Lecture 5 2009

Outline

I. Spontaneous mutation
II. Induced mutation
- **A. Chemical mutagens**
  - **a. alkylating agents (methyl nitrosoguanidine-"NG")**
  - **b. base analogues (5-bromouracii- "BU")**
- **B. Physical mutagen- (ultraviolet light- "UV")**

III. Repair
- **A. Mismatch repair**
- **B. Oxidative damage repair**
- **C. SOS response**

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

Fig of G:C—>A:T change

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

Ask: What types of mutations induced?

B. Physical mutagen
- -ultraviolet light (UV)

Fig 14- spectrum- mostly GC->A:T, but greater variety than most mutagens
- 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., OH

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

III. DNA repair

Cell isn't defenseless against DNA damage, not to mention mistakes it makes during replication. Has a variety of repair systems to limit mutations.

(DNA repair and lifespan- not in 2009)

Survival curve- first indication that repair processes existed

How to identify repair functions genetically? isolate mutants not doing it right- PHENOTYPE? Higher mutation frequency- mutators

Already discussed one type- what is? Replication proofreading exonuclease minus- doesn't catch polymerization mistakes

BOARD - screen for mutator mutants

(as in experiment 3)
How to identify

Start with cells that are $lacI^-$ $lacZ^-$ $lacY^+$

Plate on minimal glucose + lactose (or Phenyl galactoside (PG)) + Xgal

go through papillation assay (pg 475-476 3rd ed)
What is purpose of adding lactose? Lac+ mutants grow and are more visible

Once get, test other gene to be sure **GENERAL MUTATOR**

A. Mismatch repair- common

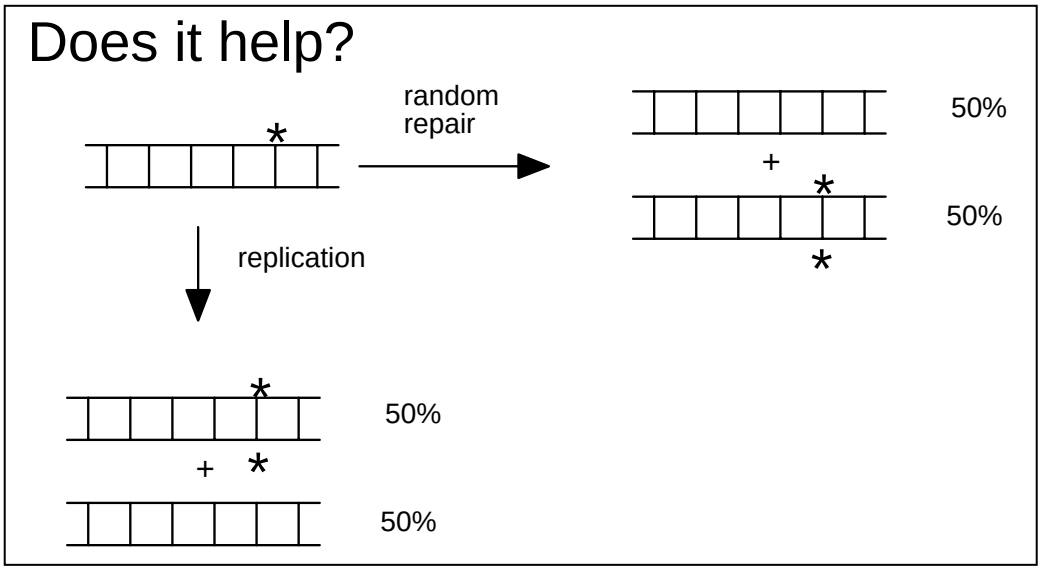
write: mutS mutH mutL- all required for <u>mismatch repair</u>

Genetic evidence that work together- all show similar spectrum, including hotspots- Fig 16

idea that some sites in DNA are more prone to replication mismatch errors than others and these are normally repaired by MM repair- even though not clear why some more prone to mm error
Point- first hint that these three gene products function together

Draw on OH of mismatch being corrected in either of two directions:

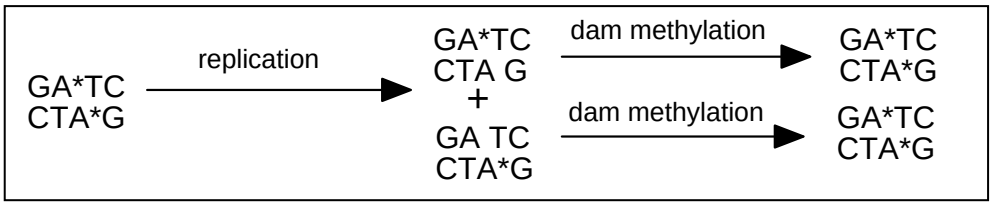
Main purpose- to catch replication errors that DNA polymerase proofreading misses. Works by identifying mismatches, excising them and repolymerizing in correct base



So mismatch repair won't help if random! Need way to distinguish which base in mismatch is wrong!!

Logic used by the cell: assumes that recently polymerized of two mismatched bases is wrong one

How to tell which one is recently polymerized?
Relative age is ascertained by methylation state of the DNA after replication- DNA methylated on A's by DNA adenine methylase (DAM Methylase)- not all A's, just GATC- DONT CONFUSE WITH CYTOSINE METHYLATION!



Note: time lag after replication before methylation- newly made strand is transiently undermethylated- this is how relative age is determined

Fig 17- mechanism
mutS binds to mismatch
mutH, L recognize nearby methyl site in DNA, cleave unmethylated strand at the site

then 3—>5 exonuclease removes, polymerase and ligase act

phenotype of Dam- mutants (mutator)- same spectrum as others

phenotype if overproduce dam methylase- also mutator, not enough time to distinguish strands

OH=> Dam- is hypersensitive to 2-aminopurine. Idea (pg 476 of 3r^{d} ed) is that 2AP is normally incorporated but causes bulge that is recognized by mm repair. If no dam function, get cleavage at random and two sites close together give fragmentation of genome. Isolate additional mutations not showing sensitivitiy- find in mutHSL so don't get cleavage

FUNCTION	ERROR RATE
DNA polymerizing	10^{-5}
3->5 proofreading	10^{-4}
mismatch repair	10^{-1}

Think about the mutational behavior of different single vs double mutants.
For the following, mutations complete nulls except for proofreading exonuclease mutant.

Mutations affect components of same "repair" system

Mutant	Mutation frequency
None (wild-type)	10–10
MutS^{-} (MM repair)	10–9 (10X increase)
MutH^{-} (MM repair)	10–9 (10X increase)
MutS^{-} MutH^{-} double	Ask =10–9 (10X increase)

Mutations affect components of different repair systems acting on same "damage" (mismatched bases arising in replication)

Mutant	Mutation frequency
None (wild-type)	10–10
MutS^{-} (MM repair)	10–9 (10X increase)
Proofreading– (partial)	10–9 (10X increase)
MutS^{-} Proofreading– double	Ask =10–8 (100X increase)

Mutations affect components of repair systems acting on different types of "damage" (replication mismatches vs cytosine deamination products)

Mutant	Mutation frequency
None (wild-type)	10–10
MutS$^-$ (MM repair)	10–9 (10X increase)
Uracil N glycosylase$^-$	10–9 (10 X increase)
MutS$^-$ Uracil N glycosylase$^-$ double	2 X 10–9 (20X increase)

Other role for mismatch repair (HSL) in blocking recombination between homeologous sequences. (=similar but not identical sequences)
interferes with formation of Holiday intermediate

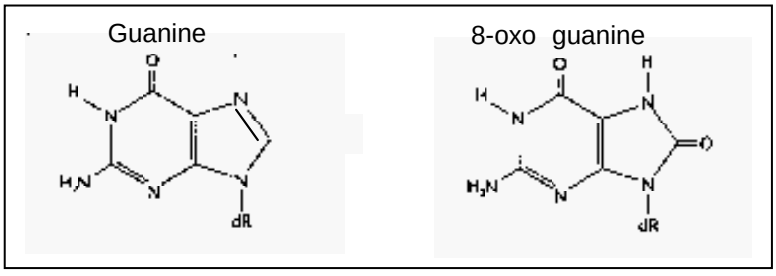
Oxidative damage

oxidants from normal metabolism- usually fairly efficient conversion of O_2 to H_20, but some of the reactive intermediates are inevitable byproducts (all organisms).

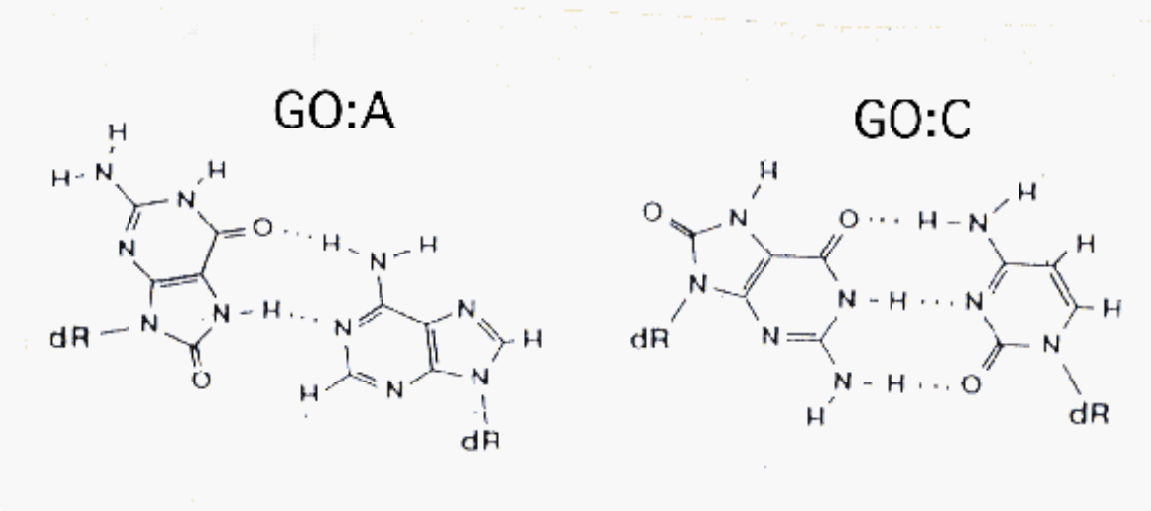
OH-reduction of oxygen to water

$$O_2 \xrightarrow{e^-} \cdot O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} \cdot OH \xrightarrow{e^-} H_2O$$

The most important nucleotide change is formation of 8-oxoguanine (="GO").
3000-5000/genome/replication



Can occur either in free nucleotide or DNA



Problem: can base pair with A as well as C!

Figure 1 showing- GO tends to flip around into syn isomer – but most of the time pairs like G

OH- Multiple lines of defense- write on OH as say

1. mutT – Fig. - cleaves OG triphosphate (DEOXY) before can be incorporated into DNA; monophosphate is further degraded

2. mutM- Figs 3and -removes GO from GO:C mismatch -Doesn't remove GO from GO:A mismatch- Why? Make 100% mutants. If replicate, have chance to rep right

3. mutY- Figs and -removes A from GO:A mismatch - If replaces A with C, mutM can correct (then it's GO:C)

mutant specificities:
mutT: A:T–>C:G
mutM: G:C–>T:A
mutY: G:C–>T:A

Although both mutM and mutY help avoid same type of damage, they function largely independently (redundant). Can see this in comparing single and double mutants

BOARD:	freq. Rif^R ($X10^{-8}$)
mutM+ mutY+	5 (1X)
mutM- mutY+	151 (30X)

mutM+ mutY-		290 (58X)
mutM- mutY-	8200	(1640X)