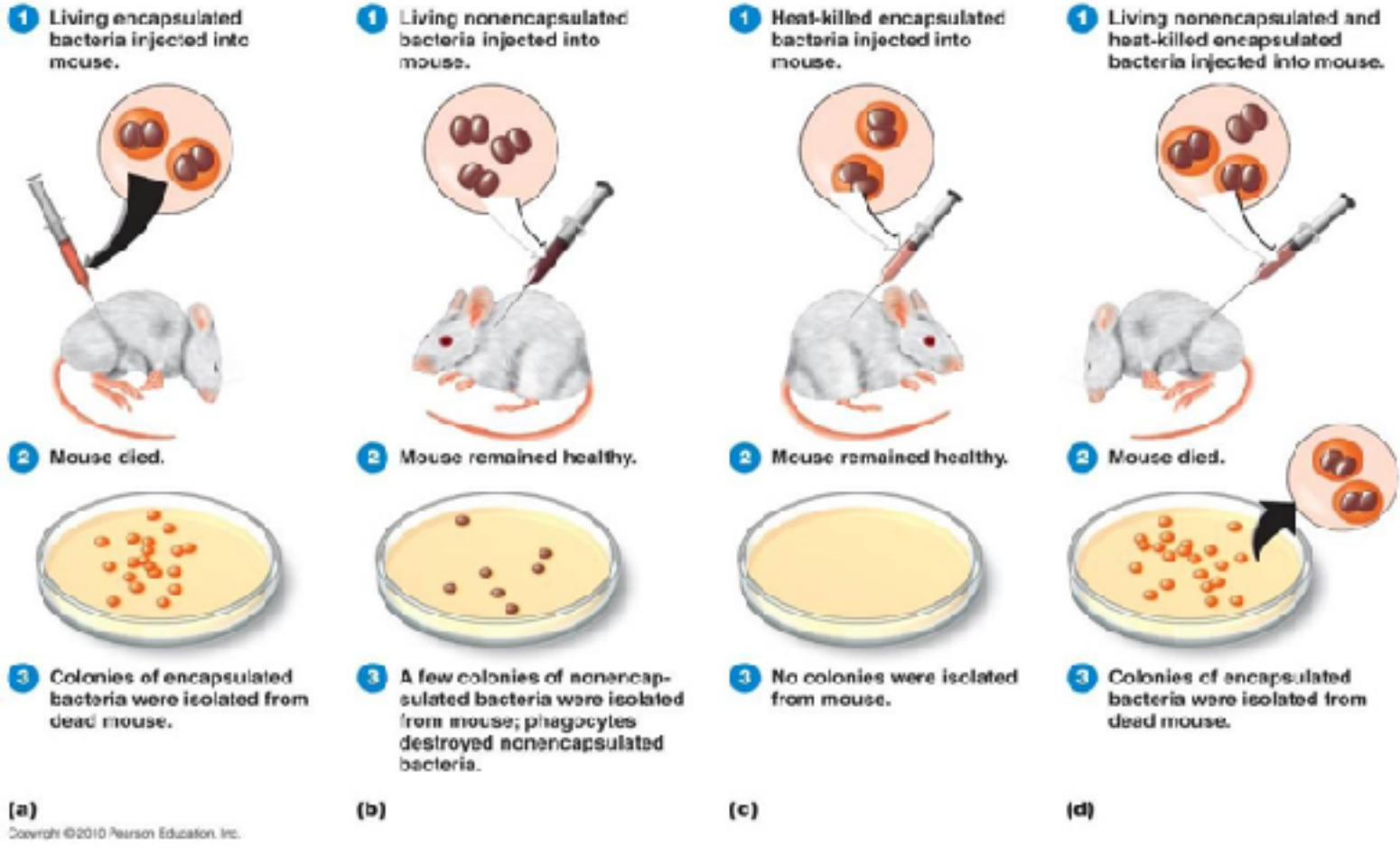
The evidence presented supports the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming principle of Pneumococcus Type III.

Avery, O. T., MacLeod, C. M., & McCarty, M. (1944). STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES : INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III. The Journal of Experimental Medicine, 79(2), 137–158.

Summary

Griffith's phenotypes

Avery's phenotypes



Not enough

- He used enzymes to degrade a specific class of molecules at a time then study the transformed cells.
- When DNA was degraded with DNase, no transformation occurred.
- Thus, DNA is the transformation principle!
- Clever right?
- Not good enough and convincing for some scientists. Why?
- Because the degrading enzymes were not pure!

We need an experiment to definitively prove that DNA is the genetic material

Hershey and Chase experiment

INDEPENDENT FUNCTIONS OF VIRAL PROTEIN AND NUCLEIC ACID IN GROWTH OF BACTERIOPHAGE*

By A. D. HERSHEY and MARTHA CHASE

(*From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island*)

(Received for publication, April 9, 1952)

The work of Doermann (1948), Doermann and Dissosway (1949), and Anderson and Doermann (1952) has shown that bacteriophages T2, T3, and T4 multiply in the bacterial cell in a non-infective form. The same is true of the phage carried by certain lysogenic bacteria (Lwoff and Gutmann, 1950). Little else is known about the vegetative phase of these viruses. The experiments reported in this paper show that one of the first steps in the growth of T2 is the release from its protein coat of the nucleic acid of the virus particle, after which the bulk of the sulfur-containing protein has no further function.

Materials and Methods.—Phage T2 means in this paper the variety called T2H (Hershey, 1946); T2*h* means one of the host range mutants of T2; UV-phage means phage irradiated with ultraviolet light from a germicidal lamp (General Electric Co.) to a fractional survival of 10^{-5}.

Sensitive bacteria means a strain (H) of *Escherichia coli* sensitive to T2 and its *h* mutant; resistant bacteria B/2 means a strain resistant to T2 but sensitive to its *h* mutant; resistant bacteria B/2*h* means a strain resistant to both. These bacteria do not adsorb the phages to which they are resistant.

"Salt-poor" broth contains per liter 10 gm. bacto-peptone, 1 gm. glucose, and 1 gm. NaCl. "Broth" contains, in addition, 3 gm. bacto-beef extract and 4 gm. NaCl.

Glycerol-lactate medium contains per liter 70 mM sodium lactate, 4 gm. glycerol, 5 gm. NaCl, 2 gm. KCl, 1 gm. NH_4Cl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.01 gm. gelatin, 10 mg. P (as orthophosphate), and 10 mg. S (as $MgSO_4$), at pH 7.0.

Adsorption medium contains per liter 4 gm. NaCl, 5 gm. K_2SO_4, 1.5 gm. KH_2PO_4, 3.0 gm. Na_2HPO_4, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.01 gm. gelatin, at pH 7.0.

Veronal buffer contains per liter 1 gm. sodium diethylbarbiturate, 3 mM $MgSO_4$, and 1 gm. gelatin, at pH 8.0.

The HCN referred to in this paper consists of molar sodium cyanide solution neutralized when needed with phosphoric acid.

* This investigation was supported in part by a research grant from the National Microbiological Institute of the National Institutes of Health, Public Health Service. Radioactive isotopes were supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, United States Atomic Energy Commission.

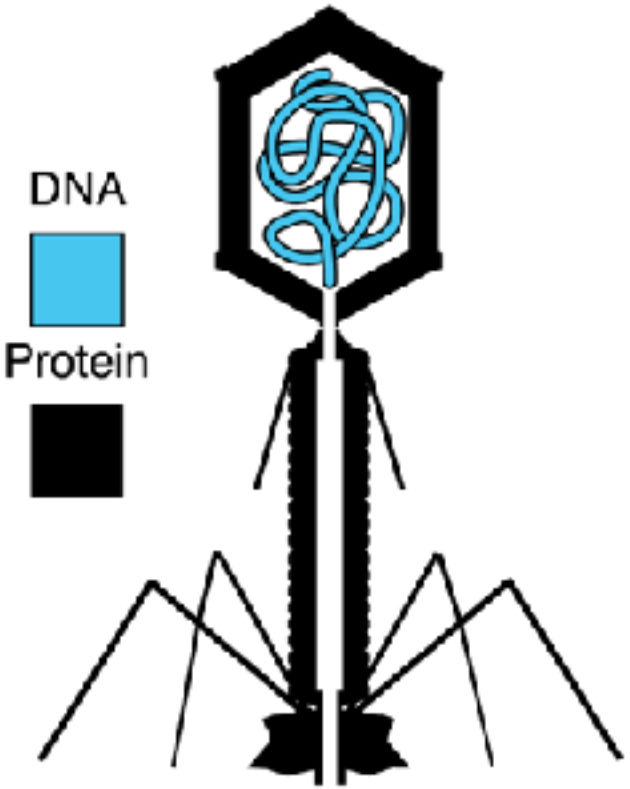
Hershey A, Chase M (1952). "Independent functions of viral protein and nucleic acid in growth of bacteriophage" . J Gen Physiol 36 (1): 39–56.

Hershey and Chase experiment

- Need more evidence that DNA is the genetic code.
- Hershey and Chase used a bacterial parasite (virus) to study the genetic code.
- **Question**: Which molecule (DNA or protein is the genetic code)?

Hershey and Chase model organism

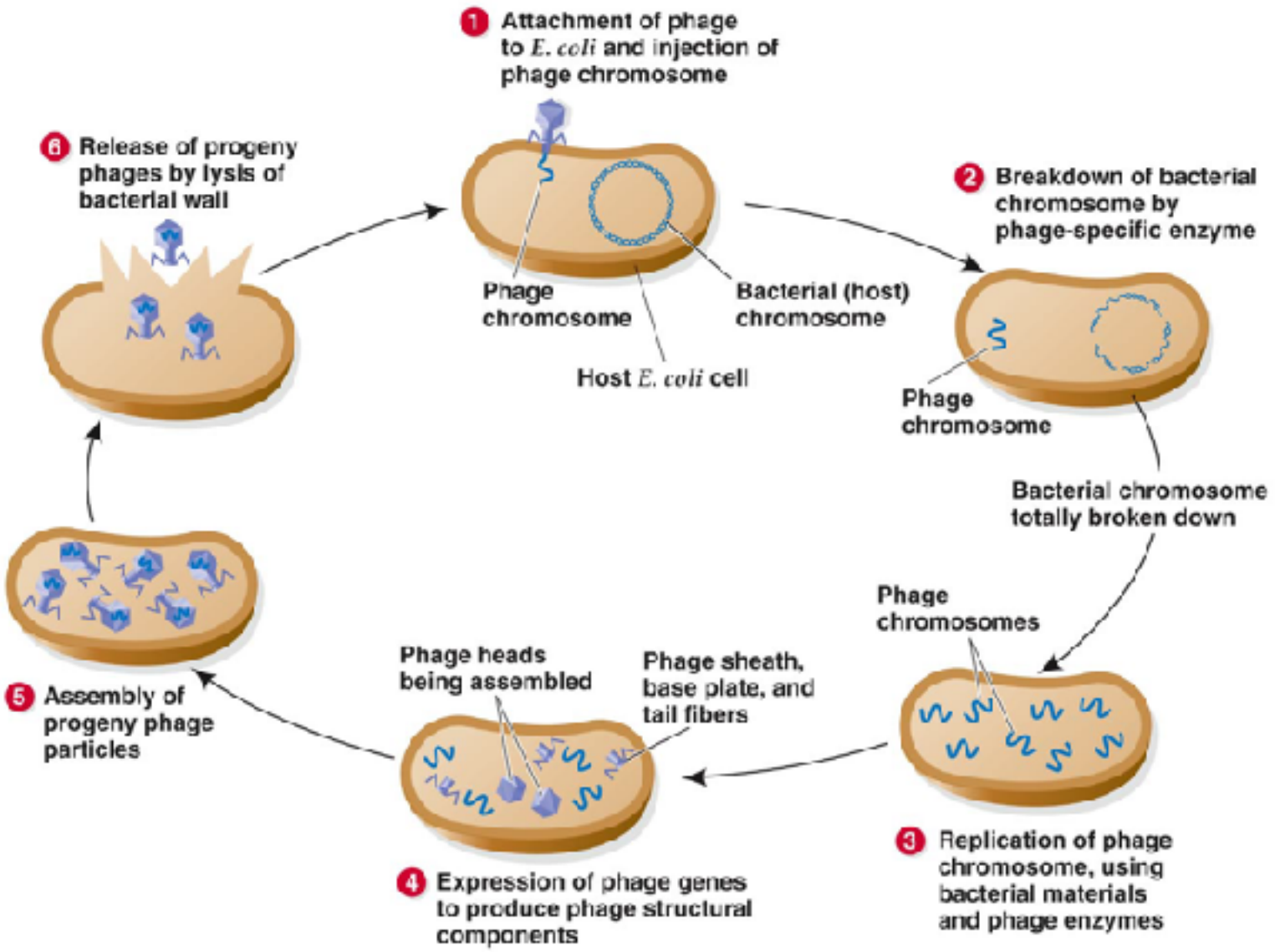
- Bacteriophage (phage):
  - Protein body.
  - DNA inside.



- Use the knowledge about the life cycle to study the genetic code.

- What is phage's life cycle?

Phage life cycle



Hershey and Chase experiment

Hershey and Chase needed phenotypes

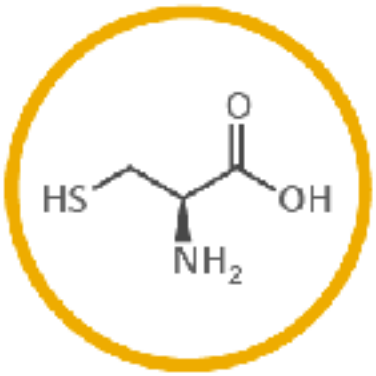
What atom is uniquely found in proteins?

What atom is uniquely found in DNA?

Can we make a phenotype or a character to observe and detect?

Hershey and Chase experiment

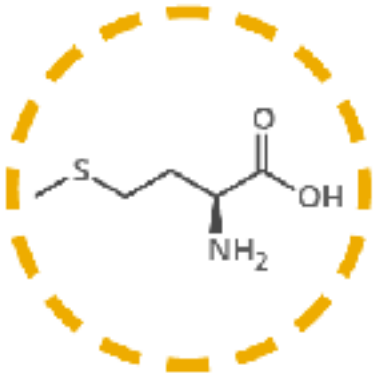
- Labeled proteins with radioactive S^{35}.



CYSTEINE C

Cys

TGT, TGC



METHIONINE M

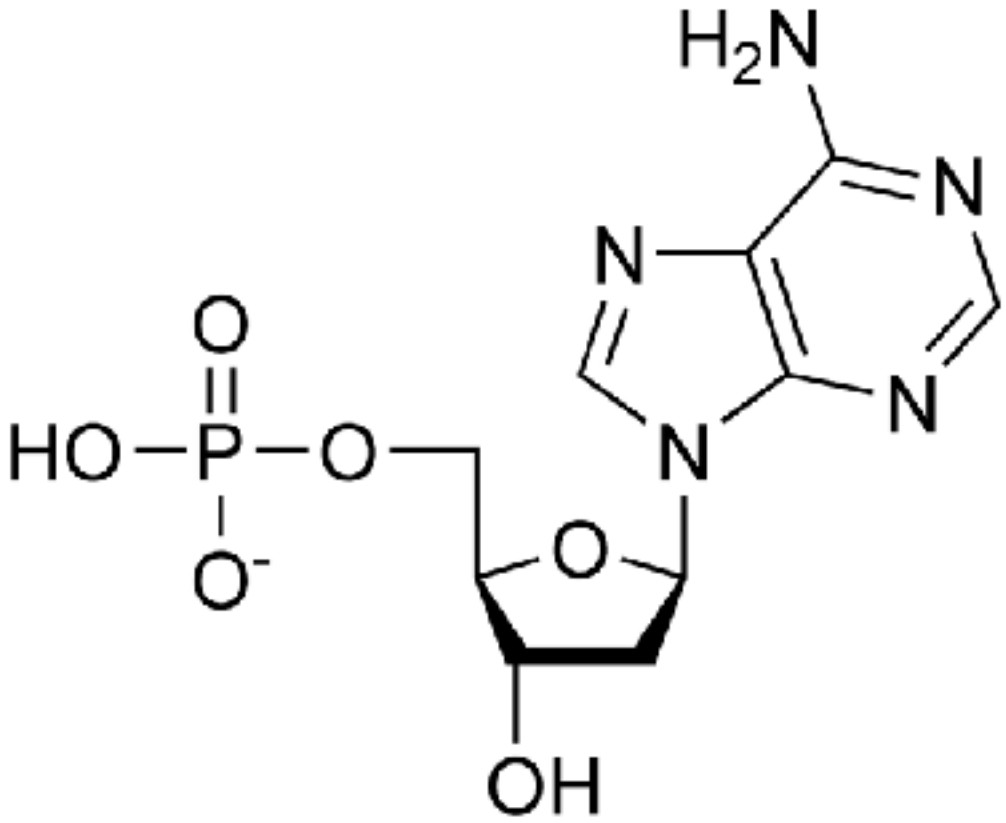
Met

ATG

^{32}S	^{33}S	^{34}S	^{36}S
31.97207	32.97145	33.96786	35.96708
95.02%	0.75%	4.21%	0.02%
Stable	Stable	Stable	Stable

Hershey and Chase experiment

- Labeled DNA with radioactive P^{32}.



Hershey and Chase experiment

How can we label a molecule with radioactive isotope?

Hershey and Chase experiment

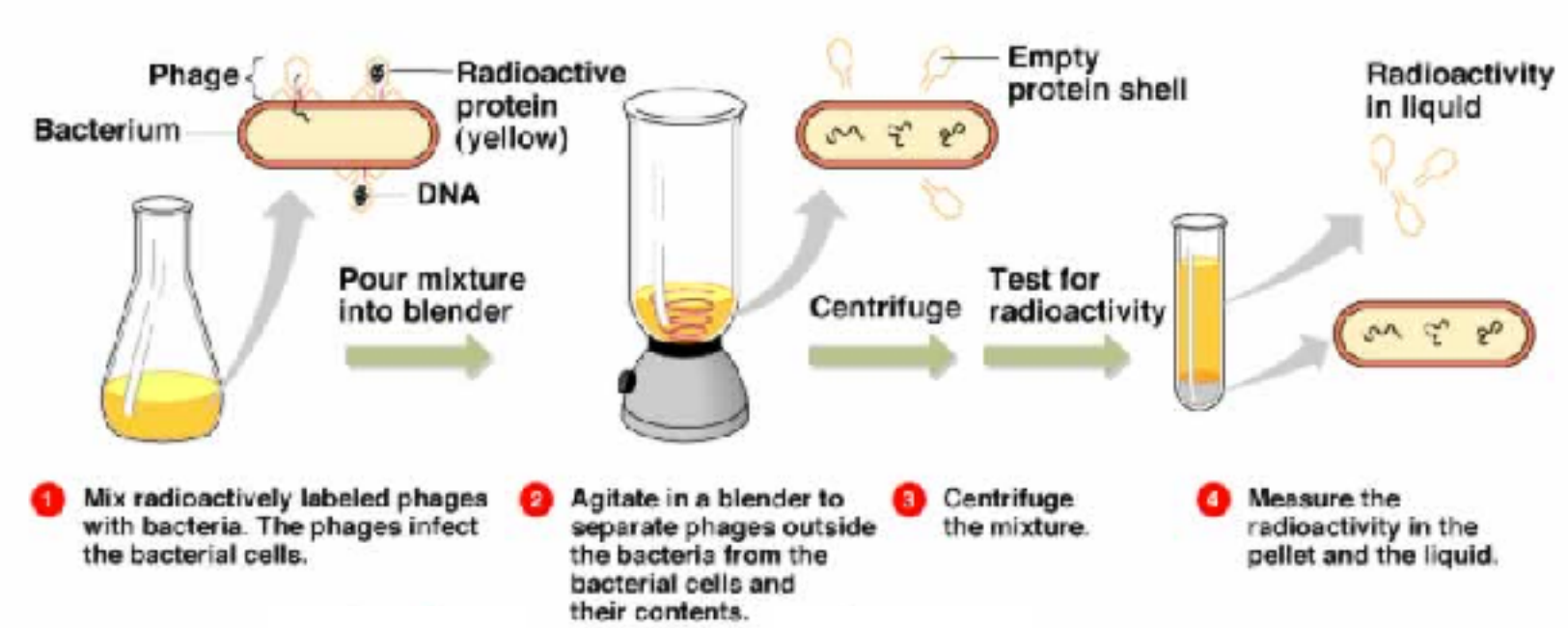
Now we have something to track!

Track where the radioactive material go!

- The molecule that would enter the cell is the hereditary molecule.
- Study where the molecule go (in pellet or in supernatant)?

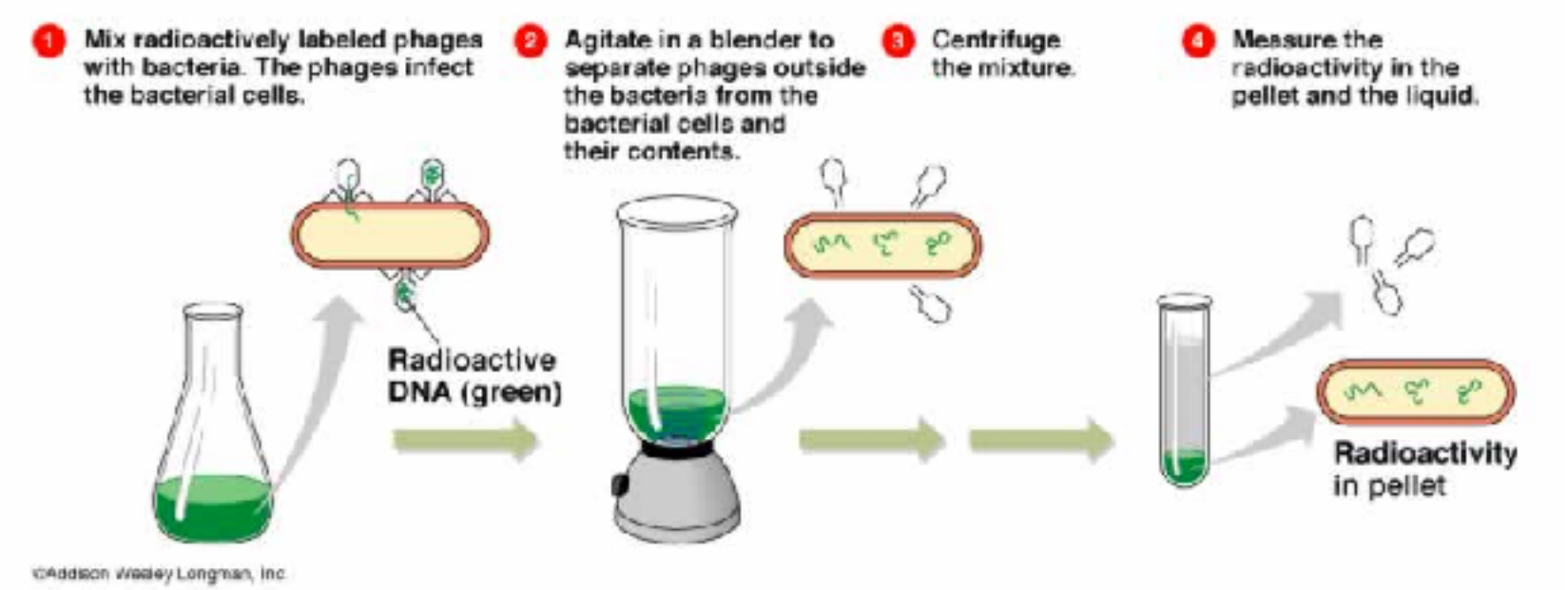
Hershey and Chase experiment 1

- Labeled proteins with radioactive S^{35}.
- Labeled proteins do not enter the bacteria cells.
- Proteins are not the genetic material.



Hershey and Chase experiment

- Labeled DNA with radioactive P^{32}.
- Labeled DNA enters the bacteria cells.
- DNA is the genetic material.

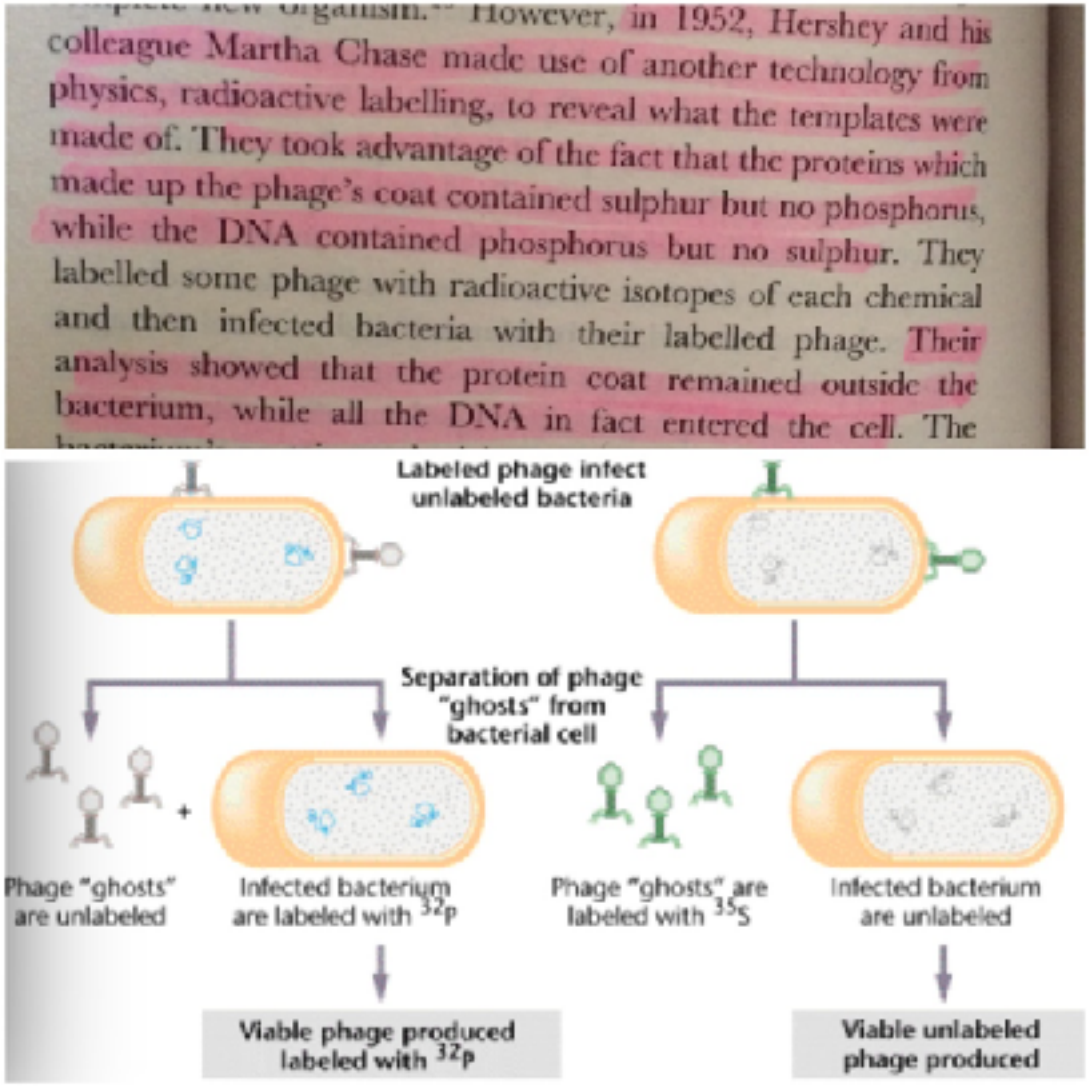


Hershey and Chase experiment

Any controls?

Hershey and Chase experiment

Track where the radioactive material go!



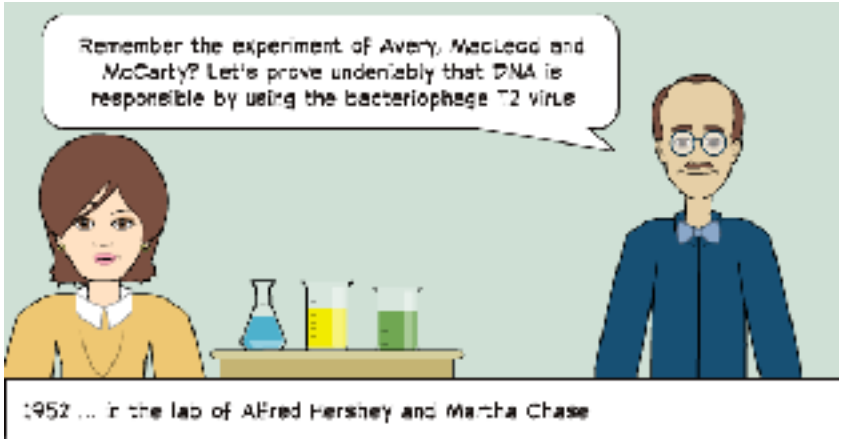
Hershey and Chase conclusion

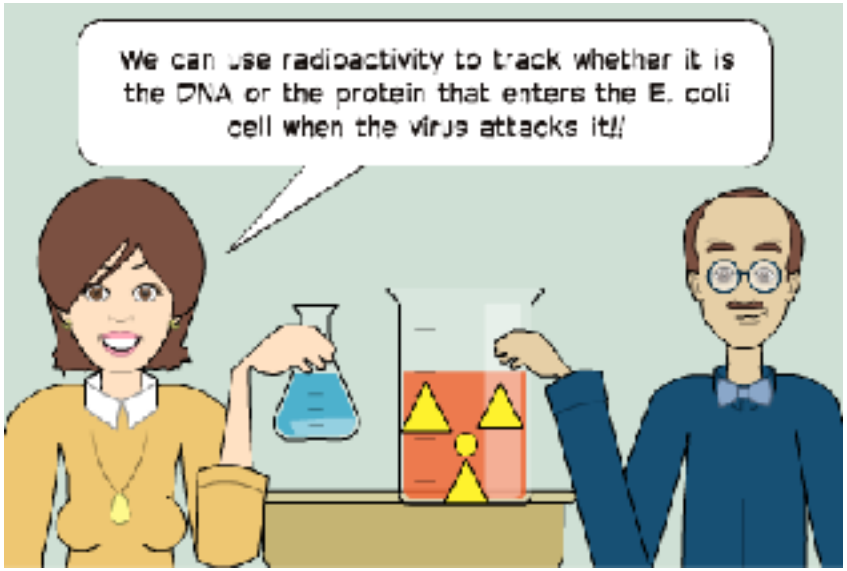
8. The sulfur-containing protein of resting phage particles is confined to a protective coat that is responsible for the adsorption to bacteria, and functions as an instrument for the injection of the phage DNA into the cell. This protein probably has no function in the growth of intracellular phage. The DNA has some function. Further chemical inferences should not be drawn from the experiments presented.

Expectations

- Know the experiments that led to the conclusion that DNA is the genetic material.
- Understand the experimental design and the controls of each experiment.
- Understand the genetic exchange between bacteria and phage life cycle.

For a smile


Remember the experiment of Avery, MacLeod and McCarty? Let's prove undeniably that DNA is responsible by using the bacteriophage T2 virus
1952 ... in the lab of Alfred Hershey and Martha Chase


We can use radioactivity to track whether it is the DNA or the protein that enters the E. coli cell when the virus attacks it!