DNA Damage and Repair I **Jason Kahn, Biochemistry 465, 4/18/06**

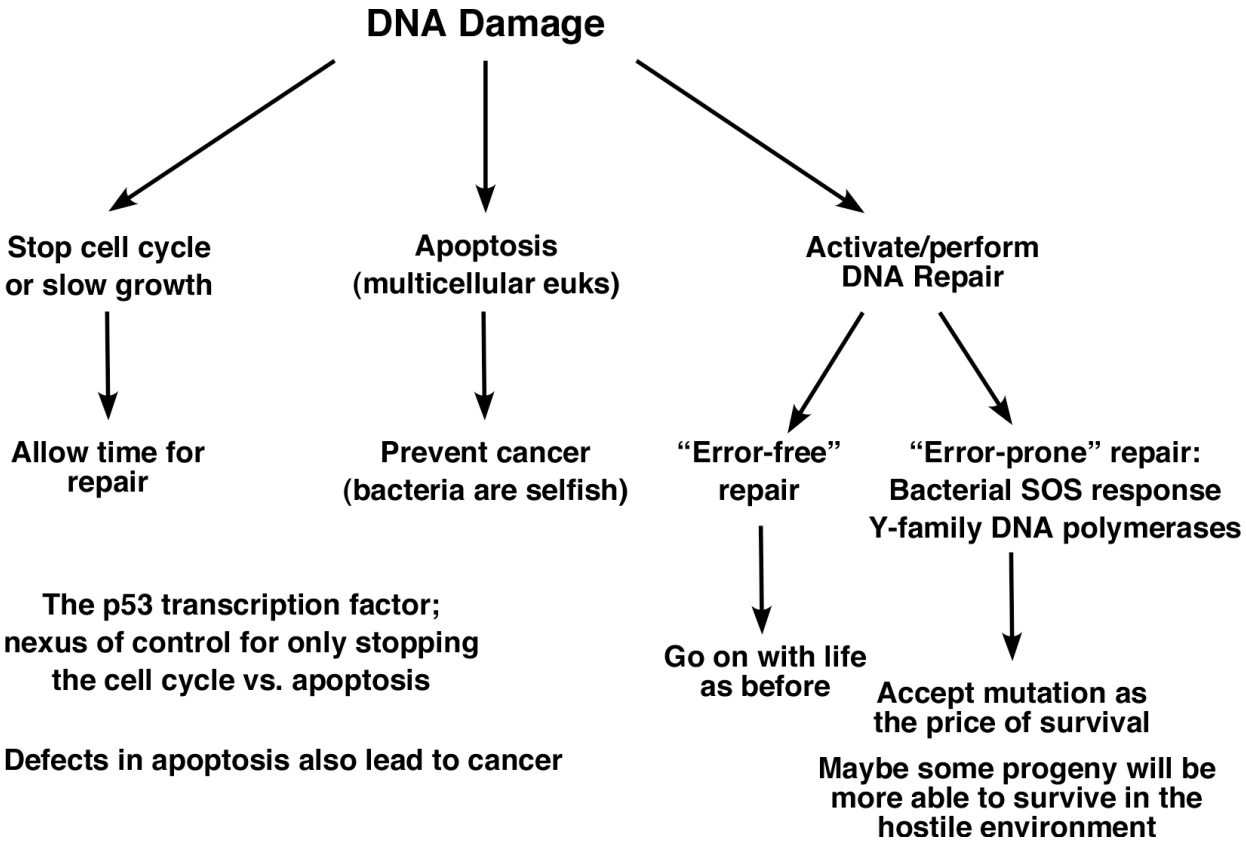
Outline:

- **General considerations about DNA damage responses**
- **Chemistry: Sources, types, and consequences of DNA damage**
- **Repair Mechanisms: Direct reversal, BER, NER (next time: Mismatch repair, recombination-mediated repair, coupling to transcription)**

General considerations

DNA damage is an inevitable consequence of using water in the cytoplasm, breathing air, eating food, and being exposed to sunlight and cosmic rays. Cells have active mechanisms to repair the damage, and defective repair mechanisms can lead to profound developmental problems as well as greatly increased cancer susceptibility. Examples of human diseases include HNPCC (human non-polyposis colon cancer, due to a defect in mismatch repair), XP (Xeroderma pigmentosa, due to defective nucleotide excision repair, NER).

We've already seen some of the responses to damage when we studied checkpoints in the eukaryotic cell cycle. Here's a flowchart for the responses:



"Error free" repair uses the other strand of the DNA or another copy available in the cell to make a perfect new version with the same information as existed before the damage occurred.

"Error-prone repair" can be trans-lesion synthesis, random joining of DNA ends: anything that doesn't use the original information in the DNA to make a clean copy. As we've said, DNA damage doesn't really matter until the cell actually tries to use the information for replication or transcription. We won't deal with it further.

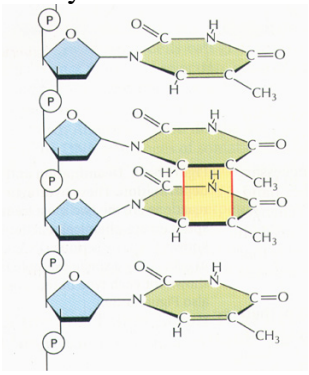
Balance between apoptosis and allowing some level of mutation is quite tricky…

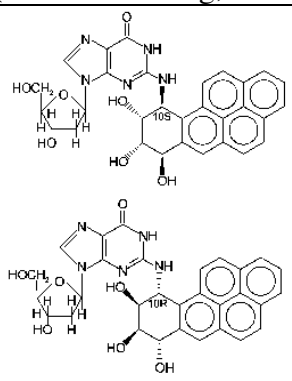
Chemistry of DNA Damage

Sources/types of DNA damage:

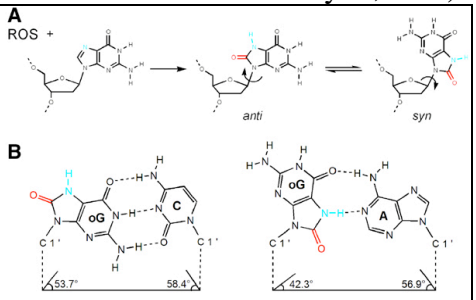
- Adducts like 8-oxo-guanosine (from ROS), thymine dimers (from UV), psoralen crosslinks (from picking vegetables in the sun; picture on web page), or benz-pyrene diol epoxide adducts to G (from barbecuing, via epoxidation in the liver. Thank you, liver).

Cis-syn T<>T





BPDE from http://www.nyu.edu/its/pubs/connect/archives/98spring/broydedna.html

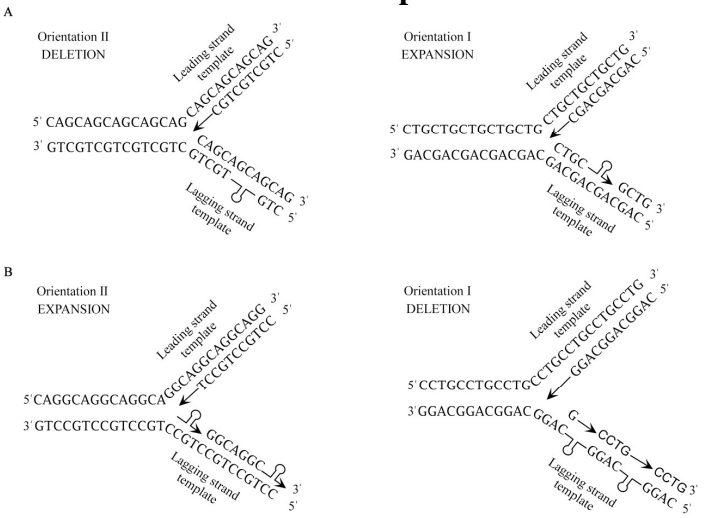


8-oxoG miscoding from Brieba et al., 2004. *EMBO J.***23**:3452-61 (Ellenberger group)

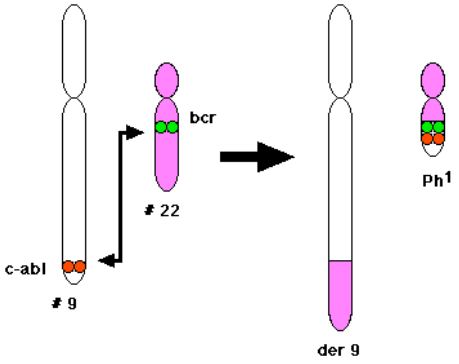
- Mismatches and small loops, from the occasional C to U deamination reaction, misincorporation or slippage error (repeated synthesis of one or a few template nucleotides, see below) in DNA polymerization.
- Strand breaks, with double-strand breaks (DSB's) being much worse, from ionizing radiation or collapsed replication forks.

Consequences of DNA damage:

- Translesion synthesis or copying of mismatches/loops leads to **substitution or frameshift mutations**. **(And it's not until the DNA is copied that this kind of damage really causes a problem.)** For example, triplet repeat expansion = repeated loop slippage is the root of many neurological diseases, including Fragile X syndrome and Huntington's disease. The diseases can be caused by misfolding of the resulting protein or because of the properties of the DNA/RNA. Interesting example of a genetic disease in which symptoms get worse with each generation as the repeat expands; initially clinicians pointed this out and basic scientists didn't believe it. Image from Wells et al., Nucleic Acids Research 2005 33(12):3785-3798; doi:10.1093/nar/gki697, no need to draw out.



- **Inappropriate end joining** due to DSBs can give chromosomal translocations: for example, chronic myelogenous leukemia is caused by a translocation that produces the BCR-ABL fusion protein, a tyrosine kinase that leads to impaired control of the cell cycle. Treated with the kinase inhibitor Gleevec. [No need to redraw picture, which is from users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/CML.html]



Reciprocal translocation between one # 9 and one #22 chromosome forms an extra-long chromosome 9 ("der 9") and the Philadelphia chromosome (Ph^1) containing the fused abl-bcr gene. This is a schematic view representing metaphase chromosomes.

- Excessive DNA damage leads to apoptosis, or plain old death if the DNA is too heavily sheared. Some bacteria are highly resistant because they have multiple copies of their genomes and can reassemble them after they are cut up.

Review general ideas and lead in to repair:

- **DNA damage is inevitable.**
- **Miscoding lesions and other types of damage can kill or dys-regulate the cell. (Many modifications, like 6-methylA, are normal, not miscoding).**
- **The consequences of a type of lesion depend on three things, something like: Consequences = (rate of appearance – rate of repair) × "seriousness"**
- **As might be expected, there are diverse repair mechanisms to handle the diverse types of damage.**
- **But…as in most of metabolism, a large number of metabolites feed in to a small number of common intermediates, so the number of pathways is much smaller than the number of adducts.**

Mechanisms of Repair

Roughly in order of how complicated they are, and in order of decreasing specialization. Common types of damage have dedicated pathways, less common types have more general pathways that may not be as fast or accurate.

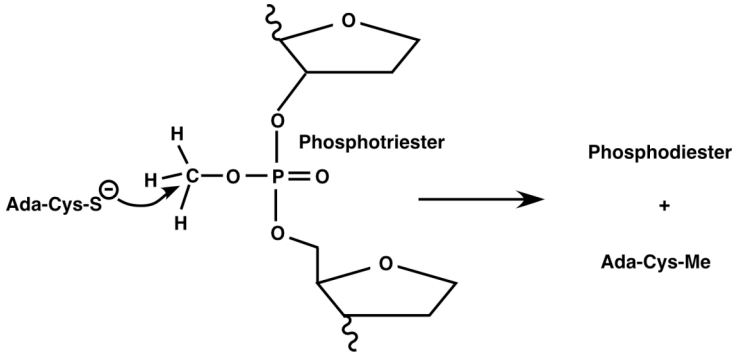
1. Direct Reversal.
2. Base excision repair = BER
3. Nucleotide excision repair = NER or short patch repair
4. Mismatch repair, in E. coli it's methyl-directed mismatch repair, MMR
5. Recombination-mediated repair of crosslinks, DSB's

1. Direct Reversal: Fix the damaged base/adduct in situ

Specialized handling of commonly-encountered simple adducts. This is the only pathway that does not require DNA polymerase activity.

Example: Ada methyltransferase

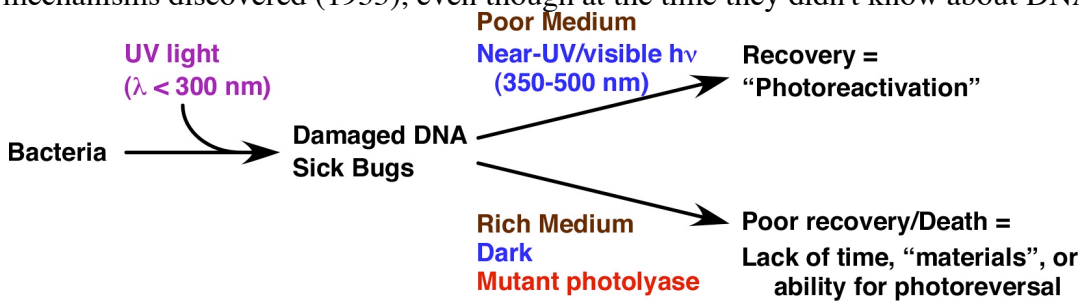
The Ada methyltransferase repairs O^6-methylG (miscoding lesion…students should draw it and figure out why, again) and phosphotriesters. It has a nucleophilic active-site Cys that ends up methylated, and the DNA is regenerated.



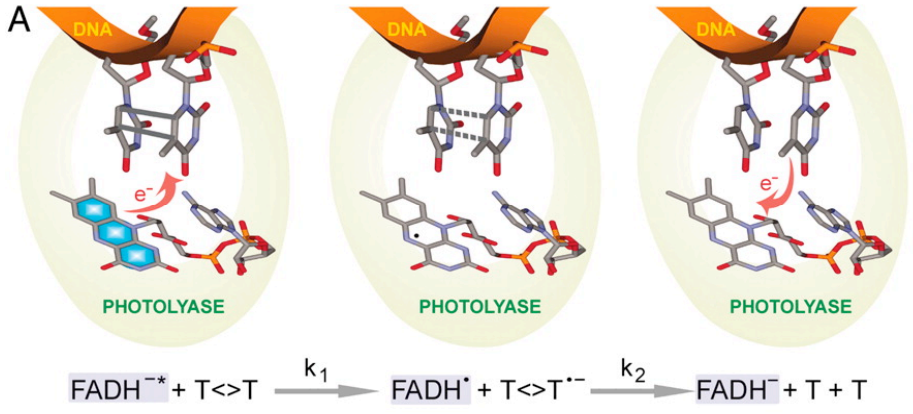
Note that the protein methylation is irreversible: strictly, this is a stoichiometric reaction, not an enzymatic one. **This illustrates a general principle of repair: it's expensive, and evolution has decided that it's worth it!** The Ada-Cys-Me is put to use, however – it's a transcription factor that activates it's own expression and other repair pathways!

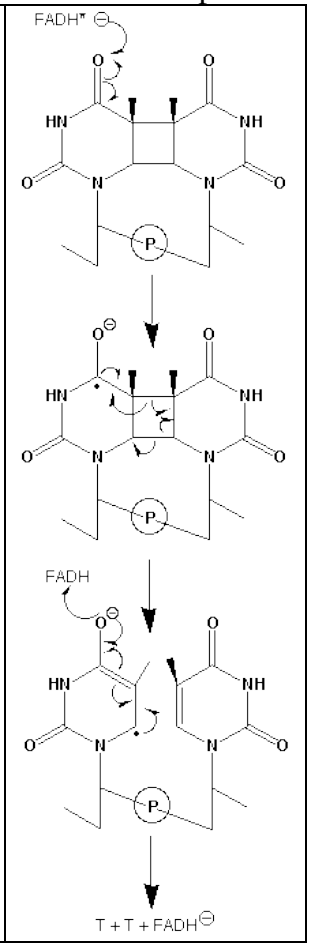
Example: DNA photolyase

This enzyme repairs thymine photodimers in all three kingdoms. We don't have it, sigh. In some cases, photolyase can block or target repair of adducts even if it is inactive. (One of?) the earliest repair mechanisms discovered (1935), even though at the time they didn't know about DNA:



Why poor media? **Slow growth avoids attempts to copy the lesions**, allows time for repair.





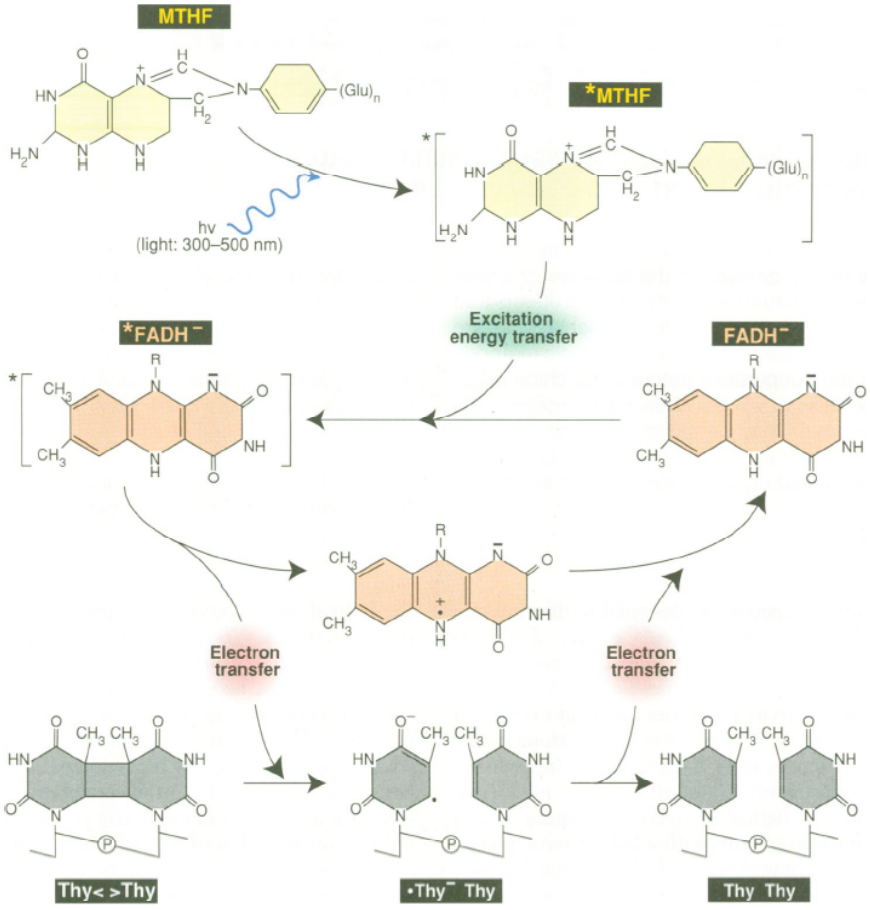
Mechanism of photolyase? Reduced FADH- absorbs blue light photon [actually it is usually excited by an "antenna chromophore," a bound MTHF = methenyl tetrahydrofolate, viz. photosynthesis]. The **excited state FADH-* is a stronger reductant than the ground state, transfers an electron to the thymine dimer, the thymine dimer anion radical breaks down to T•- + T, and the electron is transferred back to the FADH.** Arrow pushing only for the curious.

Sources:

Hearst, 1995. *Science*, **268**, 1858-1859 (Perspective)

Kao et al. (Sancar group), 2005. *PNAS*, **102**, 16128-16132 (fig. above).

Detail on the antenna chromophore from Hearst 1995 for the exceptionally curious:



The mechanism of photoreactivation by photolyase.

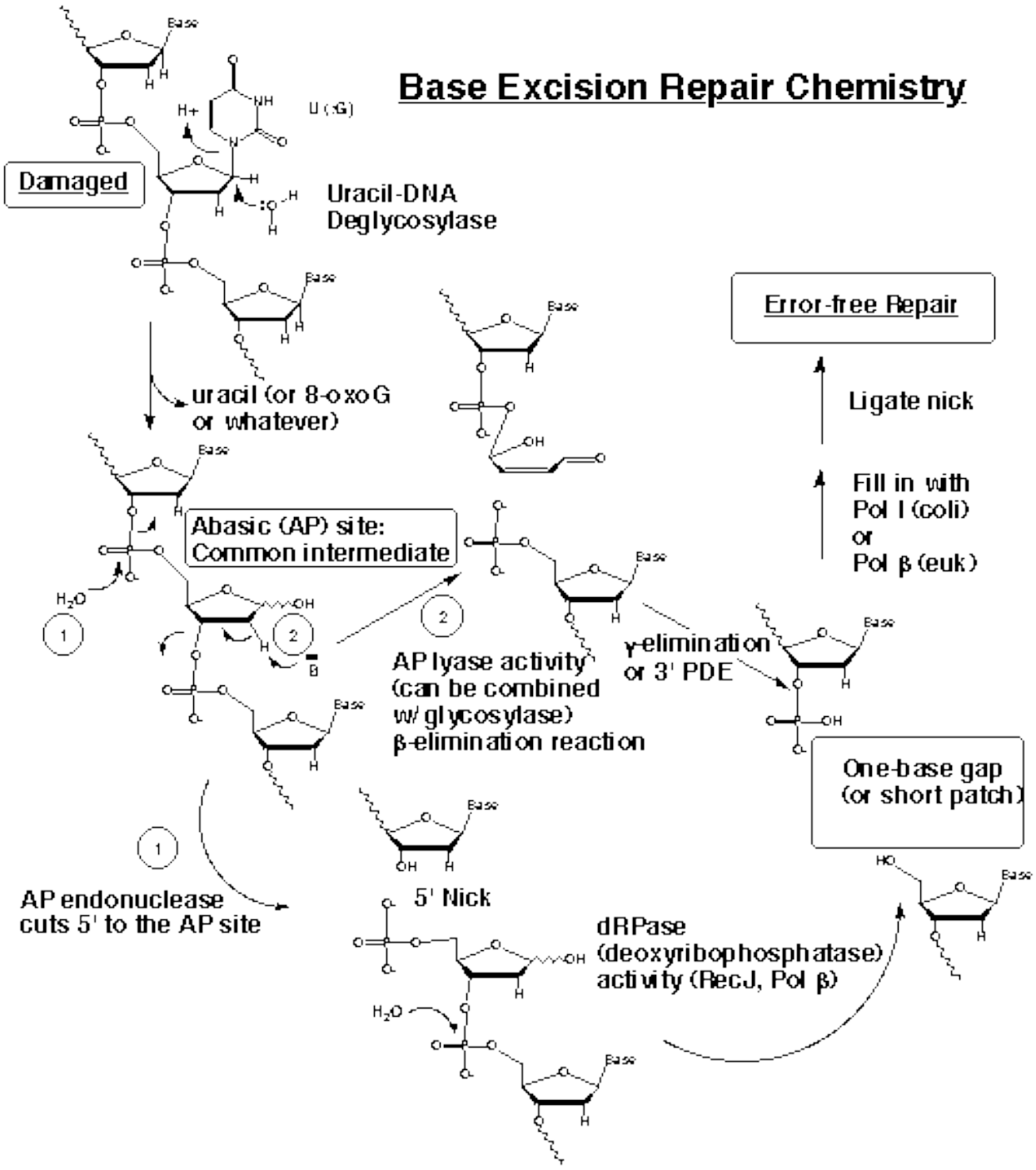
2. Base Excision Repair (BER)

Function: Repair of common small lesions, especially methylated or oxidized bases, or uracil in DNA resulting from spontaneous deamination of cytosine.

The first step is **specific recognition of the lesion by one of many specialized glycosylases, for example uracil-DNA glycosylase = UDG.**

The glycosylase removes the offending base, giving an **abasic site** or **AP site** (apurinic/apyrimidinic site). The AP site can also result from acid treatment leading to spontaneous depurination (purines are more sensitive to acid – student should protonate N-7 and ask why the base becomes a good leaving group). In footprinting with DMS, methylation promotes depurination and then base treatment cuts the backbone.

The **AP site is thus a common intermediate**. It is processed through at least two pathways, but both end up with a **missing nucleotide that is replaced by a DNA polymerase** and the nick is then sealed.

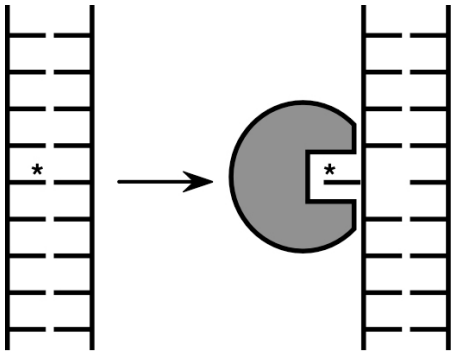


Glycosylases T4 endo V and Endo III also have AP lyase activity. The β-elimination breaks the chain. I show it here so that we see a chemical reaction other than a transesterification!

Pol β may be dedicated to repair, but repair can also be carried out by pol δ/ε and PCNA.

Key idea is that BER (and NER, below) can use **non-processive** polymerases.

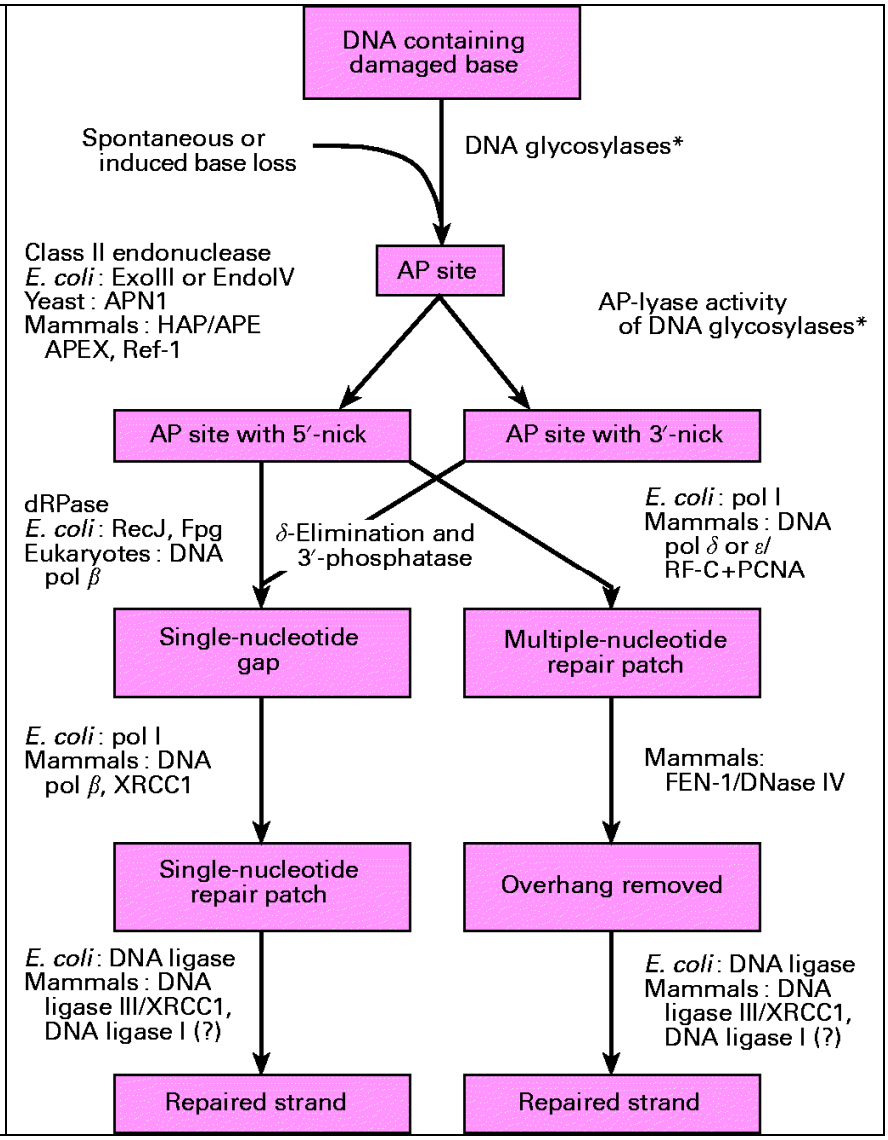
The common feature of the glycosylases, photolyase, and methylase enzymes—anything that does chemistry on the bases—is that they tend to **flip out the base** and do chemistry on the extrahelical base.



Recognition of damage is a matter of current research. The search process may be sensitive to enhanced DNA flexibility or decreased base pair stability as well as to steric effects like protrusions in the grooves.

Way too much detail on BER pathways: just for reference.
From Krokan et al.,

Biochem. J. (1997) 325, 1–16

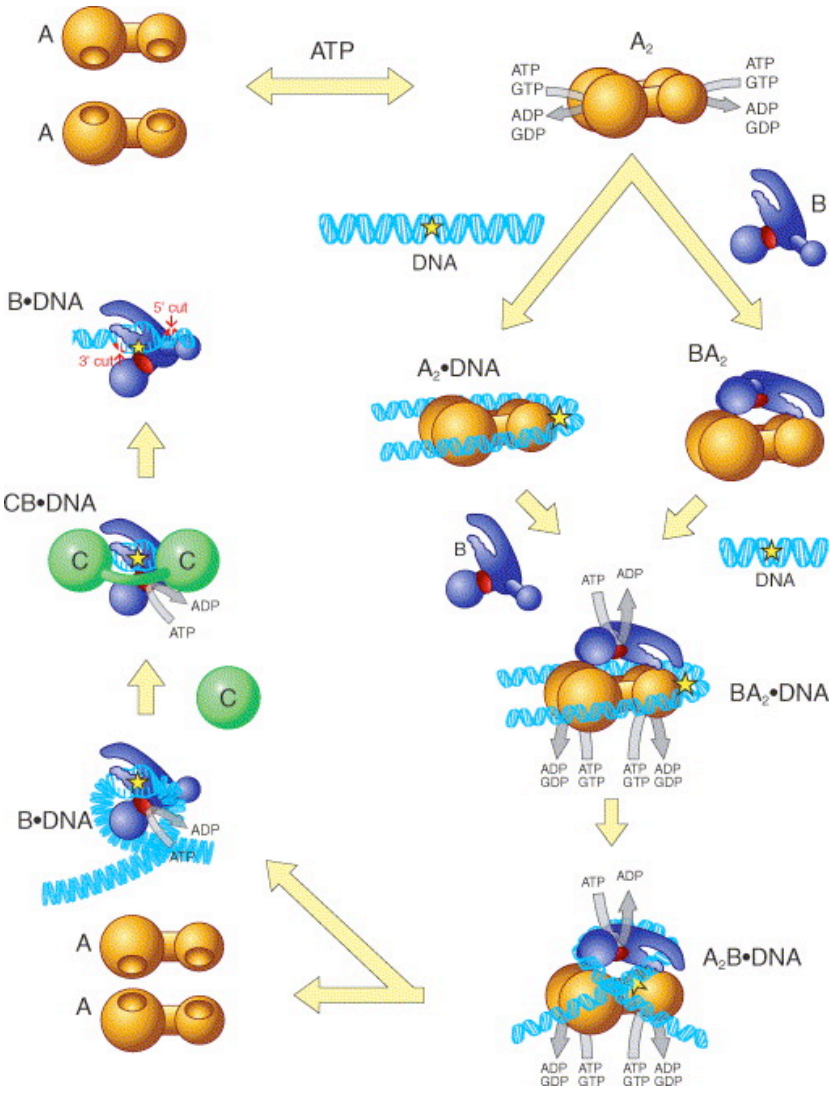


3. Nucleotide Excision repair (NER)

This is the most versatile pathway. One of the more interesting aspects of repair is that **repair, unlike most enzymatic reactions we deal with, needs to be able to deal with never-before-seen substrates**. Citrate synthase uses ……oxaloacetate and acetyl-CoA, every single time. The NER system has to deal with the latest greatest novel compound floating downstream from Novartis or Dow or whoever.
NER is also the pathway connected with transcription-coupled repair, which we will see later.

In *E. coli*, the main NER proteins are **Uvr A, B, and C.**
In humans, XP(A,B,C,D,E,F,G) and others. XPB and XPD are the helicase subunits of TFIIH.
What is the pathway? UvrA dimer tracks along DNA looking for damage, either alone or with UvrB bound. UvrB cannot bind DNA on its own. Upon locating a lesion, UvrA dissociates from the A2B•lesion complex, leaving UvrB behind on the DNA. UvrA is an example of a **molecular matchmaker**, an enzyme whose function is to deliver another protein. Some molecular matchmakers are AAA+ proteins, which we have seen before, but UvrA is an ABC-type ATPase with a fold characteristic of transporters, and UvrB is a helicase – both are ATPases.
The **UvrB-DNA complex has the DNA wrapped around UvrB. It is metastable: breakdown is irreversible. This is another example of kinetic proofreading/molecular clock.** If the UvrB has been mis-delivered to undamaged DNA, it should break up before DNA cleavage. Two independent checking steps increase the fidelity of recognition.
ATP energy is used for DNA deformation and to enable an irreversible checking step, as we have seen in general for kinetic proofreading.
Then **UvrC binds the UvrB-DNA complex and makes two nicks at the 5' side and 3' side of the lesion, excising a 12-13 nt oligonucleotide** (25 nt in euks). Sometimes the complex is called the Uvr (A)BC excinuclease because A is essential but not present during excision. Division of labor means that the **UvrC nuclease isn't running around cutting up cellular DNA randomly.** UvrC dissociates but UvrB remains until removed by Helicase II = UvrD. The resulting gap is filled by Pol I (in euks, PCNA + Pol δ or ε), and the gap is sealed by ligase.
Error-free repair.

Legend redundant with above:
Fig. 1. Graphic representation of catalytic mechanism. A hypothetical scheme for the key steps in the mechanism is shown; see the text for references and a more complete description. In solution, two molecules of UvrA form a dimer, presumably between the ABC ATPase modules and ATP binding drives dimer formation. The $UvrA_2$ complex possesses ATP/GTPase activity. UvrB can interact with this $UvrA_2$ dimer in solution or on DNA, creating the $UvrA_2B$ complex. Upon binding to DNA, the $UvrA_2B$:DNA complex undergoes conformational changes. The DNA lesion remains in close contact with UvrA and then it is transferred to UvrB. UvrB is endowed with a cryptic ATPase activity (the red nodule on UvrB) that is activated in the context of $UvrA_2B$:DNA. In this complex, the DNA is unwound around the site of the lesion because UvrB has inserted its β-hairpin structure between the two strands of the DNA to facilitate damage verification. The DNA is also wrapped around UvrB. The UvrA molecules hydrolyze ATP and dissociate from the complex, thereby creating a stable UvrB:DNA complex. UvrC recognizes this UvrB:DNA complex. We have depicted one UvrC molecule with two catalytic sites. Before UvrC can make the 3′ incision, UvrB must bind ATP, but not hydrolyze it. After the 3′ incision is generated, a second incision event on the 5′ side of the DNA lesion is produced; thus, UvrC forms a dual incision approximately twelve nucleotides apart. After the incision events, the DNA remains stably bound to UvrB until UvrD, DNA pol I and ligase perform the repair synthesis reaction.



Above from Van Houten et al., Mutation Research 577 (2005) 92–117

End of reaction: