When do Scientists Change their Minds?

Week 2 – DNA as the genetic material

EGMT-1520 Monday, Jan 24, 2022
Bill Pearson wrp@virginia.edu

Overview of this session:

- Project Groups
- Reading papers

- Early understanding of heredity:
  - plant and animal breeding ~10,000 BCE
- Discovery of genes and chromosomes in the 19th century
- 1900's – Rediscovery of Mendel, genetic diseases, chromosomes as the carriers of "genes"
- 1940's – beginning of modern molecular genetics (one-gene, one-enzyme)

Avery's demonstration of DNA as the genetic material.

1

Upcoming assignments (in groups)

- For your assigned research paper, answer questions about the paper.
- From the Avery paper, identify a result from the paper and:
  - (a) show how the data supports the conclusion
  - (b) propose an alternative explanation (powerpoint/google slides)
- Each group will submit an essay by Monday Jan 31, describing what the experiment was, why it was important, and why it might have been wrong.
- On Wednesday : Groups will be
  1. Working on "Reading a Scientific paper" questions (due Friday, Jan 28)
  2. Working on "Avery paper results" essay (due Monday, Jan 31)
  3. Working on Gravity presentation (to be presented Wednesday, Feb. 2)

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Group Assignments/Paper Assignments

- Each section (sect109_morning /sect104_afternoon) has groups of 4-6 people
- You will work in the same group throughout the quarter
- For this first group homework, the members of the group are assigned one of the six papers.
- The assignment is to look over the paper, and answer (as a group) 5 questions about the paper
  - each member of the group is expected to look over the paper, recognize the different sections of the paper, and contribute to the answer of one of the three questions in the group.

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Group Assignments (sect109_morning)

Morn_G1
Judson Faulconer (sbq3rg)
Jerlan Fish (qdt6me)
Matthew Moore (rwc5em)
Margaret Rappoport (cbz7pw)
Connor Sandall (tym4cc)
Davis Webb (ytt9sb)

Morn_G2
Colden Dorfman (ppr7as)
William Dunn (xwe8sn)
Kendall McGowan (dhk2pk)
Luke Osetek (snp4yq)
Jarin Woolfolk (uyk7hb)

Morn_G3
Marissa Coletti (pjk2bv)
Joshua Maggiano (urt6qe)
Martha Reavis (yyg8gp)
Jordan Rodgers (huq2zw)
Ella Sher (gdq9qz)
Alexis Vencill (nzp8tx)

Morn_G4
Tyler Arnold (rpa3vn)
Megan Bowen (amx5jb)
Connor Dight (yah8sv)
Emily McMahon (feu6sx)
Ian Sanders (unf5pr)

Morn_G5
Sophie Atkinson (yup6ce)
Charles Healy (bau8uq)
William Inderlied (hma6hf)
Kate McGee (hkn2bx)
Keyana Thomas (twg6uk)
Go Yabuno (mtf2wj)

Morn_G6
Ella Bathurst (kgy9gj)
Alana Forshay (qhk9qx)
Fnu Hairitai (scb9yf)
Elise Harris (fsq6sk)
Jonathan Macey (yzd8uf)
Ella Mortimer (rzq9wc)

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Group Assignments (sect104_afternoon)

>After_G1
Semin Ahn (mkb8ku)
Tyler Baroudi (dfs8xx)
Thomas Boak (ggu7zc)
Matthew Hunter (azk5cr)
Paige Lane (dxf6ru)
Liza Runyon (mry7pv)

>After_G2
Olivia Duck (swj2kq)
Tyler English (vhe6zn)
John Fay (aka9sk)
Sophia Hildebrand (dqj5hq)
Hugh Laughlin (wem2zc)

>After_G3
Liam Brennan (tvt4pu)
Abby Cebula (fgr5es)
Hailey Didion (dqg8vf)
Yuchen Huang (umf3qp)
Daniel Shea (ykd6ec)

>After_G4
Chrestine Ageeb (qps3dn)
Declan Doyle (ppa9cz)
Aidan ODonnell (chc3fa)
Gabriel Riley (qma8xv)
Mags Worden (fzp7mu)

>After_G5
Celia Laplace (ttn2gy)
Piera Molin (jzn6mf)
Anisha Ponugupati (euv7bw)
Ananya Rajkumar (uke2us)
Jack Witmer (mbu3fh)

>After_G6
William Gansereit (qkr4mx)
Savannah Gibson (dar4wx)
Jason Kim (eja8he)
Nicole Romero (jpv6vc)
Amante Smith (tvc5pn)

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Reading a Scientific Paper
Assignment Questions (in groups):
Due: Friday, Jan. 28, 5:00 pm

As a group, answer the following questions about your assigned paper. Different members of the group can be responsible for the answers to different questions. Please specify the members of the group (UVA email ids) responsible for the answer to each question. Members of the group should agree on contribution claims.

(1) List one major claim made by the paper. Explain why that claim was scientifically interesting?

(2) For one claim in the paper, cite the figure (or include it in the paper), or quote part of the text in the results, that supports that claim.

(3) For that figure, try to describe what you saw when you first looked at it (what did it seem to indicate at first glance), and what did you have to understand so that it made sense.

(4) For one claim in the paper, suggest an alternative explanation that the authors consider, and reject.

(5) What is the impact factor (average number of citations) for the journal the article was published in?

(6) List a scientific term that you are not familiar and explain what it means, and how you figured that out.

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Avery paper figure (in groups): Due: Monday, Jan. 31, 5:00 pm

- For the Avery paper, identify a result from the paper
- Write a 1-page essay (or slightly longer if you include the figure) that:
  a) Explains why the experiment was done
  b) shows how the data supports the conclusion
  c) proposes an alternative explanation
- For each paragraph, provide the computing ID of the individuals who contributed

Please submit the essay as a PDF, not a Word document or google doc link.

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For Wednesday – Feb. 2

- Gravity presentation (assignment) – 3 to 4 slides focusing on one aspect of gravity: heavy objects and light objects fall at the same rate
  – no general relativity
- Part 1 – why is the incorrect (intuitive) explanation compelling? Convince us that heavy objects should fall faster
- Part 2 – what change in perspective is necessary to understand the correct explanation
- Part 3 – with that change in perspective, why does gravity make sense
  – equations do not replace intuition

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Assignment Questions:

(1) List one major claim made by the paper:
- GST-A mRNA found in all livers, but GST-M only in tSBO+

Explain why that claim was scientifically interesting?
- correlation between tSBO activity and GST-M suggests tSBO activity encoded by GST-M mRNA (as opposed to GST-A, which is not variable)

(2) For one claim in the paper, cite the figure, or quote part of the text in the results, that supports that claim.
- Fig. 1 shows the correlation between GST-M mRNA and tSBO, and the lack of correlation with GST-A

(3) For that figure, try to describe what you saw when you first looked at it (what did it seem to indicate at first glance), and what did you have to understand so that it made sense.
- Initial look – lots of black lines in different places. Understanding: each column is a different person. +/- under top row of numbers is enzyme activity. Black lines in panels A, C correlate with +/-, panel B lines don't.

(4) For one claim in the paper, suggest an alternative explanation that the authors consider, and reject.
- The differences in restriction site bands in Fig. 4 could be due to a gene deletion in tSHO- individuals, or it could be due to a mutation that causes a restriction site to go away, thus changing band sizes.
- This was tested by breaking the human GSTM cDNA into non-overlapping pieces and showing that both ends of the cDNA clone detected multiple restriction fragments.

(5) Impact factor (PNAS): 9.3 (>900 citations to this paper)

(6) List a scientific term that you are not familiar and explain what it means, and how you figured that out.
- cDNA clone – (google is not very helpful, but Wikipedia is) – a cloned (put into bacteria) copy of a mRNA

15 min break to work on
"Reading a Scientific Paper"

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Background Info - Autism

- Question: should parents with a child with autism avoid vaccinating younger children?
- Results (from abstract):

Of 95727 children with older siblings, 994(1.04%) were diagnosed with ASD and 1929 (2.02%) had an older sibling with ASD. Of those with older siblings with ASD, 134 (6.9%) had ASD, vs 860 (0.9%) children with unaffected siblings (P < .001). MMR vaccination rates (>=1 dose) were 84% (n = 78549) at age 2 years and 92% (n = 86063) at age 5 years for children with unaffected older siblings, vs 73% (n = 1409) at age 2 years and 86% (n = 1660) at age 5 years for children with affected siblings.

MMR vaccine receipt was not associated with an increased risk of ASD at any age. For children with older siblings with ASD, at age 2, the adjusted relative risk (RR) of ASD for 1 dose of MMR vaccine vs no vaccine was 0.76 (95% CI, 0.48-1.22; P = .25), and at age 5, the RR of ASD for 2 doses compared with no vaccine was 0.56 (95% CI, 0.30-1.04; P = .07). For children whose older siblings did not have ASD, at age 2, the adjusted RR of ASD for 1 dose was 0.91 (95% CI, 0.68-1.20; P = .50) and at age 5, the RR of ASD for 2 doses was 1.09 (95% CI, 0.76-1.54; P = .65).

- Relative Risk (RR, sometimes Odds Ratio): ratio of two outcomes (in this case autism)
  - in this example, is the risk higher or lower with immunization?

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Background info – microbiomes

- most higher organisms have a massive number of bacteria living with them (the microbiome), on skin, in the gut, and in any part of the body exposed to the environment
- for people, the microbiome provides many many times as many genes (and enzymes) as the human genome
  - the human genome encodes about 10,000 enzymes
  - each different strain of bacteria encodes >3000 enzymes, and there are millions of different strains
- the diversity of the gut microbiome can be measured by sequencing fecal DNA and counting the number of different organisms by counting how many different 16S rRNA molecules are present (each strain of organism has the same 16S rRNA, so different 16S rRNA's mean different bacteria).
- For the twins obesity study – focus on Fig. 1 and Fig. 2. In Fig. 2, R^2 is the correlation coefficient.

Twins, microbiomes, and obesity

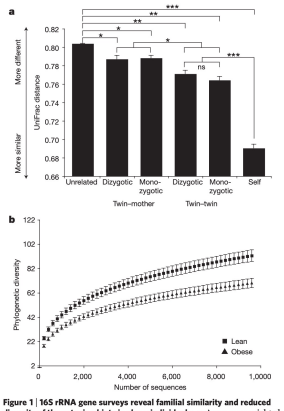

Figure 1 | 16S rRNA gene surveys reveal familial similarity and reduced diversity of the gut microbiota in obese individuals. **a**, Average unweighted UniFrac distance (a measure of differences in bacterial community structure) between individuals over time (self), twin pairs, twins and their mother, and unrelated individuals (1,000 sequences per V2 data set; Student's *t*-test with Monte Carlo; $*P < 10^{-3}$; $**P < 10^{-4.5}$; $***P < 10^{-41}$; mean ± s.e.m.). **b**, Phylogenetic diversity curves for the microbiota of lean and obese individuals (based on 1–10,000 sequences per V6 data set; mean ± 95% confidence intervals shown).

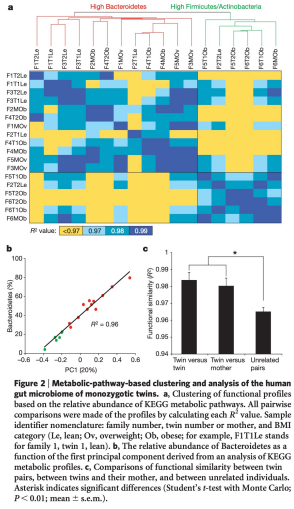

Figure 2 | Metabolic-pathway-based clustering and analysis of the human gut microbiome of monozygotic twins. **a**, Clustering of functional profiles based on the relative abundance of KEGG metabolic pathways. All pairwise comparisons were made of the profiles by calculating each R^2 value. Sample identifier nomenclature: family number, twin number or mother, and BMI category (Le, lean; Ov, overweight; Ob, obese; for example, F1T1Le stands for family 1, twin 1, lean). **b**, The relative abundance of Bacteroidetes as a function of the first principal component derived from an analysis of KEGG metabolic profiles. **c**, Comparisons of functional similarity between twin pairs, between twins and their mother, and between unrelated individuals. Asterisk indicates significant differences (Student's *t*-test with Monte Carlo; $P < 0.01$; mean ± s.e.m.).

Nature (2009) 457:480

Genome sequence of nCoV19

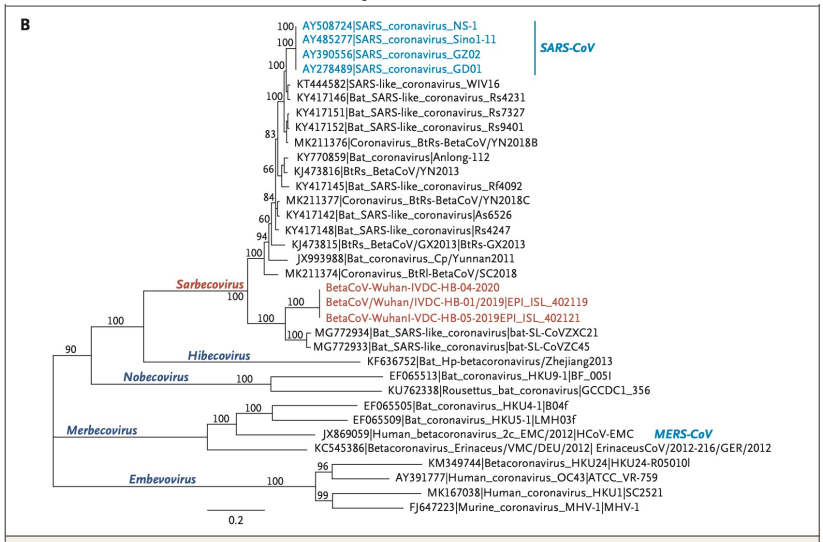

Figure 4. Schematic of 2019-nCoV and Phylogenetic Analysis of 2019-nCoV and Other Betacoronavirus Genomes.
Shown are a schematic of 2019-nCoV (Panel A) and full-length phylogenetic analysis of 2019-nCoV and other betacoronavirus genomes in the Orthocoronavirinae subfamily (Panel B).

N Engl J Med 2020;382:727-33.

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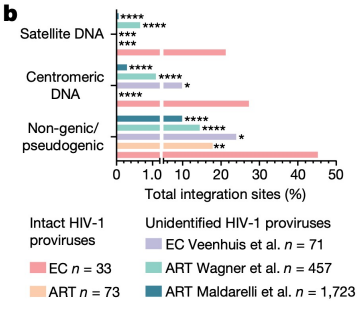
HIV resistance

- The human genome encodes about 20,000 proteins (thus has 20,000 protein coding genes), but these genes are not uniformly distributed
  - Some genes are clustered
  - Some regions of chromosomes have very few genes, including satellite regions, centromeres, and "gene deserts"
- EC – elite controllers; ART – anti-retroviral therapy
- Fig. 1 shows that EC has less HIV DNA and but more intact viruses
- Fig. 4 shows where the HIV has been inserted into the human genome
- Focus on understanding/explaining Fig. 4b.



Nature (2020) 505:261

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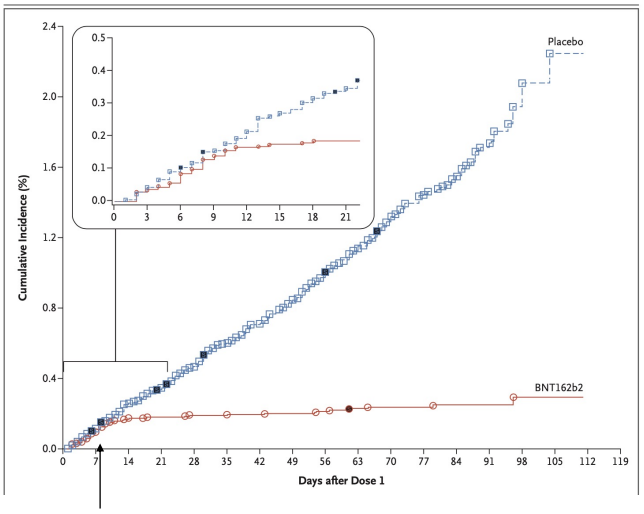
Pfizer-bioNtech Vaccine effectiveness



What is happening here?

N. Engl. J. Med. (2020) **383,** 2603–2615.

fasta.bioch.virginia.edu/egmt1520 15

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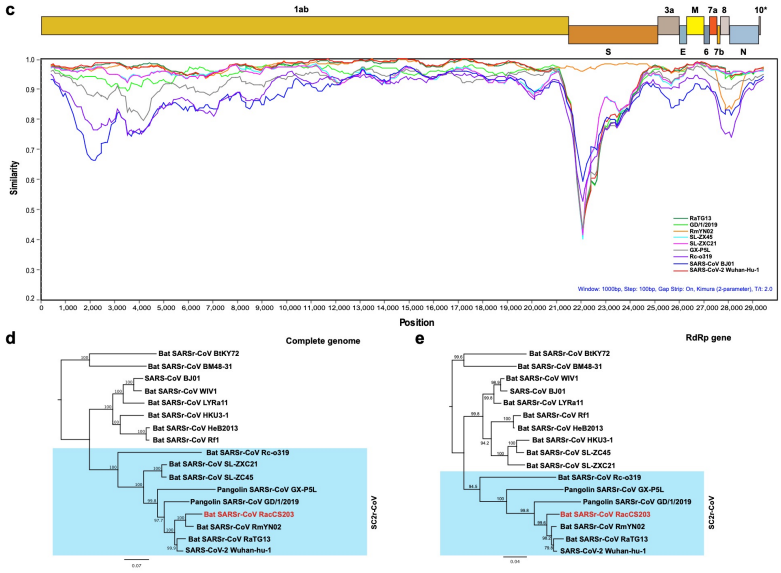
SARS viruses from Bats



Nat. Comm. (2021)12:972

fasta.bioch.virginia.edu/egmt1520 16

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In groups

1. Download the group paper (if you have not done so already)
2. Look it over and identify the different parts of the paper
3. Decide which people will be responsible for which question(s).
   - Some questions will benefit from more people working on it
   - Question 5 is the easiest; and may only need one person
4. Set a schedule to discuss your answers before submitting them for Friday

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DNA as the genetic material: Avery, McLeod, and McCarty 1944

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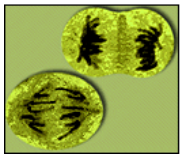
Discovery of genes and chromosomes in the 19th century

- 1865 Mendel, genotype and phenotype

When he crossed true-breeding lines with each other, he noticed that the characteristics of the offspring consistently showed a three to one ratio in the second generation. For example, for approximately every three tall plants, one would be short; for about every three plants with yellow peas, one would have green peas. Further breeding showed that some traits are dominant (like tall or yellow) and others recessive (like short or green). In other words, some traits can mask others. But the traits don't blend: they are inherited from the parents as discrete units and remain distinct. Furthermore, different traits - like height and seed color - are inherited independently of each other.

- 1869 Miescher, isolation of DNA
  - very large (long), 4 different bases (subunit types)
- 1879 Fleming, mitosis (duplication/separation of chromosomes)



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Chromosomes and Genes 1900 – 1940

1902 Inheritance of disease (alkaptonuria)
- gene not discovered until 1990's

1902 Chromosome theory of heredity
- meiosis – germ cells have ½ the chromosomes

1911 T. H. Morgan, Fruit flies and the chromosome theory

1913 Sturtevant – genetic map – genes arranged in linear order

1916 Bridges – chromosomes contain genes

1927 Muller – radiation causes mutations

1933 physical mapping of genes to chromosomes

1941 Beadle and Tatum – one gene, one enzyme

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The demonstration of DNA as the genetic material - I

- Genes are the units of heredity
- We can watch genes and mutations move from parent to offspring
- What are genes made of?
  - proteins
    - known to catalyze chemical reactions
    - built from 20 amino acids
    - have different shapes
  - nucleic acids (DNA)
    - built from 4 nucleotides
    - seemed too simple to provide the diversity of genes
- Can we "purify" a "gene", and determine its composition?

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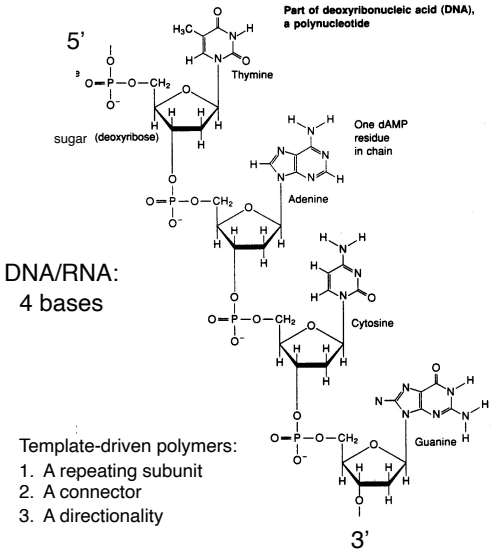
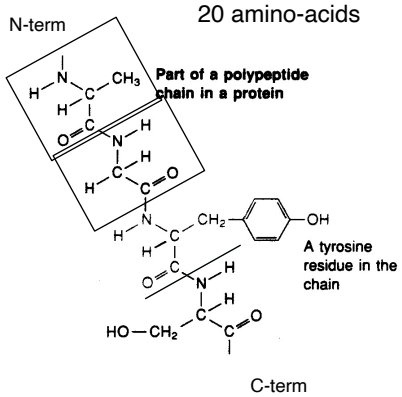
Template driven polymers : DNA/RNA and proteins



DNA/RNA:
4 bases

Template-driven polymers:
1. A repeating subunit
2. A connector
3. A directionality

Proteins:
20 amino-acids



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The demonstration of DNA as the genetic material - II

How to "purify" a gene

- genes are known by their "phenotypes" – the changes that they cause (color, wrinkled peas, pathogenicity) when they are present
- To purify something (a factor), we need
  i. an assay – some kind of change we can measure
  ii. a separation process – we need to remove the non-gene material from the gene material

An assay for a gene – dead mice with infectious blood

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) *J Hyg (Lond)* **27,** 113

An assay for a gene – dead mice with infectious blood

Inoculation experiments with heated virulent Group IV and attenuated R Type I and R Type II pneumococci.

Table V shows that three different strains of Group IV killed by steam at 100° C., when injected into mice together with living attenuated R pneumococci derived from Type II by growth in homologous immune serum, caused the R form to revert to the virulent capsulated S form. R 4, Type II, *i.e.*

Table V.

Killed S pneumococci	Living R pneumococci	No. of mouse	Result	Type of culture obtained from mouse
Pn. 85, Group IV, steamed 20 mins. Dose = deposit of 60 c.c. of broth culture	R 4, Type II. Dose = 0·25 c.c. of blood broth culture	405	Died 4 days	S colonies, Type II
		406	Killed 7 ,,	None
		407	,, 7 ,,	R colonies
		408	Died 4 ,,	S colonies, Type II
Pn. 160, Group IV, as above	R 4, Type II as above	409	Killed 7 days	S colonies, Type II
		410	Died 4 ,,	,, ,,
		411	,, 4 ,,	,, ,,
		412	,, 3 ,,	,, ,,
II B, Group IV, as above	R 4, Type II as above	413	Died 3 days	S colonies, Type II
		414	,, 2 ,,	,, ,,
		415	,, 3 ,,	,, ,,
		416	Killed 7 ,,	R colonies
None	R 4, Type II. Doses = 0·75, 1·0, 1·0 c.c. of blood broth culture	462	Killed 19 days	None
		463	,, 19 ,,	,,
		464	,, 19 ,,	,,

Control: No material/ No deaths

Griffith, F. (1928) *J Hyg (Lond)* **27,** 113

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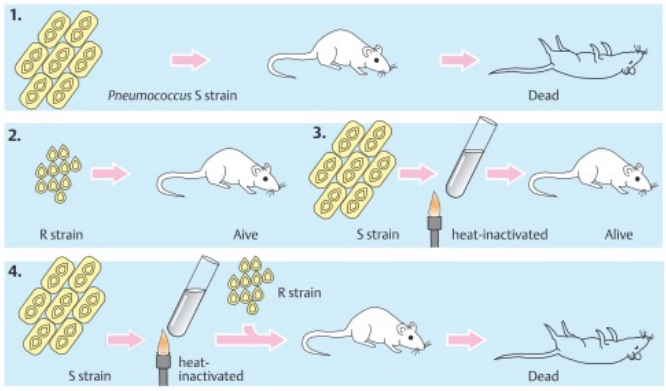
An assay for a gene – dead mice with infectious blood



www.magazinescience.com/en/biology/dna-as-carrier-of-genetic-information/

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Purification of the transforming factor

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.

J. Exp. Med. (1944) **79**, 137–158.

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Purification of the transforming factor

The present paper is concerned with a more detailed analysis of the phenomenon of transformation of specific types of Pneumococcus. The major interest has centered in attempts to isolate the active principle from crude bacterial extracts and to identify if possible its chemical nature or at least to characterize it sufficiently to place it in a general group of known chemical substances. For purposes of study, the typical example of transformation chosen as a working model was the one with which we have had most experience and which consequently seemed best suited for analysis. This particular example represents the transformation of a non-encapsulated R variant of Pneumococcus Type II to Pneumococcus Type III. (non-pathogenic)

(S-variant/S-strain pathogenic)

J. Exp. Med. (1944) **79**, 137–158.

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Purification of the transforming factor Avery et al, 1944

- Experimental –
  - nutrient broth
  - added serum (anti-R antibodies)
  - strain of R-pneumococcus
  - extraction/purification of transforming factor
- Method of titration of the transforming activity
  - alcohol precipitation/sterilization (heat destroys)
  - material is sterile (will not produce colonies)
  - assay:
    - broth + serum + purified/sterile factor + 'R'-strain bacteria
    - grow at 37 ^{o}C 18-24 hr
    - look for growth, plate out to look at colony morphology
- Results:

Purification of the transforming factor Avery et al, 1944

- Results:
  - Analysis of purified material:
    - is viscous
    - stable for 3 months in cold, decays in days at room temp in water
    - active after incubation at 60 ^{o}C for 30 min
  - Quantitative chemical tests:
    - Biuret (protein) negative
    - Diphenylamine (DNA) strong positive
    - Orcinal (RNA) weak positive
  - Chemical analysis: Nitrogen/Phosphorus ratio ~1.7 (expected for DNA)
  - Enzymatic degradation:
    - trypsin, chymotrypsin (digest protein), no effect
    - ribonuclease (digests RNA), no effect
    - serum from dog, rabbit, unheated destroys, heated no effect (unheated serum contains DNase – digests DNA; heating destroys DNase activity)

"Serum" confusion

In the Avery paper, there are two different kinds of "serum" used for two different purposes:

1. In the initial "assay" for the transforming factor, the *broth + R-strain-bacteria + transforming principle* is incubated with "*anti-R-strain*" serum to remove R-strain bacteria that have not been converted (transformed) to S-strain
2. Later, in the characterization of the *transforming principle,* the *transforming principle* is incubated with dog and rabbit serum to see if it is inactivated. These sera are simply mixtures of enzymes, similar to the "crude enzyme preparations" examined in table II.

TABLE II

The Inactivation of Transforming Principle by Crude Enzyme Preparations

Crude enzyme preparations	Enzymatic activity			
	Phosphatase	Tributyrin esterase	Depolymerase for desoxyribonucleate	Inactivation of transforming principle
Dog intestinal mucosa	+	+	+	+
Rabbit bone phosphatase	+	+	−	−
Swine kidney "	+	−	−	−
Pneumococcus autolysates	−	+	+	+
Normal dog and rabbit serum	+	+	+	+

Working in groups:

- For the Avery paper, for Monday, identify a result from the paper and:
  - (a) show how the data supports the conclusion
  - (b) propose an alternative explanation (Go over the paper Wednesday and pick a result (or set of results, e.g. Table III and Chart I)
- Each group will submit a 1 page (+figure/table) paper on Monday, Jan 31, describing what the experiment was, why it was important, and why it might have been wrong.

Remember that despite the recognized significance of this paper today, at the time, most scientists still did not believe DNA was the genetic material.

Avery paper (in groups): Due: Monday, Jan. 31, 5:00 pm

- For the Avery paper, identify a result from the paper
- Write a 1-page (+ figures) essay that:
  a) Explains why the experiment was done
  b) shows how the data supports the conclusion
  c) proposes an alternative explanation
- For each paragraph, provide the computing ID of the individuals who contributed

Please submit the essay as a PDF, not a Word document or google doc link.