411-4

OUTLINE

I. Spontaneous mutation
    A. Single base pair changes
        1. Mutational "fingerprinting"
        2. cytosine deamination
        3. depurination
    B. Other spontaneous mutations
II. Induced mutations
III. DNA repair

Main ideas of last lecture:

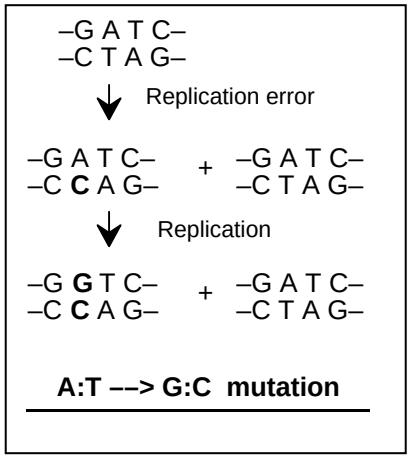
1. Trade-off in biological processes between speed and accuracy
    a. Antimutators
    b. RNA phage

2. Attain accuracy trough redundancy
    a. DNAP proofreading
    b. aa tRNA charging
    c. Codon-anticodon interaction

I. MUTATION-BOARD

DNA sequence CHANGE	Protein SEQUENCE CHANGE
1. base pair change (e.g., A:T->G:C)	missense nonsense (say protein site board binding sites, but don't write down)
2. DNA deletion	remove coding sequence frameshift (small)
3. DNA insertion	interrupt coding sequence frameshift (small)
4. DNA inversion (won't discuss)	disrupts coding sequence

Point that don't know whether initial change was T–>C or A–>G because of replication, so list as base <u>pair</u> change



<u>Transition mutations</u>
A:T->G:C
G:C->A:T

<u>Transversion mutations</u>

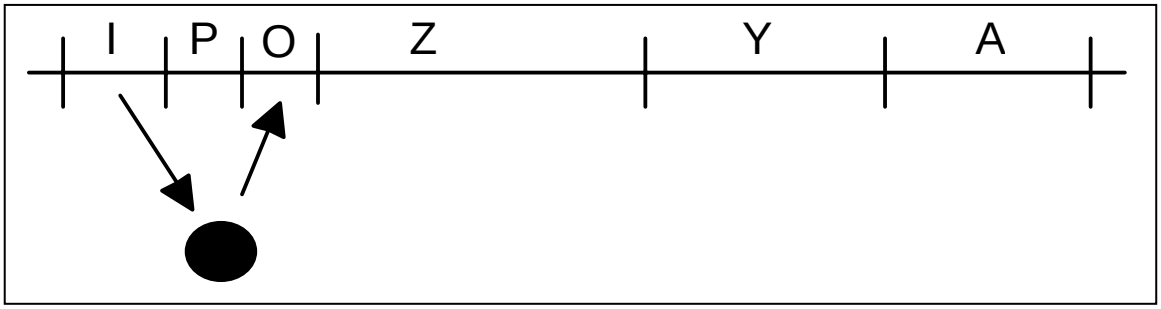
A:T->T:A
->C:G

G:C->T:A
->C:G

Describe in some detail an elegant gentic method to analyze spectrum of base-pair changes in cells- spontaneous or after DNA damage caused by mutagens. Challenging, put a number of things together

"FINGERPRINTING" point to board (OL)

BOARD- lac operon



if lactose present, release repressor

BOARD
Three features need to know to understand mutational fingerprinting:

1. Possible to select lacI- mutants from $lacI^+$ cells (growth on noninducing galactoside (phenylgalactoside)- needs to be I^- to grow on phenylgalactoside as carbon source)

1. Possible to isolate $lacI^+$ –––> $lacI^-$ mutants

2. Subset of mutations due to creation of amber codons (UAG) can be easily recognized using specific strains called amber suppressors (these are a subset of nonsense suppressors)- these have mutant tRNAs that insert an amino acid at an amber nonsense codon(pp 137-139 S/C)

BOARD::Amber codon= UAG (TAG in DNA)
ATC

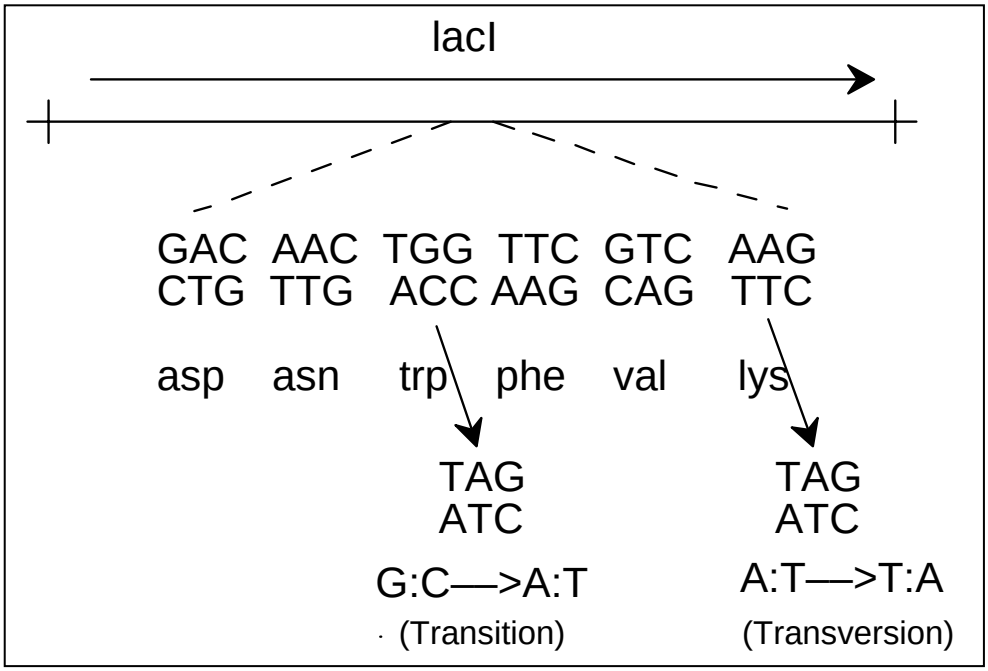
OH- codon table- point out amber and other stop codons

2. Can distinguish $lacI^-$ mutants caused by amber stop codons from other types of change

3. Map mutations finely- DELETION mapping (10 bp resolution!)- Fig 12.8 (3rd ed)

3. Can map mutant sites within lacI gene

Mutational fingerprinting based on the fact that among total set of single bp changes, some will be amber stop codons and these can be easily recognized and mapped


lacI
GAC AAC TGG TTC GTC AAG
CTG TTG ACC AAG CAG TTC
asp asn trp phe val lys
TAG
ATC
TAG
ATC
G:C—>A:T
(Transition)
A:T—>T:A
(Transversion)

-If do this for entire gene- 37 codons of 335 total can give amber codons by single base pair change

-classify according to type of bp change required

OH - positions classified

ONE BIG LIMITATION- all of the possible transitions and transversions represented except one A:T->G:C- REFER to overhead showing

Why? UAG = TAG, To detect A:T->G:C, need TAA->TAG; can't detect in this system Why?

OH-codon table TAA=>UAA, already stop codon!! None in normal (= nonmutant) lacI gene

SO this fingerprinting method can't detect- other methods have been developed

OH- Scheme for fingerprinting- go through- Why not just sequence? Harder, especially when this work was done- more than a decade ago

SPONTANEOUS MUTANTS FIRST
OH- describe what shows

Write:
Main points: 1. Mostly G:C->A:T transistions
2. Two hotspots

OH- can easily account for based on a very common type of spontaneous DNA damage- Cytosine hydrolytic deamination

deamination
OH- cytosine---------->uracil

- As you know, U bps like T, but normally only find in RNA- but if replicated over, acts like T, so get C->T = G:C->A:T

Draw out

BOARD; uracil N-glycosylase- removes most, replacing with C
residues-> those as missed =
mutations- Why would some U's be
missed?? (rep fork beats ung to site)

-REPLICATION fixes error- if get replicated over before repair, change- go through??

These hotspots helps rationalize something peculiar about DNA- Why it has T instead of U- why do cells use different bases for DNA and RNA? (other bases same)

The idea is that because C deamination is so frequent, there was selection pressure to be able to distinguish and remove deaminated C residues- from normal base

Cytosine deamination helps explain why the RNA base uracil isn't normally used in DNA (why thymine used instead)

If Uracil were used in DNA, no way to distinguish cytosine deamination product as "abnormal"

C deamination
AUGCUG---------->A UGUUG
UGCGAC UGCGAC
=>no way to distinguish U as inappropriate

C deamination
ATGCTG---------->ATGUTG
TGCGAC TGCGAC
=>mistake recognized by special enzyme recognizing U in DNA (uracil N-glcosylase)

Hotspots:
-DNA turns out to be methylated at some C residues - a few percent of total C's (not known why)

–C **C** A G G–
–G G T **C** C– bold bases methylated at 5 position

BOARD: 5-methyl C- Deamination -> 5-methyl Uracil

5-methyl U = Thymine

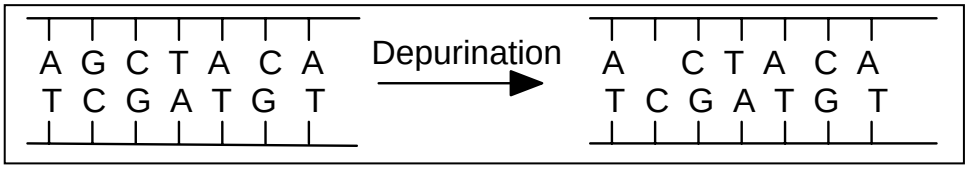
Two hotspots= 5-methyl C sites- Spontaneous deamination gives T- can't be recognized as different from normal T's by uracil N-glycosylase!

Tests:
1. cytosine methylase- mutant: hotspots decrease
2. create new methyl C sites- create hotspots
3. uracil N glycosylase- - all C's hotspots

Very short patch repair- cells attempt to limit deamination hotspot.
POINT: No repair system is 100% efficient, so still have hotspots.
SECOND main type of spontaneous DNA damage=

OH depurination

Spontaneous cleavage of base sugar bond in DNA



Replication over missing base leads to mistakes because no base to pair with
Cell has repair enzyme that recognizes= "AP endonuclease"

OH- showing ung and ap pathway

OTHER SPONTANEOUS MUTATIONS

Limitation to fingerprinting system: single base pair changes only detected=
Surprise- only 10% of spontaneous changes are base pair changes!

OH Frameshifts- one incredible hotspot- ca 2/3 of all mutations

OH- sequence at hotspot
OH showing mechanism thought responsible

ASK- what would be the result of this type of mutation if 3 bp rather than 4 bp repeated?

e.g., ACA TGA CAG CAG CAG CAG TTA TAC
TGT ACT GAC GAC GAC GAC AAT ATG

WOULD EXPECT to get increase or decrease in number of amino acids encoded by CAG

Common cause of human disease

Kennedy's disease

-adult onset- progressive muscle weakening
males show reduced fertility suggesting problem with sex hormone production or reception

ANDROGEN receptor gene

See HO

17-26 repeats normal

40-52 repeats in adults with disease- depends on individual

Idea is that adds extra residues and this screws up receptor, therefore fertility decreases- not exactly clear why muscle strength decreases

"Expansion" of CAG- replication slippage

This type of mutation seen in other genetic diseases occuring at high frequency- 13 different diseases

-fragile X syndrome (mental retardation)
-a type of muscular distrophy
-Huntington's disease- neurological disorder
-cancer

C. Spontaneous deletion events- inappropriate recombination between short sequence repeats

D. IS element insertion- simplest type of transposons- resident on chromosome (=reservoir) elsewhere can insert spontaneously into a gene- already talked about a little - will discuss later in greater depth-

II. Induced mutation

A. Chemical mutagens- DNA damaging-
1- alkylating agents
nitrosoguanidine, (NG), ethylmethane sulfonate (EMS)
-add methyl or other alkyl group to base in DNA and alter pairing

Fig - spectrum - essentially all G:C—>A:T (A:T–>G:C also increased)

Fig - showing change in base pairing

-show set of steps leading to G:C–>A:T

AGA ⟶
TCT

B. - - base analogues 5-bromouracil- same basepairing at T, but tautomerizes more frequently to C-like

Ask: What types of mutations induced?
A:T—>G:C- C-like tautomer in template when replicated

AGA ⟶
BCT

G:C—>A:T- C-like tautomer at instance of nucleotide incorp

AGA ⟶
TCT

B. Physical mutagen
-ultraviolet light (UV)

Fig 14- spectrum- mostly GC->A:T, others too
- frameshifts also common
-often at runs of pyrimidines on same DNA strand- Ts and Cs

Fig 15- mechanism

Damaged bases can't be replicated properly in DNA replication

Very often- two adjacent bases both changed- - E.g.,
GTTAC⟶ GCGAC
CAATG CGCTG **SIGNATURE** of UV damage

Skin cancer- often involves mutations altering two adjacent pyrimidines – consistent with the idea that sunlight (UV) probably culprit