A Personal (biased) Perspective on Microbiology: Some History, Some Cool Experiments, and Opinions about Where's the Action is

Eco-Evolutionary Dynamics in Nature and the Lab
Kavli Institute, Santa Barbara July 25, 2017

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Interruptions, with comments, questions, opinions or whatever are most welcome, but please speak loudly

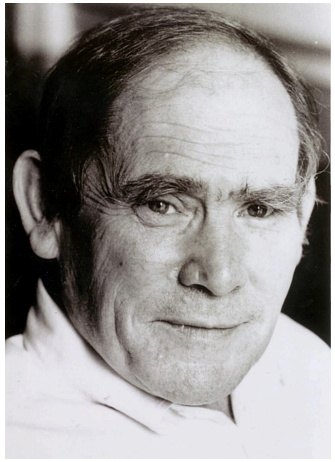
Bacteria and yeast are great tools to study ecology and evolution under well-controlled conditions in real time

I won't rant on about this subject because some of the best people using these tools to address ecological and evolutionary questions will be lecturing in this course.

In Theory

Molecular biology has been a great leveler and has made thinking unnecessary in many areas of modern biology. With the disappearance of theory has also come the decline of experimentation, and the practice of science by hypothesis and testing is not known by many students in the field. So powerful are contemporary tools for extracting answers from nature that pausing to think about the results, or asking how one might find out how cells really work, is likely to be seen as a source of irritating delay to the managerial classes, and could even endanger the career of the questioner.

Loose Ends - Current Biology 7: 3 (PR 202) 1997



Sidney Brenner
1927 -

Some recognized prejudices (aphorisms?)– *Ec*LF.net

'Doing research in population biology without mathematical and/or computer simulation models is like playing tennis without a net or boundary lines'. (Stolen with modification from Robert Frost)

'Data may be a crutch for the insecure, but really self-confident scientists subject their hypotheses to tests that can reject them'

'Just because a project may be useful, doesn't mean it's not interesting, high-quality and delicious to work on Science.'

'For us, natural and not-so-natural selection is about dN/dt and not dN/dS.'

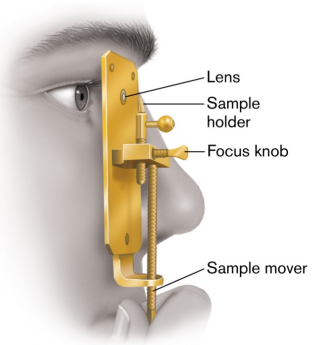
‘All models and model systems are wrong, some are useful’. (Borrowed with a friendly amendment from George Box)

‘Models are more useful when they don’t fit the data than when they do’.

Microbes and Contagion

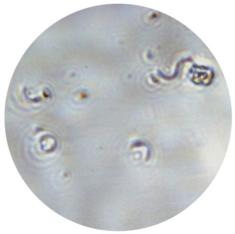
A.

B.



Leeuwenhoek microscope (circa late 1600s)

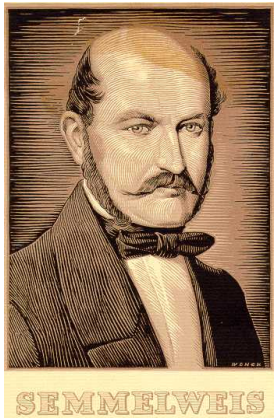
C.



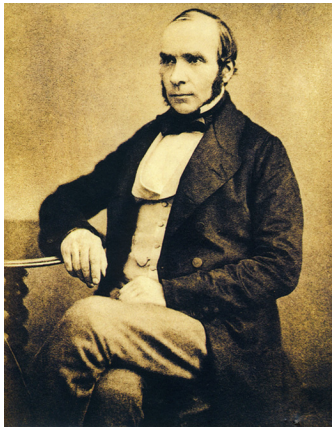
Antonie van Leeuwenhoek 1632-1723

Lazzaro Spallanzani 1729-1799

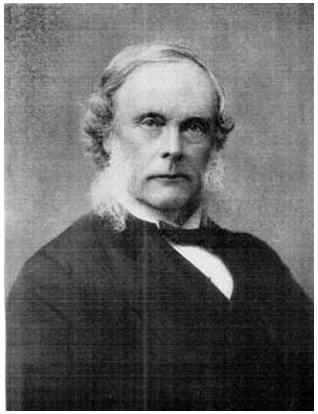
Edward Jenner 1749-1823



Ignaz Semmelweis 1818- 1865



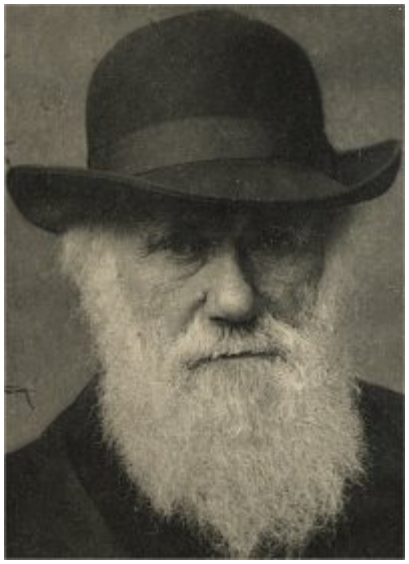
John Snow 1813-1858



Joseph Lister 1827-1912

Even ole Chuck Darwin knew of contagion

"A particle of small-pox matter, so minute as to be borne by the wind, must multiply itself many thousandfold in a person thus inoculated; and so with the contagious matter of scarlet fever[1]. It has recently been ascertained[2] that a minute portion of the mucous discharge from an animal affected with rinderpest, if placed in the blood of a healthy ox, increases so fast that in a short space of time "the whole mass of blood, weighing many pounds, is infected, and every small particle of that blood contains enough poison to give, within less than forty-eight hours, the disease to another animal."

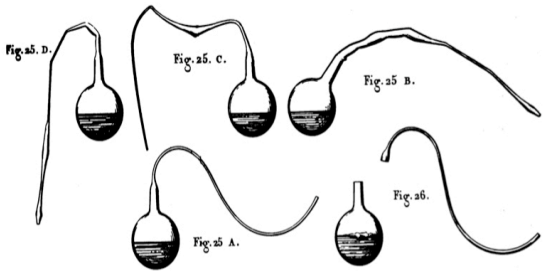


C. Darwin

The Variation in Animals and Plants Under Domestication **New York: D. Appleton & Co.** 1883
Includes citations to articles written in the middle 1860s.

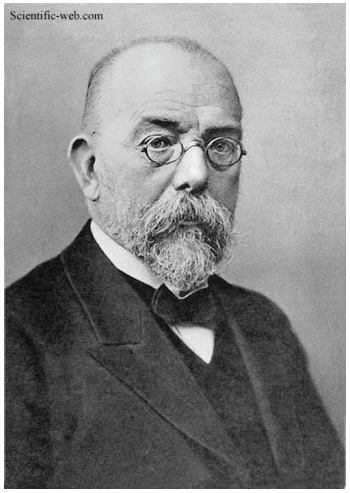
Origin of Microbiology (Bacteriology)

Louis Pasteur 1822-1895



Emil Von Behring
1854-1917

Paul Ehrlich
1854 - 1915



Robert Koch 1843-1910

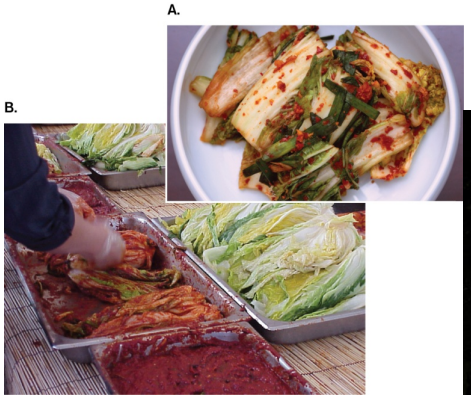
Emil Roux
1853-1933

Food and Industrial Microbiology

A major focus of applied microbiology

Louis Pasteur

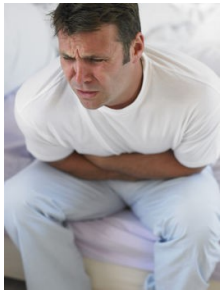
"If it doesn't rot, don't eat it". M. Pollan



Fermentation

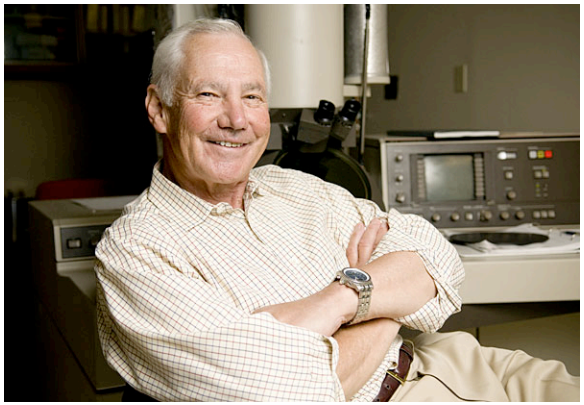
Spoilage

Preservation



Food Safety

Pathogenesis and Virulence



Stanley Falkow 1934 -

Pathogenesis and virulence are the product of a complex interaction between microbes and their host.

Much (most?) of the virulence of bacteria is consequence of failings and screw-ups in the host immune response.

Questions –

How do microbes cause disease?

A major focus of contemporary Microbiology

Why do microbes cause disease? (Bite the hand that feeds them) –

A cool evolutionary question

Conventional wisdom – a primitive character

Trade-off – virulence is necessary for transmission

Coincidental (shit happens)

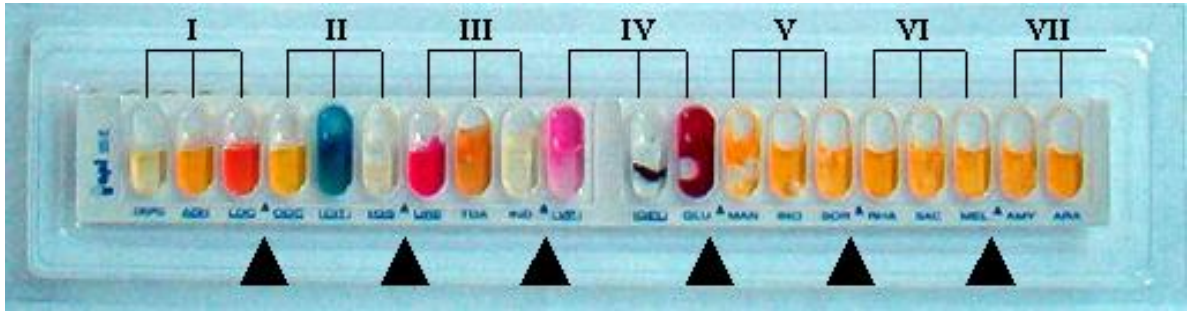
Short-sighted within host evolution

Culturing and Naming Names

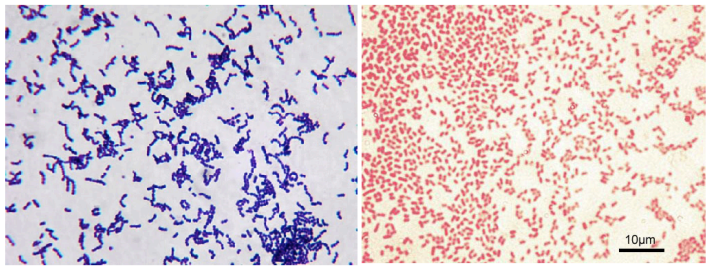
Much of late 19th and early 20th century Microbiology was devoted to developing methods to culture microbes in vitro and identify them and, to some extent determine their phylogenetic relationship.

Emphasis was given to pathogens and microbes of practical interest and not excessively fastidious, e.g. a bias for aerobes.

Morphology, serology and biochemistry (what they can eat) played a major role in the identification process and still does.

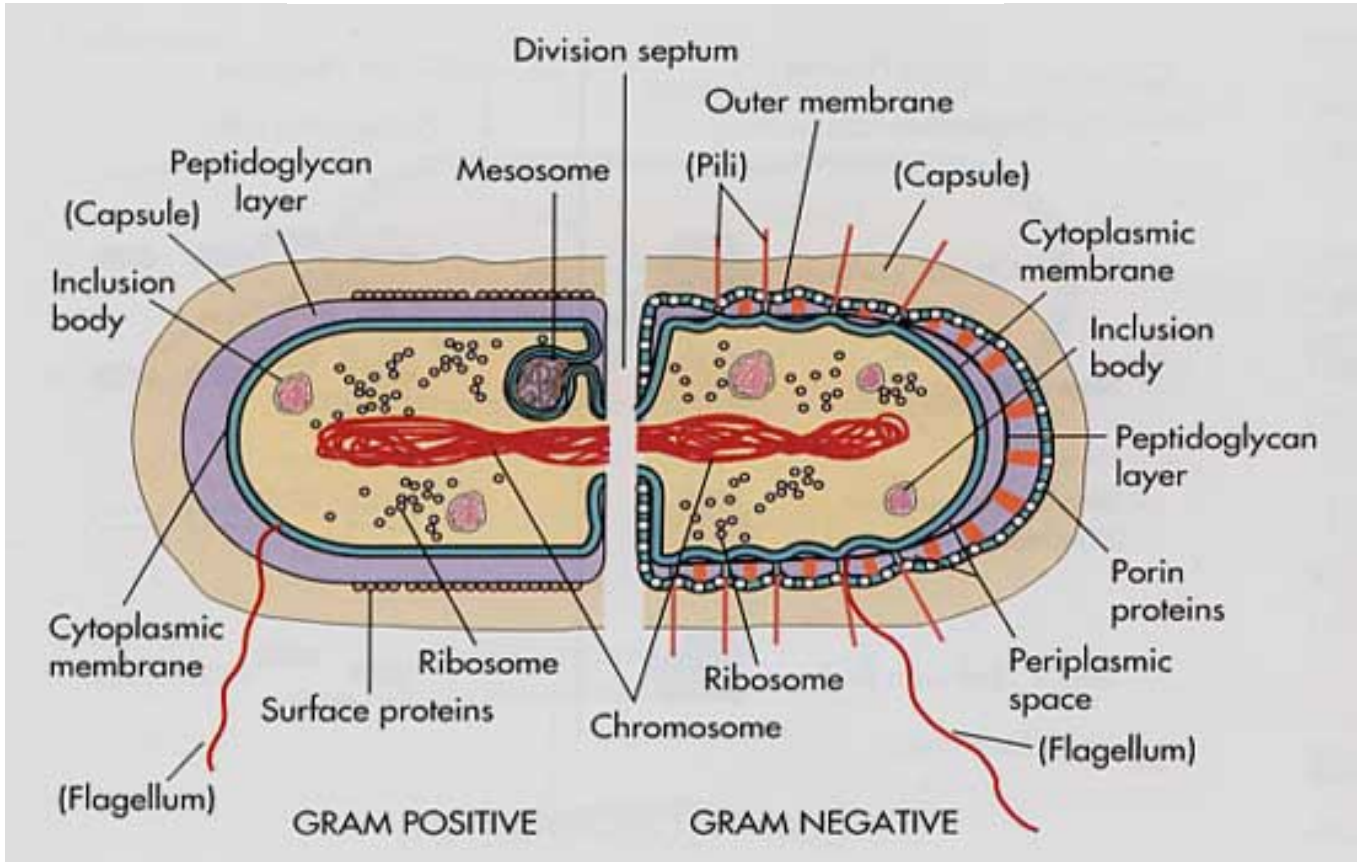


Gram Stain



Gram Positive Bacteria

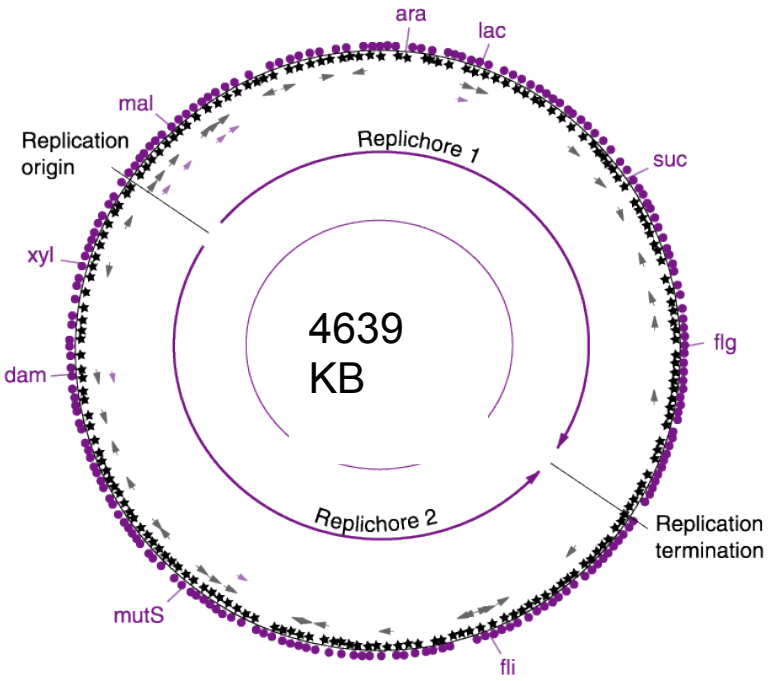
Gram Negative Bacteria

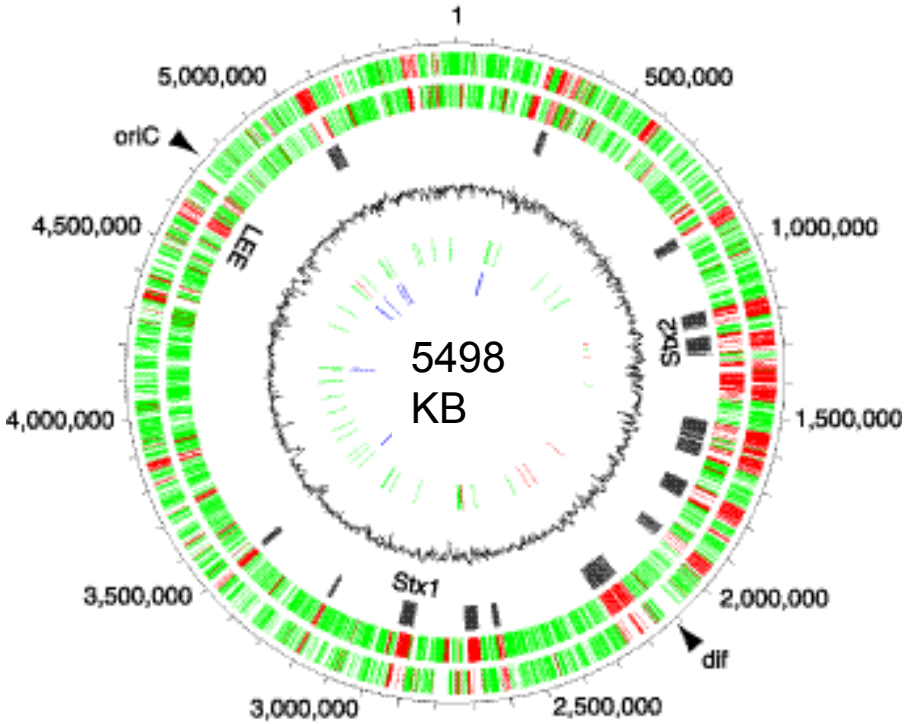


Rapid Molecular Diagnosis of the Etiologic Agents of Infections

http://www.cepheid.com/tests-and-reagents/clinical-ivd-test

Who's the real *E. coli*?



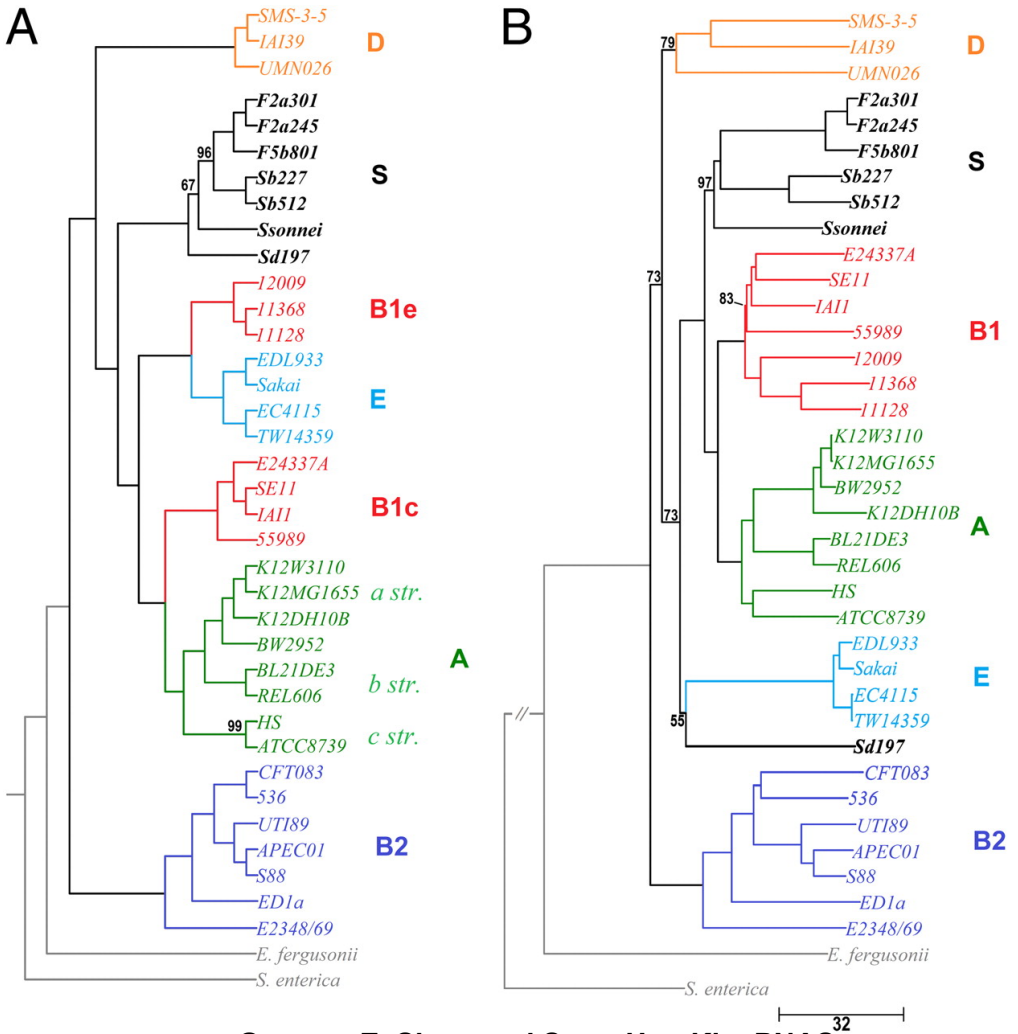


E. coli K12 (MG1655)

E. coli O157:H7

E. coli O157:H7 has some 1,346 ORFs not found in K-12

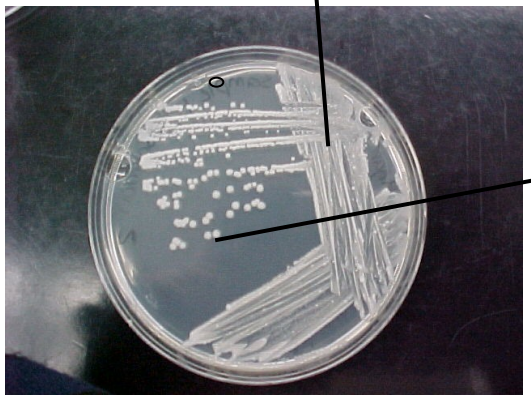
Phenetic and evolutionary alignment-free whole-genome phylogenies of *E. coli/Shigella*.

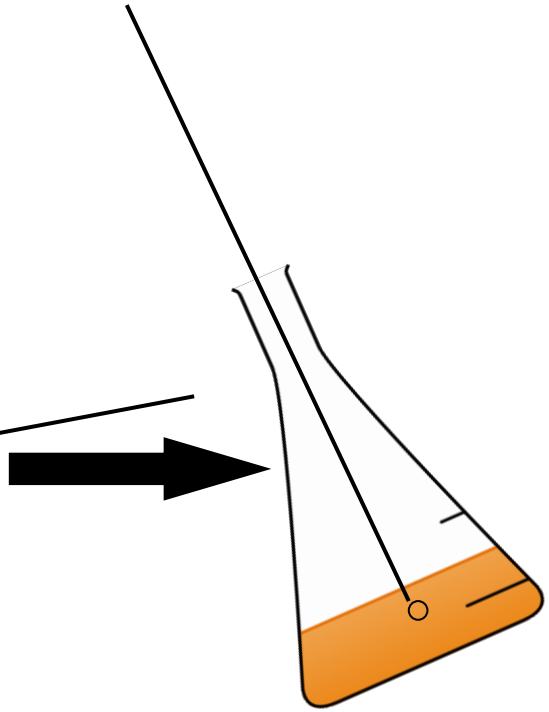


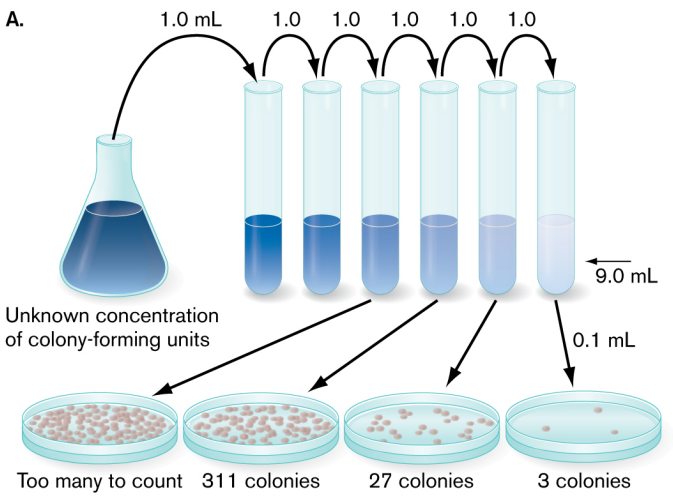
Gregory E. Sims, and Sung-Hou Kim PNAS 2011;108:8329-8334

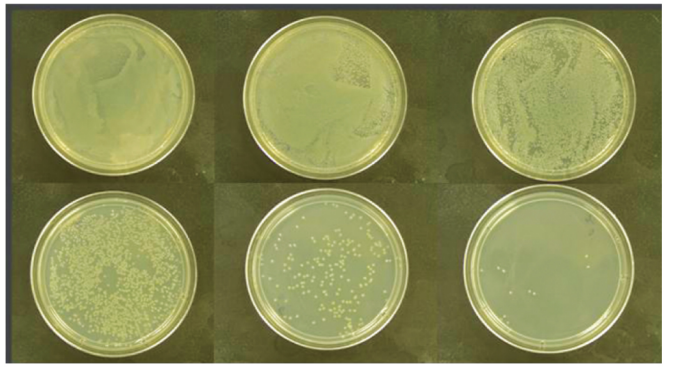
Phenetic and evolutionary alignment-free whole-genome phylogenies of *E. coli/ Shigella.* Two method were used, both constructed from FFPs of length l = 24. The compositional phylogeny in A uses all features of length l = 24 and the Jensen–Shannon divergence (the tree is drawn unscaled). The evolutionary phylogeny in B depicts the likely evolutionary history of the phylogroups. The tree was constructed from features present in all 38 genomes and a distance derived from a multistate unordered characters model of feature frequency. Distances represent the number of character feature changes. The differences between the two trees reflect lateral transfer of features or genes within Shigella and between B1 [B1 is phenetically separated into B1e (EHEC) and B1c (commensal) subgroups] and E. The A phylogroup can be divided into a, b, and c strains. Numerical values placed at internal branches represent 10% jackknife confidence values. No value represents 100% agreement among pseudoreplicates.

Pure Culture and Serial and Dilution

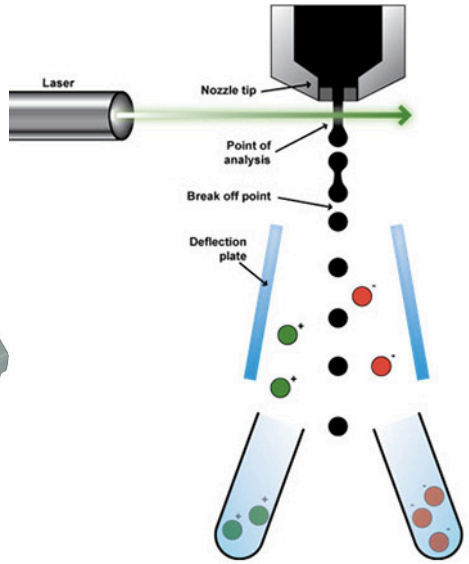






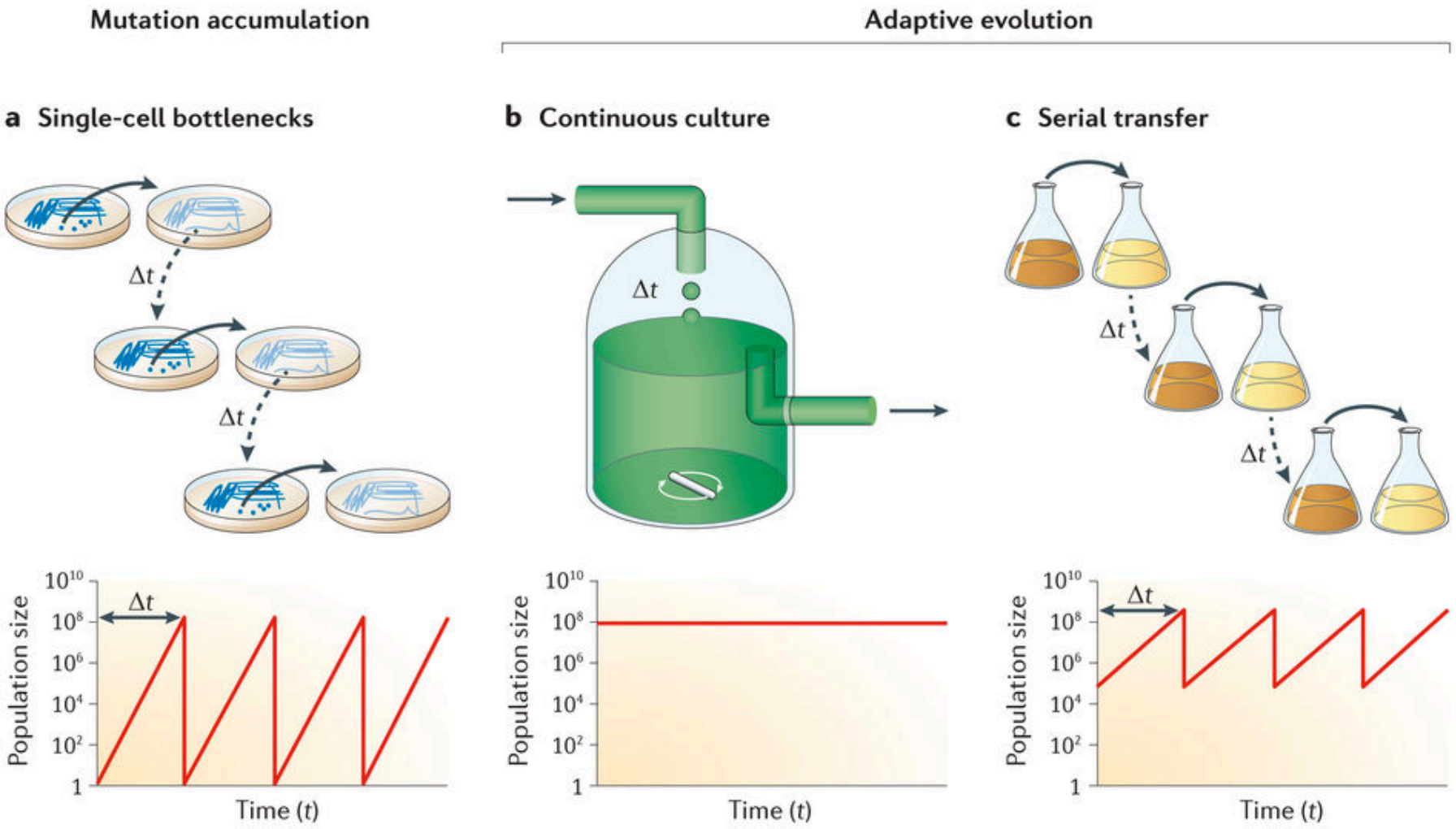


Fluorescence Activated Cell Sorting (FACS)



Gullberg E, et al. (2011) Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. PLoS Pathog 7(7): e1002158. doi: 10.1371/journal.ppat.1002158

Culture Methods Beyond Flasks



Jeffrey E. Barrick & Richard E. Lenski
Nature Reviews Genetics 14, 827–839 (2013

The Inconvenient Reality of Physical Structure

Both for modeling and for experiments, we maintain the fiction that bacteria are planktonic cells frolicking about in an essentially dimensionless habitat all with equal access to nutrients, wastes, toxic compounds, phage and antibiotics.

Unfortunately, in the real world, bacteria exist as colonies and micro-colonies on surfaces or embedded in semi solids or the polysaccharide matrices of biofilms.

The Big Question: Will qualitatively different ecological and evolutionary outcomes obtain in physical structured populations?

While we have methods to deal with this inconvenient realty of the physical structure experimentally, the theory for this is only in its nascent phase. (I will put some papers into the KITP site.) **I think, it's where the action is.**

Bacteriophage

Ernest Hanbury Hankin
1865 - 1939

"The most abundant organisms on earth" (and a great tool for molecular biology)

L'ACTION BACTÉRICIDE DES EAUX DE LA JUMNA ET DU GANGE
SUR LE MICROBE DU CHOLÉRA

Par M. E. HANKIN

Du laboratoire du gouvernement. Agra, Indo.

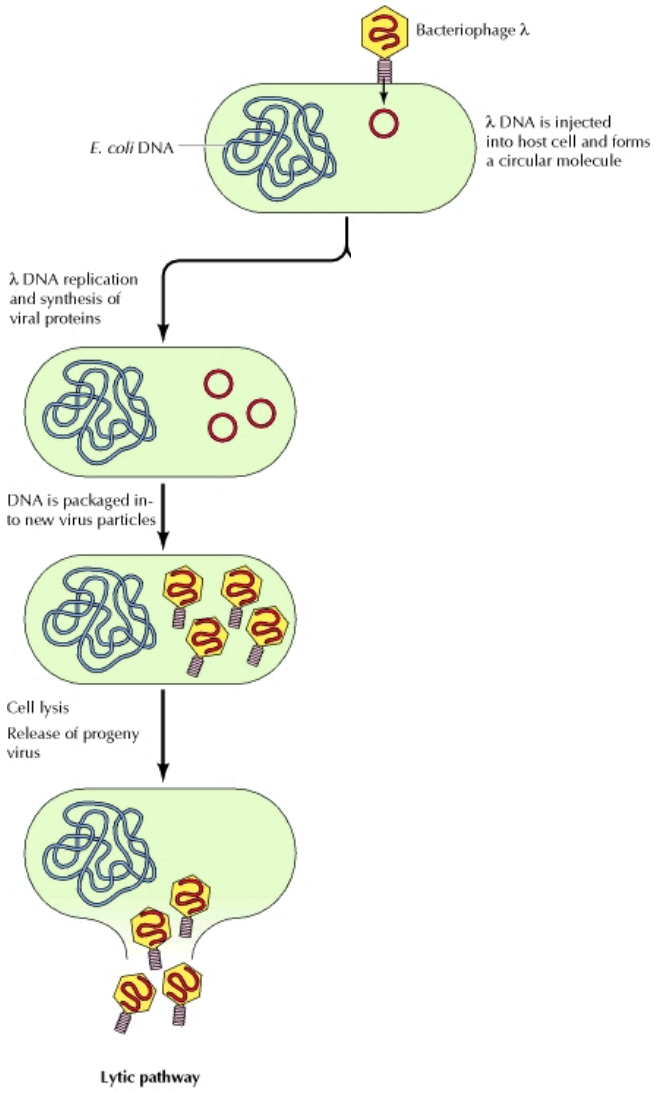
ANNALES DE L'INSTITUT PASTEUR (1896).

Nikolay Gamaleya
1859-1949

Frederick W. Twort
1877-1950

Félix d'Hérelle
1873-1949

Lytic Phage


Bacteriophage λ
E. coli DNA
λ DNA is injected into host cell and forms a circular molecule
λ DNA replication and synthesis of viral proteins
DNA is packaged in- to new virus particles
Cell lysis
Release of progeny virus
Lytic pathway

Mutations Occur at Random

Two hypotheses

1- Mutations occur only when the organism is confronted by the selecting agent, e.g. Streptomycin causes mutations for resistance to streptomycin.
Natural selection is the composer as well as the editor of evolution.
Lamarkian.

2- Mutations occur at random and are observed when they are under selection. For example, *Str-r or phage resistance* mutants are generated during the course of growth and are selected for when the bacteria confront Streptomycin or phage.

Natural selection is the editor but not the composer of evolution.

Blog by Richard Lenski

https://telliamedrevisited.wordpress.com/2013/08/29/luria-and-delbruck-1943-genetics/

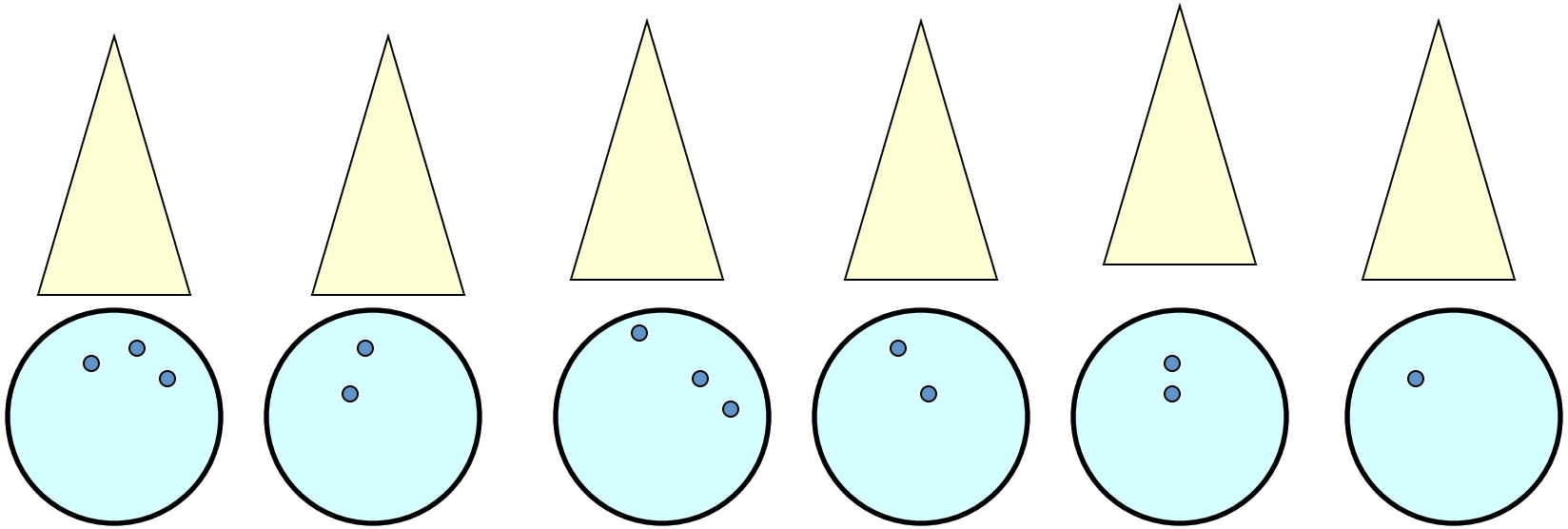
Fluctuation Test

Hyp. 1 Mutations occur only after they confront the selective agent

E. coli B and the lytic phage T1.

Salvador Luria and Max Delbruck (1943)

Many cultures started with very few cells – grow up to 10^9

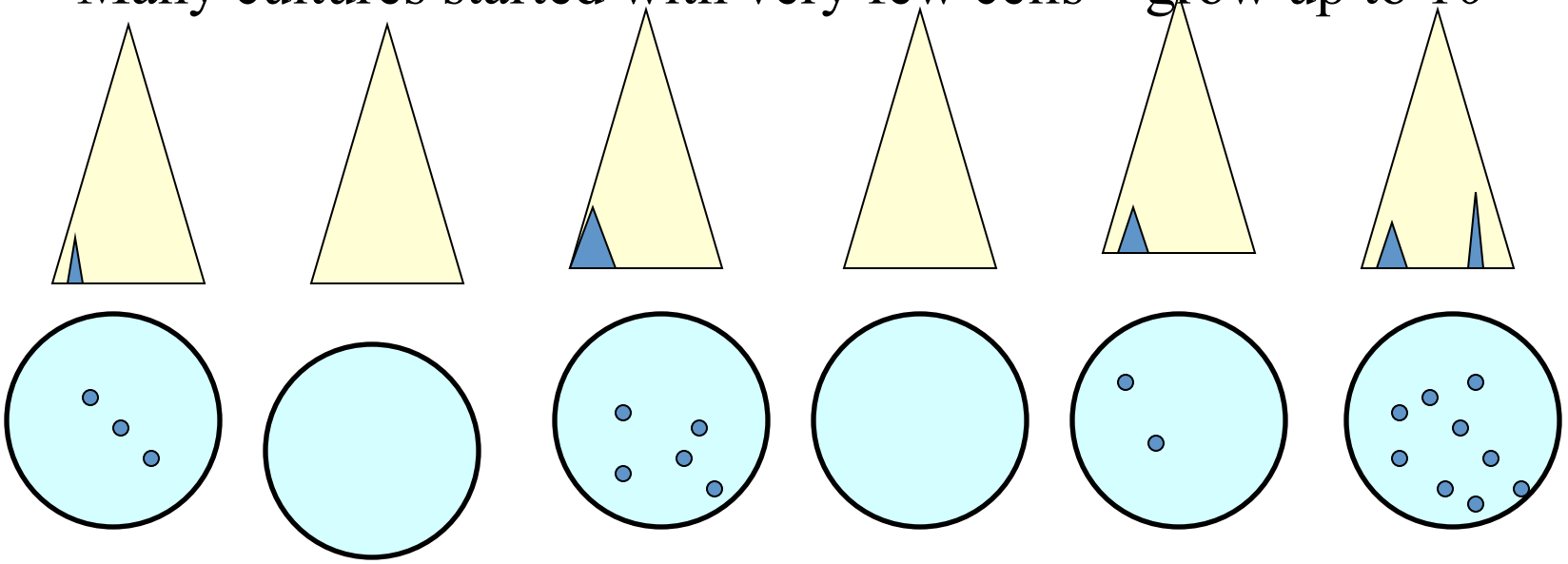


Little Variation would be anticipated

Fluctuation Test

Hyp. 2 Mutations occur at random

Many cultures started with very few cells – grow up to 10^9



Lots of variation would be anticipated

Fluctuation Test

The results of their experiments were consistent with the second hypothesis, mutations occur at random. Natural selection is the editor but not the composer of genetic variation.

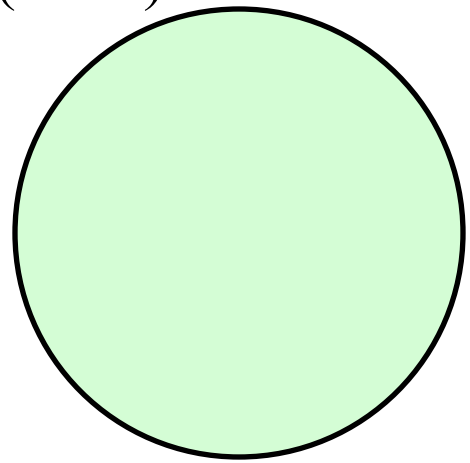
The number of resistant bacteria in series of similar cultures.

EXPERIMENT NO.	1	10	11	15	16	17	21a	21b
Number of cultures	9	8	10	10	20	12	19	5
Volume of cultures, cc	10.0	10.0	10.0	10.0	.2*	.2*	.2	10.0
Volume of samples, cc	.05	.05	.05	.05	.08	.08	.05	.05
Culture No.								
1	10	29	30	6	1	1	0	38
2	18	41	10	5	0	0	0	28
3	125	17	40	10	3	0	0	35
4	10	20	45	8	0	7	0	107
5	14	31	183	24	0	0	8	13
6	27	30	12	13	5	303	1	
7	3	7	173	165	0	0	0	
8	17	17	23	15	5	0	1	
9	17		57	6	0	3	0	
10			51	10	6	48	15	
11					107	1	0	
12					0	4	0	
13					0		19	
14					0		0	
15					1		0	
16					0		17	
17					0		11	
18					64		0	
19					0		0	
20					35			
Average per sample	26.8	23.8	62	26.2	11.35	30	3.8	48.2
Variance (corrected for sampling)	1217	84	3498	2178	694	6620	40.8	1171
Average per culture	5360	4760	12400	5240	28.4	75	15.1	8440
Bacteria per culture	3.4×10^{10}	4×10^{10}	4×10^{10}	2.9×10^{10}	5.6×10^{8}	5×10^{8}	1.1×10^{8}	3.2×10^{10}
Mutation rate	1.8×10^{-8}	1.4×10^{-8}	4.1×10^{-8}	2.1×10^{-8}	1.1×10^{-6}	3.0×10^{-8}	3.3×10^{-8}	3.0×10^{-8}
Standard deviation / Average (exp.)	1.3	.39	.95	1.8	2.3	2.7	1.7	.71
Standard deviation / Average (calc.)	.35	.33	.33	.37	.94	.67	1.04	.26

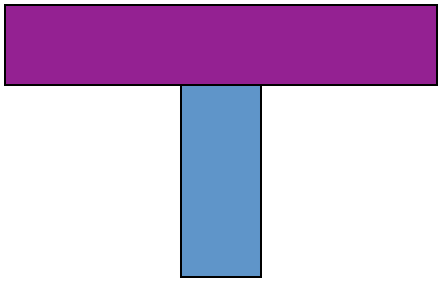
Luria, SE and M. Delbruck (1943) Mutations from virus sensitivity to virus resistance. Genetics 28:491-511

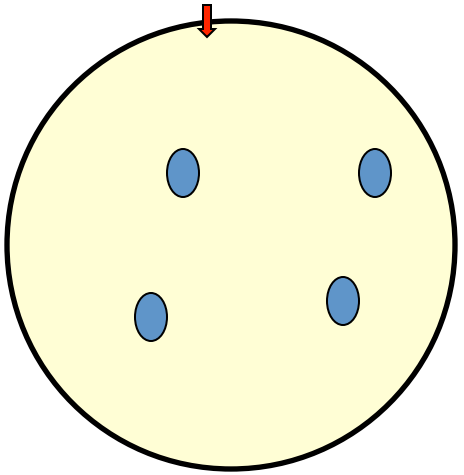
Replica Plating Demonstration of Random Mutations

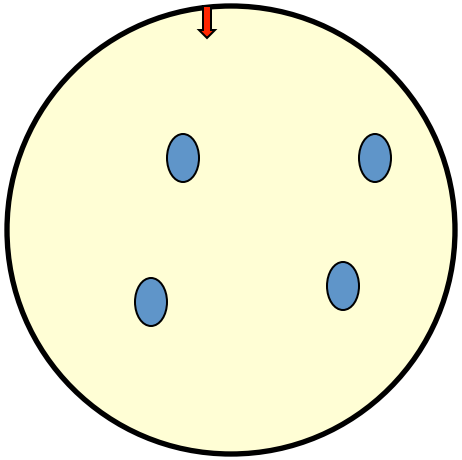
Joshua Lederberg
(1953)



Plates without Streptomycin - Oodles of bacterial colonies







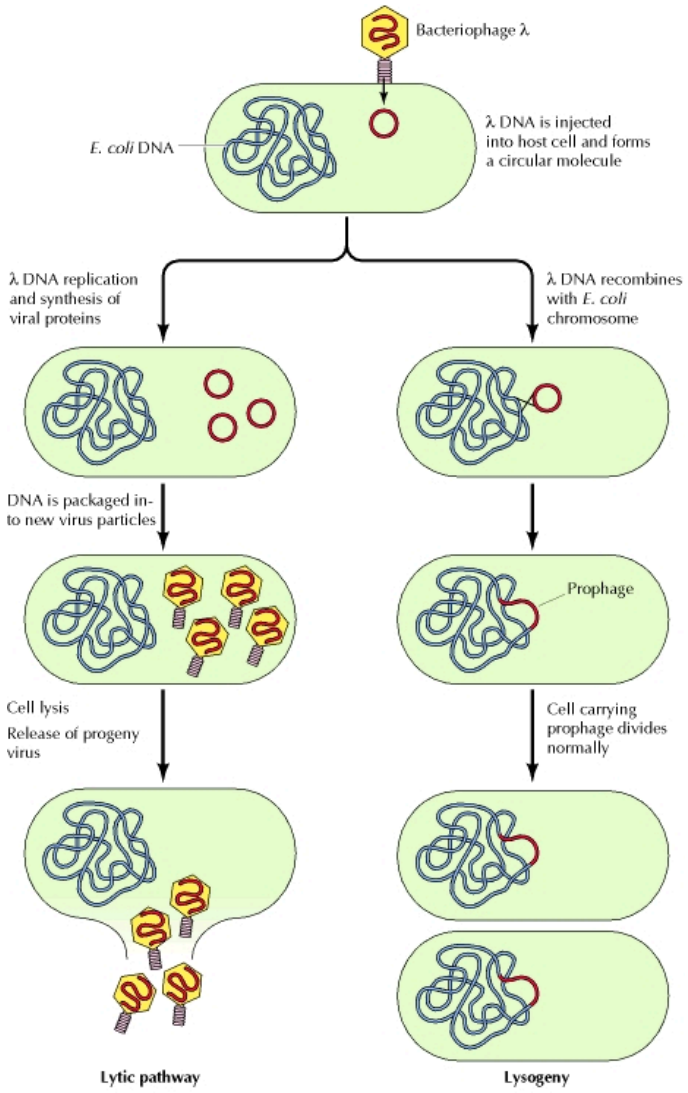
Plates with Streptomycin

Some Questions to Ponder?

Did these experiments reject the alternative hypothesis, directed mutation (Lamarkian)?

How would you **reject** the hypothesis that a some mutations are generated as a response to the selective pressure?

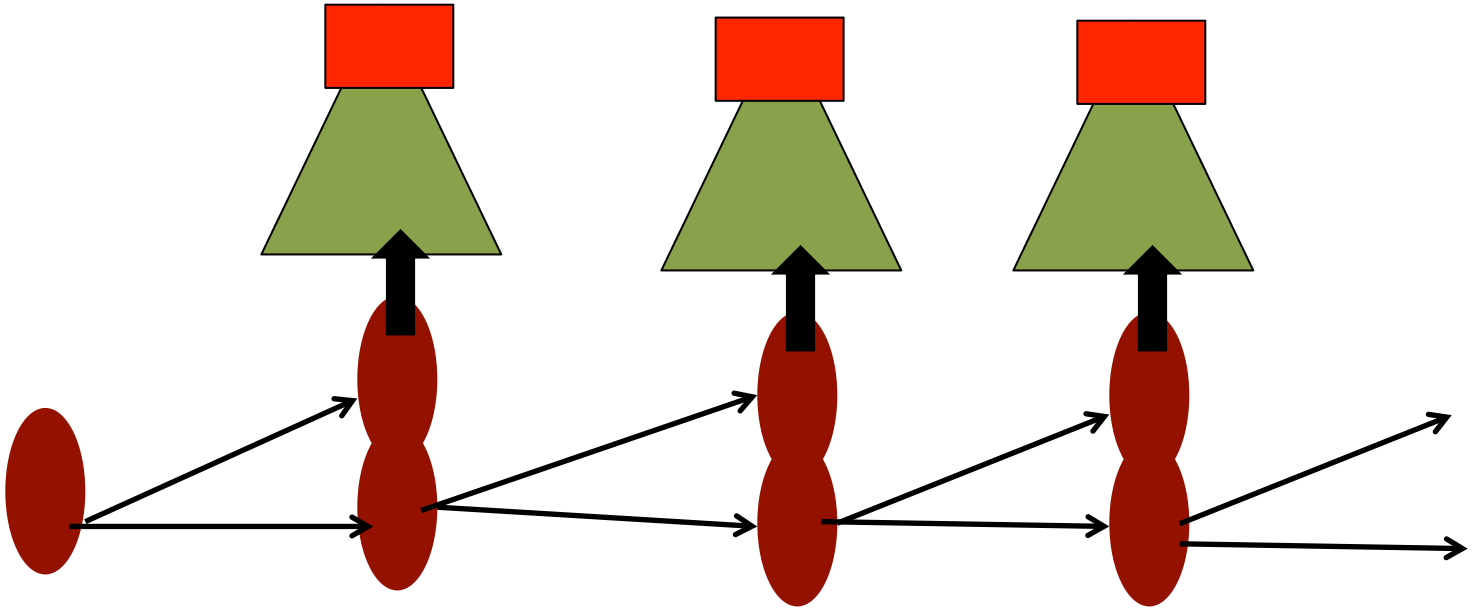
Temperate Phage and Lysogeny



Evidence for Lysogeny

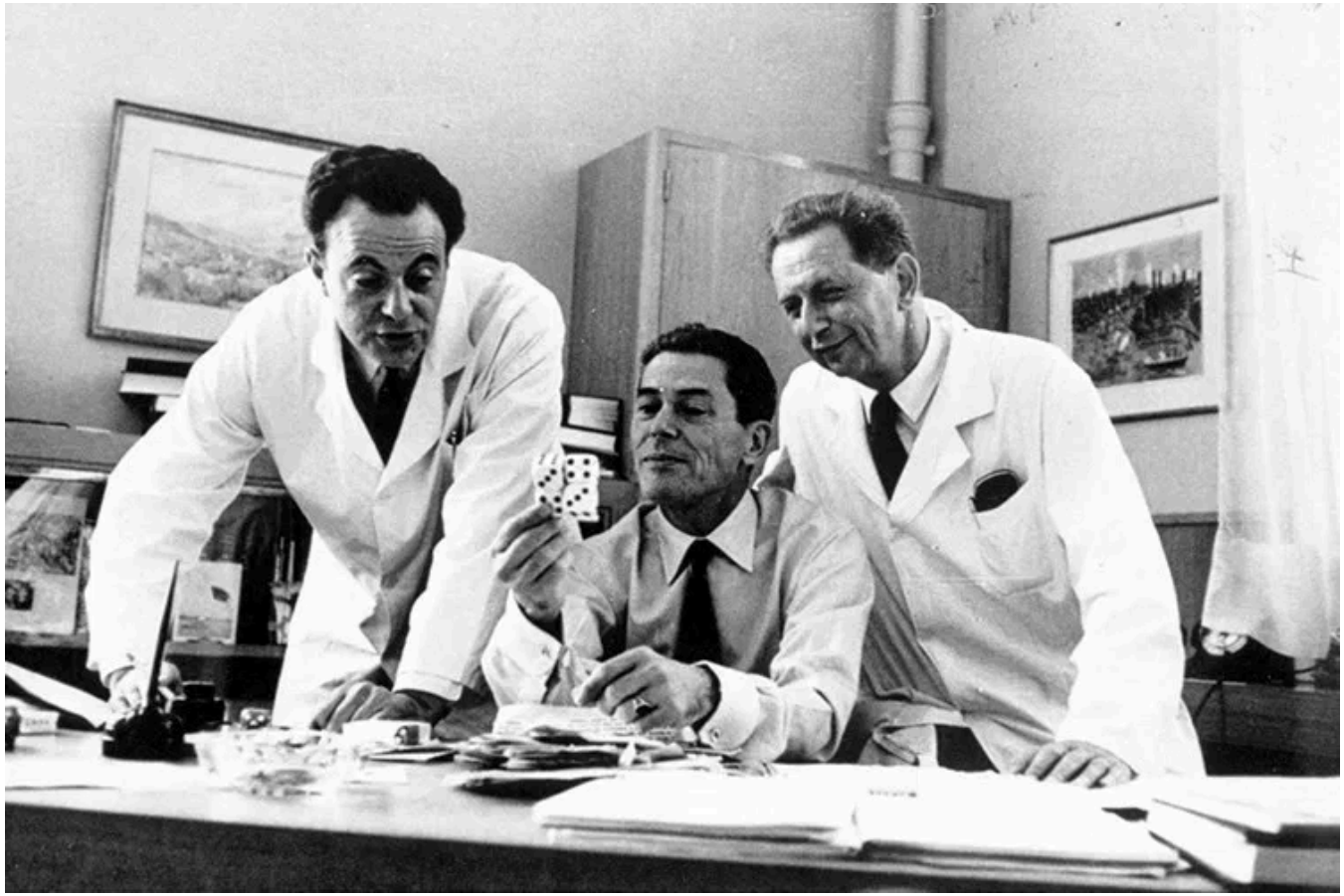
" I decided to operate on individual cells. … I was led to this decision because I don't like either mathematics of statistics. I like to see things, not calculate probabilities. " (Nobel Prize Speech, 1963)

Andre Lwoff
1902- 1994



19 Times

Not a bad Lab



Francois Jacob Jacques Mondo Andre Lwoff

Phage-Encoded Toxins and Human Diseases

Virus	Host bacteria	Virulence factor	Gene	Type	Disease (third eukaryotic organism)
β-phage	*Corynebacterium diphtheriae*	Diphtheria toxin	tox	Exotoxin	Diphteria
Phage C1	*Clostridium botulinum*	Neurotoxin	C1	Exotoxin	Botulism
Phage H-19B, Enterobacteria phage 933W	*Escherichia coli*	Shiga toxin 1A, 1B, 2A, 2B	stx1AB stx2AB	Exotoxin	Dysentery
Phage CTXΦ	*Vibrio cholerae*	Cholera toxin A, B	ctxAB	Exotoxin	Cholera
Phage ΦC3208	*Escherichia coli*	Hemolysin	hly2 stx2AB	Exotoxin	Hemolysis
Phage ΦCTX	*Pseudomonas aeruginosa*	Cytotoxin	ctx	Exotoxin	Cytotoxicity (especially on leukocytes)
Phage N315	*Staphylococcus aureus*	Enterotoxin P	sep	Exotoxin	Vomiting
Phage Mu50A	*Staphylococcus aureus*	Enterotoxin A	sea	Exotoxin	Food poisoning syndrome
Phage Φ13	*Staphylococcus aureus*	Enterotoxin A	entA	Exotoxin	Gastroenteritis
Phage ΦETA	*Staphylococcus aureus*	Exfoliative toxin A	eta	Exotoxin	Scaled-skin syndrom
Phage ΦPVL	*Staphylococcus aureus*	Panton-Valentine leukocidin S, F	lukS	Exotoxin	Leukocytes destruction
Phage T12	*Streptococcus pyogenes*	Toxin A	speA	Exotoxin	Scarlet fever
Phage CS112	*Streptococcus pyogenes*	Toxin C	speC	Exotoxin	Scarlet fever

http://viralzone.expasy.org/3967?outline=all_by_species

Recombination in Bacteria

Bacteria may not have sex often, but when they do it's really good evolutionarily (and scientifically).

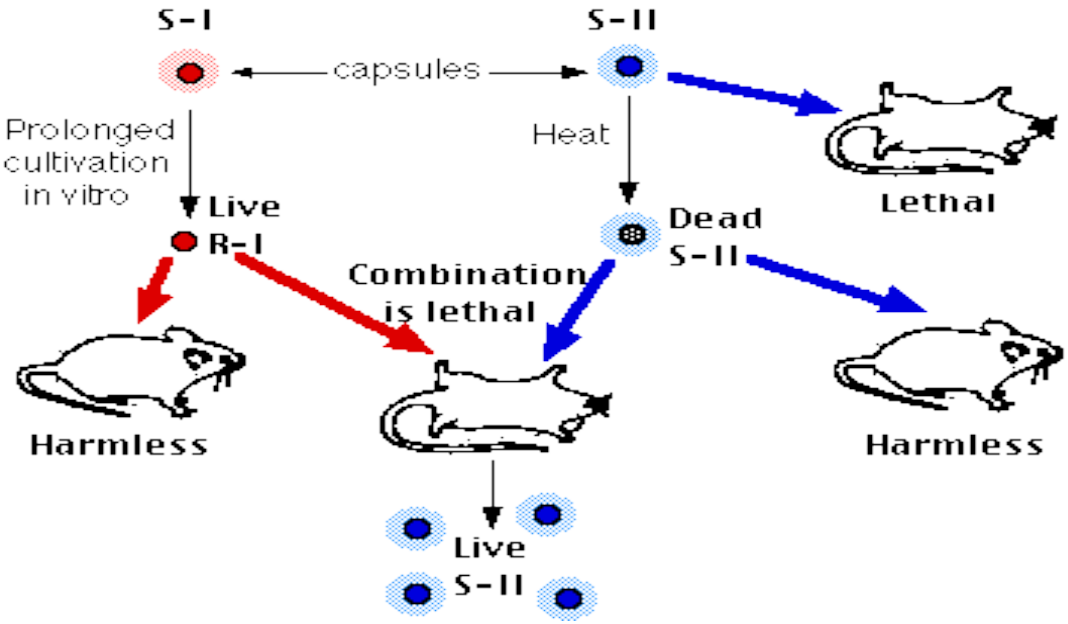
Transformation – uptake of free DNA

Conjugation – Direct cell-cell contact

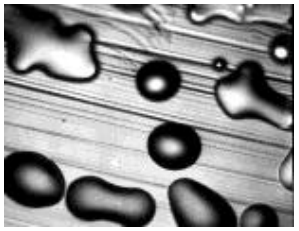
Transduction - Mediated by phage

Transformation – First evidence for Recombination in Bacteria – Evidence for DNA as the Genetic Material

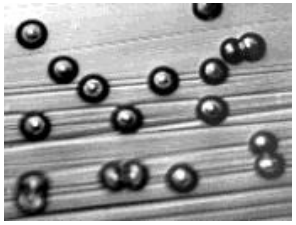
Streptococcus pneumoniae (pneumococcus)



Griffiths, F. J. (1928) J. Hygiene 27: 113



Smooth Virulent

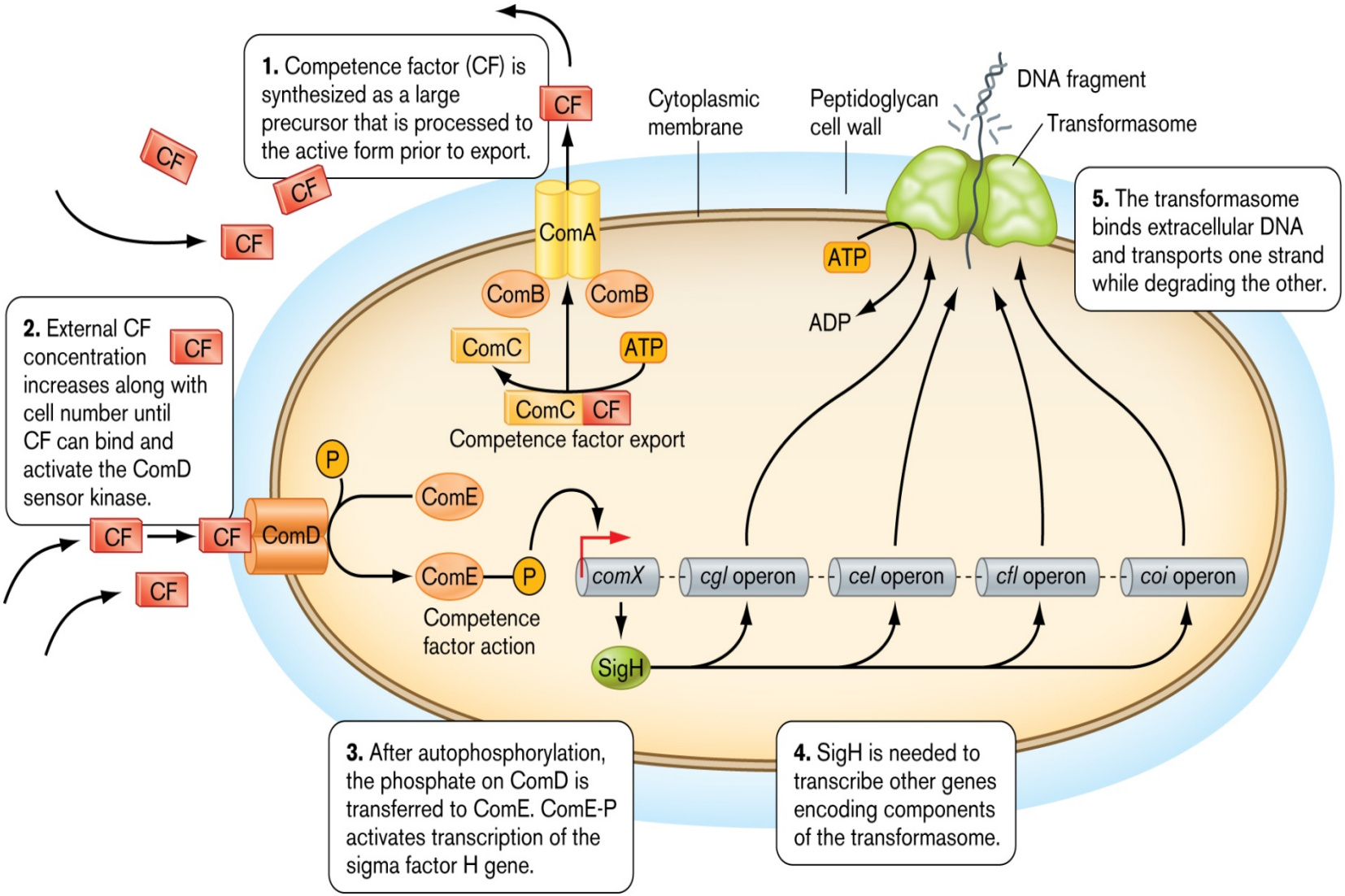


Rough Avirulent

Transformation in vitro

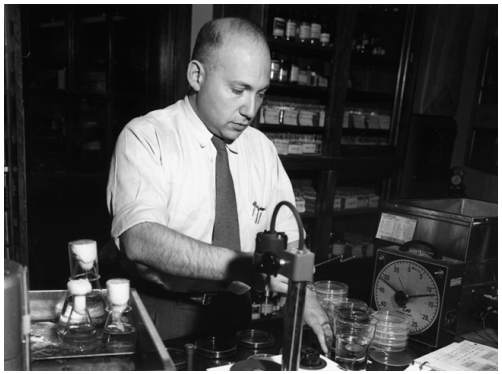
AVERY, 0. T., C. M. MACLEOD and M. MCCARTY, 1944 Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J. Exp. Med. 79: 137-158.

Transformation a complex process



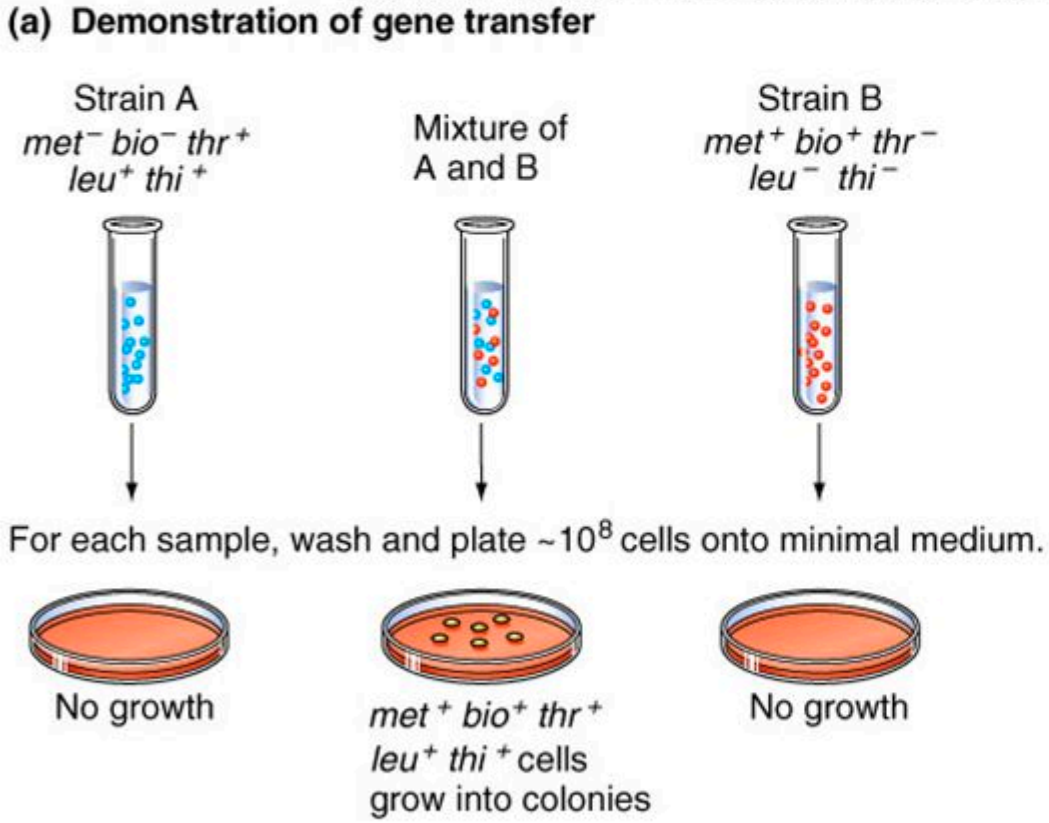
Streptococcus pneumoniae

Conjugation - 1946



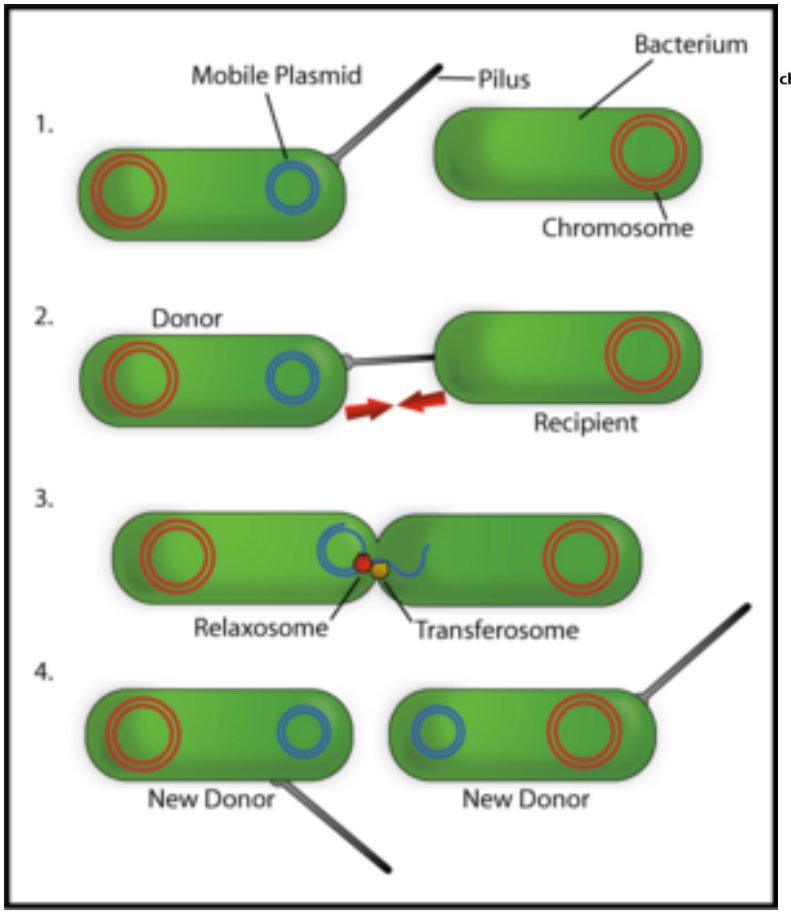
Joshua Lederberg
1925-2008

Edward Tatum
1909- 1975

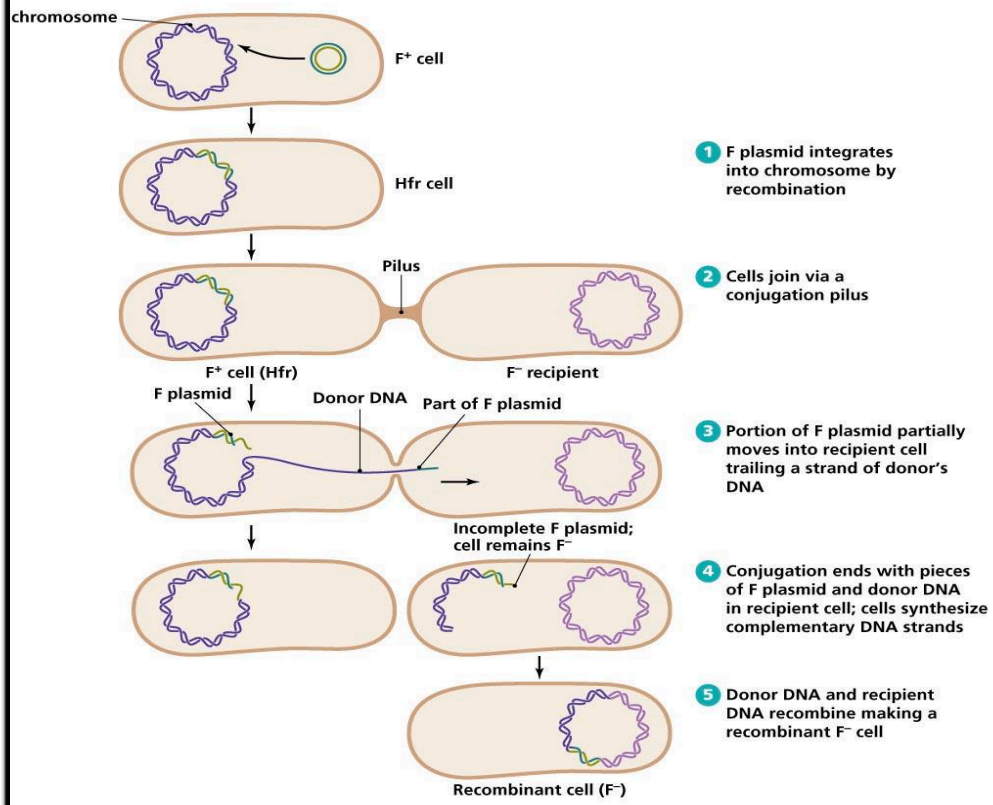


Conjugative Plasmid Transfer and Chromosomal Gene Recombination

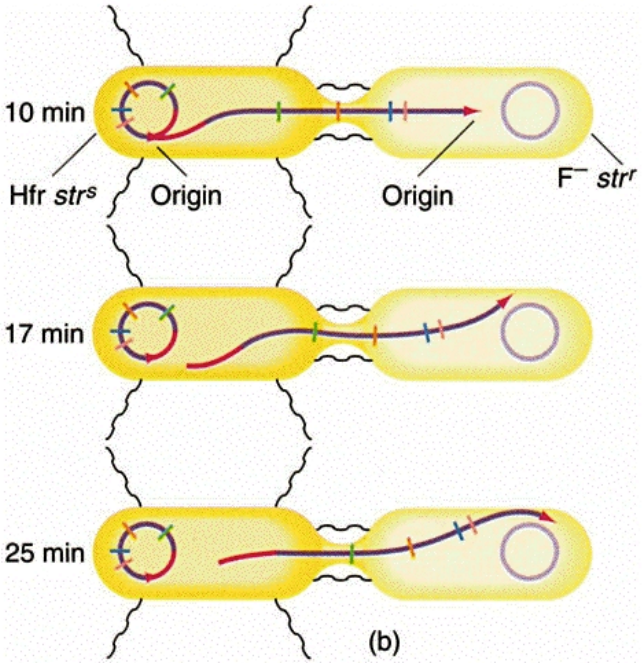
Plasmid Transfer

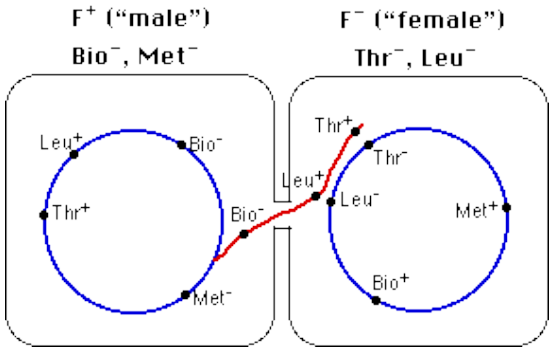


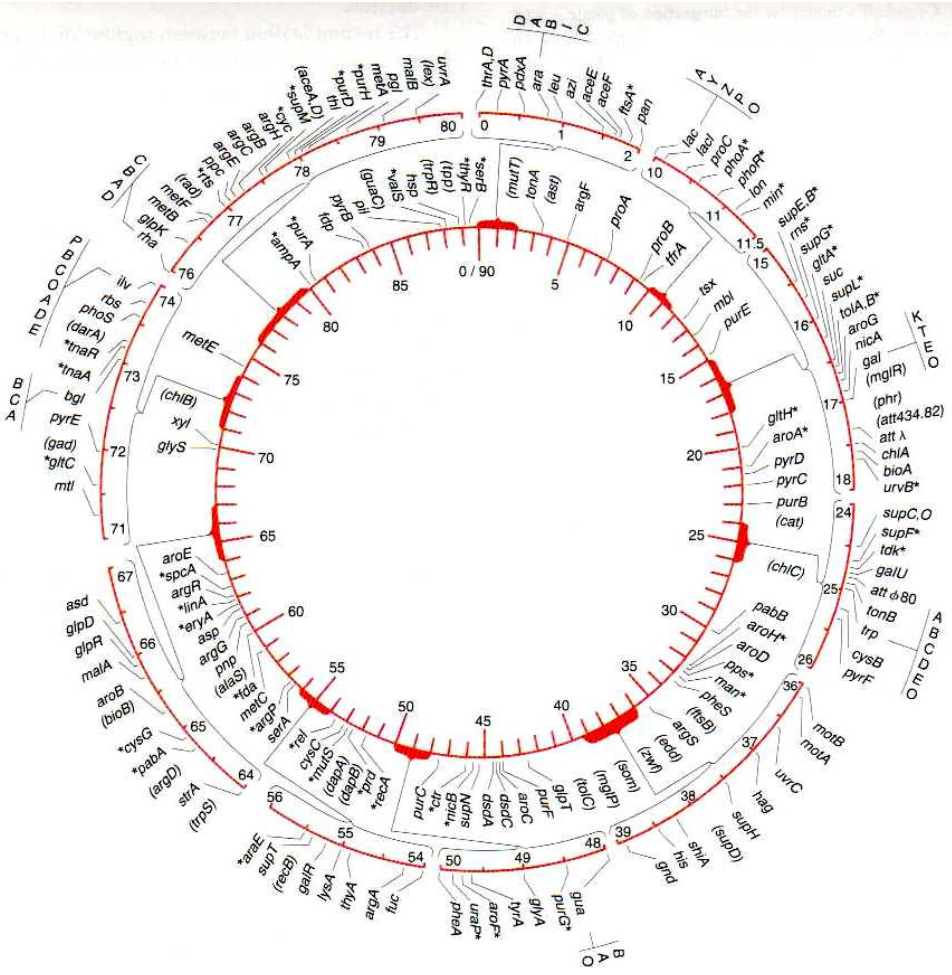
Integration and transfer of chromosomal genes



E. coli K-12 and Bacterial Genetics







A classical genetic map of the *E. coli* chromosome

Serendipity and Good Fortune

Had Luria and Delbruck been working with a temperate phage (which they didn't believe in at the time) or a bacteria with a restriction-modification or CRISPR-Cas immune system and phage susceptible to their action, they would have concluded that mutations in bacteria are directed (Lamarkian).

Had Lederberg and Tatum worked with virtually any *E. coli* other than K12, they would not have gotten the recombination.

"What is true for *E. coli* is true for Elephants, only more so." (attributed to J. Monod)

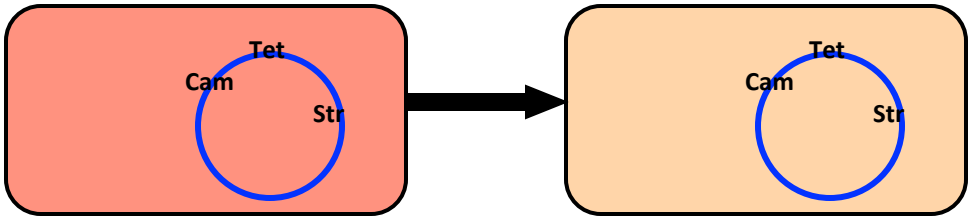
First Evidence for Transmissible Antibiotic Resistance

In the later 1950s Japanese investigators isolated Shigella (dysentary) strains that were resistant to multiple antibiotics, combinations of Str, Tet, Cam, Neo resistant.

The *E. coli* from these patients had similar resistance patterns.

By mixing resistant Shigella and sensitive *E. coli* they were able to transfer the resistance in vitro.

They postulated that the resistance was carried by a plasmid analogous to the F plasmid in *E. coli* ==> Antibiotic resistance, R- plasmids.



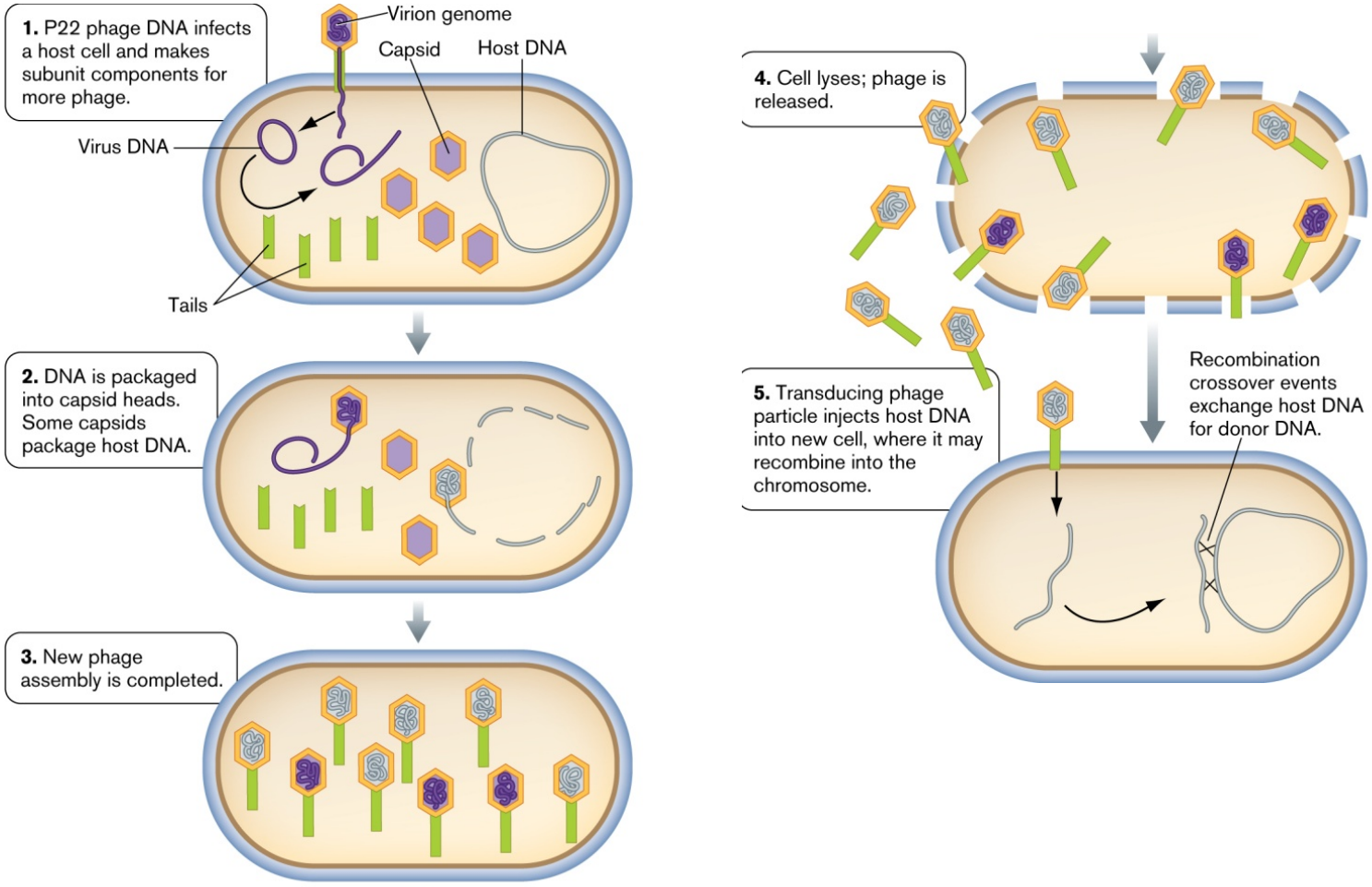
Also the first evidence for a role of horizontal gene (and accessory element) transfer in adaptive evolution in bacteria.

Ochiai K, Yamanaka T, Kimura K (1959) Studies on inheritance of drug resistance by mixed cultivation [In Japanese]. Nippon Iji Shimpo 1861: 38-42.

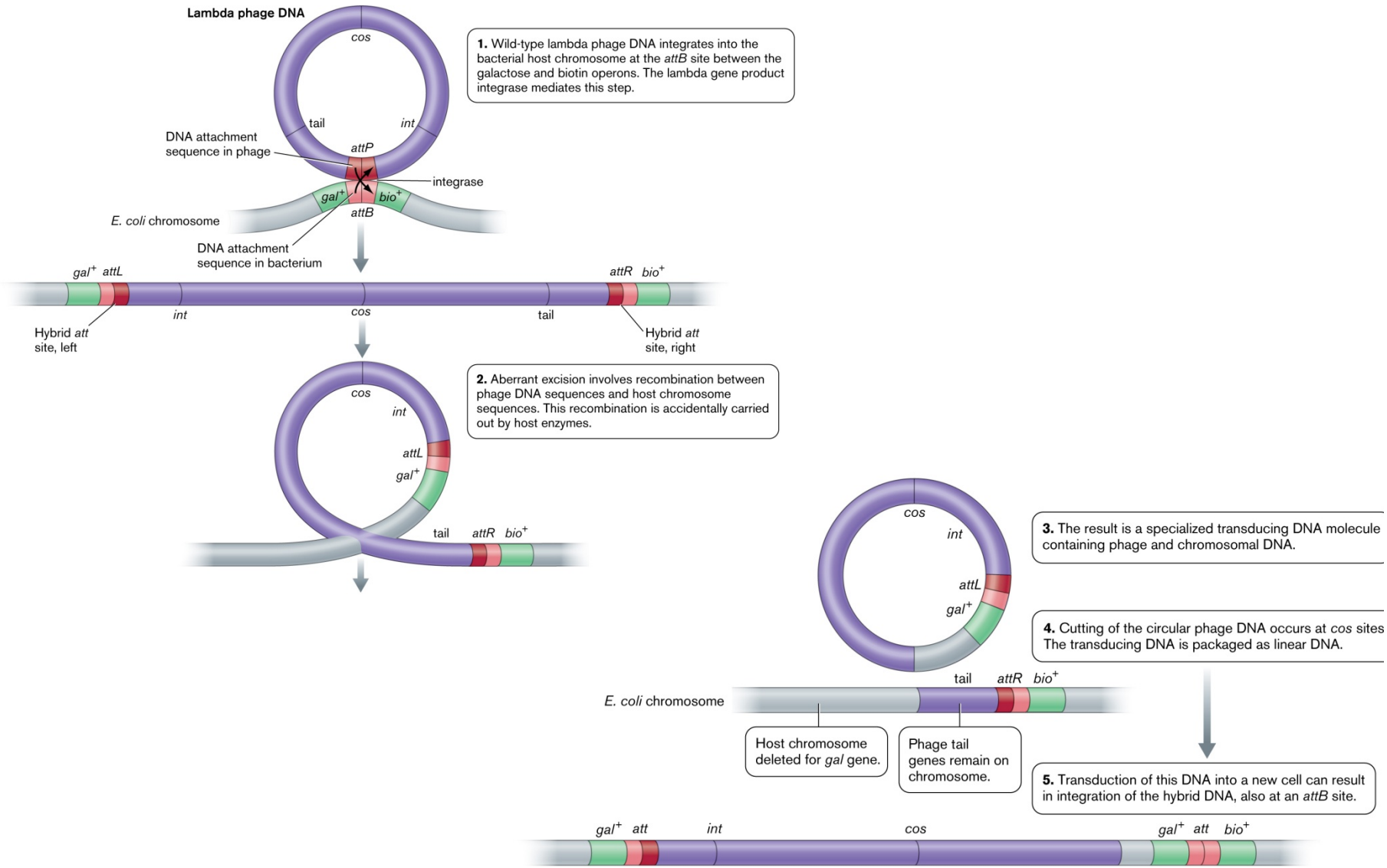
Akiba T, Koyama K, Ishuiki Y, Kimura S, Fukushima T (1960) On the mechanism of development of multiple drug-resistant clones of Shigella Japanese Journal of Microbiology 4: 219-227

Mitsuhashi S, Harada K, Hashimoto H (1960) Multiple resistance of enteric bacteria and transmission to other strains in mixed cultivation. Japan Journal of Experimental Medicine 30: 179-184.

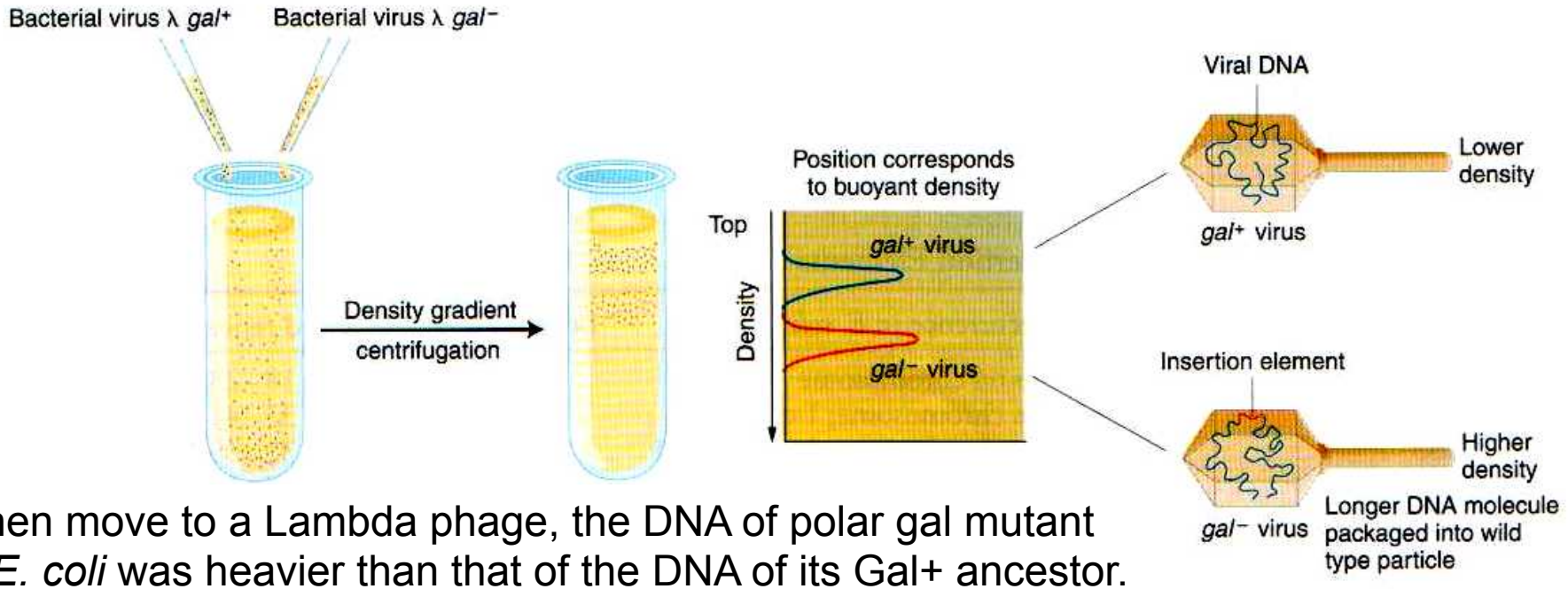
Generalized Transduction



Specialized Transduction



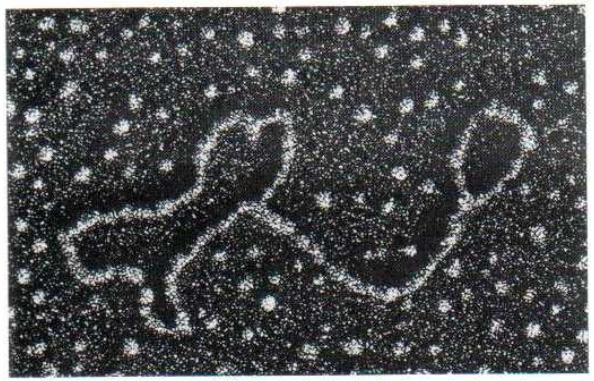
Transposons



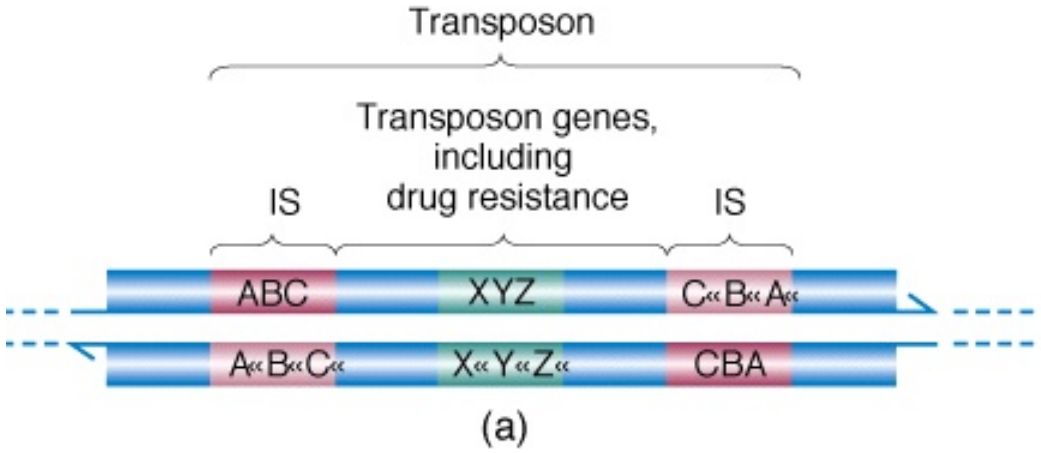
When move to a Lambda phage, the DNA of polar gal mutant of *E. coli* was heavier than that of the DNA of its Gal+ ancestor.

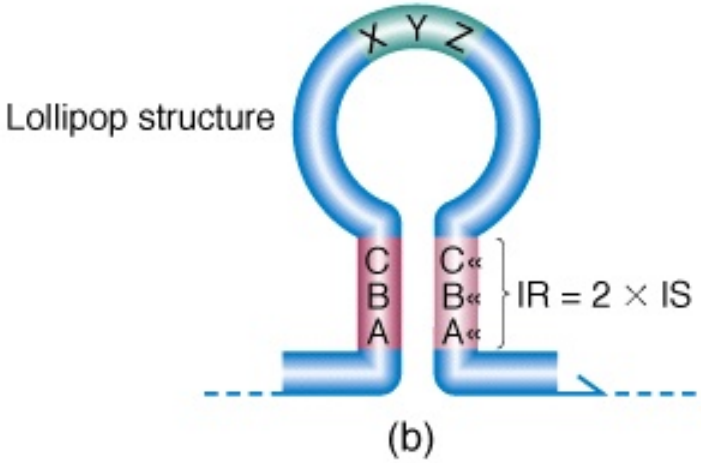
Postulated that the gal mutation was cause by the insertion of a moveable element, Insertion Sequence (IS), into the *gal* gene.

Transposon – Insertion sequences with genes

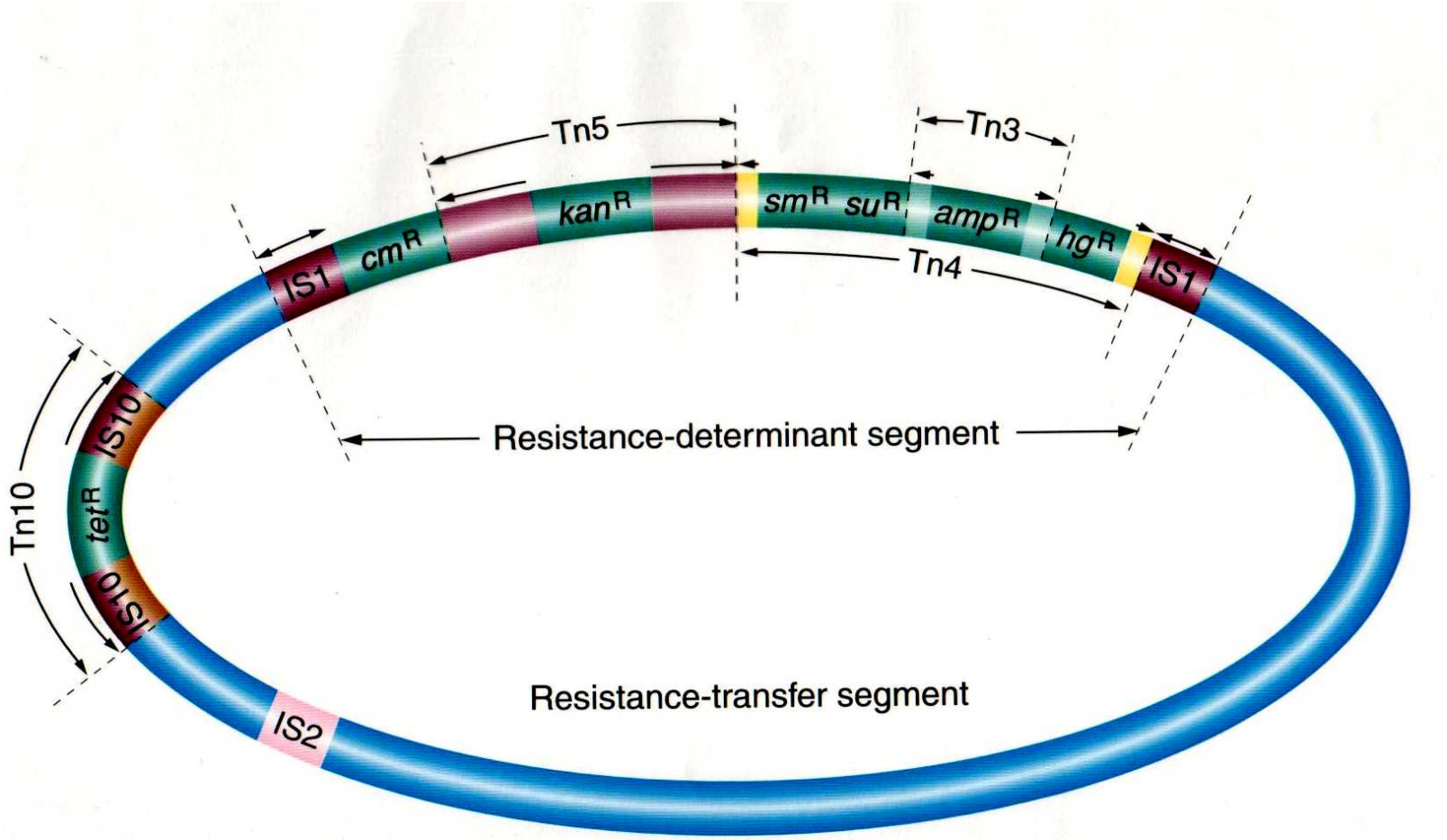


Denatured and reannealed plasmid DNA



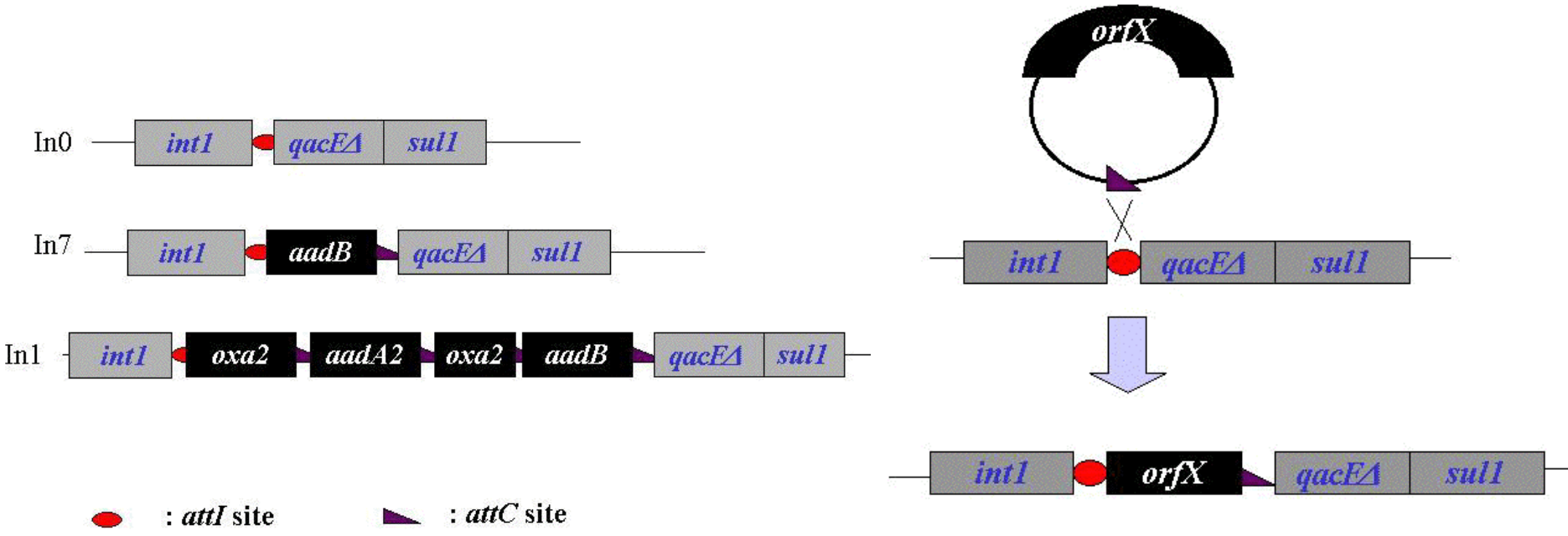


Many of the antibiotic resistance genes carried by R- plasmids are borne on transposons.



Integrons

Mobile genetic elements with the machinery to capture genes by site-specific recombination. Among the more important genes they capture are those that code for resistance to antibiotics.



int1- integrase; ***aadA, aadB*** - aminoglycoside resistance, ***oxa2*** beta-lactamase, ***sul1*** - suphonamide resistance

Some Questions to Ponder (discuss)

What are the selective forces responsible for the evolution and maintenance of "sex" in bacteria; transformation, conjugation, and transduction?

With horizontal gene transfer (HGT) how do bacteria maintain their genetic integrity; why isn't there a continuum?

The genomes of bacteria abound with plasmids, transposons, and defunct prophage with no apparent function. How do these elements become established and how are they maintained?

Why are genes borne on mobile genetic elements, rather than chromosomes?

Why are genes for producing toxins coded for by prophage, rather than chromosomal genes (or plasmids)?

The Innate and Adaptive Immune systems of bacteria

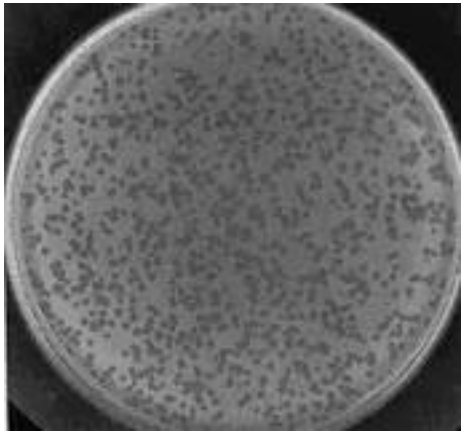
Restriction Modification and CRISPR-CAS

Tools for Capitalist Molecular Biologists and Delicious Questions for Population and Evolutionary Biologists to address

Innate Immunity in Bacteria and Archaea

Restriction and Modification

Phage Origin	Lawn A	Lawn B	Lawn C
A	1E9	1E9	1E6
B	1E6	1E9	1E6
C	1E6	1E9	1E9

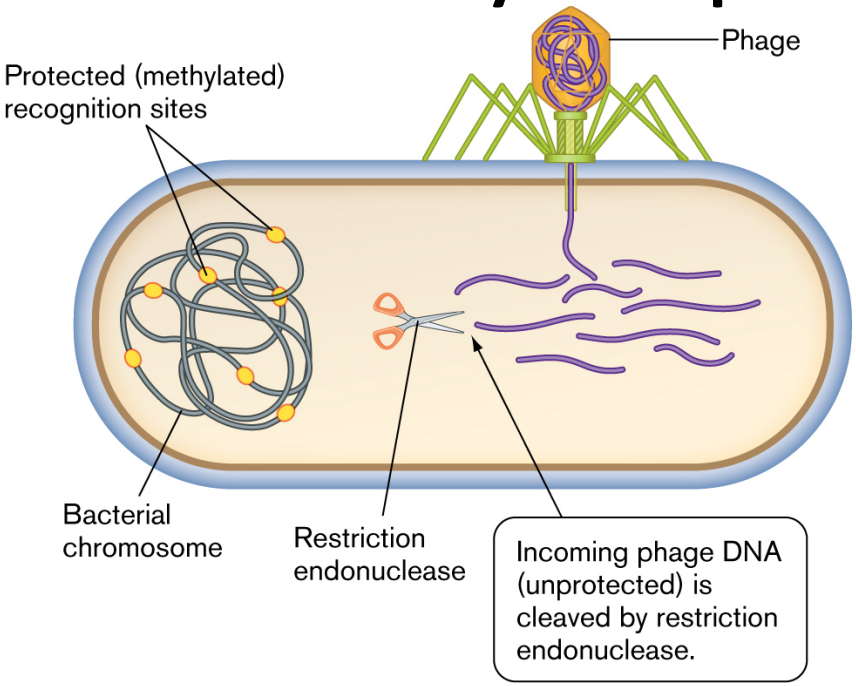


Werner Arber

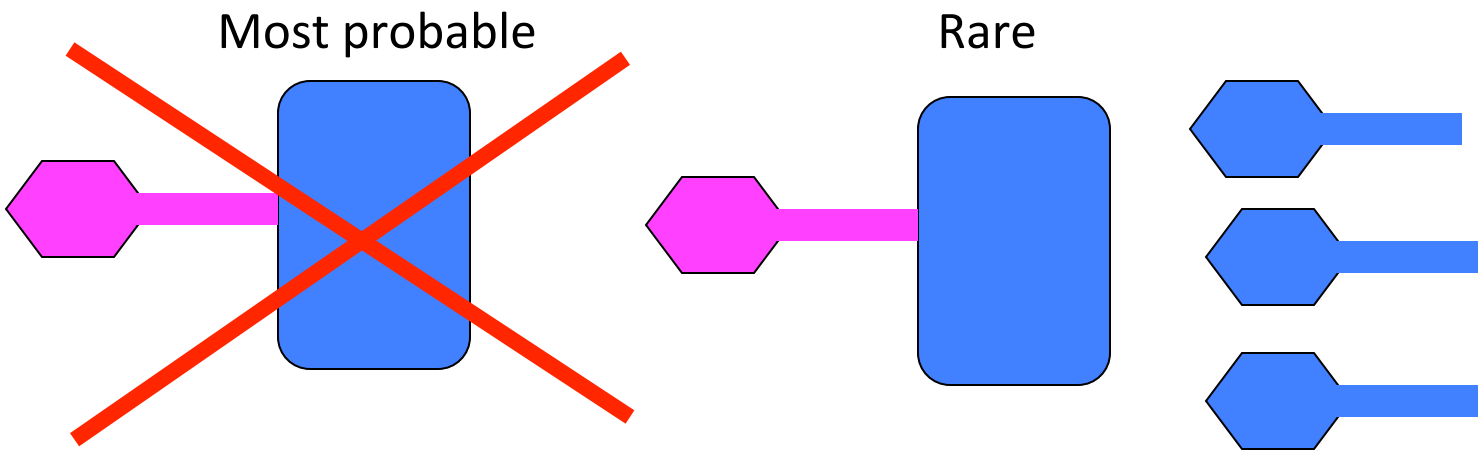
Daniel Nathans

Hamilton O. Smith

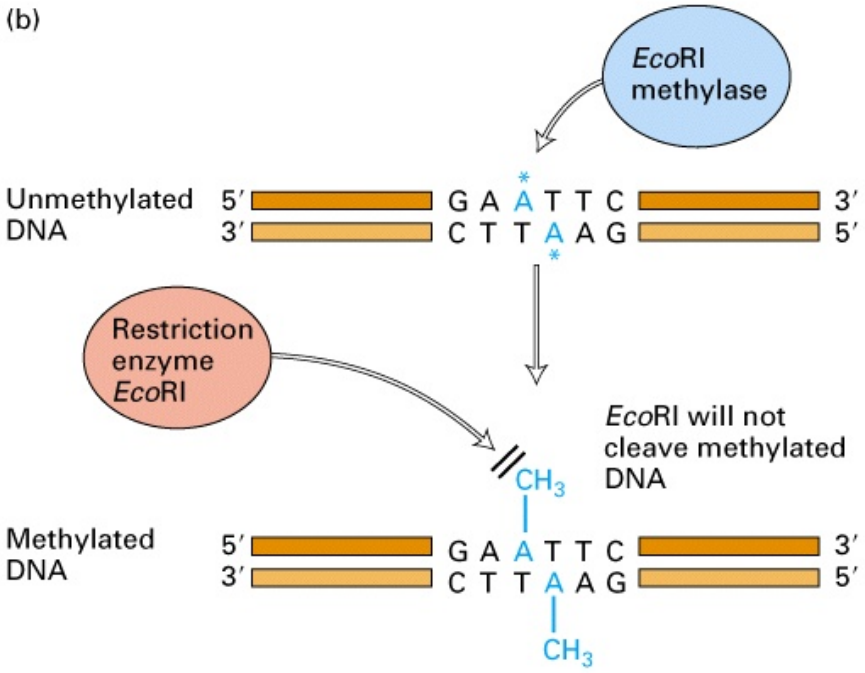
A race between two enzymatic processes

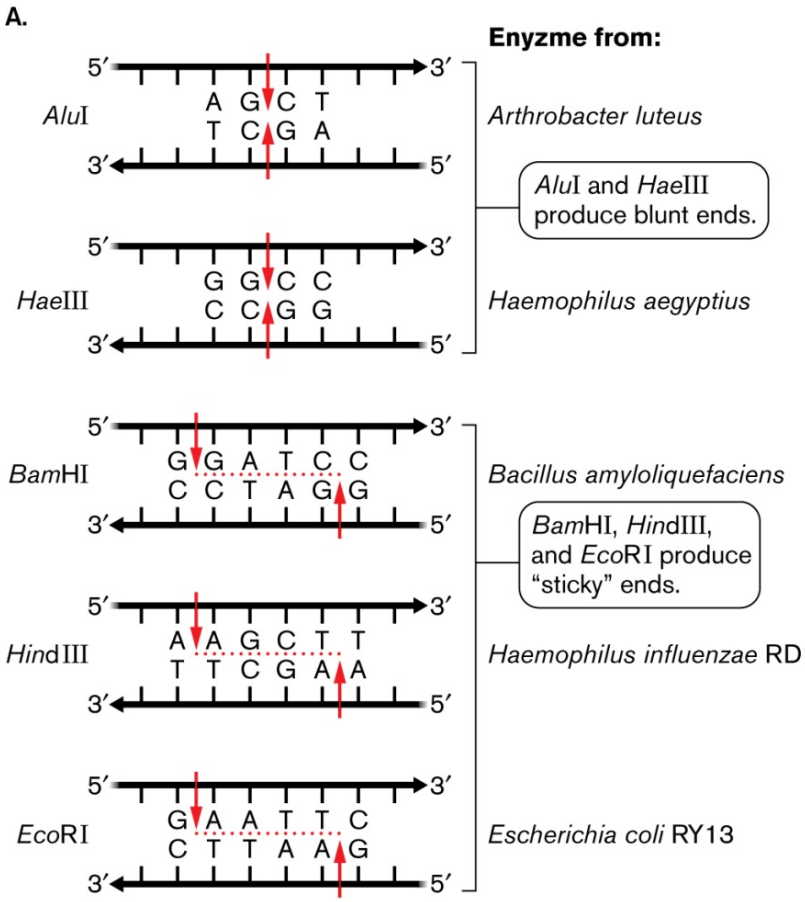


Two outcomes for infection by a phage with unmodified DNA



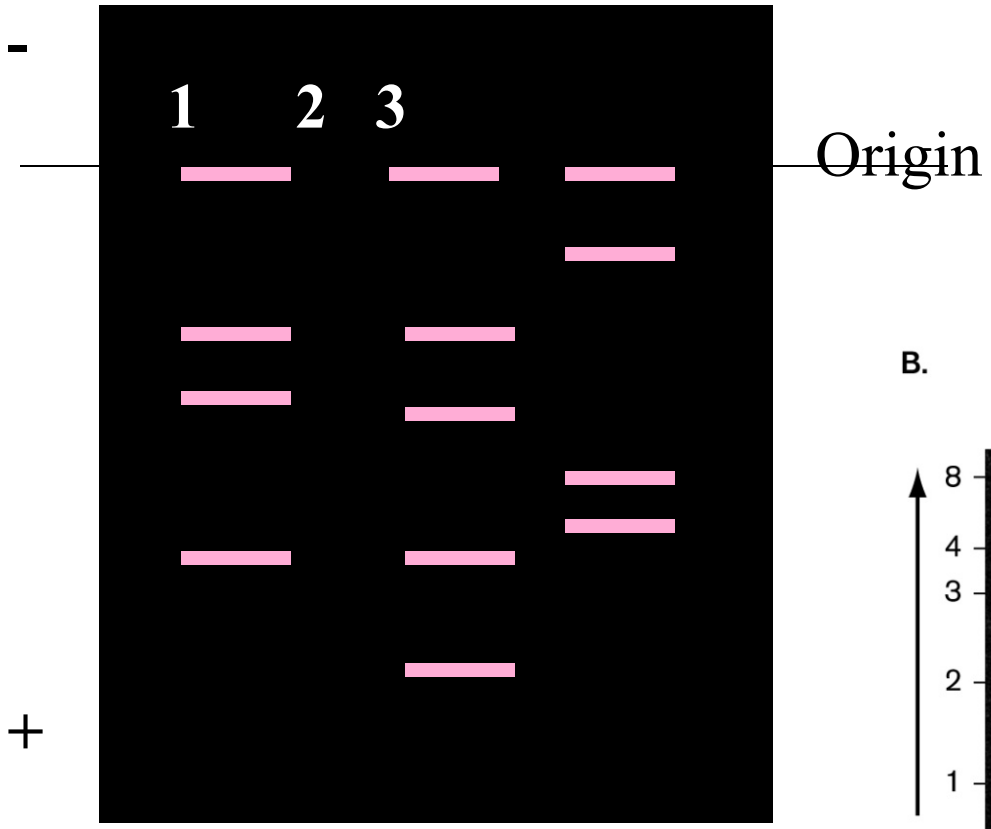
Restriction Sites and Restriction Endonucleases





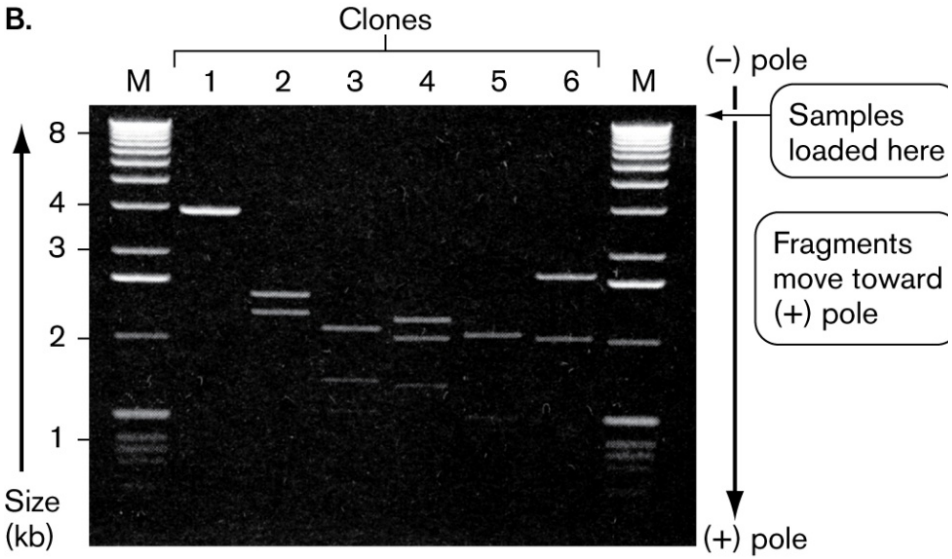
Mapping and Fingerprinting DNA by Restriction Fragment Analysis

DNA – Gel electrophoresis



Agarose gel under UV with an ethidimum bromide stain

In this gel with an electrolyte buffer, DNA will migrate at a rate inversely proportional to its molecular weight (light migrates faster than heavy).



In vitro production of recombinant DNA

Stanley Cohen

Herbert Boyer

Two major techniques.

(1) Splicing genes into plasmids in vitro (generating recombinants)

(2) Transforming *E. coli* with the recombinant plasmids.

Check out the video

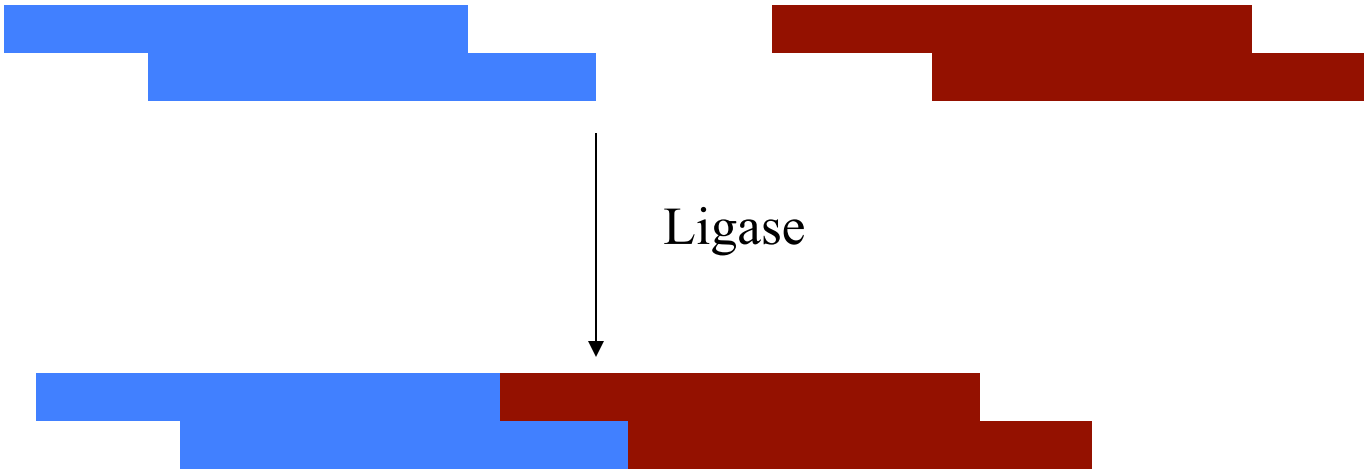
http://www.youtube.com/watch?v=G3H-Uzts108

Insertion of DNA from one organism into the DNA of another

The donor and recipient DNA are cut by the same restriction endonuclease with overlapping ends, voila:

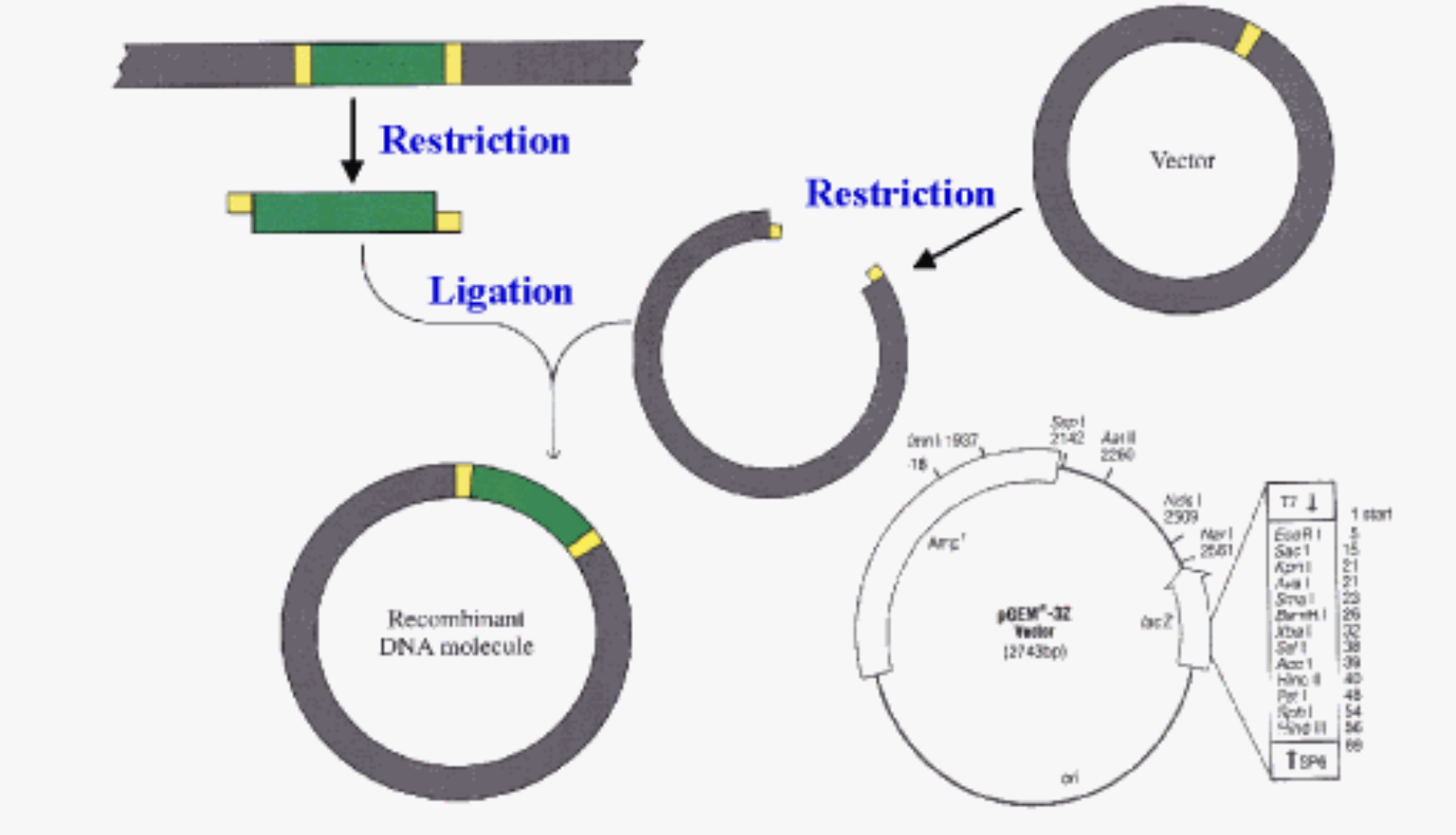
```
---G                A A T T C ----------
---C T TA A                  G----------

---G                A AT T C----------
-- -C T T A A               G----------
```

DNA from two different sources with overhung ends.

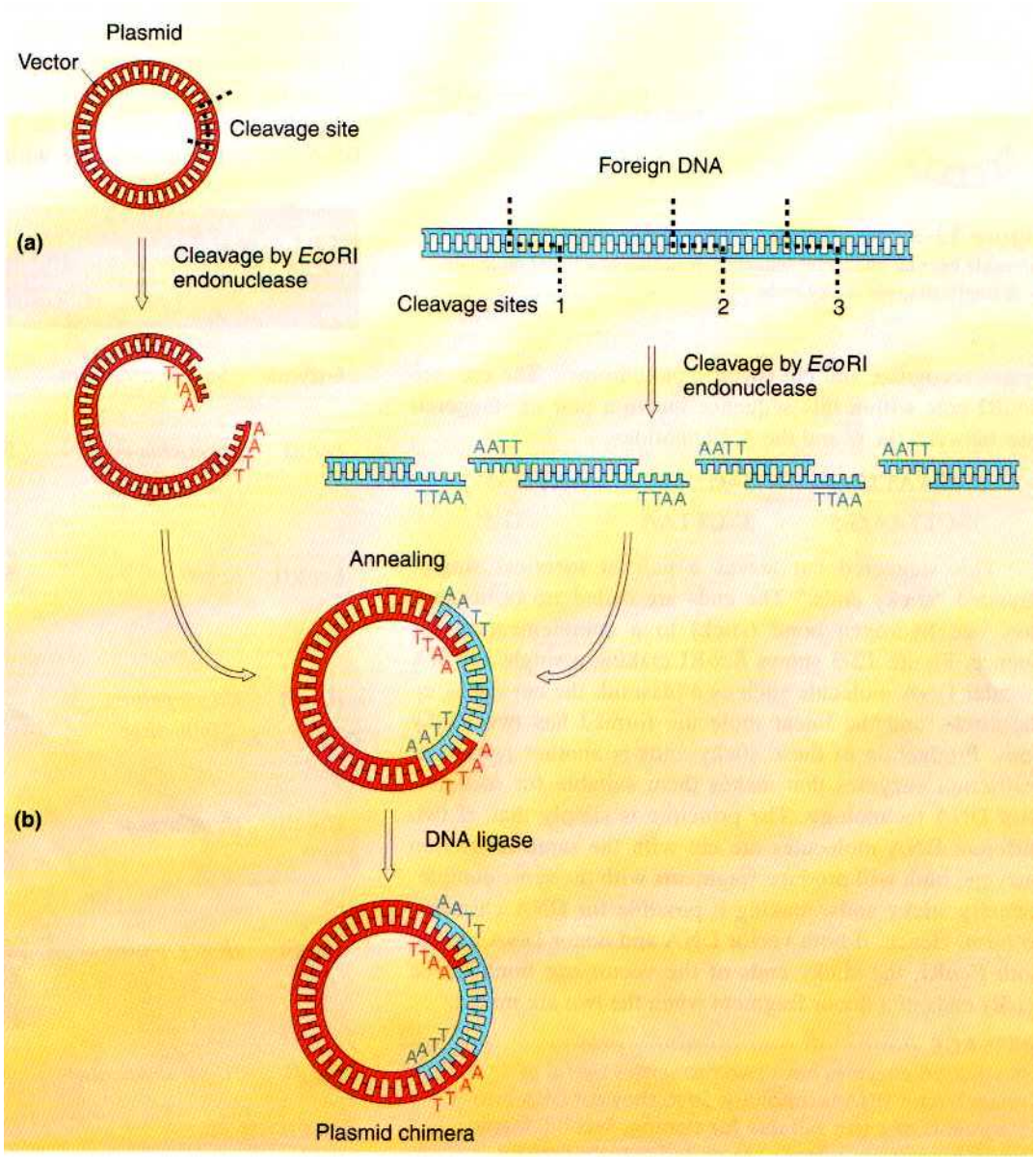


Non-conjugative plasmid vector for R-DNA

Insertion of a DNA into a bacterial plasmid

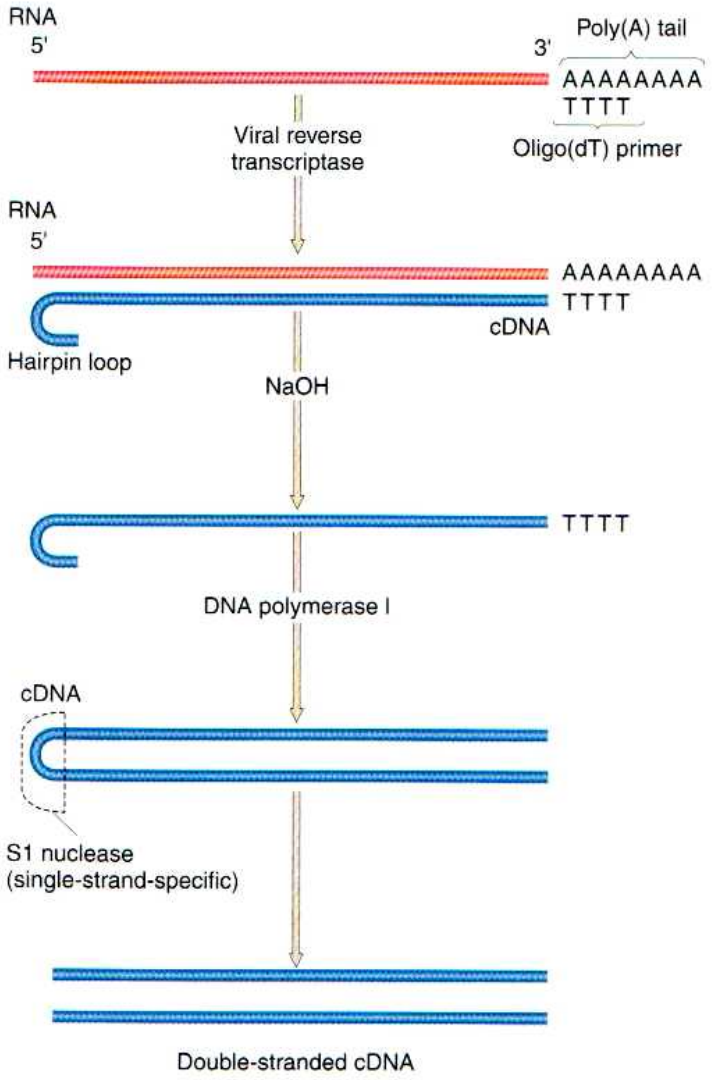


Inserting Foreign DNA into a Plasmid



Sources of DNA for cloning and producing proteins

C - DNA



C (Complementary) DNA is made from a mRNA template using an enzyme, reverse transcriptase which codes for the synthesis of RNA to DNA. Commonly for genes that code for proteins that are highly expressed and for which there would be oodles of mRNA.

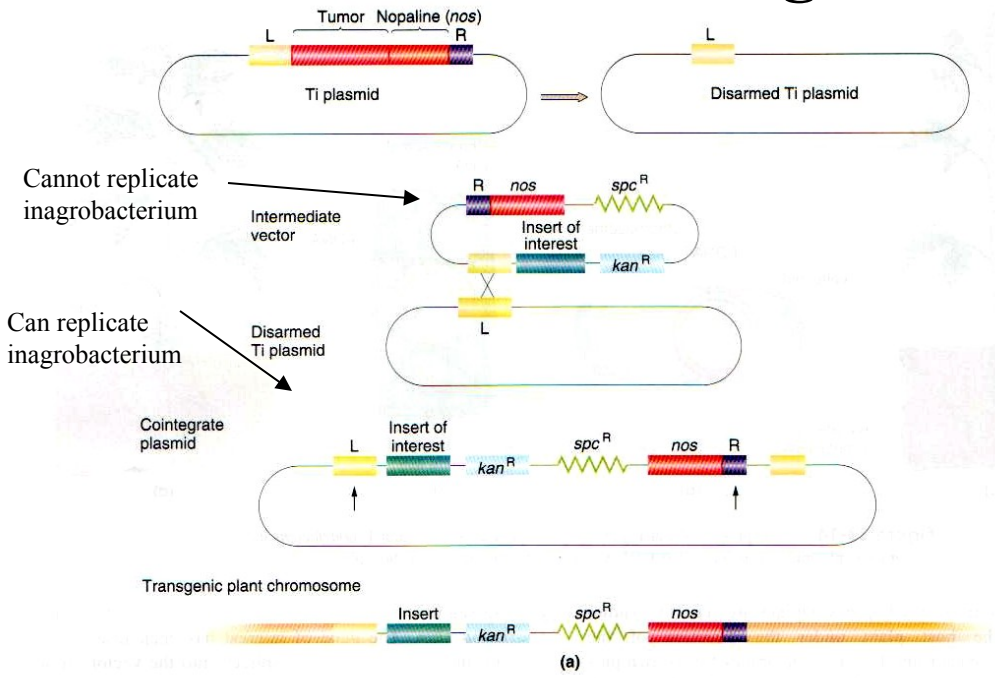
Synthetic oligonucleotides:

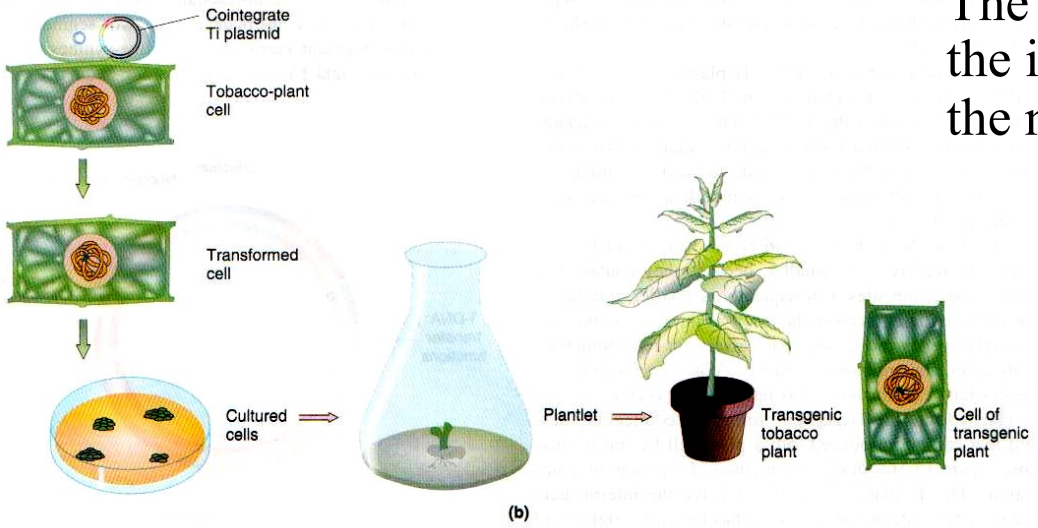
From the DNA sequence, it is possible to infer the amino acid composition of a protein.

If the protein is relatively short, a synthetic oligonucleotide can be made and inserted into a plasmid.

The growth hormone, somatistatin (14 amino acids) was the first of these products.

Agrobacterium turmorfaciens Synthetic Genetic Engineering



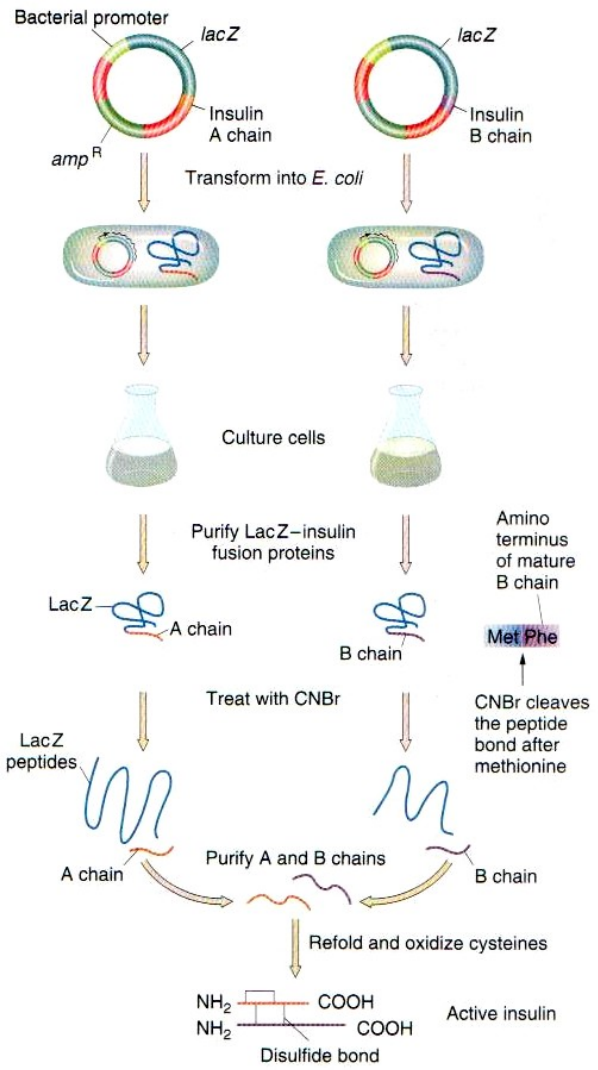


The insert of interest in is inserted into a "disarmed" Ti plasmid carrying a Kan and Spc resistance gene. The latter are needed to select for the transformed plant cell.

The Kan-r gene can be expressed in the plant cell and is used to select for cells with the plasmid.

The end product is a plant expressing the inserted region(presumably via the nos promoter.

Production of human insulin in *E. coli*

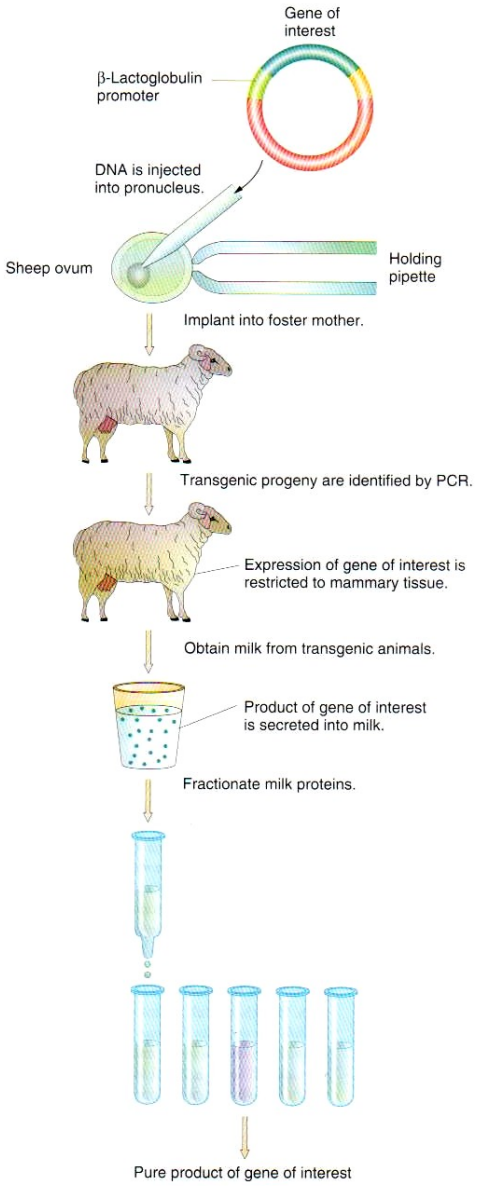


The DNA coding for the two subunits of human insulin are fused with the gene for *E. coli* β-galactosidase and separately cloned and expressed in *E. coli* using a Lac operon and promotor

The β-galactosidase is removed from the proteins produced and the two subunits of insulin are allowed to refold and, with a little chemical help, rejoin to form a functional human insulin, which is then harvested.

A number of proteins have been made this way, including with synthetic DNAs. The big problem is getting them to form the right tertiary and quaternary structure.

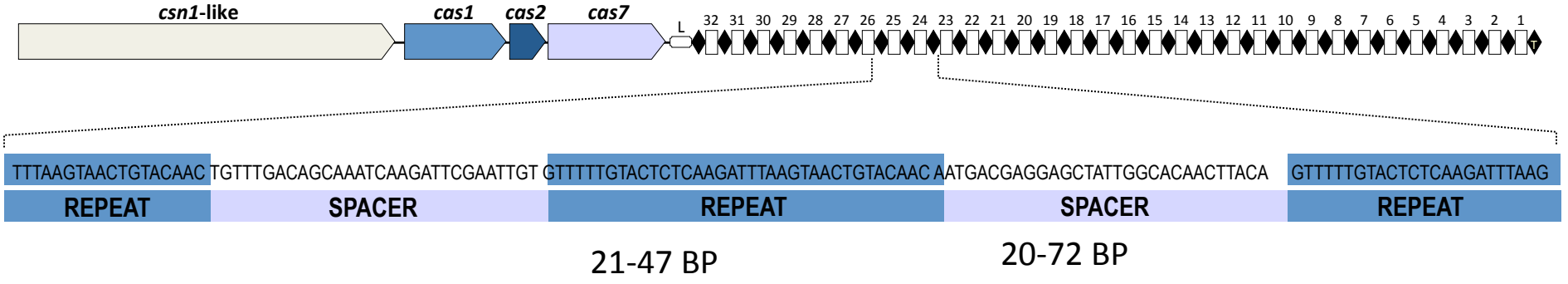
Production of useful proteins by genetic engineering in mammals



In this case, the gene is inserted into the ova and for its expression is linked to the promoter of a gene that is only expressed in mammary tissue, β-lactoglobin. The protein can then be harvested from milk.

CRISPR-Cas – Adaptive immunity in Prokaryotes

- **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
- Discovered in 1987 in K-12 (Ishino *et al.*), acronym coined in 2002 (Jansen *et al.*)
- New class of non-contiguous DNA repeats (aka SPIDR, SRSR, DR, DVR, LTRR, TREP)
- Often times, adjacent to ***cas*** genes (CRISPR-associated)
- Present in most Archaea (84%, 98/116) and nearly half of the Bacteria (46%, 668/1,441)
- Occurrence not linked to phylogeny, subject to HGT (Godde & Bickerton, 2006)



CRISPR-Cas – Adaptive immunity in Prokaryotes

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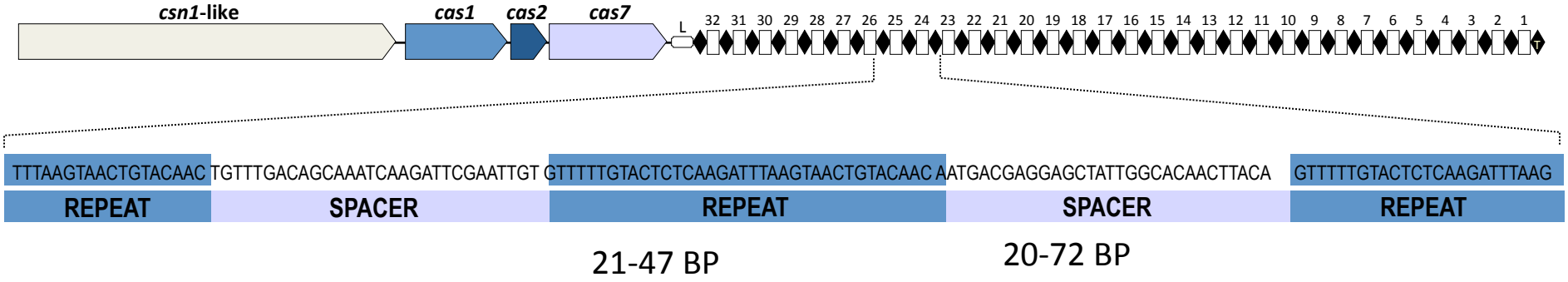

- Up to 587 repeats within a locus and 807 repeats in a genome (Wu *et al.*, 2009)
- Up to 20 loci in a single genome (Lillestol *et al.*, 2006)

CRISPR-Cas – Adaptive immunity in Prokaryotes

Initially they thought the spacers were random sequences.

They subsequently found that the sequences of these spacers were identical or nearly identical to regions of the genome of co-existing phages and plasmids.

Bolotin A, Quinquis B, Sorokin A, Ehrlich SD (2005) Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology 151: 2551-2561.

Mojica FJ, Diez-Villasenor C, Garcia-Martinez J, Almendros C (2009) Short motif sequences determine the targets of the prokaryotic CRISPR defence system. Microbiology 155: 733-740.

Pourcel C, Salvignol G, Vergnaud G (2005) CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology 151: 653-663.

Could CRISPR be some kind of immune system, an adaptive immune system, with memory, specificity, and the capacity to deal with vast numbers of different viruses plasmids and other infectious DNAs?

Surely not in prokaryotes, even Drosophila …..

Experimental Demonstration of CRISPR-Cas can be an Adaptive Immune System

CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

Rodolphe Barrangou,[1] Christophe Fremaux,[2] Hélène Deveau,[3] Melissa Richards,[1] Patrick Boyaval,[2] Sylvain Moineau,[3] Dennis A. Romero,[1] Philippe Horvath[2]*

Clustered regularly interspaced short palindromic repeats (CRISPR) are a distinctive feature of the genomes of most Bacteria and Archaea and are thought to be involved in resistance to bacteriophages. We found that, after viral challenge, bacteria integrated new spacers derived from phage genomic sequences. Removal or addition of particular spacers modified the phage-resistance phenotype of the cell. Thus, CRISPR, together with associated *cas* genes, provided resistance against phages, and resistance specificity is determined by spacer-phage sequence similarity.

SCIENCE (2007) 315: 1709

Rodolphe Barrangous
~1975 -

CRISPR Interference Limits Horizontal Gene Transfer in Staphylococci by Targeting DNA

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Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2205 Tech Drive, Evanston, Illinois, 60208, U.S.A.

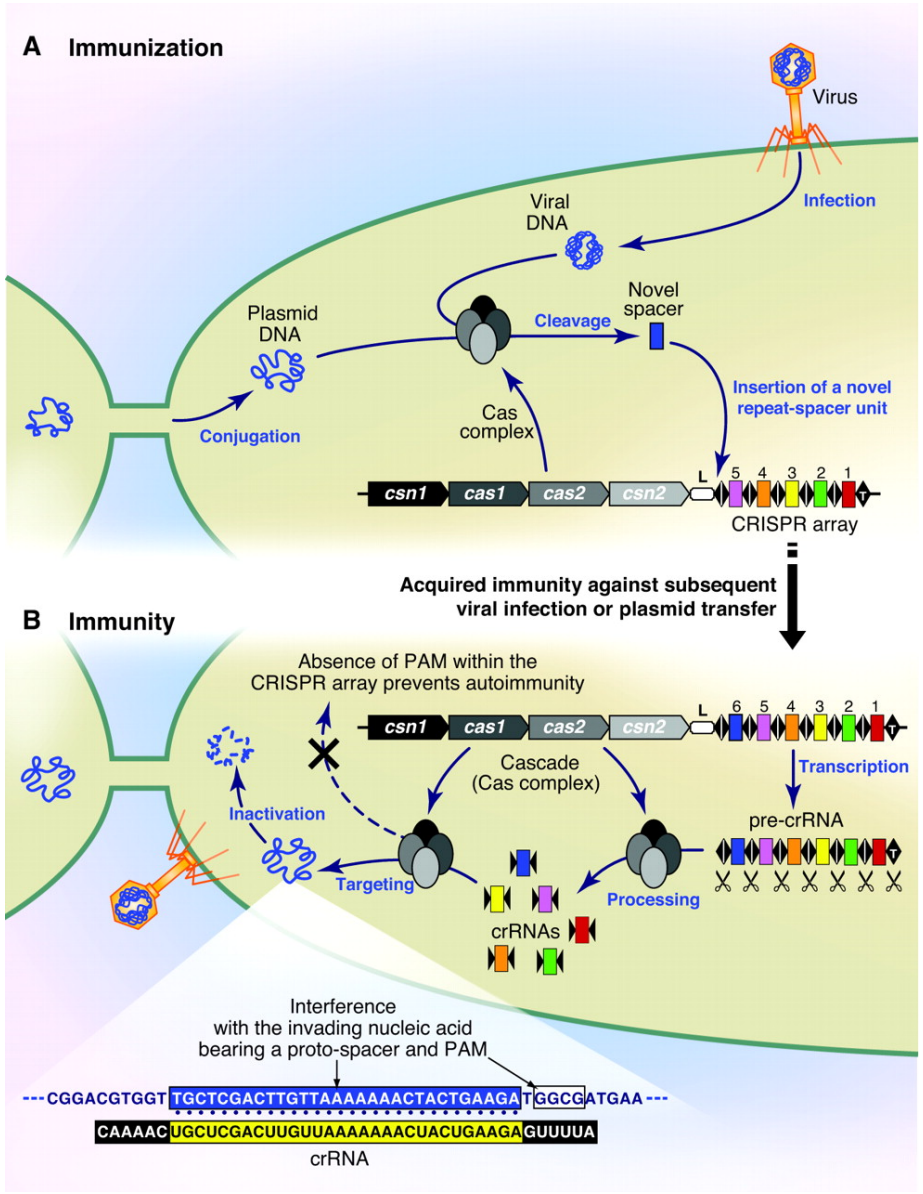
Abstract

Horizontal gene transfer (HGT) in bacteria and archaea occurs through phage transduction, transformation, or conjugation, and the latter is particularly important for the spread of antibiotic resistance. Clustered, regularly interspaced short palindromic repeats (CRISPR) loci confer sequence-directed immunity against phages. A clinical isolate of *Staphylococcus epidermidis* harbors a CRISPR spacer that matches the *nickase* gene present in nearly all staphylococcal conjugative plasmids. Here we show that CRISPR interference prevents conjugation and plasmid transformation in *S. epidermidis*. Insertion of a self-splicing intron into *nickase* blocks interference despite the reconstitution of the target sequence in the spliced mRNA, indicating that the interference machinery targets DNA directly. We conclude that CRISPR loci counteract multiple routes of HGT and can limit the spread of antibiotic resistance in pathogenic bacteria.

SCIENCE (2008) 322: 1843

Luciano Marraffini
~ 1978 -

CRISPR/Cas – as an Adaptive Immune System



P Horvath, R Barrangou Science 2010;327:167-170

Published by AAAS

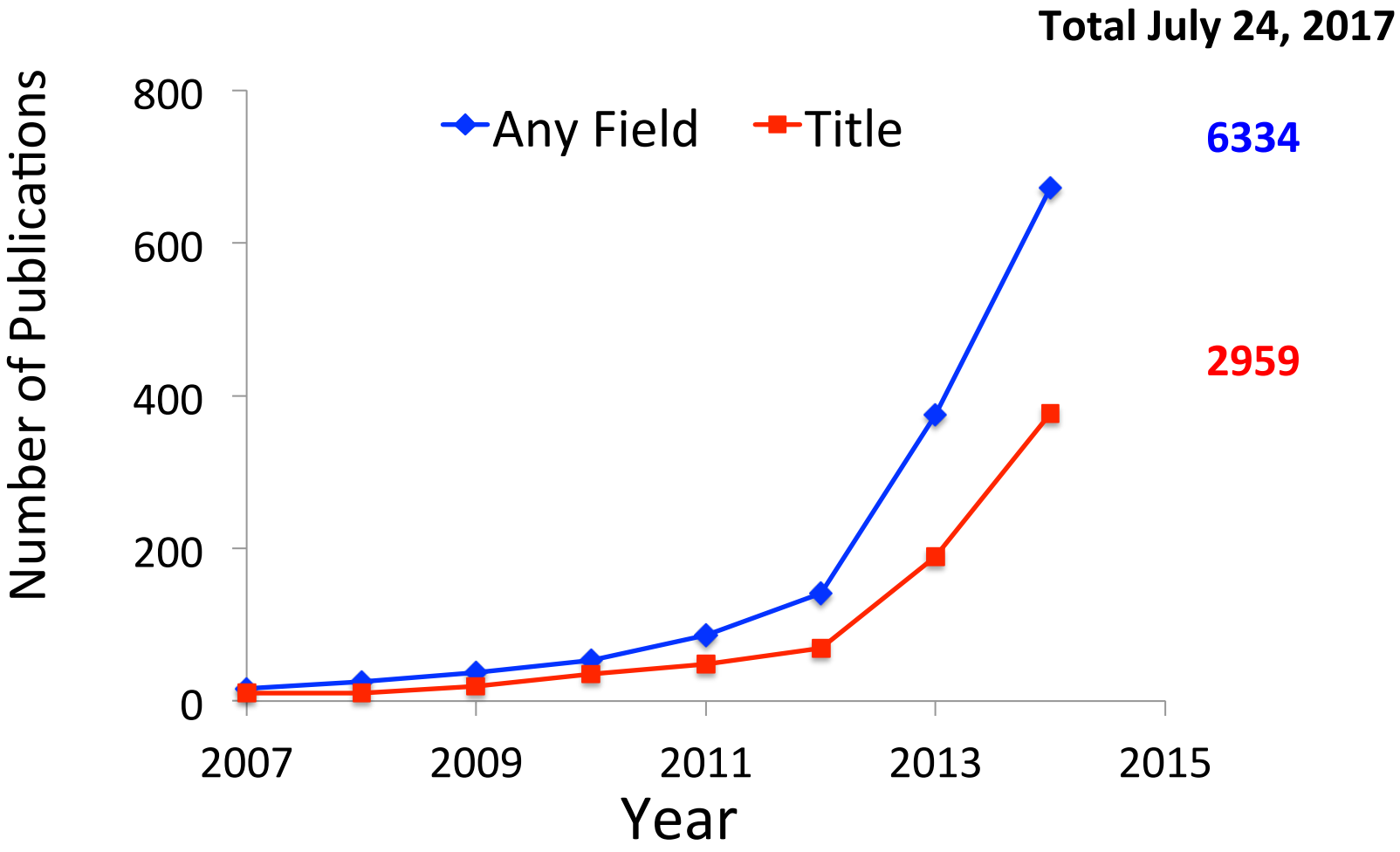
Check out

Deveau H, Garneau JE, Moineau S (2010) CRISPR/Cas system and its role in phage-bacteria interactions.Annu Rev Microbiol 64: 475-493.

Wiedenheft B, Sternberg SH, Doudna JA RNA-guided genetic silencing systems in bacteria and archaea. (2012) Nature 482: 331-338

Ratner, H.K., T.R. Sampson, and D.S. Weiss, *Overview of CRISPR-Cas9 Biology.* Cold Spring Harb Protoc, 2016. **2016**(12): p. pdb top088849.

A Growth Industry – Career Opportunity



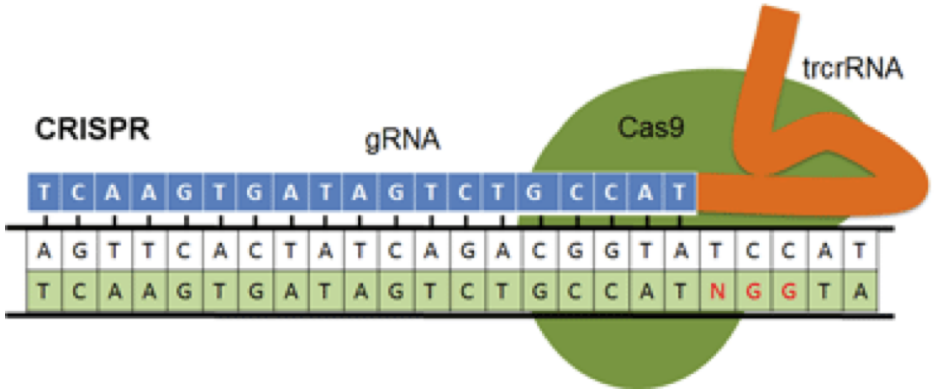
PubMed Data

A Powerful New Way to Edit DNA

By ANDREW POLLACK MARCH 3, 2014

addgene

A better way to share plasmids



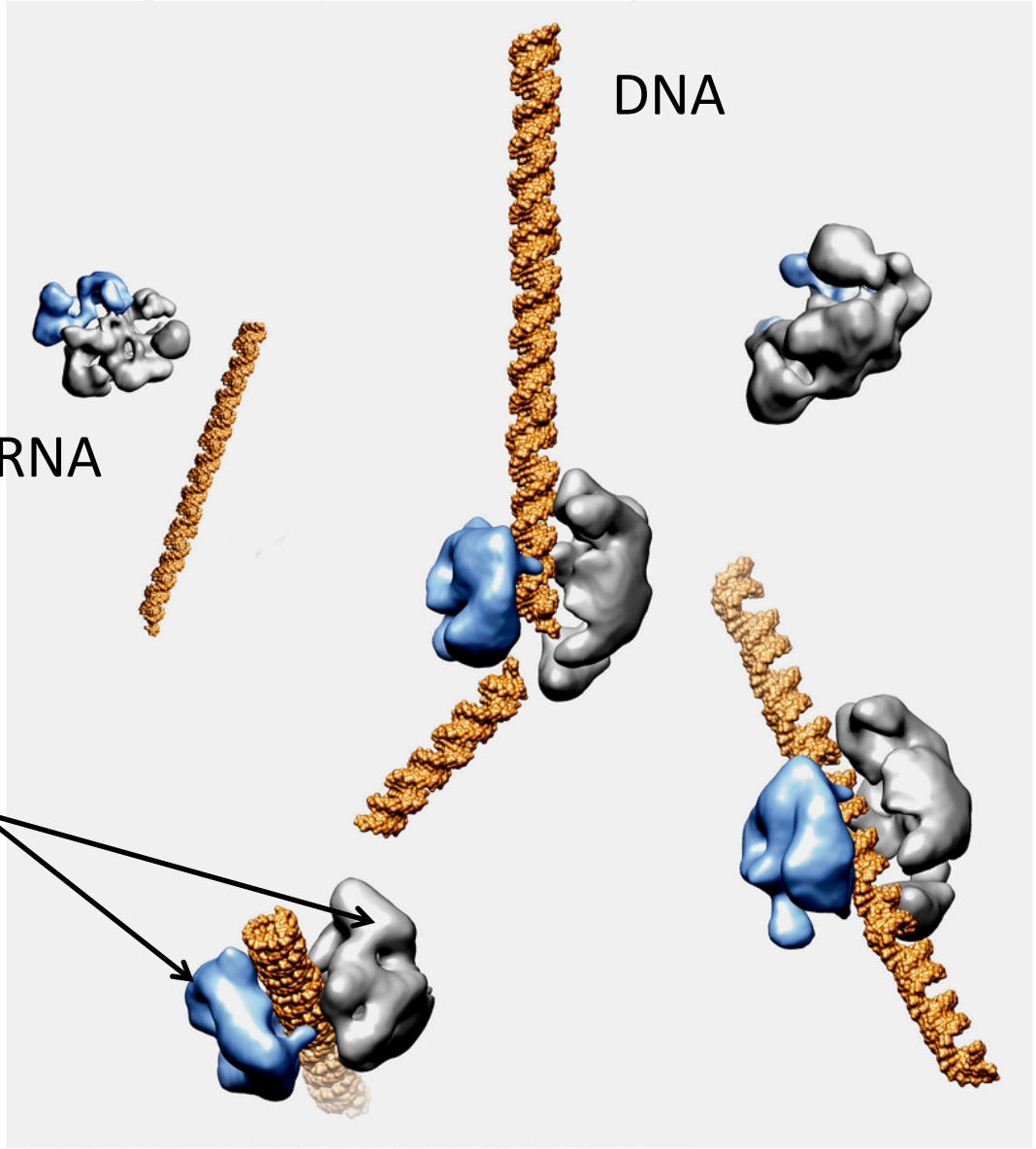
http://www.addgene.org/

CRISPR genome editing systems allow users to design short guided RNAs, (gRNA) which target their DNA sequence of interest. When expressed intracellularly in conjunction with a CRISPR associated endonuclease (Cas9), the gRNA directs Cas9 to the target sequence where it unwinds and cleaves the double stranded DNA.

JIANG, W., D. BIKARD, D. COX, F. ZHANG and L. A. MARRAFFINI, 2013 RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat Biotechnol **31:** 233-239.

SUPER VIDEO ABOUT CRISPR-MEDIATED GENE EDITING AND THEIR USE
https://www.youtube.com/watch?v=2pp17E4E-O8

CRISPR-Cas and CRISPR-mediated Genome Editing



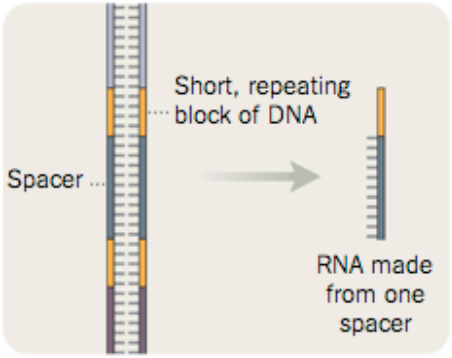
The enzyme Cas9, shown in blue and gray, can cut DNA, in gold, at selected sites, as seen in this model from electron microscope images.

David Taylor and Jennifer Doudna

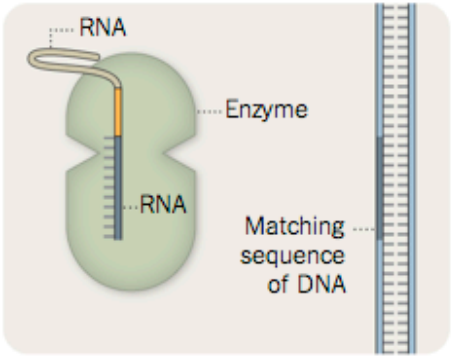
CRISPR-Cas and CRISPR-mediated Genome Editing

Breaking the Chain

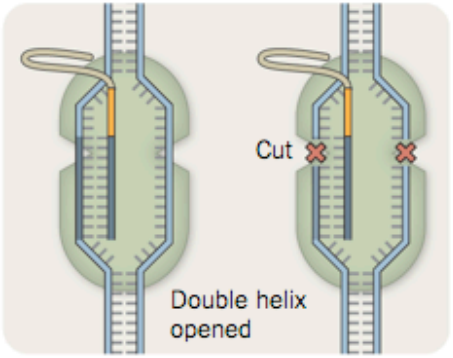
A complex immune system found in bacteria is already proving useful in editing DNA and may lead to future therapies.



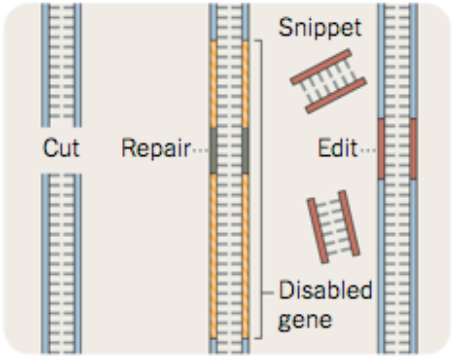
STORAGE Researchers in the 1980s noticed that bacteria had small blocks of palindromic DNA repeated many times, with nonrepeated spacers of DNA stored in between. This pattern is a sophisticated immune system known by the acronym Crispr, for "clustered regularly interspaced short palindromic repeats."



RECOGNITION These spacers match pieces of DNA from viral invaders that bacteria or their ancestors have faced before. When needed, the DNA contained in the spacer is converted to RNA. An enzyme and a second piece of RNA latch on, forming a structure that will bind to strands of DNA that match the spacer's sequence.



CUTTING When a matching strand of DNA is found, the enzyme opens the double helix and cuts both sides. The double cut breaks the strand and disables the viral DNA. If a bacterium survives an attack by an unfamiliar virus, it will make and store a new spacer, which can be inherited by future generations.



EDITING Researchers are learning how to use synthetic RNA sequences to control the cutting of any piece of DNA they choose. The cell will repair the cut, but an imperfect repair may disable the gene. Or a snippet of different DNA can be inserted to fill the gap, effectively editing the DNA sequence.

Sources: Nature; Addgene

By The New York Times

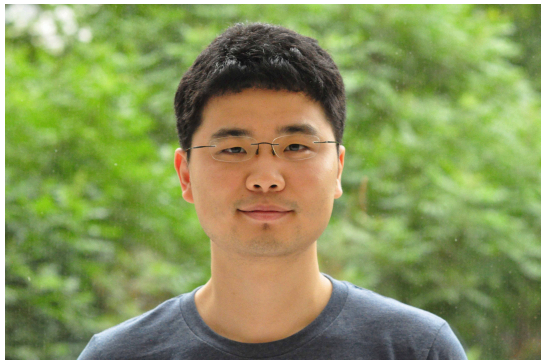
Population Dynamic and Evolutionary Questions about R-M and CRISPR-Cas

What are the ecological and genetic conditions responsible for the evolution and maintenance of Restriction Modification and CRISPR-Cas in populations of bacteria and archaea? ‘

Are R-M and CRISPR-Cas commonly immune systems?

What role do R-M and CRISPR-Cas play in maintaining the genetic integrity of bacteria and archaea by limiting horizontal gene transfer?

Why do bacteria put up with R-M and CRISPR-Cas for immunity when they can evolve envelope resistance?



Wenyan Jiang (Rockefeller/Columbia)
CRISPR-Cas and the rates of plasmid transfer.

Gregory Goldberg (Rockefeller)
CRISPR-Cas and the dynamics of temperate phage and lysogeny

Maros Pleska (IST Vienna)
R-M and the dynamics of temperate phage and lysogeny

Mechanisms: A cure for the Microbiome Mania

High Tech, High Throughput, Community Ecology

Raises some delicious quantitative population dynamic, ecological and evolutionary questions

What are mechanisms responsible for limiting the densities of the component populations and determining their relative numbers.

- Resources (nutrients)?
- Predation (phage, protozoa)?
- The physical habitat?
- Allopathic interactions between the component populations?
- Synergistic interactions between the component populations?

ROLF FRETER, M.D.

Rolf Freter
1926 -2009

INFECTION AND IMMUNITY, Feb. 1983, p. 676–685
0019-9567/83/020676-10$02.00/0

Vol. 39, No. 2

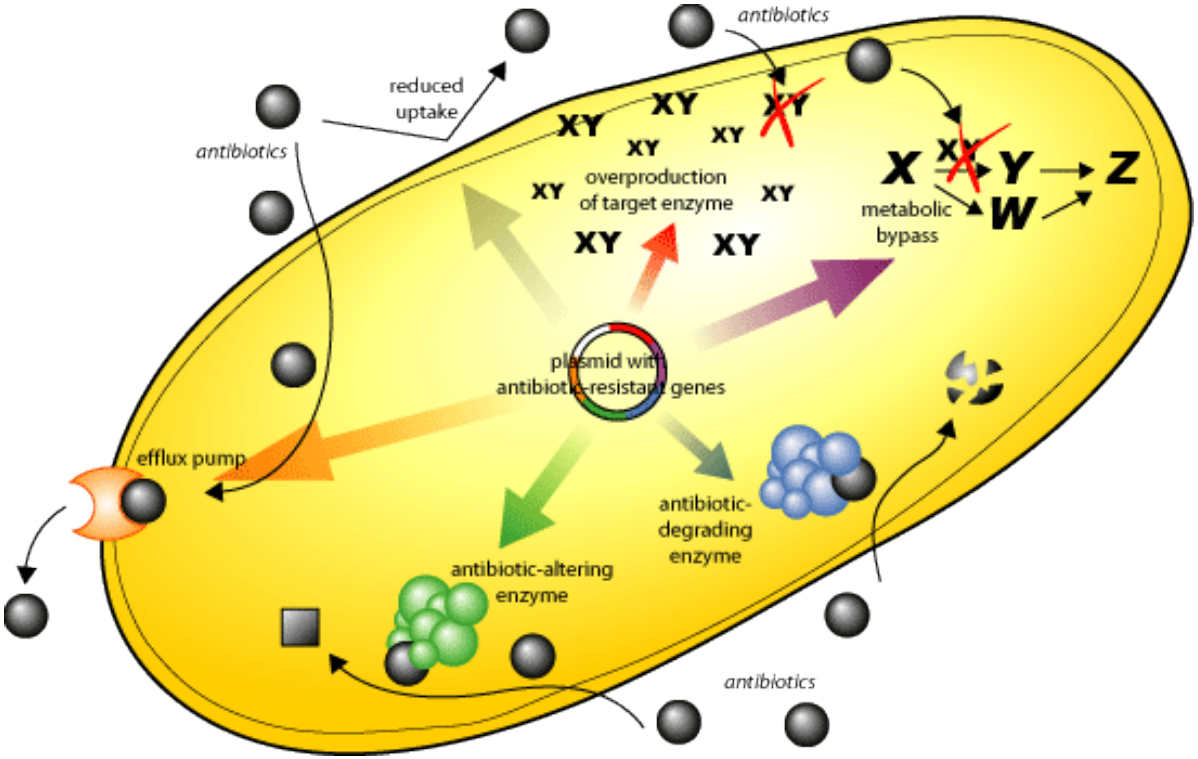
Mechanisms That Control Bacterial Populations in Continuous-Flow Culture Models of Mouse Large Intestinal Flora

ROLF FRETER,* HOWARD BRICKNER, MITCHELL BOTNEY, DIANE CLEVEN, AND ALEXANDER ARANKI

Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, Michigan 48109

Received 6 August 1982/Accepted 15 October 1982

Antibiotic Resistance a Problem and a Great Career Opportunity



- Reduced drug uptake
- Active pumping of drugs out of the cell
- Enzymatic alteration of the antibiotic
- Modification of targets
- Drug sequestering by protein binding
- Overproduction of the target
- Metabolic bypass of the targeted pathway

Apocalyptic Predictions

Almost Certainly Bullshit

Review on Antimicrobial Resistance

Tackling drug-resistant infections globally

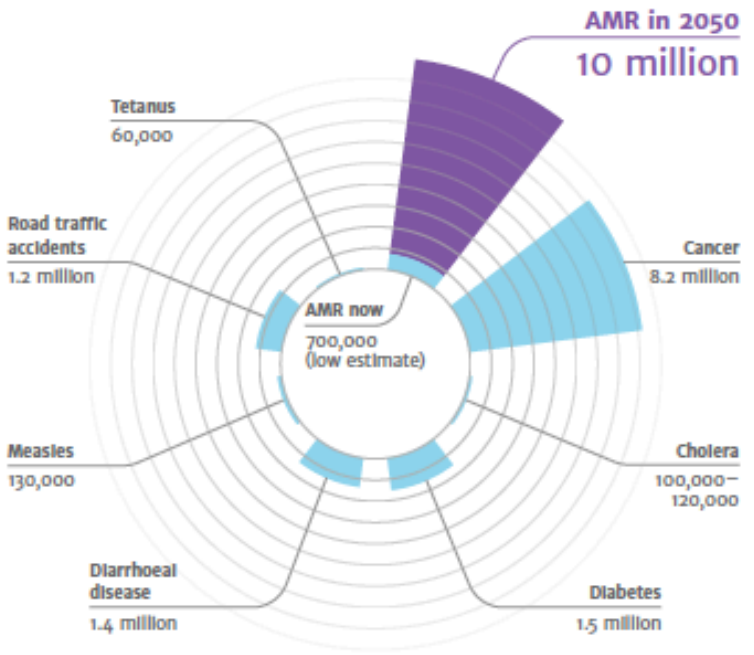
TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: FINAL REPORT AND RECOMMENDATIONS

THE REVIEW ON ANTIMICROBIAL RESISTANCE

CHAIRED BY JIM O'NEILL

MAY 2016

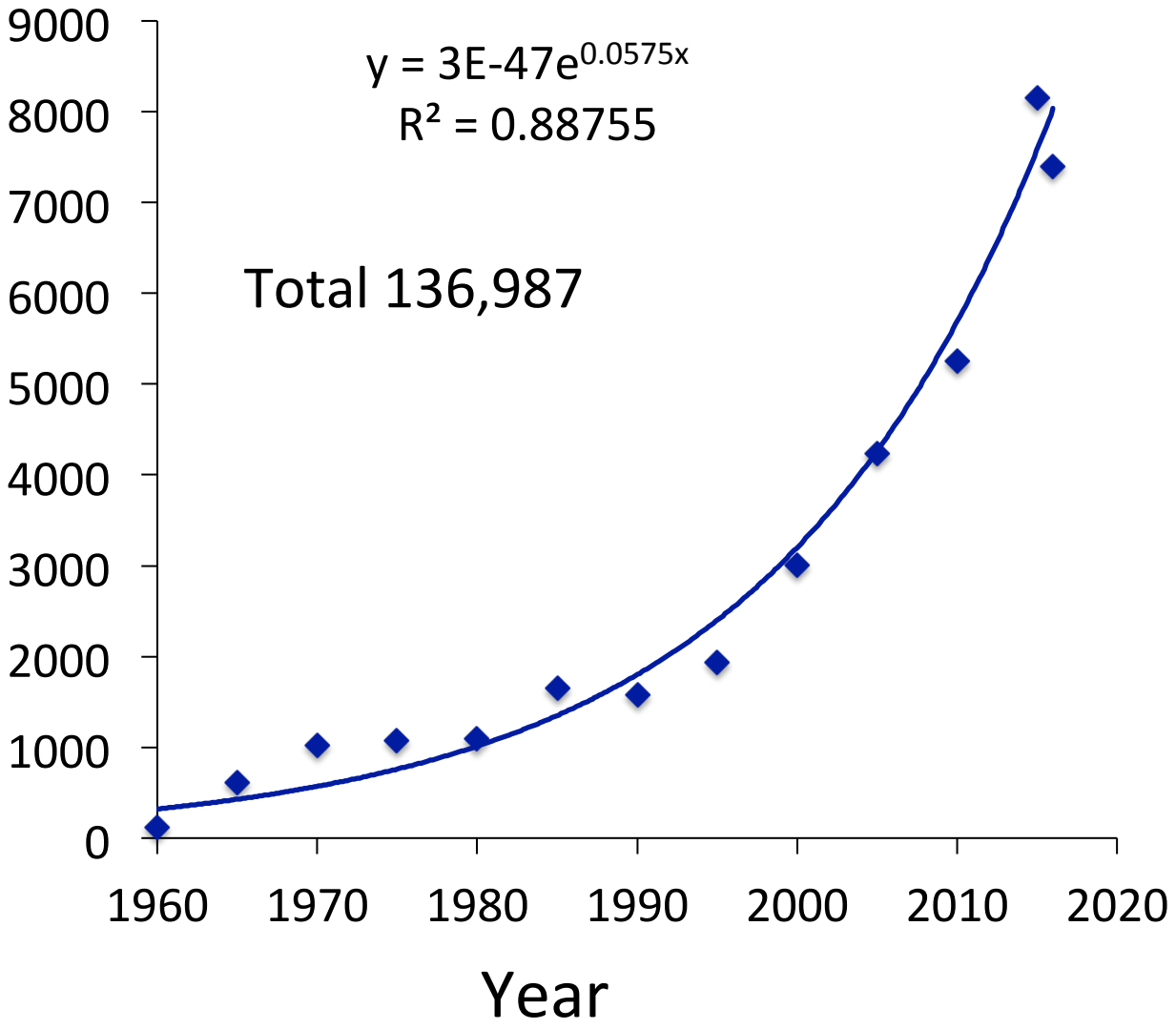
DEATHS ATTRIBUTABLE TO AMR EVERY YEAR



Sources:

Review on Antimicrobial Resistance

Articles with Antibiotic Resistance “Anywhere”



An Artists Conception of a Bacterium Artist's