DNA and RNA Structure, Protein Interaction, Damage and Repair

Required Reading:

- ✓ DNA-Protein Interaction – Chpt 24 pt4
- ✓ DNA Damage – Chpt 25 pt 4

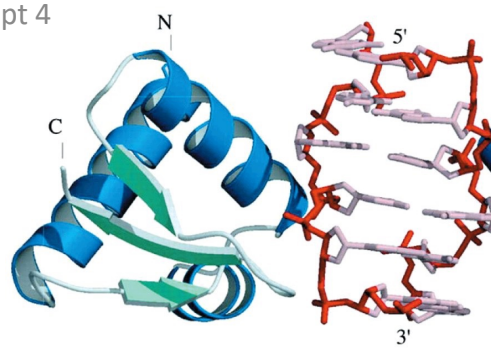
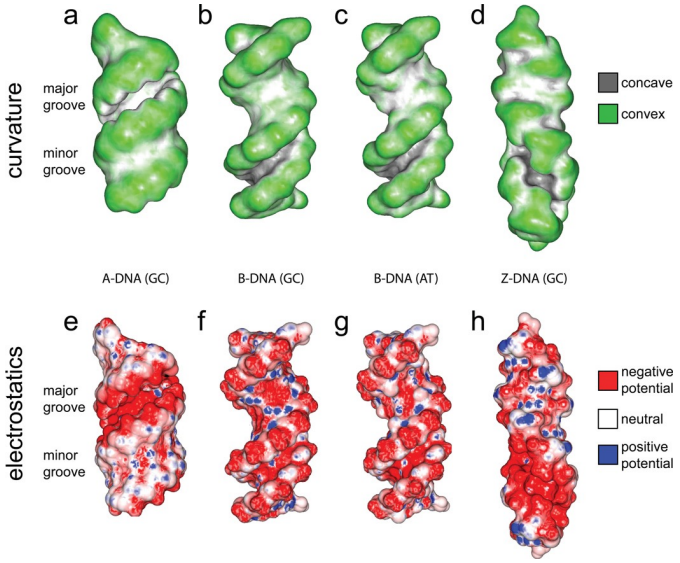


Figure 24-3
Courtesy of Alexander Rich, MIT

1

Reminder and New Info on DNA

- Get your groove back (it HAD to be said)



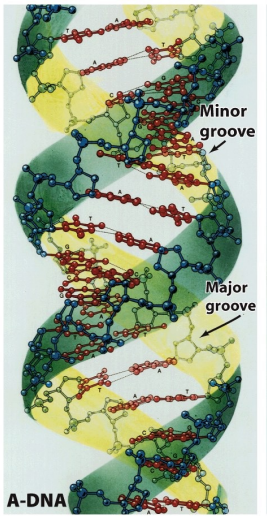
2

Protein-DNA interactions – very specific themes

- Concept of "scanning" is not fully correct. Some proteins bind independent of sequence (histones…) based primari on pos side chains and phosphate backbone and some ribose H bonding. Specific protein binding is $10^3 - 10^7$ X stronger than non-specific…
- Loose association means a constant "on" and "off" interaction mixed with random movement = scanning. Think of equilibrium of binding – dissociation constant *of course this is a simple non-cooperative description*

$$DNA_{(free)} + Protein_{(free)} \underset{K_{off}}{\overset{K_{on}}{\Leftrightarrow}} DNA\text{-}Protein_{(bound)}$$

$$K_d = K_{off} / K_{on} = [DNA_{(free)} + Protein_{(free)}] / [DNA\text{-}Protein_{(bound)}]$$



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Specificity is due to binding… What kind of binding???

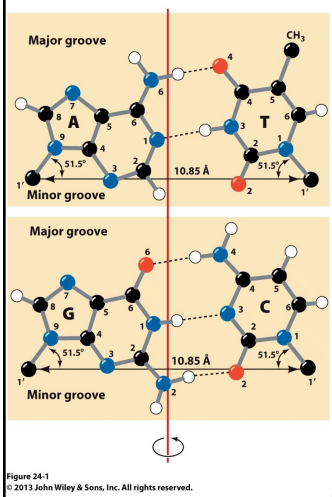

Figure 24-1

- Access and numbers of potential interactions with protein side chains is greater in major groove than minor groove
- For most of the 129 DNA binding proteins, ~2/3 of contacts are van der Walls, rest are H bonding (with or without intervening water) and a few ionic P backbone/Arg, Lys or N terminus.
- Protein-DNA interface involves on average 24 residues and 12 nucleotides
- Protein binding can accompany a torsion of DNA "induced fit" that can also open site for a different protein or a subunit of the same protein.

- Prokaryotic Motifs include:
  - Restriction enzyme – unwinding, opening up groove
  - Helix-turn-helix (repressor and others)
  - Indirect Readout

- Eukaryotic Proteins
  - HTH Zinc Fingers
  - Leucine Zippers

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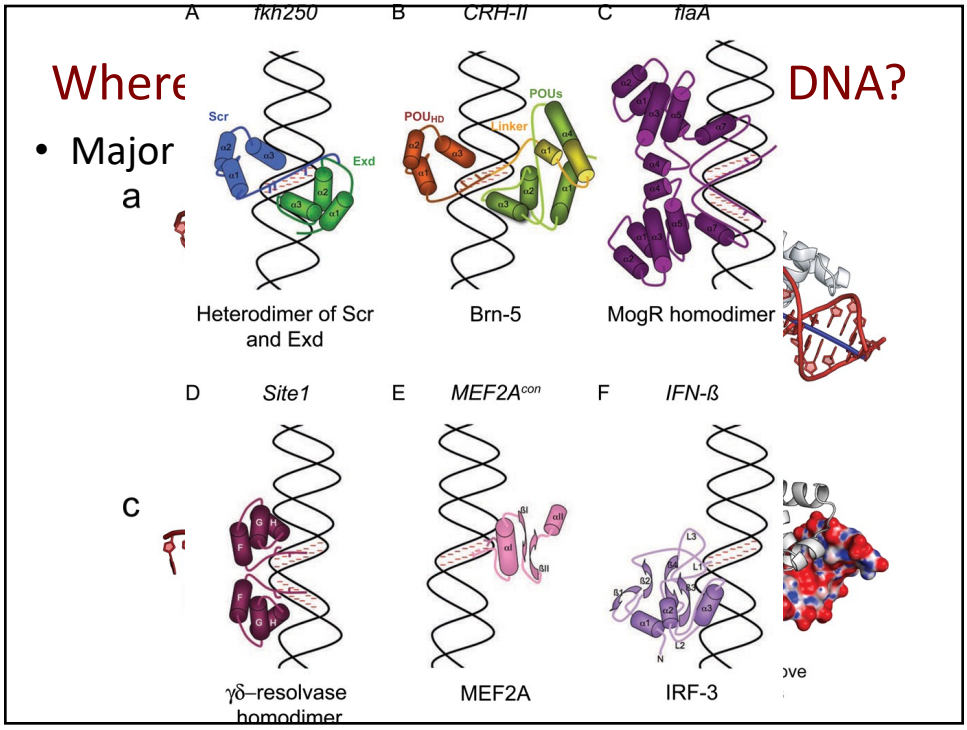

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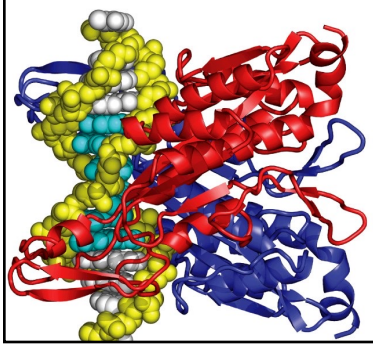
Prokaryotic Protein-DNA Interactions

Altering DNA to open binding sites

- Motifs include unwinding and bending DNA to open major or minor groove to protein binding
- Example of this type of protein interaction is the restriction enzymes
  - Bind and recognize palindromes –
    - Dimer to fold into major groove and force base pairs to separate by ~50o (unstacking)
    - Rest of the DNA compensates keeping the molecule from bending but the opening of the bases unwinds DNA widening minor groove where the rest of the protein can then bind and attack phosphate backbone
    - SOME proteins bind without distortion indicating the sequence alone is important. BamHI uses every possible H binding spot via water bridge



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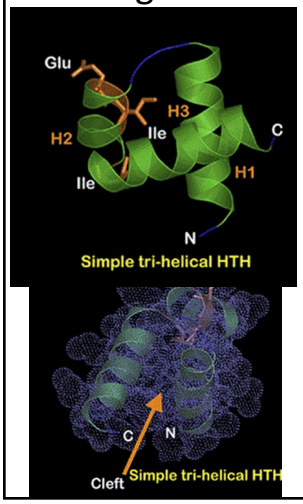
Helix-turn-helix (HTH)

- Typical in prokaryote repressor proteins but the motif is common to many DNA binding proteins
- Most HTH involve three helixes with a partially open configuration.



- Sharp turn between H2 and 3 are characteristic for HTH while N and C term vary greatly.
- Surface view shows cleft for binding
- 3rd helix is recognition helix forms interaction by inserting itself into major groove. Other residues will interact giving additional specificity
- Conserved Ile (2) and Glu bind to backbone and ribose.

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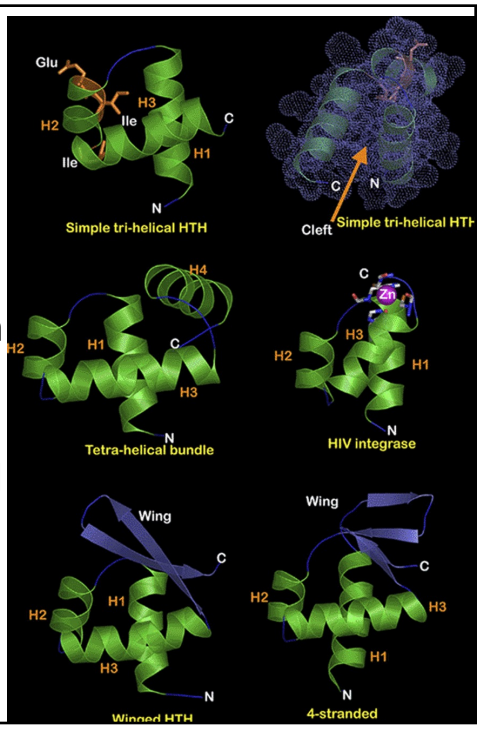
Helix-turn-helix (HTH)

- Grouped into two main classes simple three-HTH bundles and HTH with extensions
- Some versions (extensions) include beta sheets "wings" of a winged HTH motif
- Wings provide additional interface for DNA contact by binding to minor groove in the hairpin of sheet



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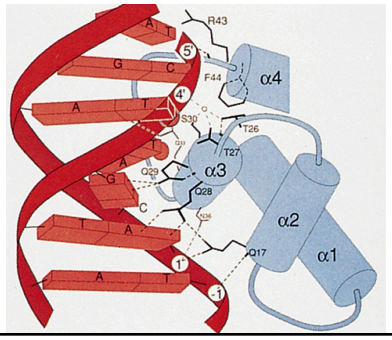
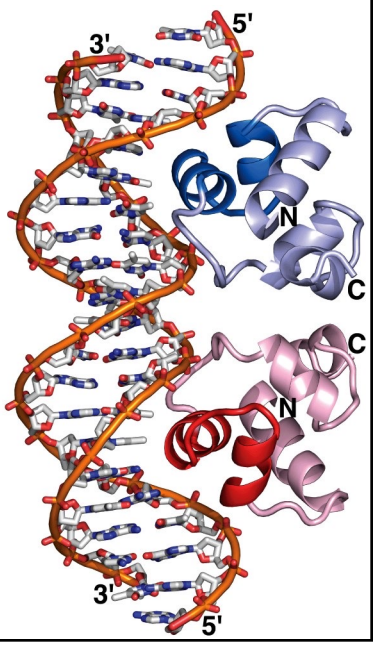
HTH : bacteriophage 434 repressor

- Direct binding with 2nd and 3rd HTH helix.
- Two dimers binding in major groove on same side
- Complex opens DNA by widening major groove, decreasing minor groove





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Indirect Readout Protein-DNA binding

- **Direct readout** – Short DNA sequences that serve as binding sites specifically read by complementary sequenced/structured amino acid side chains. (>2000 solved structures)
- There is not a simple recognition code or a one-to-one correspondence between DNA and protein sequences, thus direct readout can not be sufficient to account for DNA-protein binding
- **Indirect readout** – protein-DNA interactions that depend of base pairs that are not in direct contact with the protein; in other words, bp that create a specific structure that is re subsequently recognized by a protein. Often non-B form helix. ***The book states "that the protein senses the base sequence of DNA through the DNA's bakebone conformation and or flexibility"***.
  - *In "other other words" Indirect readout recognizes the shape and or flexibility of the DNA sequence – can be caused by protein binding.*

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E. Coli trp Repressor is an indirect readout DNA binding protein

- Trp repressor binds but doesn't have enough interactions to explain specificity
  - Mutation of "non-binding" aa mutate ability of repressor to bind – indicating their importance
  - The DNA assumes a sequence specific structure upon protein binding to backbone that opens base pairs.

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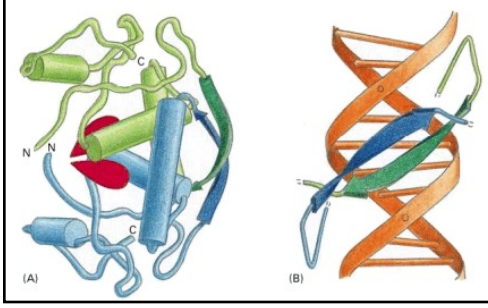
The met Repressor binds via β sheets

- Last major class or prokaryotic DNA binding proteins – demonstrated by met repressor
- Two-stranded beta sheet binds DNA
- Homodimeric protein regulated by substrate
- Two stranded beta sheet lies into major groove
  - Small conformational changes to both protein and DNA allow the helix to bind
  - This is an example of indirect readout



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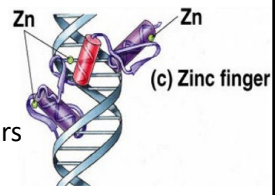
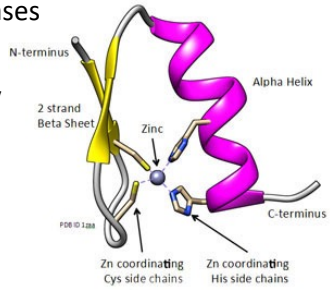
Eukaryotic TF involve two major motifs

- Zinc Fingers: ~30 aa binding loops, minimal binding domains.
  - Short a helix, two stranded antiparallel b sheet and a Zn^{2+} coordinated by Cys or His combination
  - Organized by chelating aa (eg. Cys_2His_2, Cys_4, Cys_6)
  - Metal holds complex together.
  - Often occur as tandem repeats with 2 or more fingers binding in major groove spaced at 3 bp intervals
  - A helix of each domain is the "recognition" sequence contacting 4 or more bases
  - Fingers also bind to phos via Arg and His
  - Similar to HTH – but these are larger with many
  - fingers making a full protein.





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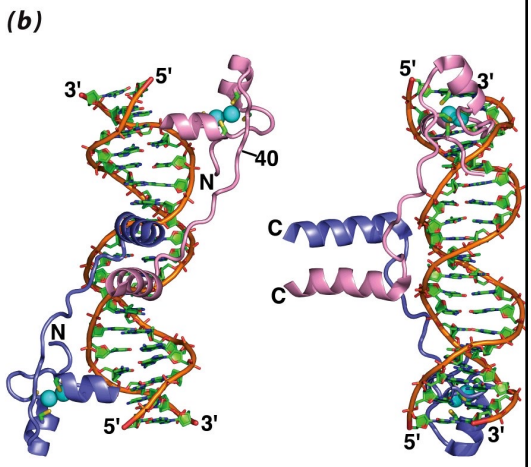
Gal4 transcriptional activator – Zn finger example

- Binds to DNA substrate as a dimer that forms in presence of target (monomer w/o DNA)
- N terminal binds into minor groove increasing sequence specificity
- Linkers allow a "wrap around" for protein.



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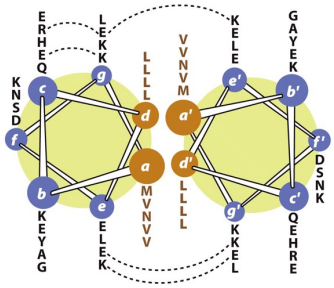
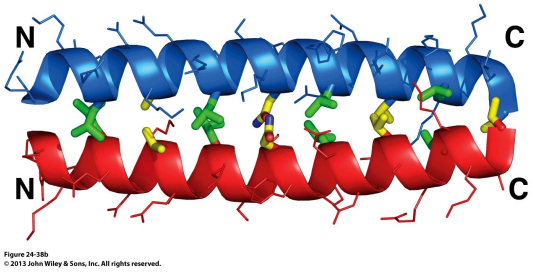
Leucine Zippers

- Proteins dimerize via hydrophobic interactions of leucines - every 7th position
- This mediates dimerization to allow rest of the protein to fit into major groove from opposite side





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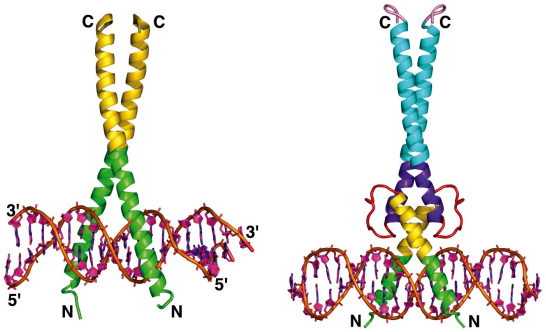
Leucine Zipper

- Coiled coil domain of supercoil helices.
- Zipper does NOT bind to DNA, but mediates the formation of a binding dimer
- Often include basic amino acids N term to the zipper and called bZIP proteins – found in many organisms – helps bind to phosphate backbone



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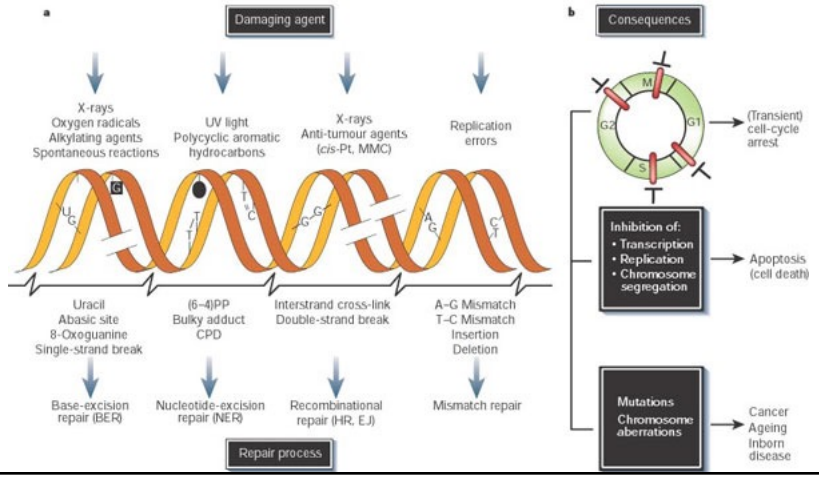
DNA Damage

Mutations in nucleotide arise spontaneously from replication errors, and external (natural and other) agents. Some of these mutations are carcinogenic.



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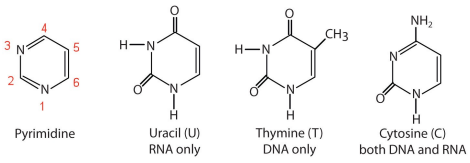
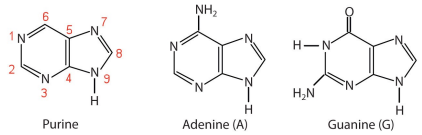
Uracil – why not in DNA?

Ever wonder why DNA uses ATG&C but RNA includes U (uracil)? T and U bp the same. AT / UA paring is more complicated and takes additional resources (several enzymes needed for production of T from U)… so why not have U in DNA?





- Cytosine is easily mutated (loss of amine group) to Uracil. DNA that included U could not be distinguished from a mutant C-U mutation. And U in DNA came from C and not an OG C, thus recognized and removed.
- RNA is shorter lived than DNA so it is better for fidelity of message

FIX the YELLOW to be more clear – look at the data
Frame why this info is in this set of slides- mutation

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Classes of Chemical Mutations

- Point Mutation (one bp is exchanged for another)
  - Transition (Purine-Purine or Pyrimidine-Pyrimidine)

    C<->U, C->T or A<->G conversions
  - Transversion (Purine-Pyrimidine)
- Insertion/Deletion – addition or loss of one or more bp.

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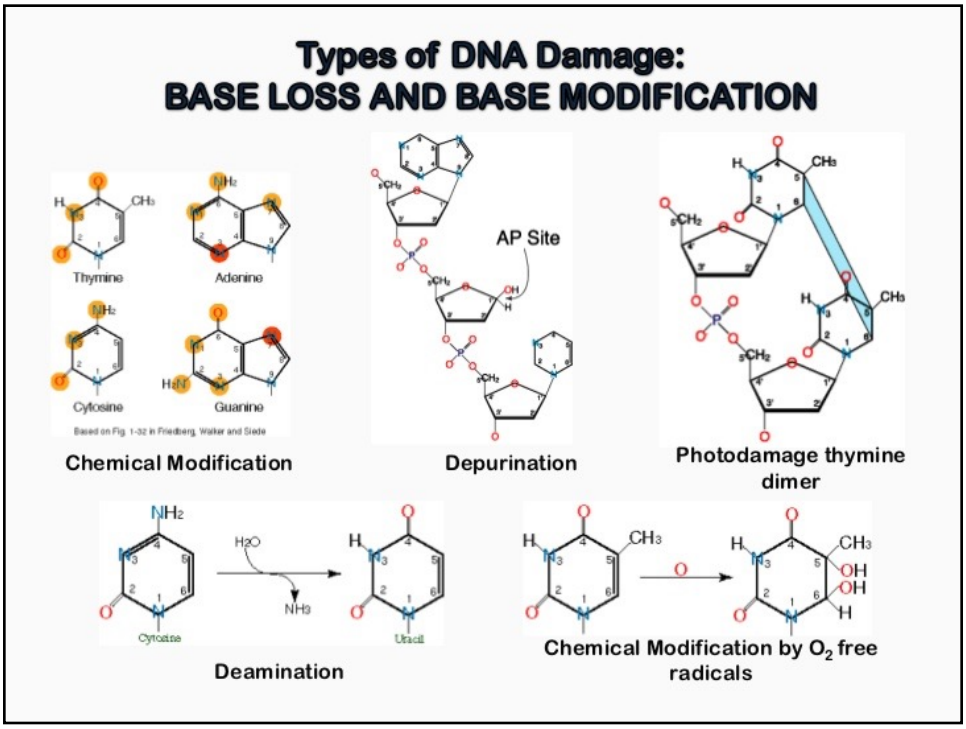

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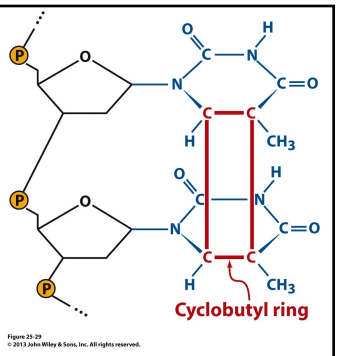
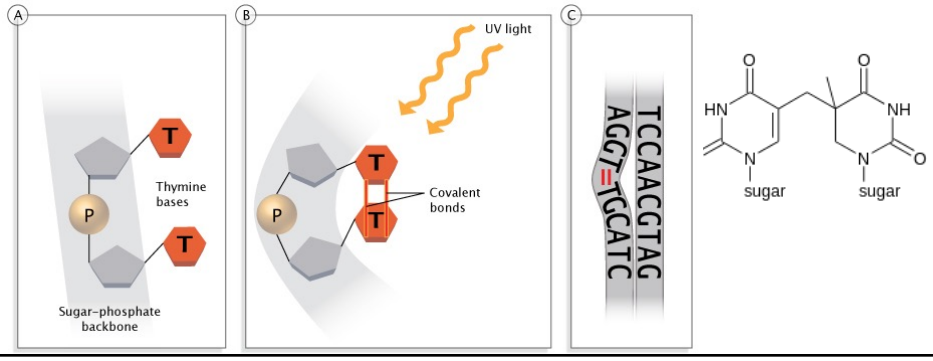
UV Damage

UV radiation causes two classes of DNA lesions

- Cyclobutane pyrimidine dimers (T:T)
- 6-4 photoproducts
- Both lead to bulge or kinks in one strand impeding transcription and replication.
- Flexible areas of the chromosome are highly susceptible to UV damage (p53 oncogene and skin cancer)





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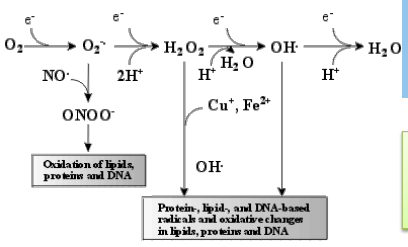
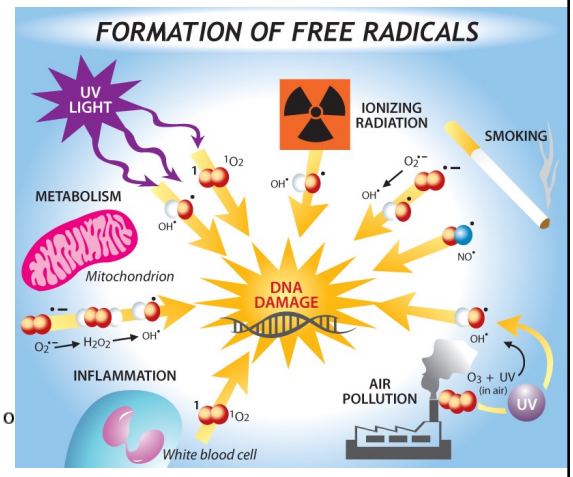
Free Radical Formation and DNA

- Single electron acceptance to oxygen from metabolism
- Smoking and cellular activity also generates nitric oxide radical.
- Intermediates include several reactive nitrogen and oxygen radical





How will a free radical alter DNA structure?
Is a free radical an electrophile or nucleophile?
What part of DNA is susceptible to attack?

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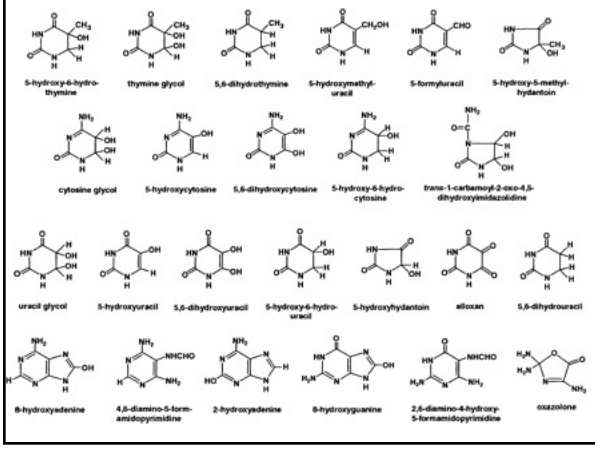
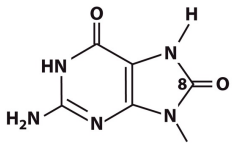
Oxygen Radical DNA Damage

- Altered bases lead to transversions and transitions – 100s of possible oxidized products





8-Oxoguanine (oxoG) residue

Common guanine alkylating product

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Point Mutations: a common result of alkylation

Radicals, Nitrous Acid and Alkylating Agents: Base modification leading to point mutations.

- Nitrate ($NaNO_3$) is converted to Nitrous Acid (HNO_2) in some cases can cause DNA damage.
- Deamination by nitrous acid leads to transitions

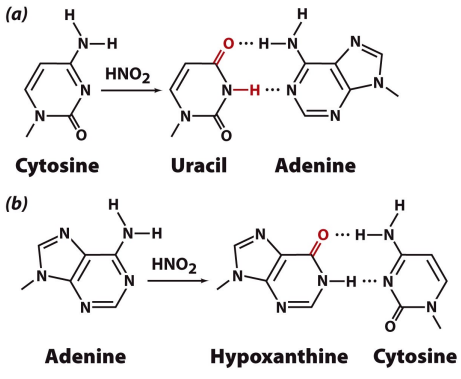

Figure 25-30
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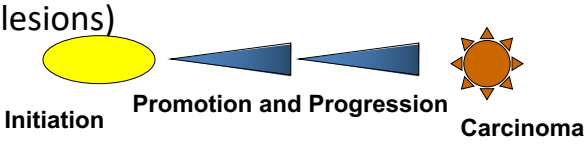
Chemical Carcinogenesis

•Carcinogen - any substance or agent that significantly increases tumor incidence. - any dose, any route

•1 -4 % of cancers in the US are in due to industrial produced chemicals, there are many more naturally occurring compounds.

•Initiating events occurs when a carcinogen interacts with DNA causing a strand break or forming an altered nucleotide called an adduct. DNA replication without repair leads to mutation.

•Promoters stimulate initiated cells to form begnin tumors (hyperplastic lesions)



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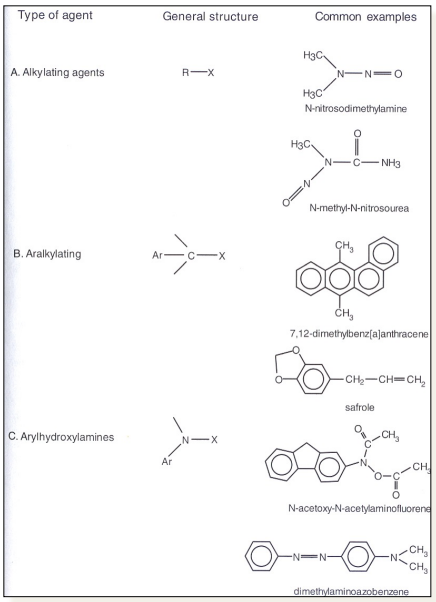
Most carcinogens fall into 3 categories

•**Alkylating agents** - agents that add methyl or ethyl groups to nucleotides particularly at the N or O atoms not in the ring of the base.

•**Aralkylating agents** - cause the transfer of aromatic compounds to nucleotides to form an adduct.

•Polycyclic aromatic hydrocarbons (PAH) are at the root of several industrial carcinogens. This includes benso[a]pyrene, found in tobacco and in charcoal grilled meats



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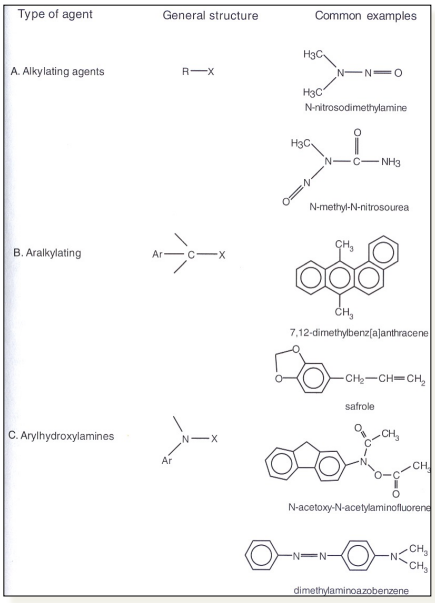
Most carcinogens fall into 3 categories

- **Arylhydroxylamines** - chemicals that transfer aromatic amines to nucleotides.

- Many of the dyes used these compounds. Analine dyes caused high rate of bladder cancer. These also require metabolism by P450 before an active nitrogen containing cation reacts with DNA.



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Three Classes of Alkylating agents

- **Classical Agents** – Nitrogen mustards (mechlorethamine, chlorambucil, cyclophosphamide-cytoxan), Nitrosoureas (streptozocin, carmustine, lomustine), Solfonates (busulfan)…
- **Alkylating Like** – Platinum drugs (cisplatin, carboplatin, oxalaplatin) more likely to cause secondary cancer – leukemia (carcinogenic/mutagenic)
- **Nonclassical** – mixed method of action. Includes: *Dacarbazine* – activated by p450 acts as both a purine analoge inhibiting DNA synthesis, alkalyates and interacts with –SH. *Procarbazine* crosses CNS barrier, inhibits DNA synthesis, RNA and protein synthesis, alkylates and is a monoamine oxidase inhibitor…

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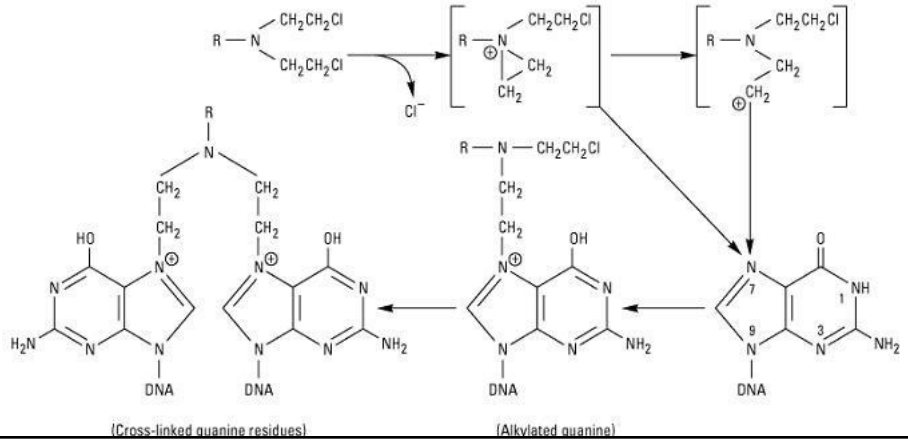
Alkylating Agents

- Generate transversions and loss of base

Direct transfer of an alkyl group (R-C_nH_{2n+1}) to DNA – typically a N atom on guanine base leading to cross-linking between strands



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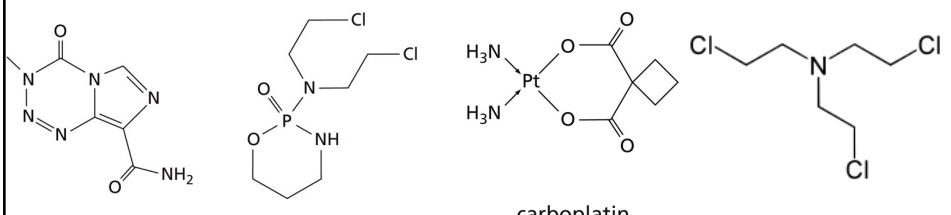
Mechanism of Alkylation

- Alkyl groups forms covalent bonded carbon atom – in DNA to N7 of purines. Form via carbonium ion or carbon radical.
- Nucleophilic alkylation: Organometalic compounds contribute to electron-deficient carbon atoms - often involve halide substituents on a carbon atom.
- Electrophilic alkylation: Alkyl halides with lewis acid catalyst – can react directly with amines (guanine N) to form C-N bonds. Attack on nucleophilic atoms/functional groups include amino, sulfhydrile and nitrogen of guanine



temozolomide | cyclophosphamide | carboplatin

alkylating | alkylating-like | Mustard

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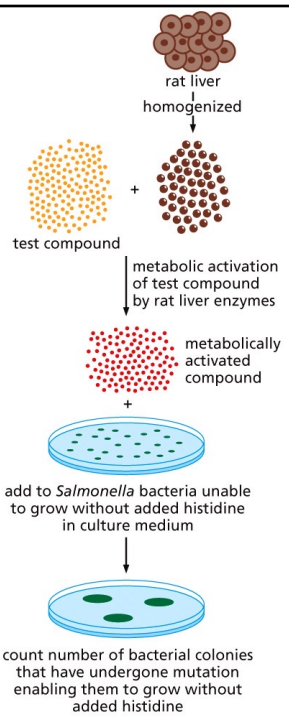
Chemical Carcinogenesis

•Ames test - determine mutagenicity of Test uses a Salmonella strain that is readily mutable to histadine independent growth. Only tests for active forms of carcinogens.

•Many compounds are inactive until metabolized - often in the ER by the cytochrome P450 enzymes. P450 is a superfamily of genes (>100) normally responsible for degrading xenobiotics. P450 has many variations between species and individuals.

•Many of the tobacco carcinogens are activated by various P450 system enzymes. Thus differences in smoking and lung cancer.

•Many of the active carcinogens are electrohilic intermediates that react with DNA bases. The modified bases are then mis-read by polymerases leading to oncogene activation or loss of tumor promoter expression.



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