PERSISTENCE: Molecular Mechanisms of Evolution

Announcements:
- Please fill out course questionnaire

MMN
- How do epigenetic modifications of genes change transcription?
- What are the implications of the broad occurrence of transposable elements?

DKN
- Caltech connections: extra credit iClicker point opportunity
- Do mutations arise spontaneously, or in response to their environment?
- How do mutations form?
- How can we apply the power of bacterial genetics to solve problems?

Both iClicker Qs at the end
GENE STRUCTURE AND TRANSCRIPTION/TRANSLATION IN EUKARYA

transcription factor (a must)/polymerase binding

transcription start

start codon (ATG)

stop codon (TGA)

Promoter 5' UTR INTRON EXON INTRON EXON INTRON EXON 3' UTR

DNA

transcription

polyA tail

transcription stop

splicing (alternative)

5' cap

Pre-RNA 5' UTR INTRON EXON INTRON EXON INTRON EXON 3' UTR

RNA 5' UTR Open reading frame 3' UTR

protein

translation
Mendelian genetics

PUNNETT Square

How genes encode for traits. Is it really that simple?
Just over 10 years ago, the full human genome sequence was determined.

**Why so few genes?**

~20,000 – 30,000

[aren’t we more complex than worms, fish and plants?]
Other mysteries:

Identical twins

IDENTICAL?

EMBRYOGENESIS

--HOW????

Fertilized egg

8 cell embryo

differentiation

nerve cells

cardiac muscle

blood cells
Epigenetics

Transposable elements (TEs)
EPIGENETICS: ‘ABOVE THE GENOME’

STUDY OF THE INHERITANCE OF CHANGES IN GENE FUNCTION THAT OCCUR WITHOUT CHANGES IN THE DNA SEQUENCE

Some major epigenetic mechanisms of control:

**Methylation**
- DNA itself
- Histones

**Acetylation** of histones

![Chemical structures](image)
HISTONE ACETYLYATION
(usually enhances transcription – opens up the chromatin)
EPIGENETICS IN HEALTH AND DISEASE

pancreatic cancer (and other cancers)
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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.

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 change its relative position within the genome of a single cell.

Likely originally viral, then passed in the genome.
Transposable element (TE) inserts

CAN DROP IN ANYWHERE
replicate

TE excises

Promoter 5’ UTR INTRON EXON INTRON EXON INTRON EXON 3’ UTR
NO PHYLOGENETIC PATTERN TO TE LOAD – significance – higher ‘evolvability’?

Proportion of transposable elements (% of genome)
The human genome TE load

- Non-LTR retrotransposons: 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
Somatic retrotransposition alters the genetic landscape of the human brain

J. Kenneth Baillie1*, Mark W. Barnett1*, Kyle R. Upton1*, Daniel J. Gerhardt2, Todd A. Richmond2, Fioravante De Sapiò1, Paul Brennan1, Patrizia Rizzu4, Sarah Smith1, Mark Fell1, Richard T. Talbot1, Stefano Gustincich5, Thomas C. Freeman1, John S. Mattick4, David A. Hume1, Peter Heutink4, Piero Carninci7, Jeffrey A. Jeddeloh2 & Geoffrey J. Faulkner1

doi:10.1038/nature10531
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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!

Folding, structure, function

Pierce

Tirell

Gray

Rothemund

Clemons

Shan

Mayo
Examples of Caltech research in Biology applying/probing aspects of the Central Dogma: 2 extra credit iClicker point—see website!
What types of mutations are there?

- Single nucleotide 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

  (DNA) A A G A C T G C A
  (RNA) U U C U G A C G U
  aa Phe TERM Arg

  ribosomal error
  Ala Tyr Arg

- Deletion (frameshift, partial, clean deletion)

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

- Insertion (insertion elements: transposons)

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

  * differences in GC content suggest horizontal gene transfer
How do mutations form?

“Vertical” inheritance:

- DNAP, RNAP, ribosome mistakes [lower proofreading fidelity]
- environmental stressor  
  e.g. chemical mutagens, UV

“Horizontal” gene transfer:

→ Transformation (natural, chemical, electrical “competence”)

→ Transfection (phage infection)

→ Conjugation (exchange of DNA between bacteria)
Why you should care about this: antibiotic resistance!

Dangerous liaisons – conjugative transposons

→ Some transposons hop at random into the genome; others integrate at specific sites!
Do mutations arise spontaneously, or in response to their environment?

Darwin (theory of natural selection)    Lamarck (adaptive mutation)
“Phage group” (almost entirely comprising physicists)

Max Delbrück, Caltech

Salvador Luria, MIT

Nobel Prize 1969 Physiology and Medicine (with A. Hershey)
Why phage and bacteria?

→ Simplest systems that could be studied

→ Easy assays, requiring no more than “toothpicks and logic”
The plaque assay

Lytic phage create a burst (clearing) on bacterial lawn

→ 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
Luria–Delbrück experiment (1943) Fluctuation Test

Experimental Design & Results:
- Draw out 1 flask split into 20 volumes at the end
- Draw out 20 parallel flasks
- Plate them all (normalizing # of cells) on a saturating T1 phage background, select for resistant colonies
- Compare the number of resistant colonies
- What would the implications be if you saw equal numbers of colonies regardless of how you grew up the cultures? What if you saw different numbers?
- They observed different numbers, showing that mutations arose randomly in some cultures (“jackpot”) but not in others—had nothing to do with the exposure to the phage!

Conclusion:
- Mutations spontaneously arise in the absence of selection, not a response to environment
How can we apply the power of bacterial genetics to solve problems?

Draw out cartoon of microbe respiring As(V) to As(III)

Discuss As(III) more mobile and Toxic

How can we know when microbes are catalyzing this activity?

GOAL: FIND A ROBUST MARKER!
Isolating a good “model organism” to find the gene(s) specifically conferring the ability to respire As(V)

- Tell the story of isolation
- Explain how yellow mineral assay works
- Explain identified a new strain of *Shewanella*
ANA-3 is metabolically versatile, and a close relative of other strains that CANNOT respire As(V)

Why do you think these properties would help us find the As(V)-respiratory genes?
How did we find the gene? What approaches could have been taken?

Gain of function

Loss of function

→ Actual approach posted in supplementary readings on website
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 (prions)*
- How can we apply the power of bacterial genetics to solve problems? *To identify genes controlling a process via mutational analysis.*