Ch 13. Mitochondrial and chloroplast DNA, Extranuclear inheritance

37 genes
2 rRNAs
22 tRNAs
13 polypeptides
Genes of the mt

- Cellular respiration to produce ATP (+ CO2)
mt genome

- Circular (most)
- Double stranded
- 16,500 bp
- 37 genes
- Have own ribosomes!

- Other proteins required for cellular respiration and mt replication are encoded by nuclear genes and imported into the mt
mt genome
Evolution of mt and chloroplasts = Endosymbiont Theory Box 23.1

- primitive bacteria formed symbiotic relationship with early eukaryotic cells
- gradual transfer of mt genes to nucleus
- mt genes similar to prokaryotic genes
mt inheritance

- Extranuclear = non-Mendelian = maternal = uniparental inheritance
  - All progeny have phenotype of mother with respect to mt genes
  - mitochondria you tube → why don’t sperm contribute mt?
mt mutations

- **Neurospora**
  - [Poky] mutation (1952)
  - mutation in promoter of small subunit rRNA gene (protein synthesis)
  - Poky strains grow slowly for days, then growth rate accelerates reaching wild type rate after ~3 days.
  - How can they do this?
- Human mt mutations
  - Leber’s hereditary neuropathy
    - Adult optic nerve degeneration $\rightarrow$ blindness
    - Electron transport chain protein mutations
DNA sequencing gel – locate the mutation responsible for LHON.

LEBER'S HEREDITARY OPTIC NEUROPATHY

NORMAL

MUTANT

MITOCHONDRIAL GENOME nt11778 G > A

© CELTEK CORPORATION
mt inheritance pedigree
Heteroplasmy – mixed population of mitochondria in a cell
Effects of heteroplasmy on egg/offspring

- **Mother with mild or no symptoms**: small number of mother’s mitochondria, selected randomly, goes into each early egg cell.

**Contribution from mother**
- **80% mutant**: child with severe disease?
- **50% mutant**: child with mild disease?
- **20% mutant**: child with no disease?

**Contribution from father**
- **Sperm cells (no mitochondria)**

**Possible outcomes**
- Child with severe disease?
- Child with mild disease?
- Child with no disease?
mt inheritance - exceptions

- A few paternal mt may be in sperm
First sequenced *Haemophilus influenzae*

- 1.83 million bp
  - 1,743 protein coding sequences found, 736 are unknown proteins
Escherichia coli (1997)

- Lower intestines of animals
- Pathogenic strains (ex. *E. coli* 0157)
- Genome
  - 4.6 million bp (4.6 megabases)
  - ~4000 genes, ~88% of genome open reading frames
Single circular chromosome

http://www.sinauer.com/cooper/4e/micrographs0603.html
http://www.emc.maricopa.edu/faculty/farabee/biobk/bactchromo.gif
E. coli biology

- Prokaryote
  - nucleoid region contains the chromosome

Neisseria gonorrhoeae.
E. coli reproduction

- Binary fission -> Exponential growth

~ 3 microns
Bacterial growth

- **colony** - visible cluster of clones
  - about 1 million cells /colony

- **lawn** – entire plate covered, no individual colonies

Growth on agar plate
Growth of bacteria (E. coli)

- **Lag phase** - slow or no apparent growth
- **Log phase** – double every 20’ to \(1 \times 10^9\)/ml
- **Stationary phase**
  - nutrient and/or oxygen limited
  - Cell number remains constant
- **Death phase**
  - Nutrients gone, toxic products build up, cells die
Bacterial growth curve

Bacillus subtilis video
Generation times for bacteria vary

- *Escherichia coli*  
  Glucose-salts  
  17 min.

- *Streptococcus lactis*  
  Milk  
  26

- *Streptococcus lactis*  
  Lactose broth  
  48

- *Staphylococcus aureus*  
  Heart infusion broth  
  27-30

- *Rhizobium japonicum*  
  Mannitol-salts-yeast extract  
  344-461

- *Mycobacterium tuberculosis*  
  Synthetic  
  792-932

- *Treponema pallidum*  
  Rabbit testes  
  1980
How to determine the titer of bacteria

Titer = number of colonies (cfu) per ml liquid culture

1. plate 100 ul of culture on an agar plate – why 100 ul?
2. count colonies
   ~ 400

3. 100 ul plated yielded
   400 colonies
   = $4 \times 10^3$ cfu /ml
If there are too many colonies to count, then the original culture must be diluted before plating.

Dilute culture 1 :100. Plate 200 ul. Observe 120 colonies.

Titer (cfu/ml) ? 1000 ul
Growth media

- **minimal media** = essentials
  - Sugar (carbon source) + salts
  - bacteria synthesize aa, nucleotides, vitamins

- **complete media**

- **selective media**
  - Allows one species to grow while selecting against another
Solid and liquid culture

Growth in liquid media

Growth on agar plate
Phenotypes

- **Prototroph**
  - Organism synthesizes requirements from minimal media

- **Auxotroph**
  - nutritional *mutant*
  - Requires one or more supplements to grow
    - Ex. amino acids
symbols

- Resistant to ampicillin = $\text{Amp}^r$
- Sensitivity to streptomycin = $\text{Str}^s$
- Auxotroph mutant requires tryptophan = $\text{Trp}^-$

$\text{trp}^-\text{leu}^-\text{thi}^+\text{tet}^r$

Wildtype = +
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Character or phenotype associated with symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(bio^-)</td>
<td>Requires biotin added as a supplement to minimal medium</td>
</tr>
<tr>
<td>(arg^-)</td>
<td>Requires arginine added as a supplement to minimal medium</td>
</tr>
<tr>
<td>(met^-)</td>
<td>Requires methionine added as a supplement to minimal medium</td>
</tr>
<tr>
<td>(lac^-)</td>
<td>Cannot utilize lactose as a carbon source</td>
</tr>
<tr>
<td>(gal^-)</td>
<td>Cannot utilize galactose as a carbon source</td>
</tr>
<tr>
<td>(str^r)</td>
<td>Resistant to the antibiotic streptomycin</td>
</tr>
<tr>
<td>(str^s)</td>
<td>Sensitive to the antibiotic streptomycin</td>
</tr>
</tbody>
</table>

**Note:** Minimal medium is the basic synthetic medium for bacterial growth, without nutrient supplements.
Bacterial mutants

- **Nutritional mutants**
  - Auxotrophs that require supplement to grow

- Ex. bio-
**Conditional mutants**

- mutation is expressed in a certain condition

- **temperature-sensitive** \((ts)\) mutants.

- Ex. *E. coli* protein can function at 25\(^\circ\) C but not at 42\(^\circ\) C. Grow cells at 25\(^\circ\) C and can examine loss of protein function at 42\(^\circ\) C
**Resistance mutant**

- Antibiotic resistance in bacteria

- $\text{amp}^r$
quorum sensing

- What are *Vibrio harveyi*?
- What happens when *V. harveyi* gather to “quorum”?
- What is bioluminescence?
- What is meant by bacteria “talking” to each other?
- How does Bassler plan to use her work in the field of medicine/ antibiotic resistance?
How do bacteria undergo genetic recombination?
Noble Prize for bacterial genetics

- Lederberg, Beadle and Tatum 1946 > Nobel 1958
- Nutritional mutants in E. coli
1. Conjugation

- parasexual mating

- one-way transfer of genetic information from “male” to “female” bacteria
Plasmid $\rightarrow$ DNA from donor to recipient bacterium

- Plasmid
  - circular, episomally maintained DNA
- F factor plasmid
  - Encodes F pilus
- Conjugation video

1953 Hayes

F+ cell

F- cell
94,000 bp
origin of replication (ori)
F pilus

Donor F+  Recipient F-

Pilus cannot form between 2 F+ cells
Conjugation

F+ + F- = 2F+

1. Pilus attaches to recipient cell
2. 2. nick DNA -> transfer DNA
3. DNA polymerase makes dsDNA
4. break pilus

Note: no chromosomal DNA transferred
Conjugation flash
Lederberg and Tatum experiment

Mix 2 auxotrophs \(\rightarrow\) grow in minimal media

Strain A \(\text{met}^{-}\ \text{bio}^{-}\ \text{thr}^{+}\ \text{leu}^{+}\)
Strain B \(\text{met}^{+}\ \text{bio}^{+}\ \text{thr}^{-}\ \text{leu}^{-}\)

OBTAIN ---\(\rightarrow\) a few prototrophs form!

What would the genotype of this prototroph be?
rare 1/10,000,000

Genetic recombination has occurred
Lederberg and Tatum experiment
Davis U-tube \(\rightarrow\) conjugation requires cell/cell contact

Fig. 15.3

-media  -bio-

-thr-  -leu-

Filter prevents cell contact

Media can pass \(\rightarrow\) no prototrophs obtained
Show that cell-cell contact is required
Rarely, the plasmid integrates into the bacterial chromosome = Recombination

What happens when an Hfr strain conjugates?
Hfr conjugation flash
- The first DNA to be transferred is the chromosomal DNA!
- Pilus is broken before F factor is transferred
- Recipient cell remains F-
The transferred DNA MAY undergo homologous recombination
Comparing an Hfr to F\(^+\) strain
Lederberg’s experiment explained
Fig. 15.7

$Hfr \ H \ (azi^R \ ton^R \ lac^+gal^+str^S)$

$X$

$F^- \ (azi^S \ ton^S \ lac^-gal^-str^R)$
**E. coli** minute map = 4,639,221 base pairs (4377 genes)

Clock face.... Gene controlling
---
Noon+ threonine synthesis
1 o'clock lactose degradation (lac-operon)
2 o'clock galactose -> glucose (gal-operon)
3 o'clock tryptophan synthesis (trp-operon)
5 o'clock histidine synthesis (his-operon)
7 o'clock lysine synthesis
8 o'clock streptomycin resistance
9 o'clock mannitol degradation
10 o'clock Place where chromosome synthesis begins in both directions ("OriC")
11 o'clock methionine synthesis
Noon- "F"-episome (where "F" is inserted)

Genes encode 4288 proteins and 89 RNAs.
FYI  E. coli

The complete sequence of the genome of E. coli (K-12) was reported in the 5 September 1997 issue of Science.

E. coli Originally isolated in 1922 from a diphtheria patient
Circular chromosome

4.6 million bp (4.6 Mb)