Illinois Institute of Technology

Radiation Biophysics
Lecture 13: Genetics
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Plan for this lecture

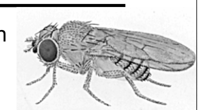
- Genetics
  - Chromosome Breakage
    - Single hits
    - Double hits
  - Gene mutations
    - Frameshifts
    - Introns
  - Molecular Biology 102

Generalizations about Genetics

- Main focus here is on ways that ionizing radiation can damage the physical and replicative properties of DNA
- We'll look at three length scales:
  - The individual base-pair (~0.5 nm)
  - The gene (1000 bp - many nm)
  - The entire chromosome (200 nm or more)
- Focusing only on single-base mutations (substitutions, deletions, insertions) does not tell the whole story

Early studies

- T.H. Morgan studied chromosomes in Drosophila
- *Tradescantia* (spiderwort) microspores: Sax, 1938-1950
- More *Tradescantia*: Lea et al, 1946: Provided quantitative data on chromosomal aberrations during first mitotic cycle



Chromosome Breakage

- Quite common result of radiation exposure
- Can happen before replication:
  then the structural defect will be replicated
  (if the cell survives)
- Can happen afterward:
  then one of the two chromatids
  will differ from the other one.
- Types of damage:
  Subchromatid, Chromatid, Chromosome
- Repair tends to be rapid

Single-Hit Breaks

- Cartoons show * as centromere and ^ as break
- Single-Hit:
  A B C * D E F^G H I → A B C * D E F + *G H I*
- This can recombine in a number of ways:
  A B C * D E F *I H G*
  I H G A B C * D E F
  G H I A B C * D E F
- … or a circle plus a fragment without a centromere, which won't be able to use the spindle machinery to get sorted.

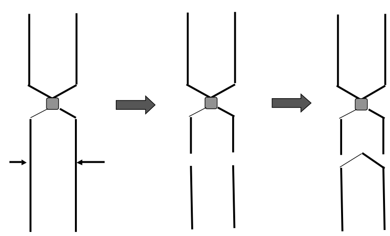
Double Hits

- A B C * D^ E F^ G H I →
  A B C * D + *E F* + *G H I*
- Numerous ugly combinations can arise, e.g.
  E F A B C * D *I H G*
- Acentrics can readily arise

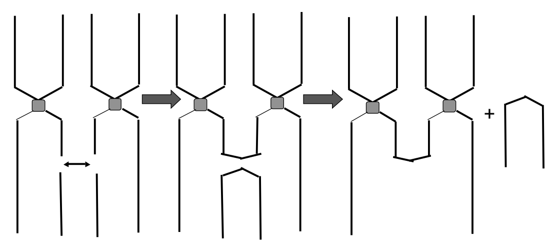
Multiple Hits in Replicated Chromosomes

- See figs. 13.1 and 13.2
- Major losses of genetic information possible
- Balanced Translocations: no harm done!
- Dicentrics--two centromeres in one pair of chromosomes.
- Sister chromatid exchange: exchange of DNA fragments from one chromatid to another between the two chromatids of a single chromosome
  - Important for chemical mutagens
  - Not common with radiation

Double-stranded breaks



Recombination



Breakage and Exchange Hypotheses

- Breakage hypothesis:
  Types of aberrations that require one break follow linear dose-response; those requiring two or three follow quadratic or cubic dose-response.
- Exchange hypothesis:
  emphasizes reciprocal exchanges among chromosomal materials
  Data don't support this one much.

Gene Mutations

- We've discussed these in detail previously
- Types:
  - Deletions (frameshift)
  - Additions (slightly less likely) (frameshift)
  - Substitutions (e.g. C for T; no frameshift)
- Remember that three bases code for an amino acid!
- If we skip a single base, we can throw off every single amino acid that is coded for downstream of the error.
- Mutation frequencies are often linear with dose

Effect of Frameshift

- Suppose the DNA duplex looks like this:
  5'- A - T - C - G - C - A - A - 3'
  3'- T - A - G - C - G - T - T - 5'
- Now we delete one base, so that the coding strand is
  3'- T - A - G - G - T - T - 5'
- Now the amino acids coded for will be different.

Frameshifts (continued)

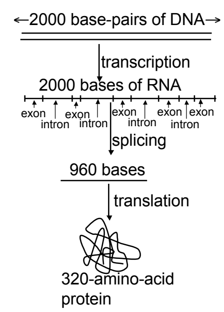
- Most common form of (local) DNA damage
- Defined as any form of DNA damage that leads to a loss of one base during replication or transcription.
- Chemistry
  - Deletion of bases
  - Fragmentation of sugar-phosphate backbone
  - Distortion of base-base hydrogen bonds and other 3-D elements
- Results in complete misreading of remainder of message

Genes

- A gene is a unit within a chromosome (i.e., a stretch of DNA) that codes for a specific function.
- Most genes ultimately code for proteins; *but*
- Some code for transfer RNAs
- Eukaryotic genes often have exons and introns:
  - Exons are segments of a gene that are ultimately turned into proteins
  - Introns are segments of a gene that are removed from the message before translation

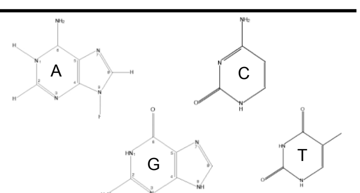
How Introns are Removed

- Suppose we begin with a 2000-base-pair gene.
- It will be transcribed to make a 2000-base messenger RNA molecule
- That gets chopped up to make up a ~960 base ultimate message
- This will produce a 320-amino acid protein (960/3 = 320)



Structural components of genes

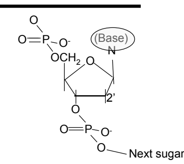
- Bases
  - Adenine
  - Cytosine
  - Guanine
  - Thymine



- Phosphate-deoxyribose backbone
- Double helix enables the replication and transcription processes and offers physical stabilization

Review of DNA backbone

- Sugar-phosphate backbone on each strand has a nitrogenous acid base attached at the 1' position of the ribose ring
- The lack of an -OH group on the 2' position prevents formation of a reactive intermediate; this makes DNA more stable than RNA
- DNA's double-helical structure also helps its stability.



RNA hairpins

- DNA is almost always double helical; Watson-Crick base pairing contributes to stability
- RNA sometimes has complementary bases that allow for bases to pair up around a (hairpin) loop

```
5'-A-U-G-G-C-C-U-A-A-U-G-C-C-A-U-3'
              |
              v
5'-A-U-G-G-C-C-U\
3'-U-A-C-C-G     A
            \U A/
```


Special features

- Code is redundant
- 4^3 = 64 codons
- Only 20 amino acids plus a few control codes; most amino acids have more than one codon each associated with them.
- Middle base is generally conserved; all the codons for any given amino acid generally have the same middle base.
- First RNA base may be sloppy

A problem to ponder

- Explain why a frameshift of 3 bases is less likely to be fatal than a frameshift of 1 or 2 bases.

Errors in Fidelity and Their Consequences

- How do chemicals & radiation affect fidelity (rate of mutation)?
- Increase likelihood of replication error
- Radiation: bond disruption in bases (or sugars)
  - Direct (radiation breaks bonds in DNA)
  - Indirect (radiation causes free radicals; free radicals break bonds in DNA)

Chromosomal Instability

- The observation is that cell death continues for several generations after the initial exposure
- The explanation is that the chromosome becomes unstable, i.e. it loses some of its ability to remain structurally sound.
- This instability is heritable and therefore accounts for some of these long time-scale effects
- Little (1990): decreases in cloning efficiency lasts for 20-30 population doubling times in mammalian cells

Results in intact eukaryotes

- Not as easy to study as in culture
- Most work historically has been done in *Drosophila*
  - Rate of production of lethal mutations is linear with dose
  - Dose rate is irrelevant
  - Male gamete's sensitivity varies greatly with the stage of development at which irradiation occurs
  - Rate of mutations ~ $1.5*10^{-8}$ - $8*10^{-8}$ per locus per cGy.
- n.b.: a locus is a testable genetic element; it's typically one gene, although it could be a group of genes controlled by a single promoter

Results in Mammals

- Concern was raised in the 1960's that we shouldn't base mammalian exposure risks on *Drosophila* data
- Mouse specific locus test: suggests
  - $2.2*10^{-7}$ mutations/locus/cGy.
  - Dose-rate matters: fewer observed mutations at lower dose rate.
- Difficult to extrapolate these mouse results to humans
- Rates around1 - $5*10^{-7}$ is typical.

Repair

- Cell has mechanisms to recognize & replace faulty bases before they have a chance to be replicated
- Some injury may disrupt a large enough segment of DNA that repair either fails or is error-prone