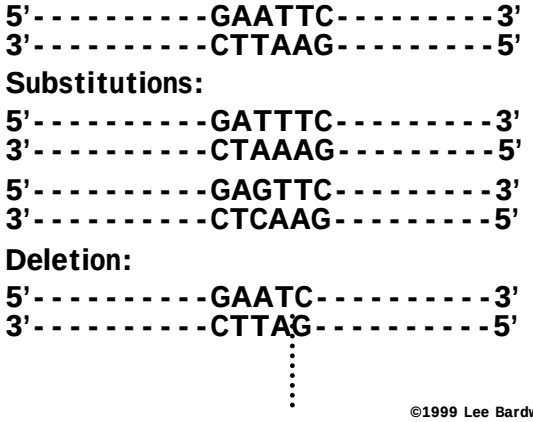
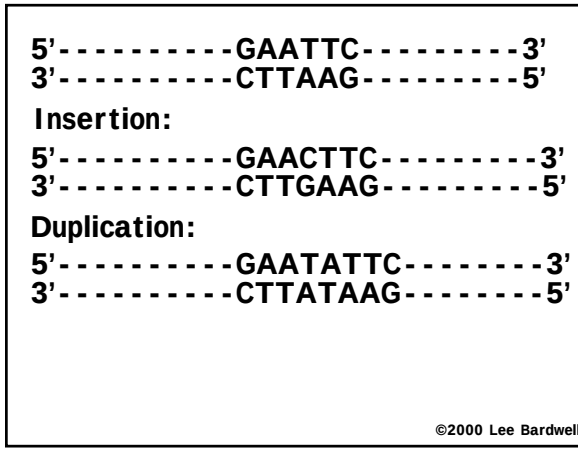
Lecture 10

DNA REPAIR CONTINUED

Types of mutations

- Deletions a part of the DNA is missing anywhere from 1 base pair to parts of chromosomes.
- Insertions of new DNA again ranging from 1 to many base pairs
- Point mutations; a change in the nucleotide. Two types
  - Transitions Purine to other purine or pyrimidine to other pyrimidine.
  - Transversions: Purine to Pyrimidine or Pyrimidine to Purine.





Substitutions that occur in protein-coding sequences

- **Silent** - changes a codon, but not the encoded amino acid residue
  - possible because the code is degenerate
- **Missense** - changes the encoded residue
- **Nonsense** - an amino acid-encoding codon becomes a stop codon

EXAMPLES - substitutions

- **Silent -** TGT (Cys) --> TGC (Cys)
  GCA (Ala) --> GCN (Ala)
  (N = any)
- **Missense -** TGT (Cys) --> TGG (Trp)
- **Nonsense -** TGT (Cys) --> TGA (STOP)

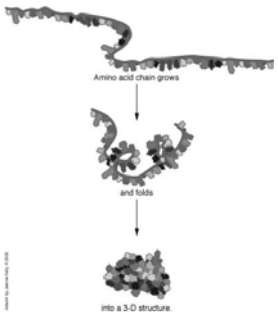
Frameshift example

NH2- Met-Thr-Leu -Lys -COOH
5'-ATG-ACC-TTG-AAA-TAA-3'

Delete A

NH2-Met-Pro -COOH
5'-ATG-CCT-TGA-AAT-AA-3'

SNPs



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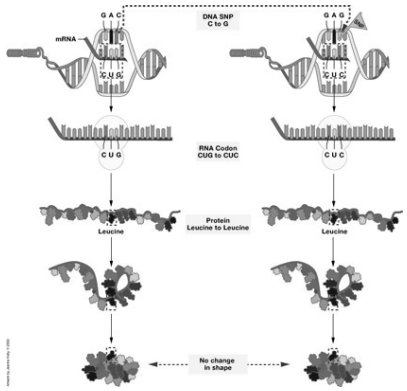

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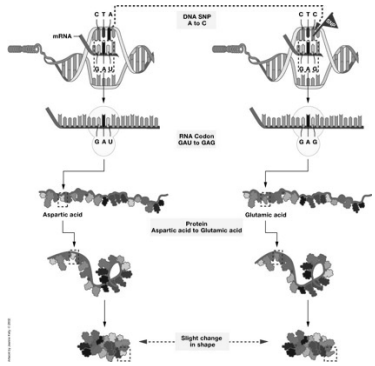
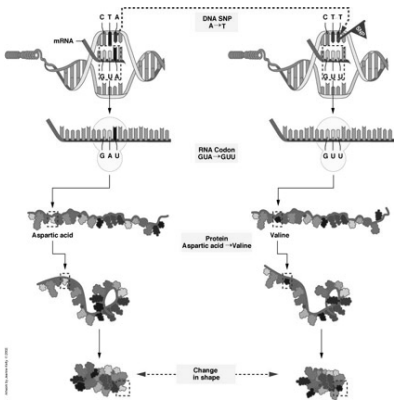


SNPs



SNPs can have variable effects

- SNPs can have no effect. Their change can be neutral to the protein, e.g. a silent mutation.
- SNPs can have a subtle effect, e.g lys to arg (both are polar basic). This is what we suspect is happening in complex genetic diseases
- SNPs can have measurable effects (a pronounced reduction in activity).
- SNPs can change protein function. A new substrate might be recognized.
- SNPs can complete eliminate the proteins ability to function.

Fate of DNA damage

- Tolerated (ignored)
- Repaired
- Can kill the cell or cause the cell to kill itself
- Can become fixed, resulting in a mutation

  (Note: fixed <> repaired)

Examples of mutation fixation

- Replication of an unrepaired misincorporation
- Replication of an unrepaired cytosine deamination

  (deaminated cytosine = uracil)

Human Genome

- Haploid size = 3300 Megabase pairs

  = 3.3 x 10^9 (= billion) base pairs
- Diploid size = double that
- Misincorporation (10^{-5}) x not proofread (10^{-2}) x escape mismatch repair (10^{-3}) = 10^{-10}
- Thus, less than one replication error is fixed per cell division

Mutation Rate per bp

From all sources (misincorps, damage):

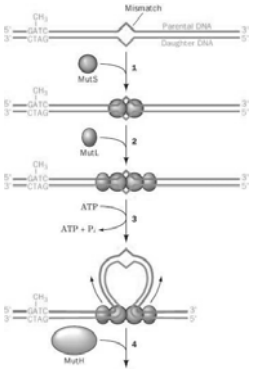
- 10^{-9} per base pair per cell division
- This refers to mutations that are not repaired (i.e. they're *fixed*)
- Thus, there are at least six new base changes in each kid that were not present in either parent, but this is an underestimate as there's more since they accumulate in the germ line stem cells as the father ages
- Remember, most of these are not in genes

Mutation rate per gene

From all sources (misincorps, damage):

- Approx 10^{-5} per gene per cell division
- Human genome contains 30,000-100,000 genes
- Thus, roughly one new mutation (allele) is created per cell division (most likely recessive)

DNA mismatch repair



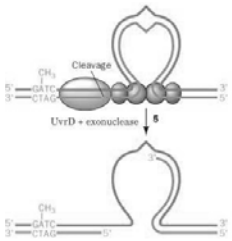
1. **MutS** binds to a mismatched bp

2. MutS-DNA complex binds **MutL**

3. The MutS-MutL complex translocates along the DNA in both directions, forming a loop.

4. On encountering a hemimethylated GATC palindrome, the MutS-MutL complex recrutes **MutH**

MMR cont



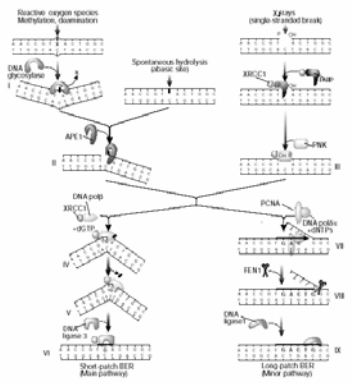
5. MutS-MutL recruits UvrD helicase, which in concert with an exsonuclease, separates the strands and degradates the nicked strand from the nick to beyond the mismatch.
If nick is 3' side: exsonuclease I (3'->5' exsonulcease
If nick is 5' side: RecJ or exsonuclease VII (5'->3' exsonuclease)

6. DNA pol III fills gap and DNA ligase seals nick

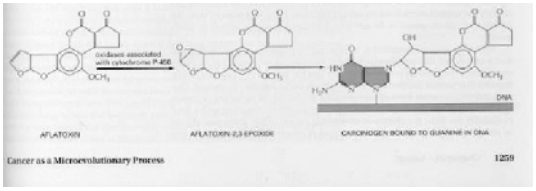
Uracil DNA glycosylase and BER

- **An enzyme that removes Uracil from DNA**
- **Resulting abasic site is filled in by polymerase**
- **Uracil in DNA comes mainly from deamination of cytosine**
- **That may be why DNA uses thymine instead of uracil**
- **If the uracil isn't removed, it will pair with A, causing C/G --> T/A transition.**

BER



Environmental DNA damage



The discovery of NER

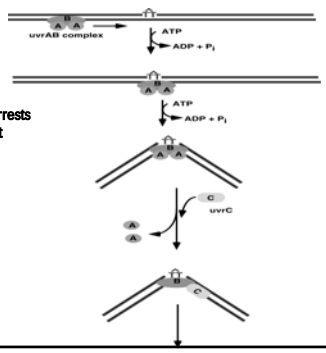
- Setlow found three mutations in E. coli that rendered the cells sensitive to UV damage.
- The genes were named UvrA, UvrB and UvrC for UV resistance.
- Using cell-free extracts, Sancar determined the mechanism of uvrABC which has been refined over the years.

The process of uvrABC excision of DNA damage is called nucleotide excision repair.

uvrA is a ATP-helicase that moves along the DNA looking for damage

When DNA damage is encountered the complex arrests at the site. The DNA is bent

uvrA is released and uvrC is recruited to the complex



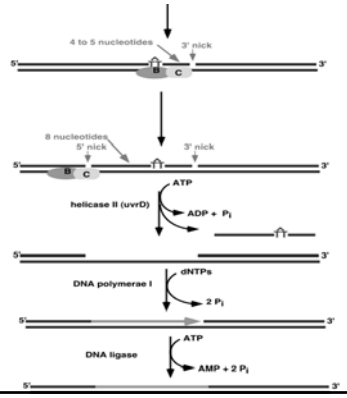
NER in E. coli continued

two single strand nicks are placed to the 3' side of the dimer (4 to 5 nucleotides) and to the 5' side of the dimer (8 nucleotides)

helicase II (uvrD) unwinds 12 - 13 b fragment containing the dimer, ATP hydrolysis required

DNA polymerase I fills in the gap dNTPs required

DNA ligase seals the single strand break ATP hydrolysis to AMP required



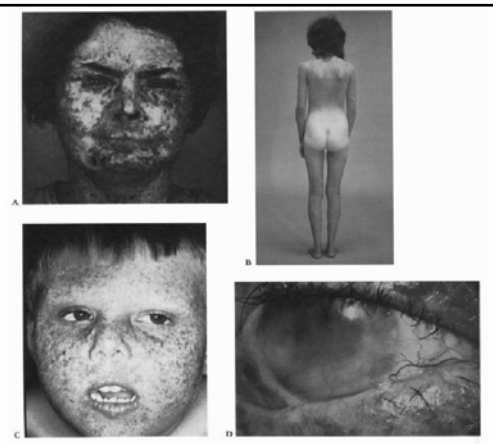
NER in mammalian cells

A disease in humans known as Xeroderma Pigmentosum XP is a rare inherited disease of humans which, among other things, predisposes the patient to

- **pigmented lesions on areas of the skin exposed to the sun and**
- **an elevated incidence of skin cancer.**

It turns out that XP can be caused by mutations in any one of several genes - all of which have roles to play in NER.

James Cleaver went around and collected cells from hundreds of these patients. He then figured out that the disease was made up of eight genes named XP-A through XP-G plus one called XP-V for variant.



There are 8 XP complementation groups

- XP-A participates in photoproduct recognition and DNA binding This binding may be followed by the formation of a quasi-stable complex consisting of XPA, XPC, human single-strand binding protein (RPA/HSSB), and TFIIH, which then acts as a nucleation site for binding of the incision/excision enzymes.
- XP-B is a 3'-> 5' DNA helicase that may be involved in unwinding the DNA 5'-ward of a damaged base
- XP-C is a single-stranded DNA binding protein that is essential for repair of the nontranscribed regions of the genome, that acts in the initial step of damage recognition.
- XP-D is a 5'–3' helicase, a component of transcription factor TFIIH may be involved in 3'-ward unwinding of the DNA in the vicinity of a damaged base

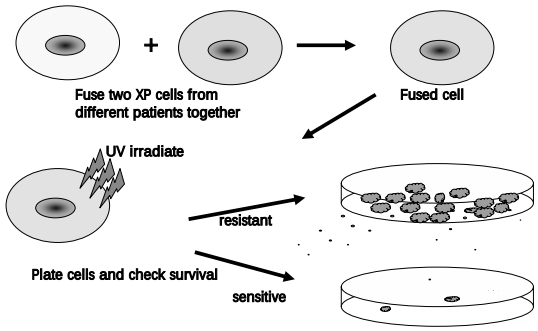
XB Genes continued

- XP-E is thought to be involved with the recognition of damaged DNA because it has the capacity to bind to UV-damaged DNA
- XP-F in association with the ERCC1 protein, incises DNA on the 5' side of the damaged site
- XP-G incises DNA 3' to the damaged site
- XP-V protein is a low-fidelity class Y DNA polymerase, that can replicate UV-induced pyrimidine dimers in vivo with the insertion of the correct bases in the daughter strand
- CSA likely participates in a CSB/RNA polII complex stalled at damaged sites in transcriptionally active DNA that helps remove the stalled RNA polII from the DNA damage site.
- CSB is believed to be a DNA helicase that is required for ubiquitinating RNA polII for its remove and degradation at sites of DNA damage.

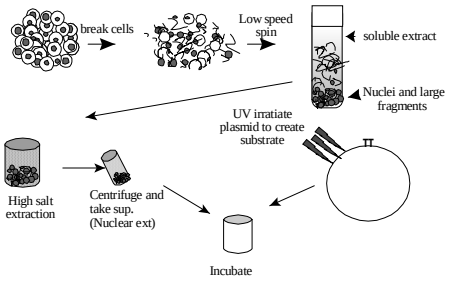
Some XP proteins are

- **XPA, which encodes a protein that binds the damaged site and helps assemble the other proteins needed for NER.**
- **XPB and XPD, which are part of TFIIH. Some mutations in XPB and XPD also produce signs of premature aging.**
- **XPF, with ERCC1 cuts the backbone on the 5' side of the damage**
- **XPG, which cuts the backbone on the 3' side.**
- XPC interacts with HR23B in GGR and recognizes damage
- XPD is a DNA helicase in in transcription complex
- VPV is a by-pass polymerase

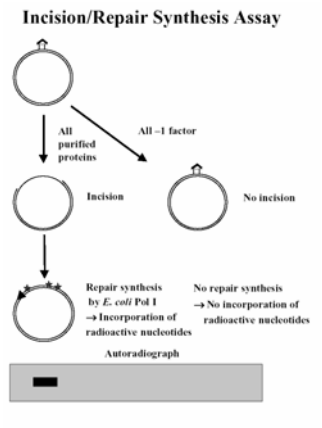
Cleaver's study: Complementation groups of XP



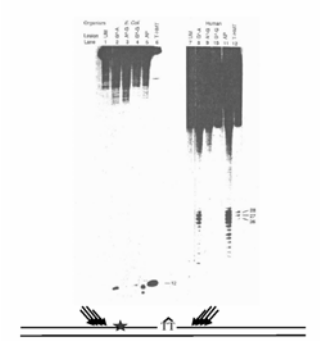
Developing an in vitro DNA repair system



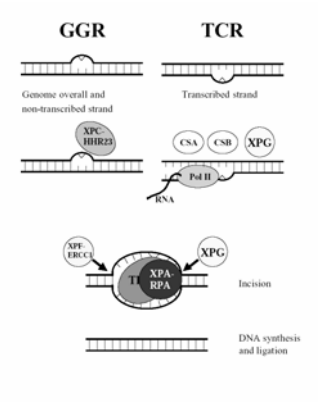
Incision/Repair Synthesis Assay

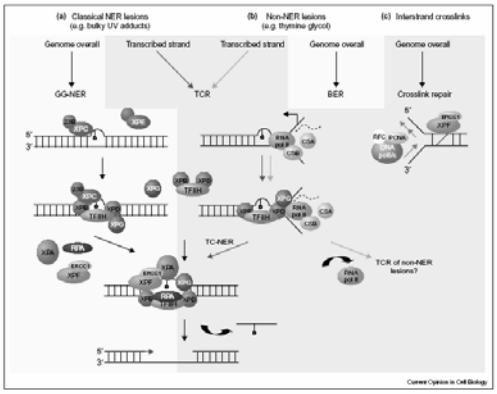


How the incision product is detected



GGR TCR





Global Genomic Repair

- Human global genome NER. (a) In the damage recognition step, the XPC-hHR23B complex recognizes the damage (a pyrimidine dimer in this case), binds to it, and causes localized DNA melting. XPA also aids this process. RPA binds to the undamaged DNA strand across from the damage. (b) The DNA helicase activity of TFIIH causes increased DNA melting. (c) RPA helps position two endonucleases (the ERCC1-XPF complex and XPG) on either side of the damage, and these endonucleases clip the DNA. (d) With the damaged DNA removed on a fragment 24-32 nt long, DNA polymerase fills in the gap with good DNA and DNA ligase seals the final nick.

There are endogenous and exogenous sources of mutagens

Mutagens are any reagent that causes changes in DNA (often referred to as DNA damage) that can ultimately lead to a change in the DNA sequence.

Examples of endogenous reagents are; free radicals generated during oxidation reactions, pH changes that can lead to changes in DNA, errors in DNA replication and recombination errors.

Examples or exogenous reagents are UV radiation, ionizing radiation, chemicals such as benzopyrene and and natural compounds such as aflatoxin.

What do mutagens do?

- Mutagens primarily affect DNA by causing a physical change in the structure, which ultimately alters the sequence, leading to changes in genes such that the information is altered. This leads to loss of a protein, a change in the sequence (and likely structure) of a protein or a change in the level of proteins found in cells.

Types of Mutagens

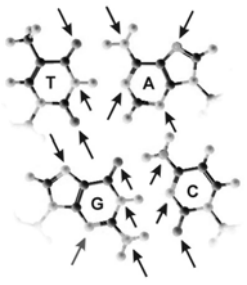
- A variety of chemicals react directly with DNA. Alkylating agents are electrophiles that add methyl, ethyl and more complicated alkyl groups to nucleic acid bases. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in vivo becomes a highly reactive methylating agent. Electrophilic reactants can also be generated by cytochrome P450 oxidation of xenochemicals. These chemicals include benzo[a]pyrene, acetylaminofluorene and aflatoxin. Bulky adducts result. Nitrogen and sulfur mustards (used in chemical warfare) link bases on opposite DNA strands, creating cross-links.

Alkylating agents

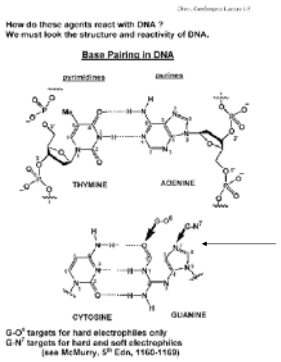
$ClCH_2CH_2NMeCH_2CH_2Cl$

- HCl

Reactive sites on nucleotides



Alkylating agents



Nitrogen mustard reacts with the N^7 position of guanine

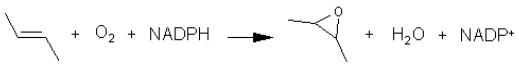
Mutagens don't always start out that way

- Many compounds that enter out cells are lipophilic (typically organic compounds). These compounds are not reactive with DNA. A system of enzymes called P450 monooxygenases add oxygen molecules in order to make them more soluble but this also makes them reactive with DNA.

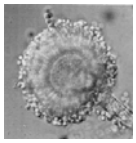
Cytochrome P450 monooxygenase system

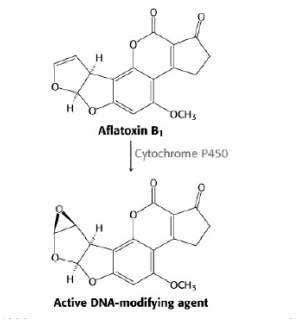
- Xenobiotics are chemical compounds that do not belong to the normal composition of the human body. These compounds enter the body via the diet, air and medication. The principal route of elimination of xenobiotics from the body is biotransformation. They are eliminated by microsomal phase I and microsomal and cytosolic phase II drugmetabolising enzymes. These enzymes add functional groups to make lipophilic molecules more hydrophilic and hence easier to eliminate. The oxidative reactions are mainly catalysed by cytochrome P450 (CYP or P450) enzymes. The CYP superfamily of microsomal hemoproteins catalyses the monooxygenation of a large number of endogenous and exogenous compounds. They play a key role in the metabolism of a wide variety of xenobiotics, such as drugs, pesticides and (pre)carcinogens.

General reaction

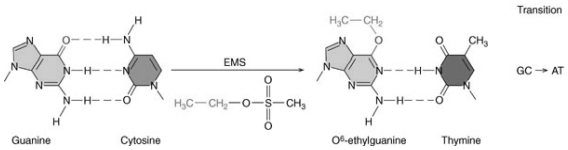


Aflatoxin reaction

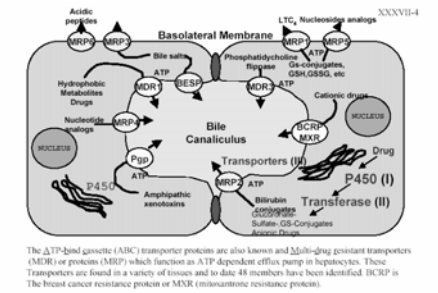




EMS modification of DNA

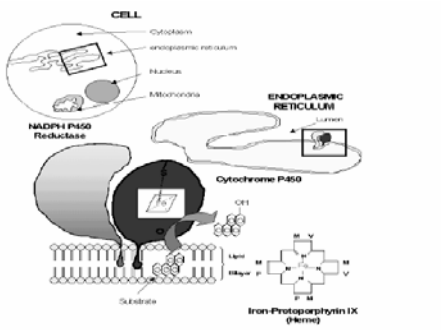


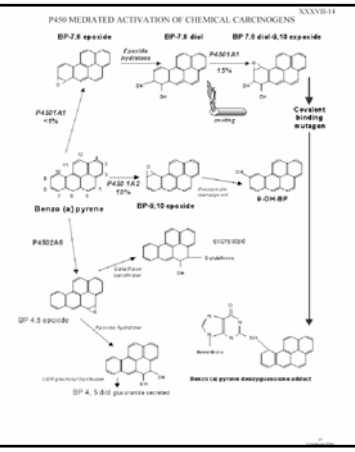
P450s are found in all cells but mostly in liver and small intestine



The ΔTP-bind cassette (ABC) transporter proteins are also known and Multi-drug resistant transporters (MDR) or proteins (MRP) which function as ATP dependent efflux pump in hepatocytes. These Transporters are found in a variety of tissues and to date 48 members have been identified. BCRP is The breast cancer resistance protein or MXR (mitoxantrone resistance protein).

P450 at work

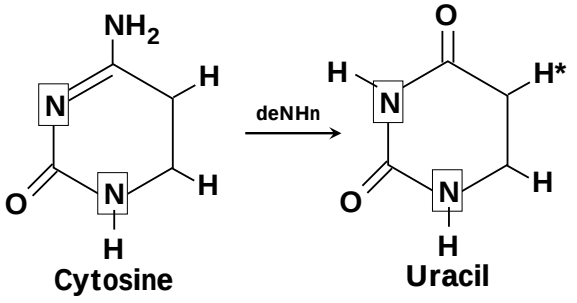




Endogenous DNA damage

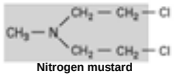
- **Replication errors**
  - **Polymerase misincorporation**
- **Recombination errors**
  - **Unequal crossing over, etc.**
- **Spontaneous base damage**
  - **Deaminations, depurinations**
- **Byproducts of metabolism**
  - **Oxygen radicals**

Deamination of Cytosine



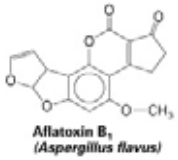
***Thymine has CH3 here**

Sources of exogenous DNA damage



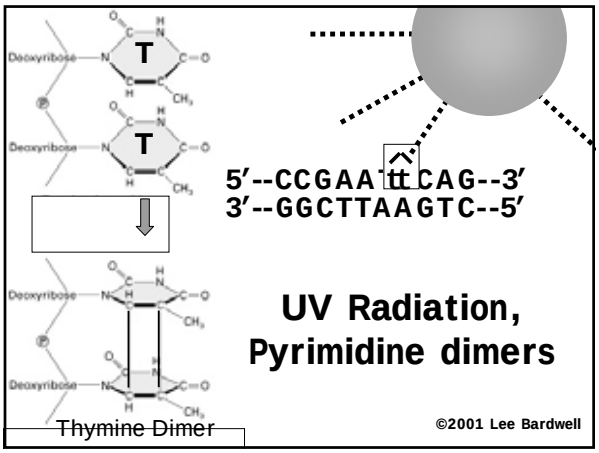
Nitrogen mustard

- **Chemicals**
  - **Natural**
    - **In foods, e.g. aflatoxin**
  - **Man-made/man-increased**
    - **Nitrogen Mustard - WWI nerve gas**
    - **Benzopyrene - smoke from coal, autos, cigs**
- **Ultraviolet (UV) Radiation (from sun)**
- **Ionizing radiation**
  - **Natural: radon gas, cosmic rays**
  - **Man-made: x-rays, nuclear tests**



Aflatoxin B_1 (*Aspergillus flavus*)

Benzo(a)pyrene



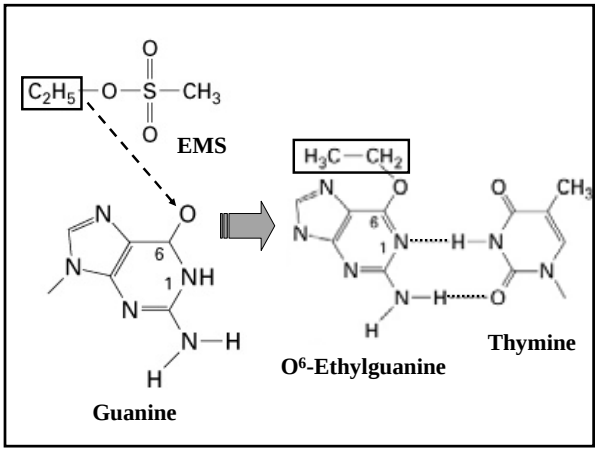
How does DNA damage cause mutations?

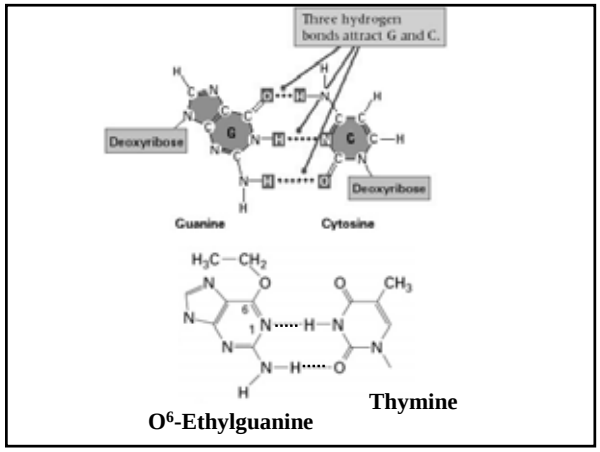
In many ways

One example:

When a damaged base mispairs

- **O^6-ethyl-guanine pairs with thymine**
- **Deaminated cytosine pairs with adenine**





Frameshift

See the man pat the pet cat

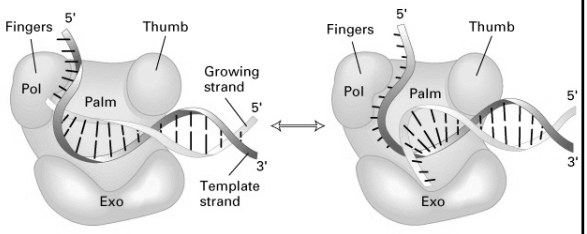
See tem anp att hep etc at

Examples of repair mech's

- **Polymerase proofreading**
- **DNA mismatch repair**
- **Uracil DNA glycosylase**
- **Nucleotide excision repair**

DNA polymerases

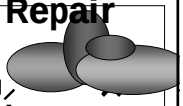
- Are proteins that replicate DNA
- Have multiple domains or subunits
- A good polymerase domain has a misincorporation rate of 10^{-5} (1/100,000)
- Any misincorps are clipped off with 99% efficiency by the "proofreading" activity of the polymerase



Nucleotide Excision Repair

- Carried out by a multi-protein complex
- Removes bulky adducts from DNA, e.g.
  - Pyrimidine dimers caused by UV
  - Benzopyrene-DNA adducts
- Nearby nucleotides are also excised
- Resulting single-strand gap is filled in by polymerase

Nucleotide Excision Repair



5′--------CCGAAttCAG-------3′
3′--------GGCTTAAGTC-----5′

DNA Damage

1. Damage Recognition
2. Dual Incisions
3. Excision
4. Gap-Filling

-CCGAATTCAG-

5′--------CCGAAttCAG-------3′
3′--------GGCTTAAGTC--------5′

INCISION

5′-------CCGAAttCAG------3′
3′--------GGCTTAAGTC--------5′

EXCISION

5′-------- -------3′
3′--------GGCTTAAGTC--------5′

GAP-FILLING (BY A POLYMERASE)

5′--------CCGAATTCAG-------3′
3′--------GGCTTAAGTC--------5′

There are many other types of DNA damage, and many other DNA repair enzymes/pathways.

DNA Repair part 2

Repair of other DNA damage

How big is the problem?

- Consider this:

10 bp is one helical turn which is 0.34nm (3.4×10^{-10} m)

There are $3X10^9$ bp of DNA per haploid human genome

There are 2 genomes/cell (diploid)

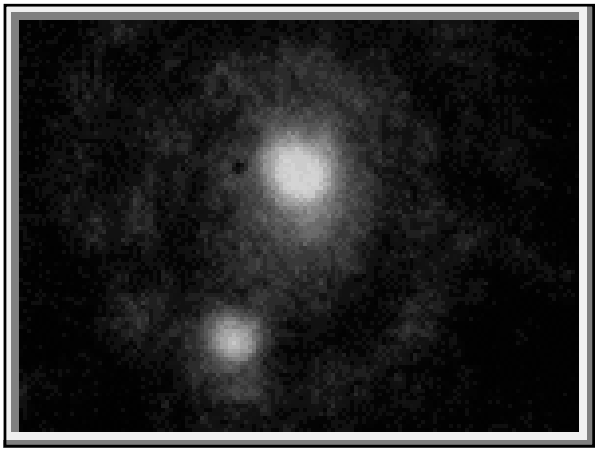
There are approximately 10^{14} cells/individual

So:

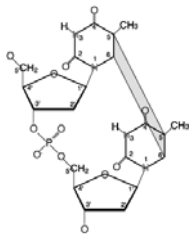
(3.4×10^{-10}) $(3X10^9)$ (2) (10^{14}) = 2×10^{14} meters

2×10^{14} meters/ 3×10^8 m/sec = 6.7×10^5 light sec

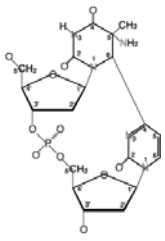
6.7×10^5 /60sec/60min/24hr = 7.7 light days or 1 light week or 6 round trips to pluto.



Types of UV damage

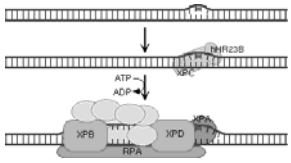


cyclobutan pyrimidine dimer -CPD

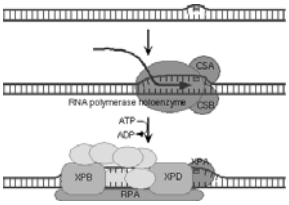


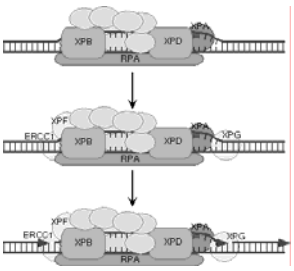
6-4 pyrimidine pyrimidone –6-4PP

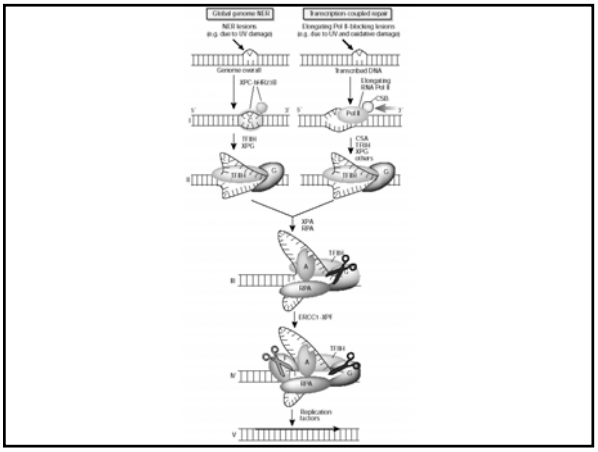
GGR

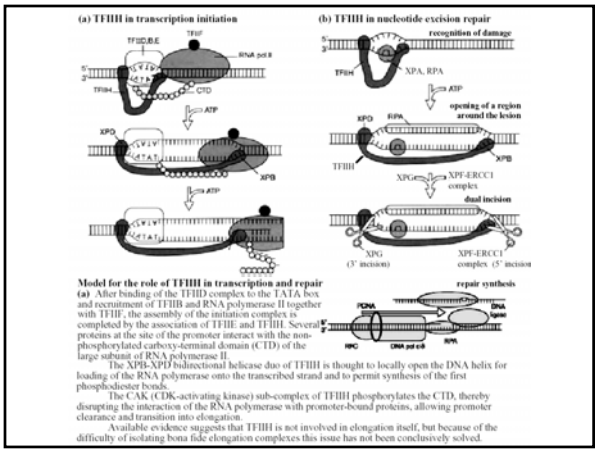


TCR









Model for the role of TFIIH in transcription and repair

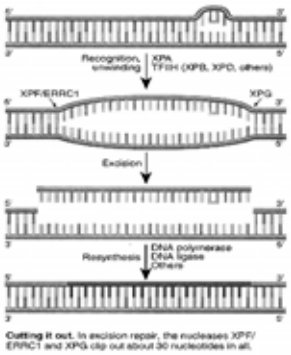
(a) After binding of the TFIID complex to the TATA box and recruitment of TFIIB and RNA polymerase II together with TFIIF, the assembly of the initiation complex is completed by the association of TFIIE and TFIIH. Several proteins at the site of the promoter interact with the non-phosphorylated carboxy-terminal domain (CTD) of the large subunit of RNA polymerase II.

The XPB-XPD bidirectional helicase duo of TFIIH is thought to locally open the DNA helix for loading of the RNA polymerase onto the transcribed strand and to permit synthesis of the first phosphodiester bonds.

The CAK (CDK-activating kinase) sub-complex of TFIIH phosphorylates the CTD, thereby disrupting the interaction of the RNA polymerase with promoter-bound proteins, allowing promoter clearance and transition into elongation.

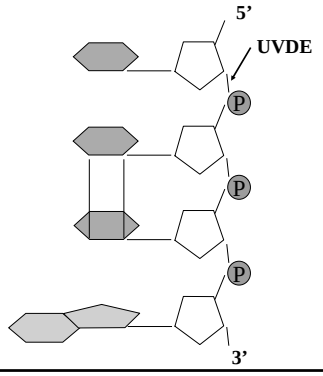
Available evidence suggests that TFIIH is not involved in elongation itself, but because of the difficulty of isolating bona fide elongation complexes this issue has not been conclusively solved.

Nucleotide Excision Repair

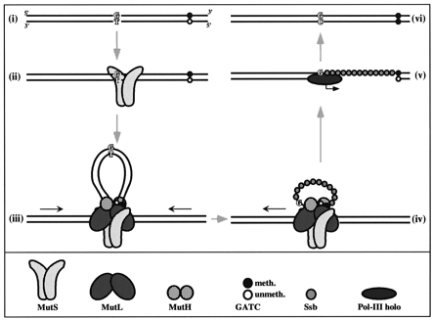


Cutting it out. In excision repair, the nucleases XPF/ERRC1 and XPG clip out about 30 nucleotides in all.

How UVDE works



Mismatch repair

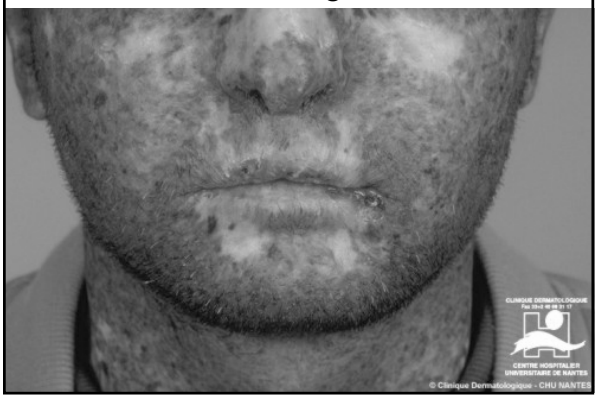


DNA repair diseases

Xeroderma Pigmentosum

- **Autosomal recessive, multigenic, very rare**
- **Symptoms:**
  - **Dry scaly skin (xeroderma)**
  - **Freckling; pigmentation abnormalities (pigmentosum)**
  - **Extreme sensitivity to sunlight**
  - **Greatly increased incidence of skin cancer (1000 X)**
  - **Neurological abnormalities**
- **Defect in nucleotide excision repair**

Xeroderma Pigmentosum



DNA repair diseases

HNPCC

- **Heriditary nonpolyposis colorectcal cancer**
- **Autosomal dominant, multigenic, up to 1/200**
- **Symptoms:**
  - **High frequency of colon and several other cancers**
- **Defect in mismatch repair**