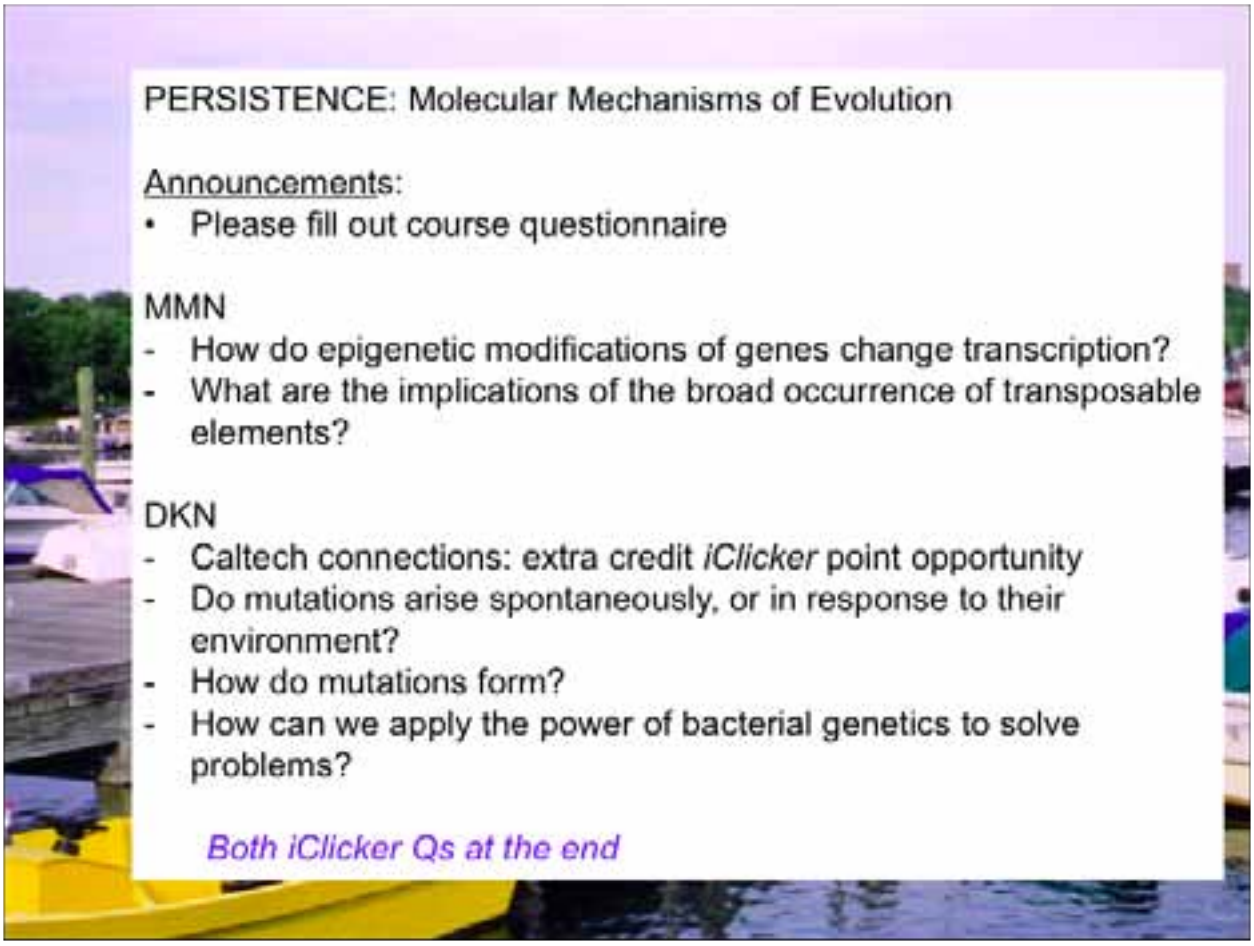

PERSISTENCE: Molecular Mechanisms of Evolution

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MMN

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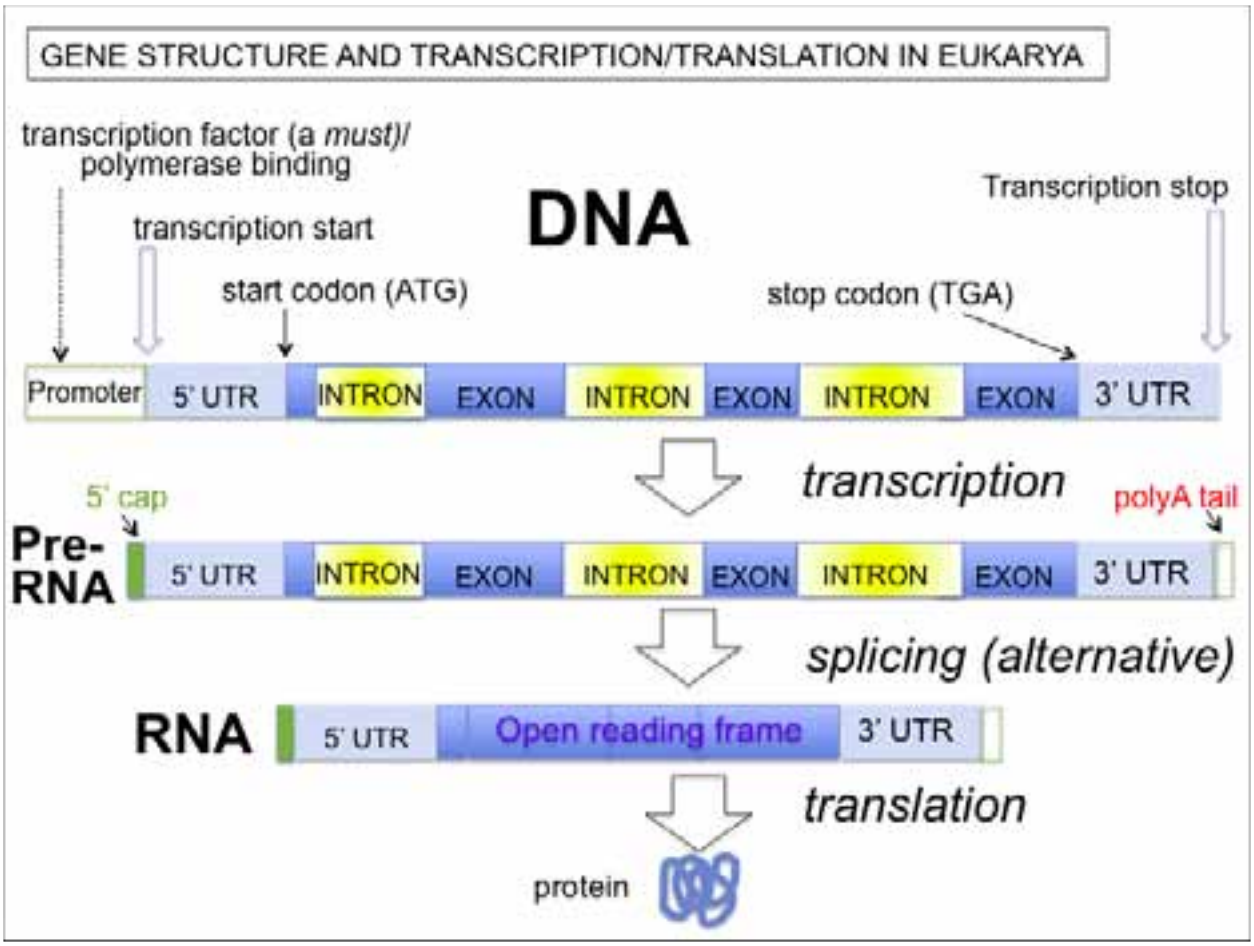
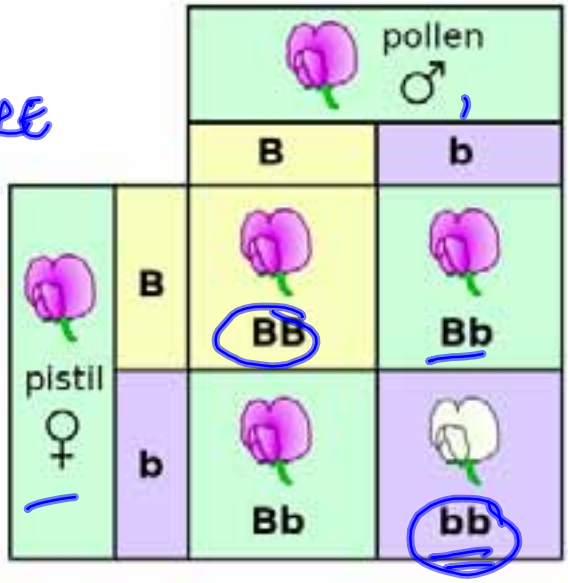
Both iClicker Qs at the end


GENE STRUCTURE AND TRANSCRIPTION/TRANSLATION IN EUKARYA
transcription factor (a *must*)/ polymerase binding
transcription start
start codon (ATG)
DNA
Transcription stop
stop codon (TGA)
Promoter
5' UTR
INTRON
EXON
INTRON
EXON
INTRON
EXON
3' UTR
transcription
5' cap
polyA tail
Pre-RNA
5' UTR
INTRON
EXON
INTRON
EXON
INTRON
EXON
3' UTR
splicing (alternative)
RNA
5' UTR
Open reading frame
3' UTR
translation
protein

Mendelian Genetics

PUNNETT SQUARE



Bb = hetero zygous

B - dominant

b - recessive

How genes encode for traits.

Is it really that simple? No!

Just over 10 years ago, the full human genome sequence was determined.

20,000 - 50,000
(~ 24,000
why so few?

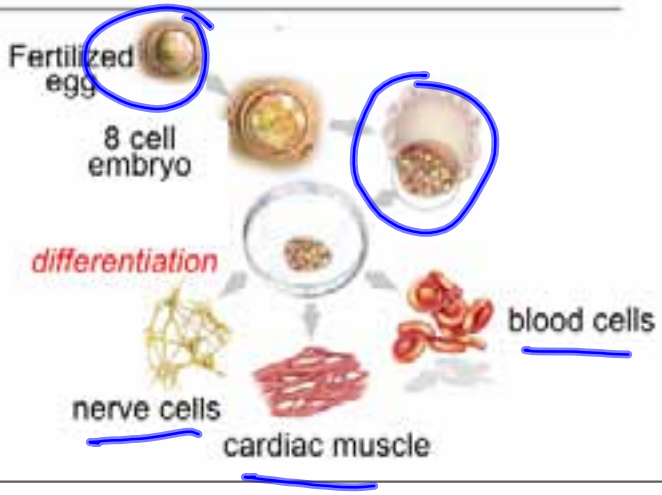
Other mysteries:

Identical twins

identical

EMBRYOGENESIS

How?



Epigenetics

Transposable Elements (TEs)

EPIGENETICS: 'above the genome'

Study of the inheritance of changes in gene function that occur w/o Δs in DNA sequence

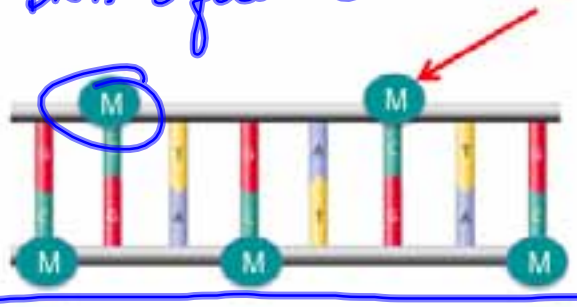
EPIGENETIC MECHANISMS

METHYLATION

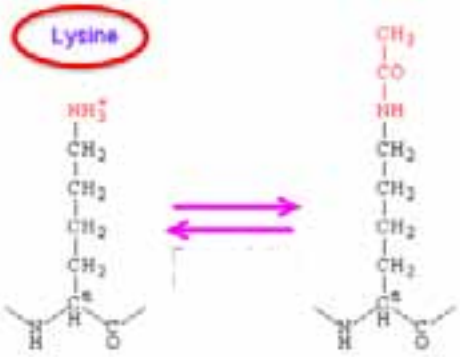
↓

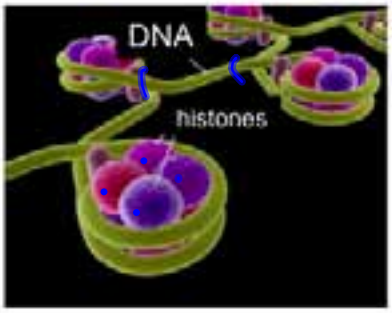
DNA itself

histones



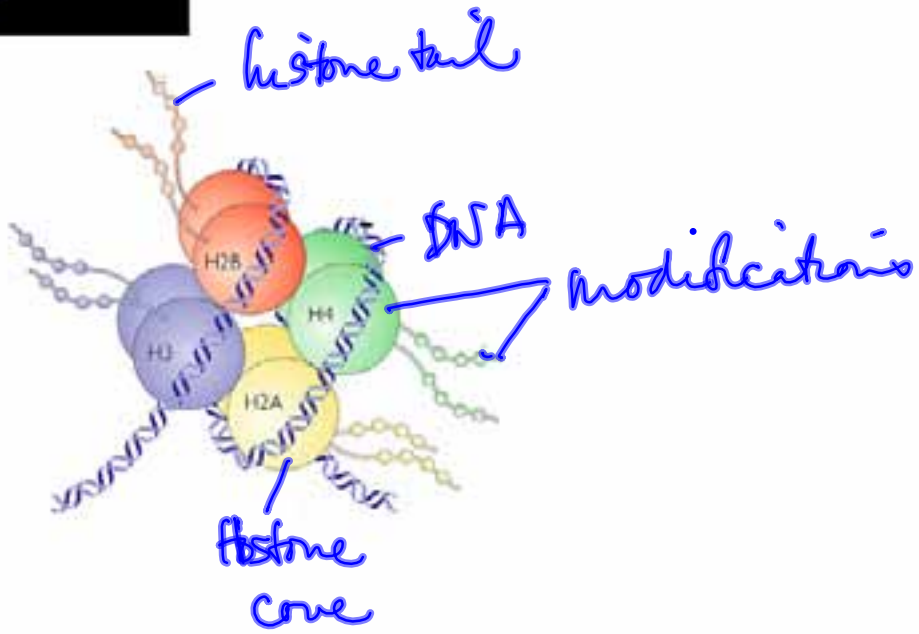
Acetylation of histones





HISTONE ACETYLATION

(usually enhances transcription – opens up the chromatin)



EPIGENETICS IN HEALTH AND DISEASE

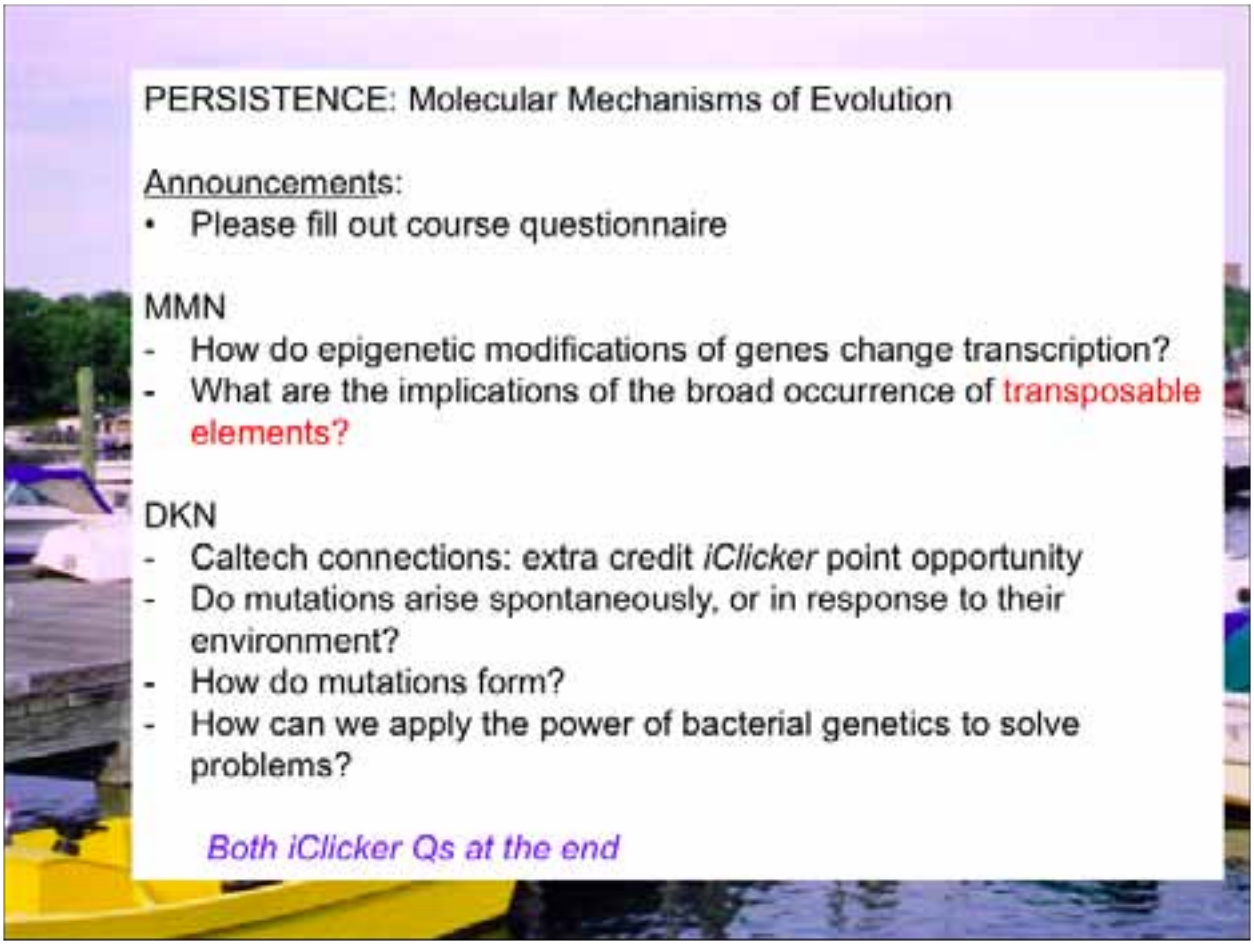
?

pancreatic cancer
(& other cancers)

DEPRESSION AUTISM
SCHIZOPHRENIA

MICROBIOME

Steve Jobs



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Both iClicker Qs at the end

Barbara McClintock (1902-1992)
Nobel Prize in Physiology/Medicine 1983

DISCOVERIES

Among others

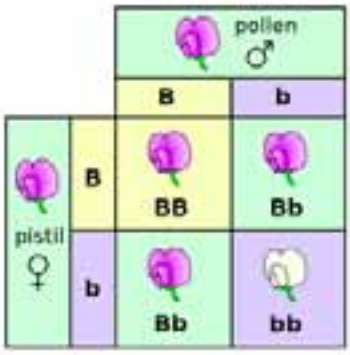
Transposition
(this lecture)

Crossing over during
cell division
(next lecture)

McClintock studied corn genetics – abundance of visible traits, due to (we now know) a diverse genome

Why are these corn kernels spotted?

Mendelian genetics could not explain the phenomenon.



no mechanism

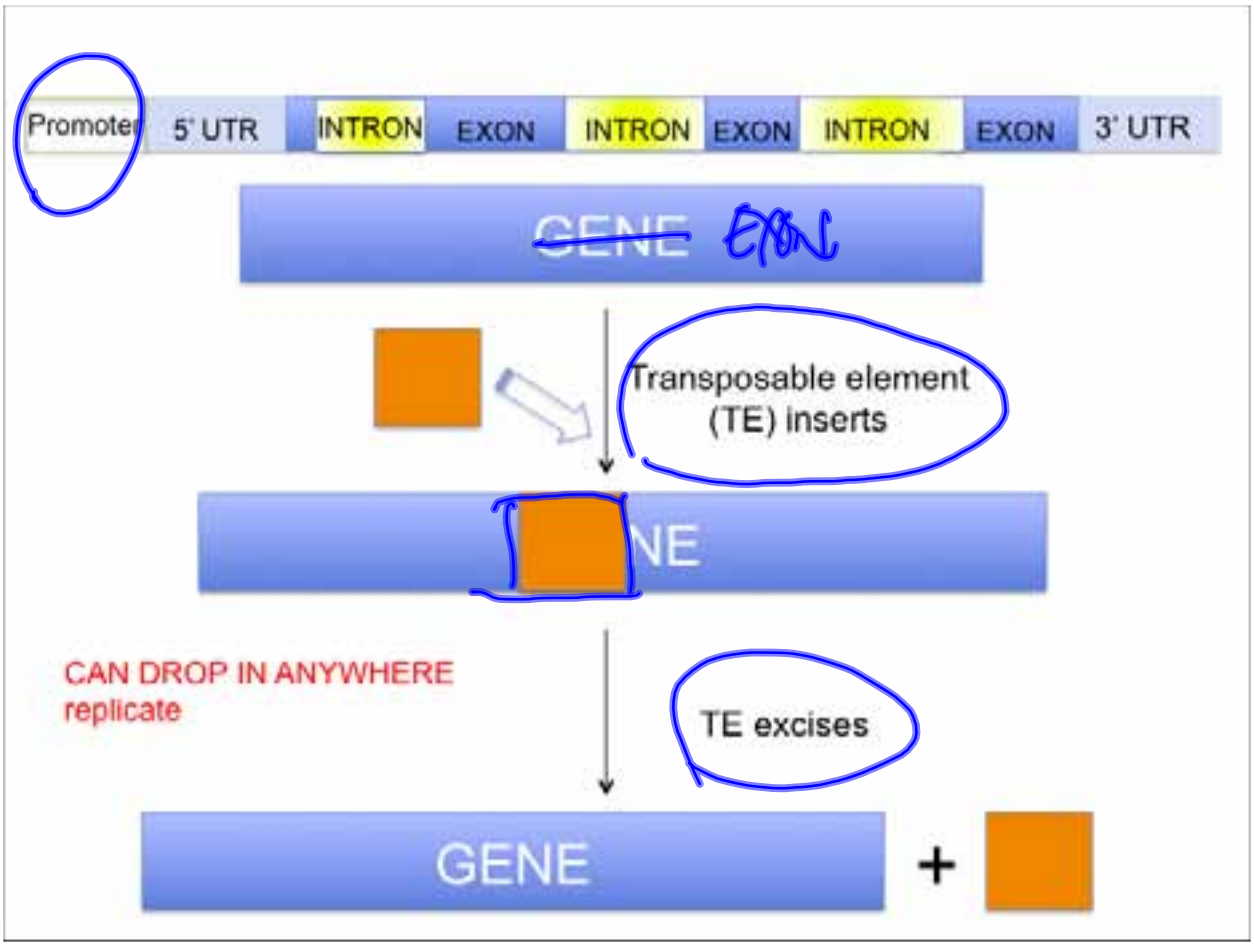
1940's discovered transposition
-such criticism that didn't publish
again on the subject after 1953

Transposable Elements (TEs):

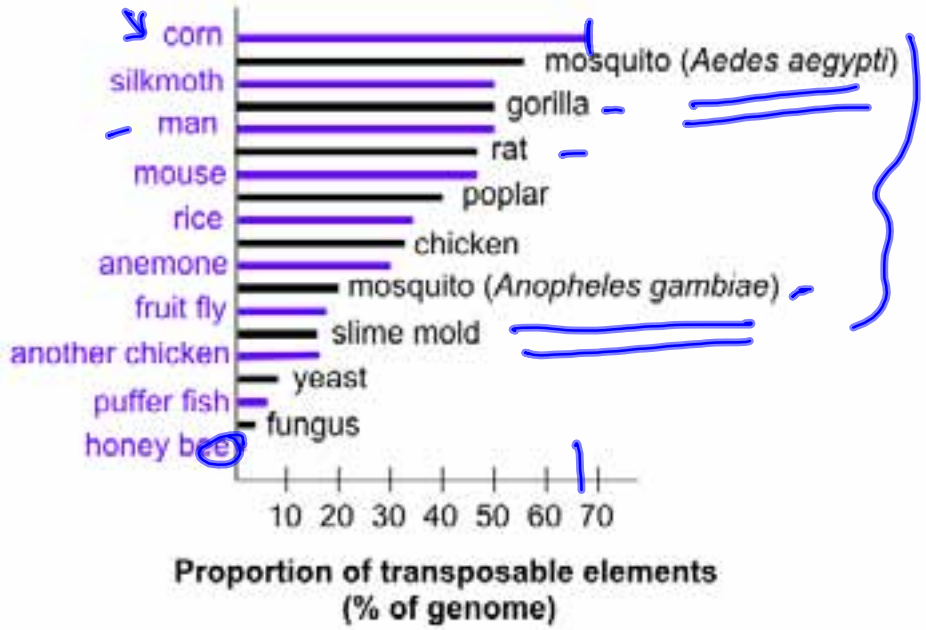
'junk DNA'

DNA sequence that can Δ its relative position w/in the genome of a single cell

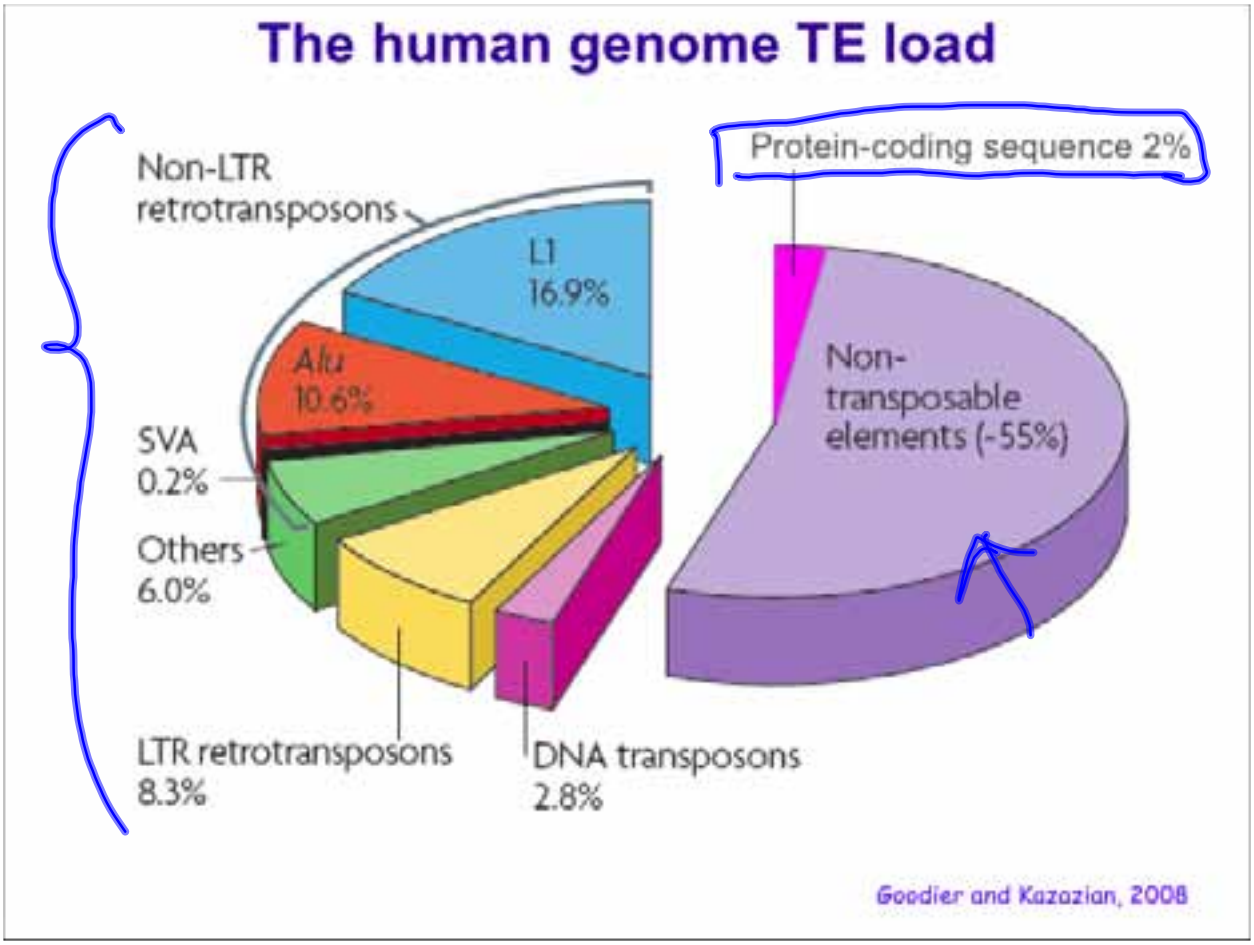
originally viruses


Promoter
5' UTR
INTRON
EXON
INTRON
EXON
INTRON
EXON
3' UTR
GENE
Transposable element
(TE) inserts
CAN DROP IN ANYWHERE
replicate
TE excises
GENE
+

NO PHYLOGENETIC PATTERN TO TE LOAD – significance – higher 'evolvability'?



evolvability


The human genome TE load
Non-LTR retrotransposons
L1 16.9%
Alu 10.6%
SVA 0.2%
Others 6.0%
LTR retrotransposons 8.3%
DNA transposons 2.8%
Protein-coding sequence 2%
Non-transposable elements (~55%)
Goodier and Kazazian, 2008

A REVOLUTION

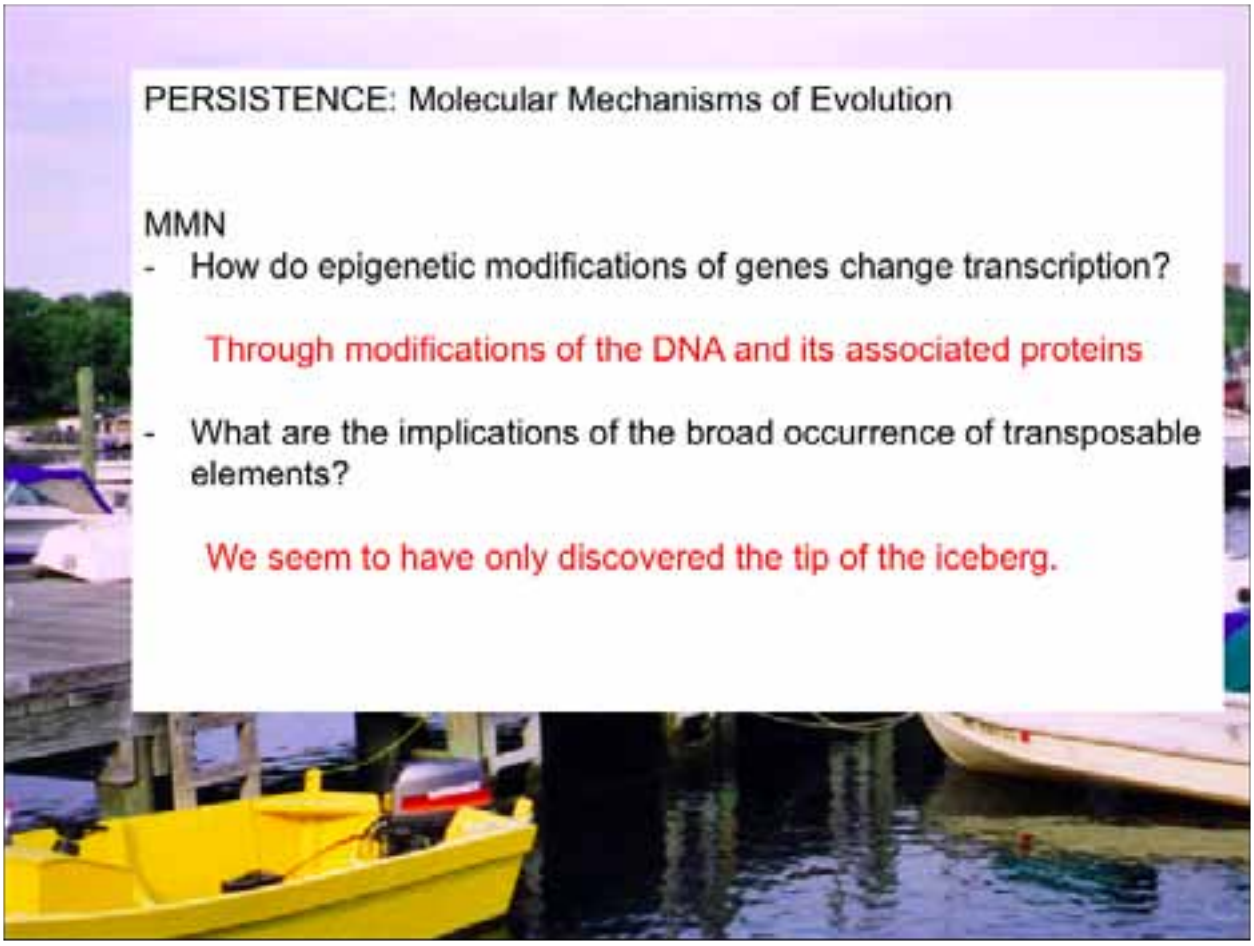
ONE EXAMPLE:

LETTER

doi:10.1038/nature10531

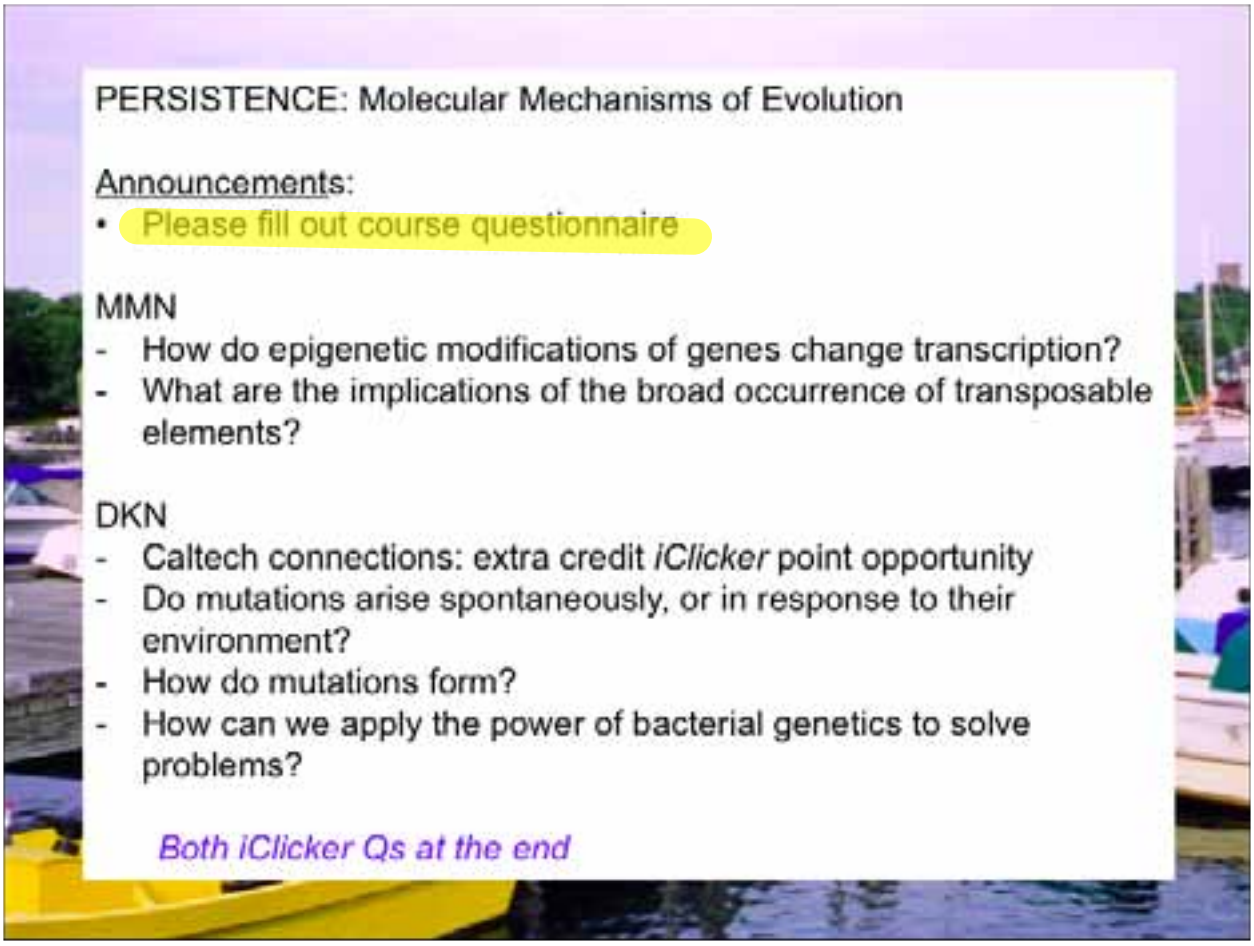
Somatic retrotransposition alters the genetic landscape of the human brain

J. Kenneth Baillie[1]*, Mark W. Barnett[1]*, Kyle R. Upton[1]*, Daniel J. Gerhardt[2], Todd A. Richmond[2], Fiorentino De Sapio[2], Paul Brennan[3], Patrizia Rizzu[4], Sarah Smith[3], Mark Fell[3], Richard J. Talbot[1], Stefano Gustincich[5], Thomas C. Freeman[1], Allan E. Mattick[6], David A. Thaine[1], Peter Heutink[4], Piero Carninci[2], Jeffrey A. Sekhobo[2] & Geoffrey J. Faulkner[2]


PERSISTENCE: Molecular Mechanisms of Evolution
MMN
- How do epigenetic modifications of genes change transcription?
Through modifications of the DNA and its associated proteins
- What are the implications of the broad occurrence of transposable elements?
We seem to have only discovered the tip of the iceberg.

Barbara McClintock (1902-1992)
Nobel Prize in Physiology/Medicine 1983

Transposable Elements - Rediscovered in Bacteria in Late 1960s &1970s



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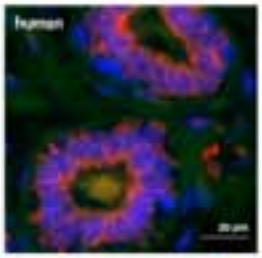
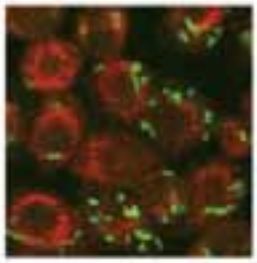
Both iClicker Qs at the end

Examples of Caltech research outside of Biology applying/probing aspects of the Central Dogma: 2 extra credit iClicker point—see website!





DNA

RNA

proteins

Folding, structure, function

Examples of Caltech research in Biology applying/probing aspects of the Central Dogma: 2 extra credit iClicker point—see website!


Aravin

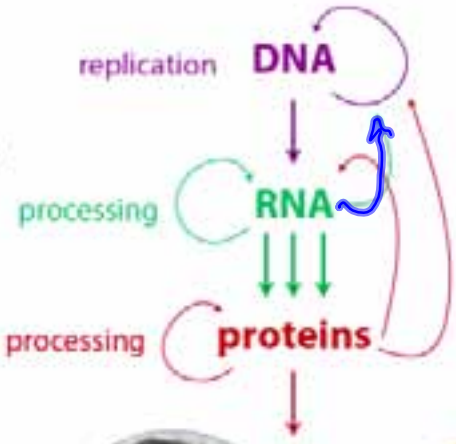

Fejes-Toth


Deshaies


Varshavsky


Campbell


Baltimore



RNA interference
(i.e. RNA-mediated gene silencing)

Reverse Transcriptase
(e.g. HIV)

What types of mutations are there? Universal mechanism

→ Single mismatch

(DNA) ...A A G A C A G C A...
(RNA) U U C U G U C G U
aa Phe Cys Arg

→ Δprotein properties
→ Δ length

(DNA) A A G A C A G C A
(DNA) A A G A C T G C A ← DNAP
(RNA) U U C U G A C G U
aa Phe TERM Arg
(DNA) A A G A C A G C A
(RNA) U U C U A U C G U ← RNAP
aa Phe Tyr Arg
Ala Tyr Arg ← Ribosome

→ DNA Deletion ← -2

(DNA) A A G A C A G C A
(RNA) U U G U C G U
aa Leu Ser

"(frameshift, in-frame)"
-1, -2 ↳ sets of 3

→ DNA Insertion (insertion elements transposons) agents of insertion

(DNA) A A G G G G C C C G C C G C C A C A G C A

* differences in GC content suggest horizontal gene transfer

G-C vs. A-T

How do mutations form?

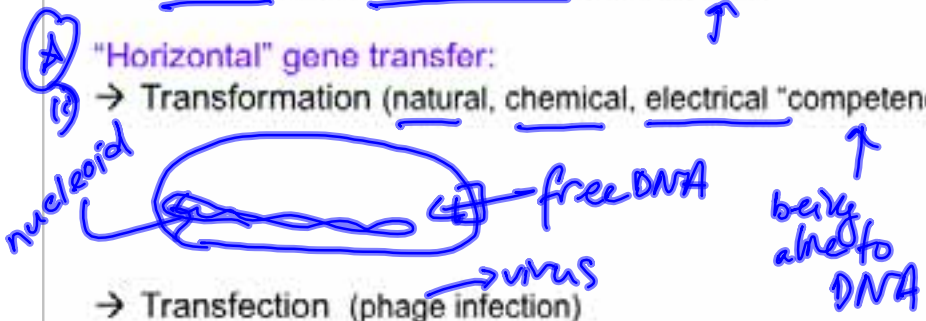
"Vertical" inheritance:

DNAP, RNAP, ribosome mistakes [lower proofreading fidelity]

Chemical, physical mutagens (*e.g. UV*)

"Horizontal" gene transfer:

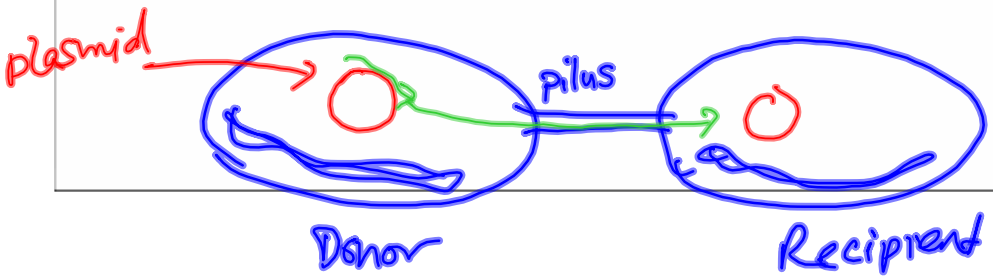
→ Transformation (natural, chemical, electrical "competence")



Recombination integrates as a spot where sequence matches

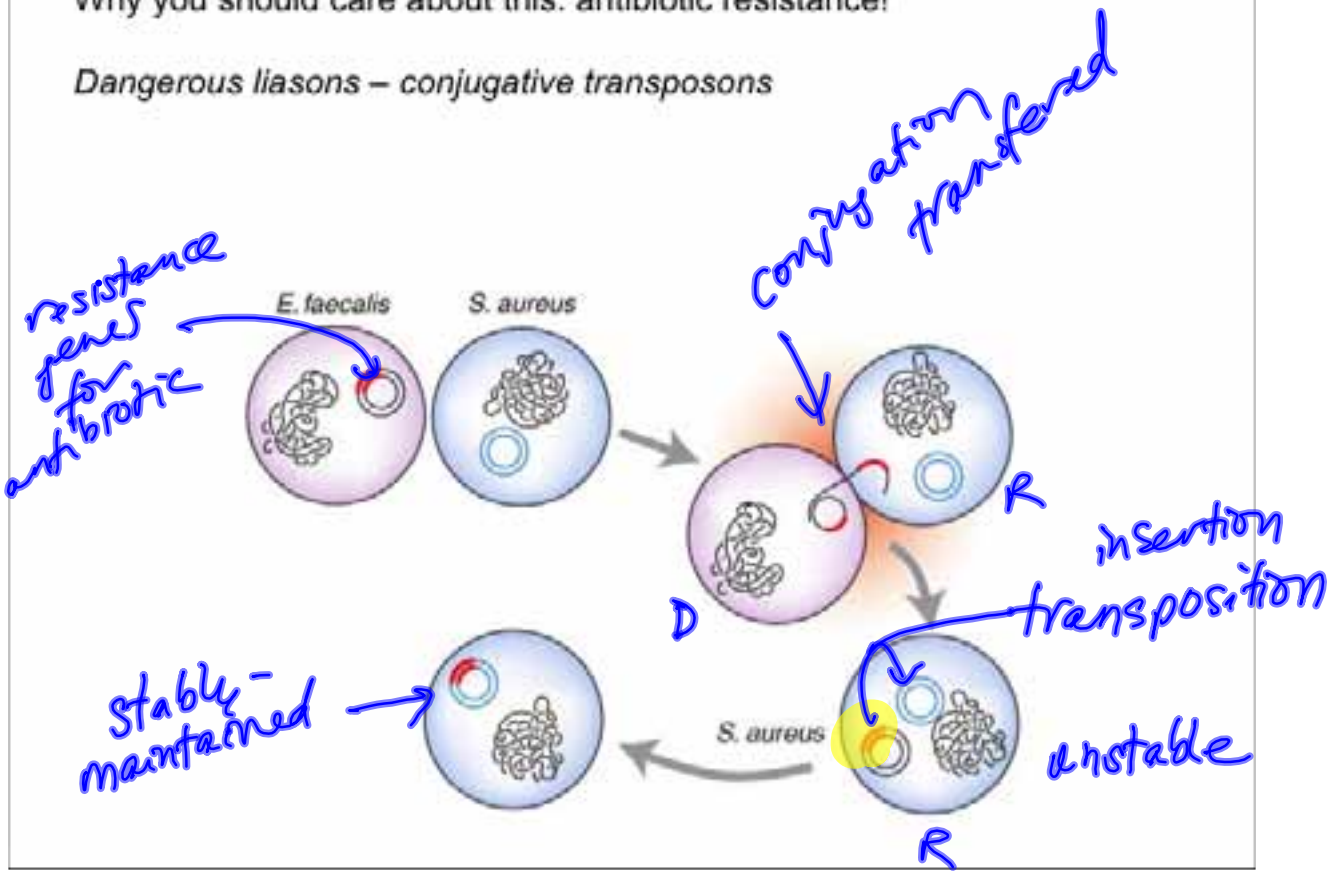
→ Transfection (phage infection)

→ Conjugation (exchange of DNA between bacteria)



Why you should care about this: antibiotic resistance!

Dangerous liasons – conjugative transposons

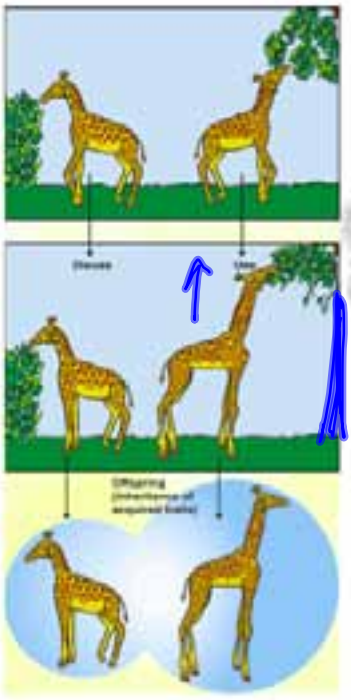


Do mutations arise spontaneously, or in response to their environment?

Darwin natural selection

Finches from Galapagos Archipelago

Lamarck adaptive mutation



How can we test?

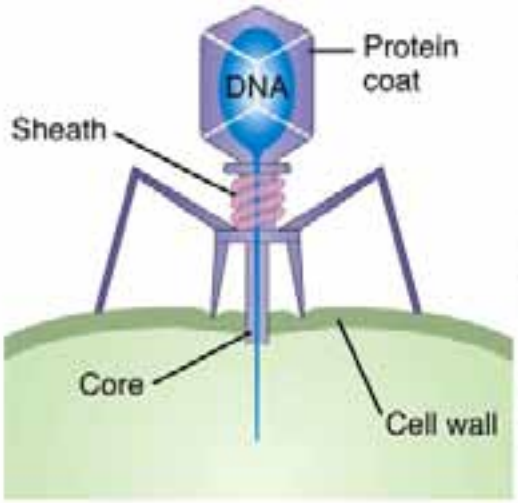
"Phage group" (almost entirely comprising physicists)

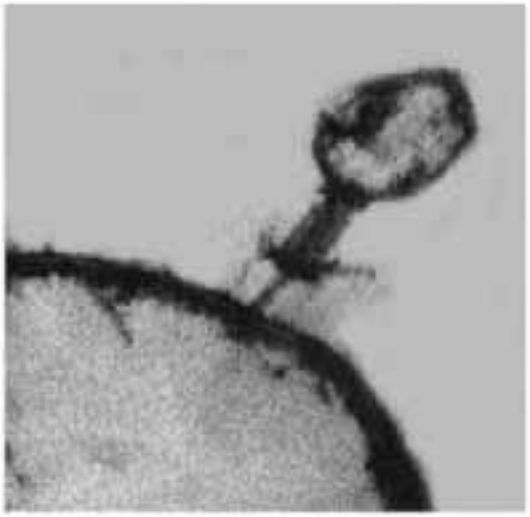
Nobel Prize 1969 Physiology and Medicine (with A. Hershey)

Why phage and bacteria?

→ Simplest biological system

→ Easy assays ("toothpicks & logic")





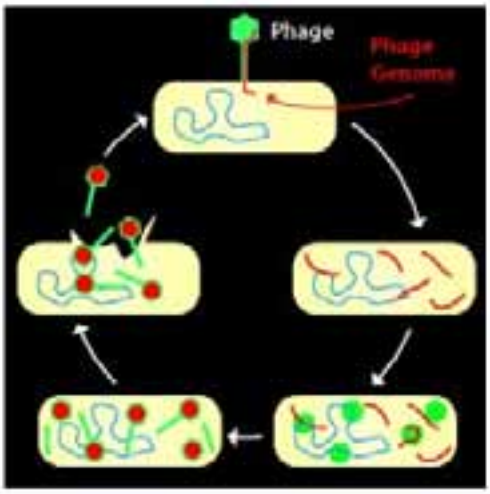
phage = bacterial virus

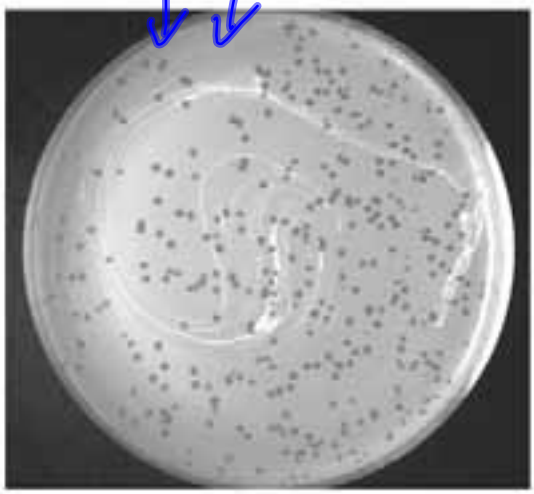
The plaque assay

lytic → lysis

holes = bursts

lawn of bacteria

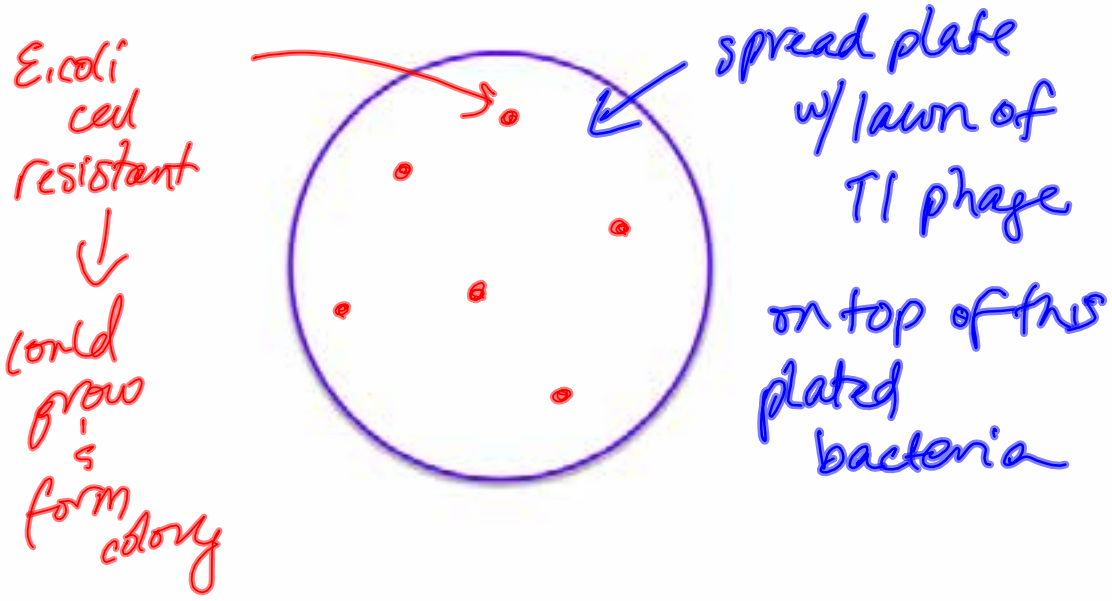




→ A simple assay to measure mutation frequency is to score the numbers of bacteria that can grow in the presence of the phage (*i.e. phage resistant*)

Question: Do mutations arise spontaneously, or in response to their environment?

Specifically: Does *E.coli* acquire resistance to phage T1 by "induced immunity" or by spontaneous mutation?

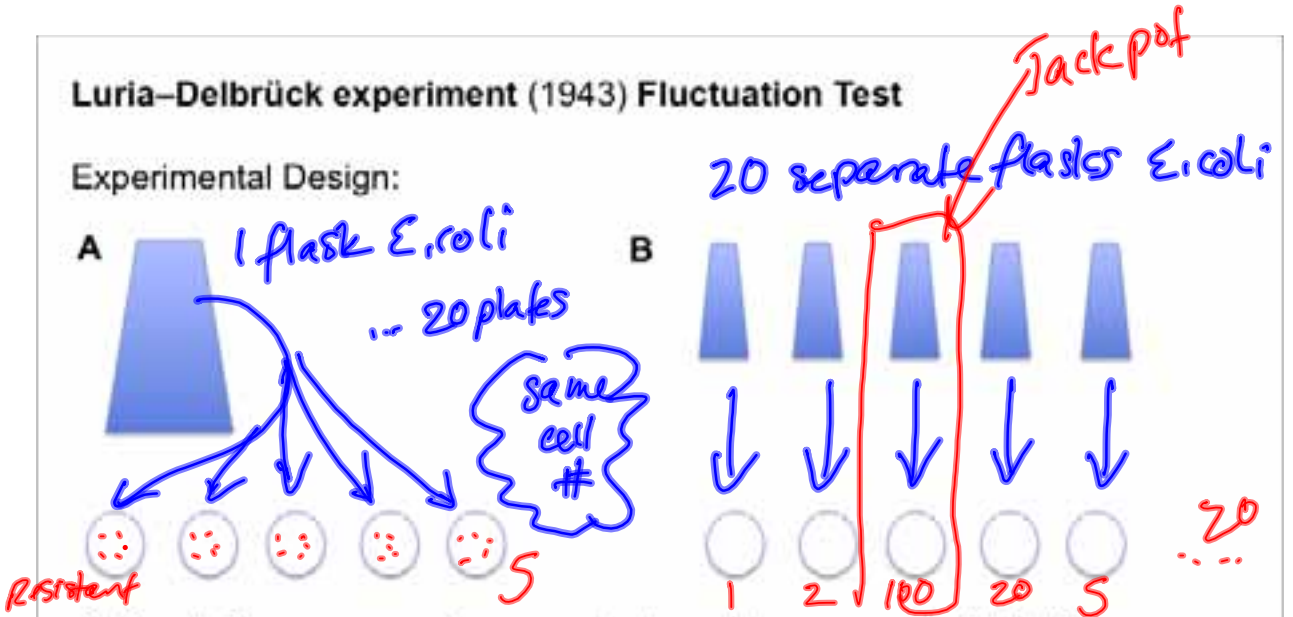


Luria's key insight at a faculty party → hitting the jackpot is random

This inspiration provided the basis for a truly elegant experiment…

Luria–Delbrück experiment (1943) **Fluctuation Test**

Experimental Design:



→ If mutations are spontaneous, what would you expect distribution frequency to look like (same or different between A & B sets)? differences

→ If they are adaptive, what would you expect? same

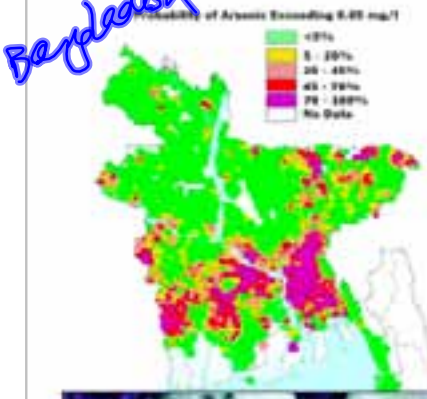
Results: differes

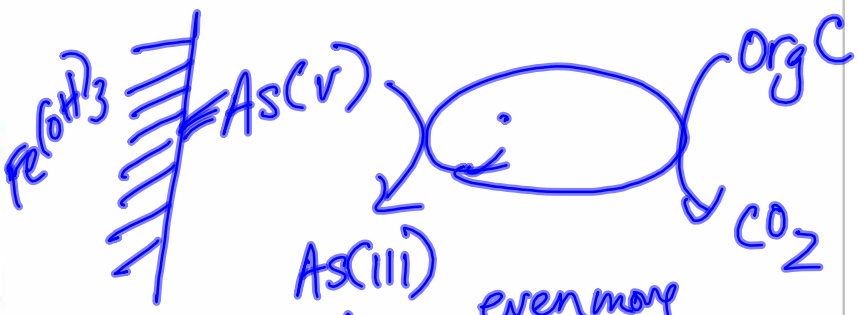
Conclusion: spontaneous! → Darwin] Epigenetic ↓ remarkion

As(V) Respiration

How can we apply the power of bacterial genetics to solve problems?

Bangladesh



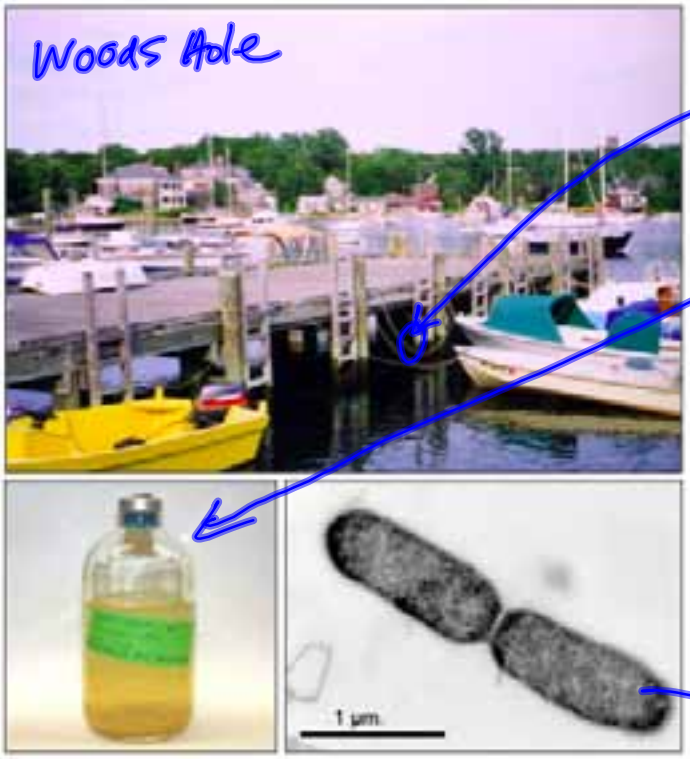


How can we assess whether bacteria are catalyzing arsenic mobilization into drinking water?

→ identify gene (arr) arsenate respir reductase

→ look for its expression (mRNA) in environment

Isolating a good "model organism" to find the gene(s) specifically conferring the ability to respire As(V)



air/water interface?
O_2, & other e^- acceptors

Simple assay

As(V) → As(III) + S(-II)

yellow = As_2S_3

→ Shewanella ANA-3

ANA-3 is metabolically versatile, and a close relative of other strains that CANNOT respire As(V)

Chad Saltikov

Davin Malasarn

Why do you think these properties would help us find the As(V)- respiratory genes?

Gain of function assay

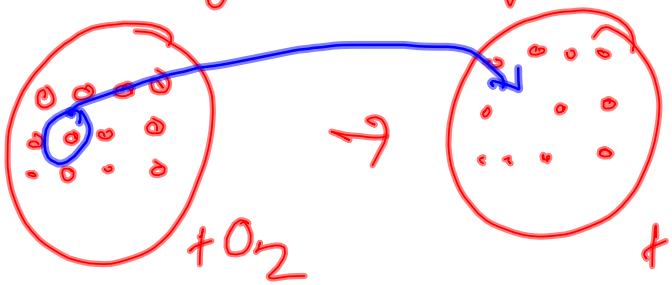
+As(V) resp → ? → plasmid → -As(V)

select for gain of trait

plasmid DNA

Loss of function assay

→ Creating the ability → Screen



+O_2

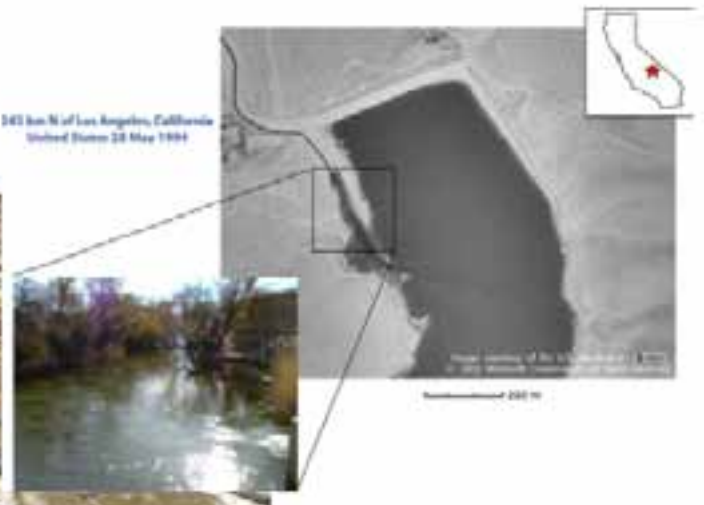
+As(V)

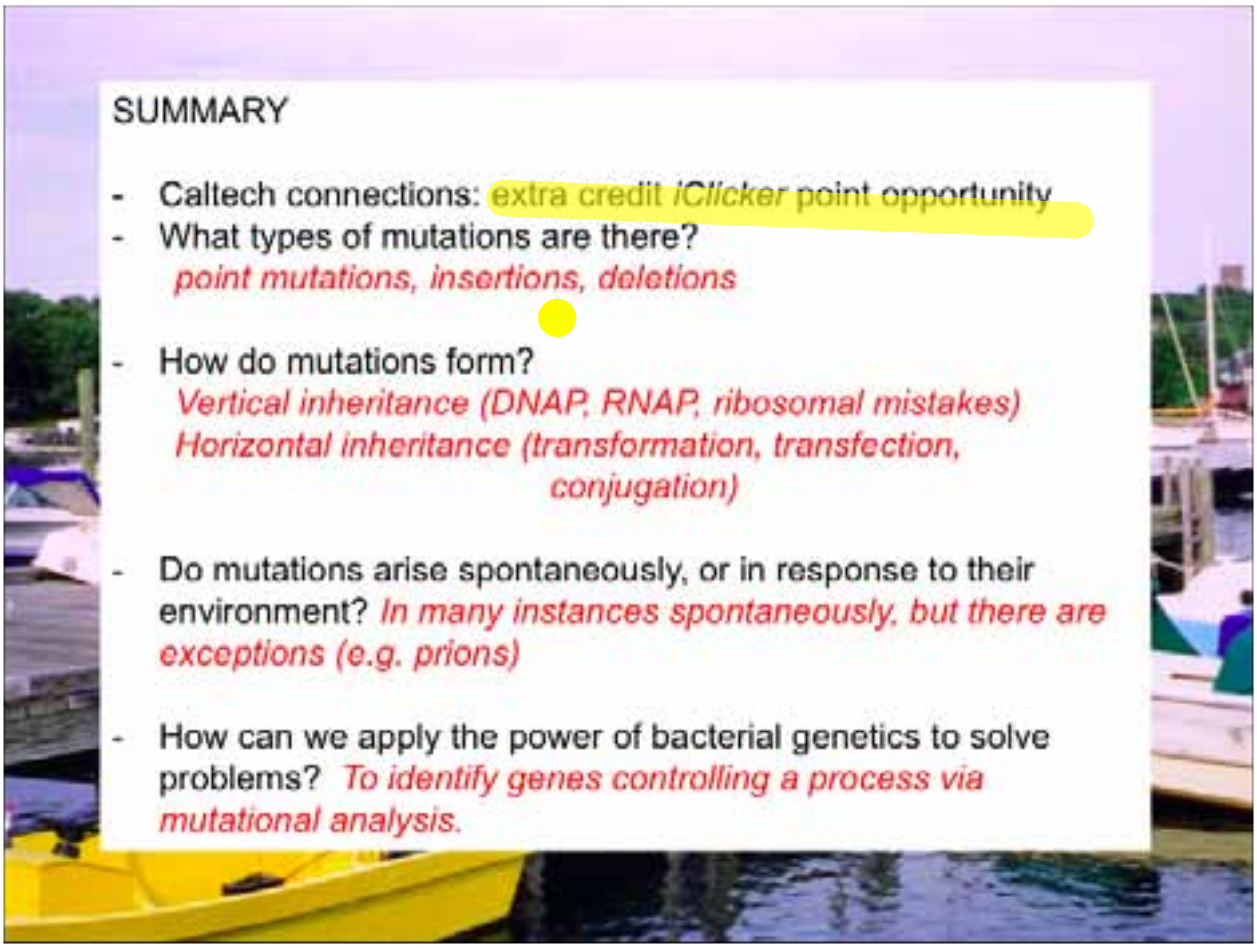
- transposons
  - ① insert at random
  - ② specific sites

Turns out every As(V)-respiring strain has the *arr* genes—robust biomarker

→ Encode a highly conserved protein family!

→ Can detect genes in the environment using PCR




SUMMARY
- Caltech connections: extra credit *iClicker* point opportunity
- What types of mutations are there?
point mutations, insertions, deletions
- How do mutations form?
Vertical inheritance (DNAP, RNAP, ribosomal mistakes)
Horizontal inheritance (transformation, transfection, conjugation)
- Do mutations arise spontaneously, or in response to their environment? *In many instances spontaneously, but there are exceptions (e.g. prions)*
- How can we apply the power of bacterial genetics to solve problems? *To identify genes controlling a process via mutational analysis.*