411-6 2009

-mention other way of getting mutators (Miller review)

Outline:

III. DNA repair
- A. Mismatch repair (mutH, mutL, mutS)
- B. Oxidative damage repair (mutT, mutY, mutM)
- C. SOS response (recA, lexA)
  1. excision repair (uvrA, urvB, uvrC, uvrD)
  2. recombinational repair (recA)
  3. error-prone repair (umuC, umuD)

IV. Transposition

Two levels of redundancy:

1. Process- multiple repair systems act on same DNA damage
2. Informational- DNA double helix

3. SOS response

The repair systems mentioned so far are present all the time because they deal with mistakes made in replication (mismatch repair) or damage that occurs all the time (uracil deamination, depurination, base oxidation)

Some special repair systems turned on during time of severe DNA damage- e.g., exposure to UV light (remember that under normal conditions, UV exposure minimal)

Best characterized- SOS response

Key components (write on OH)

1. RecA protein- senses DNA damage (pyrimidine dimers, X-linked DNA, broken DNA)(also involved in homologous rec)

2. LexA protein- repressor of repair genes

Fig. : top: Normal case- no damage
bottom: +DNA damage- cleavage of LexA repressor allows expression of repair genes

Fig <u>Genes repressed (partially or wholly) by LexA</u>

1. Excision repair (uvrA, uvrB, uvrC)
2. RecA gene (recombinational repair)
3. Error-prone repair (umuC, umuD)
4. Cell division inhibitor (SulA=SfiA)
5. lexA gene

Go through one-by-one

1. Excision repair (uvrA, uvrB, uvrC, uvrD)
used to remove bulky adducts in DNA that can't be read during replication- most important of these are pyrimidine dimers caused by UV light

Also PHOTOREACTIVATION repairs these- pg 386 in SC2

Fig – mech- UvrAB complex scans for helix distorted b damage- UvrC displaces UvrA and UvrB cuts 4 nt 3' of damage. Then UvrC cuts 7 nt 5' of damage. UvrD is helicase which then removes oligo, and poly I resynthesizes strand

logic of system similar to MM repair- mistake recognized, SS with damage excised, SS gap filled in using information on opposite strand

Very important point- both mismatch repair and excision repair show how redundancy in the DNA double helix is used to prevent mutation. Imagine if there were no redundancy- if DNA were single-stranded!

2. Recombinational repair = (RecA)- another way to cope with problem of pyrimidine dimers (Fig. 11-14 in SC2) "Damage tolerance" rather than true repair- antoher example from replication lecture – isomerization at fork

Fig

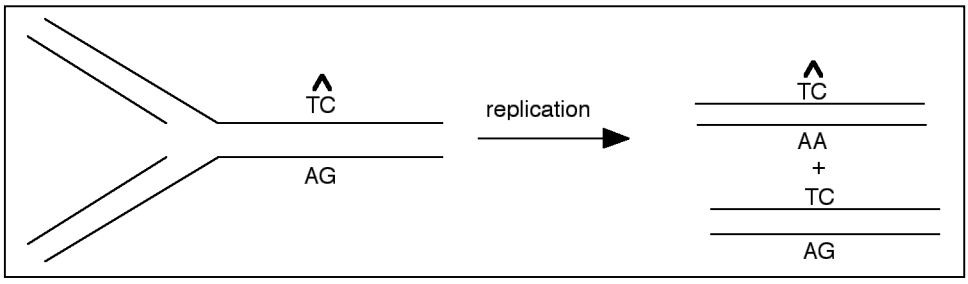
3. Error-prone repair or mutagenic repair (umuC, umuD); also dinB as redundant system

Special system to deal with damage encountered at the replication fork- say if overload the cell with UV damage and get so many pyrimidine dimers that excision repair and photoreactivation can't remove them all

OH TT----->TT
CT---->CT

Simple logic- system allows replication fork to replicate anyway- if can't read base, some such polymerases A residue preferentially (don't mention mutation generated yet) (different lesion bypass polymerases differ in what bases they add)

OH- show fork encountering TC dimer and replicating past with AA



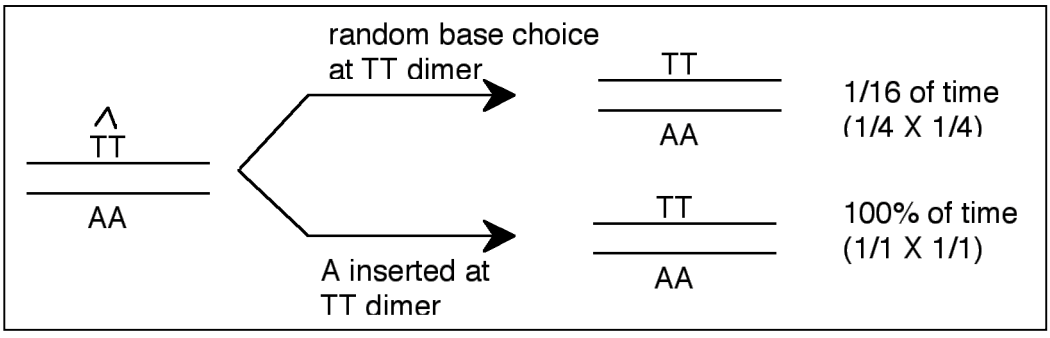
system sometimes generates mutations at sites where A inserted (when original base or bases weren't As)

Why not random? Any advantage to A?

Probably is- back to BOARD

DNA damage often involves pyrimidine dimers from UV
-show how inserting A often corrects damage

OH-Compare replicating past TT dimer with AA always added vs random

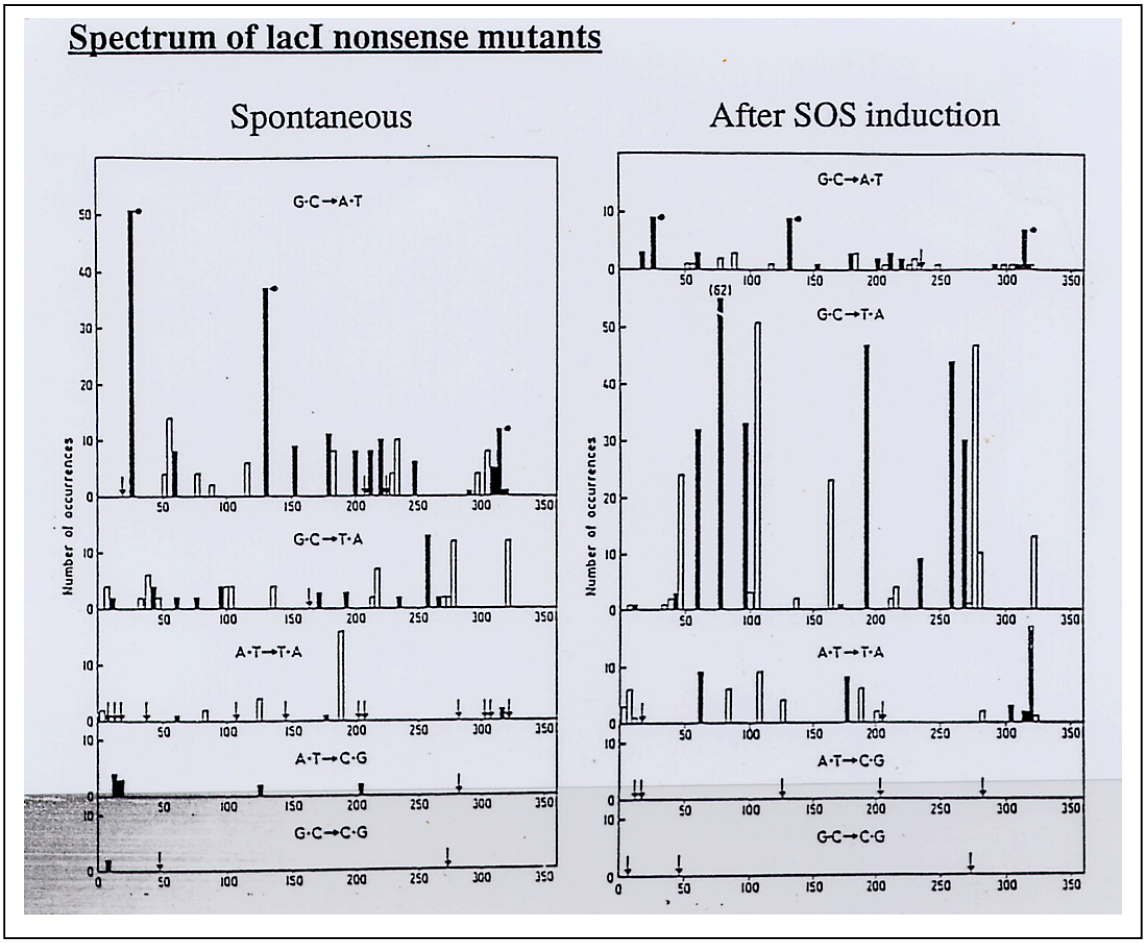


Why error prone?- system makes errors when replicating across unreadable DNA damage. Recent work has shown that system encodes a special DNA polymerase that substitutes for normal DNA polymerase III when unreadable DNA encountered in replication. So important that there's a second such polymerase dinB as well doing similar thing

EXAMPLE- Induce SOS response including error prone replication without DNA damage

HOW?- lexA$^-$ or recA*

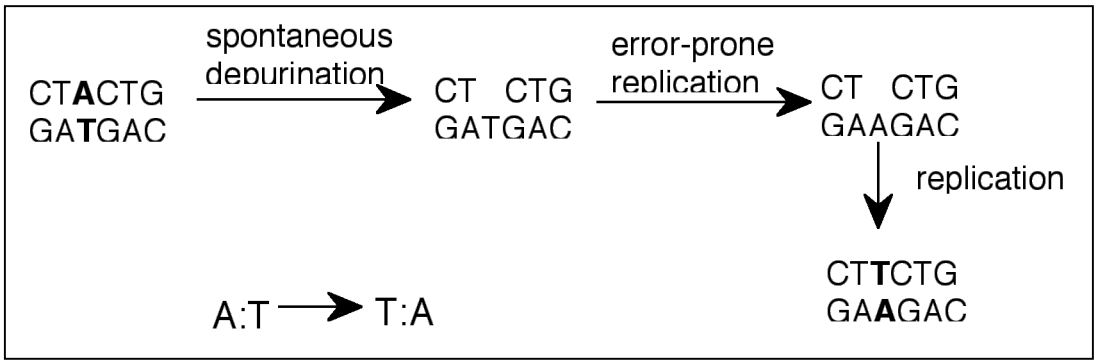
1. 25X increase in lacI- mutation freq
2. Mostly transversions adding A -Fig 9.



Probable explanation return to earlier type of spontaneous DNA damage- spontaneous depurination- loss of A or G. Most common type of unreadable spontaneous DNA damage (uracils from cyt deamination can be read)

AFTER BREAK:
Start with lifespan as function of repair-
mice die after ca 2 yrs full of cancerous
tumors (short and miserable lives)

OH:



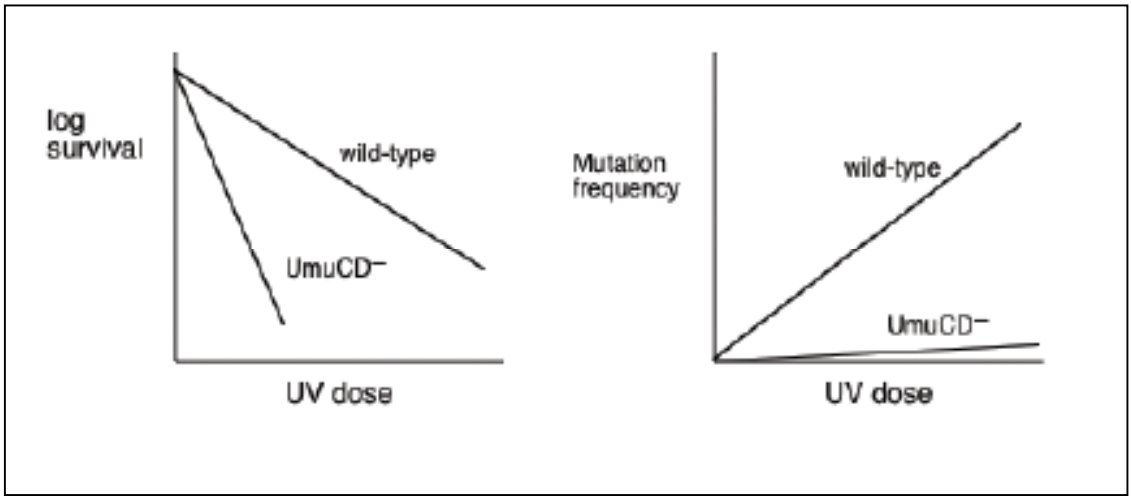
AGAIN- Making mistakes better than stalling replication fork- last ditch mechanism to avoid death

Recent experiment- asks how permissive error-prone repair really is with respect to template. Sustitute C12 for phosphate backbone.

OH- ds DNA with gap with C12 opposite gap-Replicate in SOS-induced cells: get bases added- usually two A's. So system doesn't even require Po4 backbone

ASK Consider error prone repair- mutant: survival and mutation rate after UV irradiation

OH- plots of mutation frequency and killing



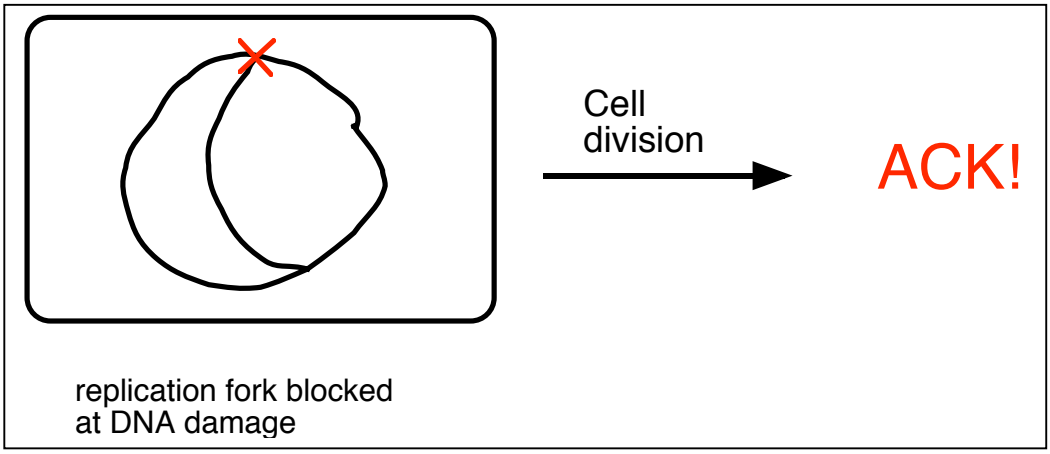
UV sensitivity increased- cant rep and dies
no. mutant cells after UV treatment- DECREASED!- Why?

Cells that would have given mutants by error prone polymerization die instead- so get a decrease in mutation rate due to UV-

Error-prone polymerization is Nature's way of saying that being mutant is better than being dead

Back to BOARD

4. Cell division inhibitor- sulA
blocks cell division until damage repaired- imagine chaos if cell tried to divide and partition chromosomes with stalled replication forks



Draw cell division and FtsZ ring (tubulin-like) and SulÅ inhibition (filament forms)

5. LexA gene- get more LexA during SOS response, tends to limit response

Point is that turning off at the right time is as important as turning on- after all, cell division inhibited, error-prone repair)- require sustained DNA damage to maintain SOS response

BOARD: Minimizing replication errors
proofreading
mismatch repair

UV induced pyrimidine dimers
excision repair
recombinational repair
error prone repair
photoreactivation (book)

Have multiple systems for correcting same mistakes or damage because no single system is fully efficient

Infect mice with wild type or LexA noninducible mutant of *E. coli*, then treat with ciprofloxacin or rifampicin- get resistant mutants taking over population only for wt- indicates SOS mutagenesis important for generation of resistance (the rif resistance is curious one- not due to abt inducing SOS!)