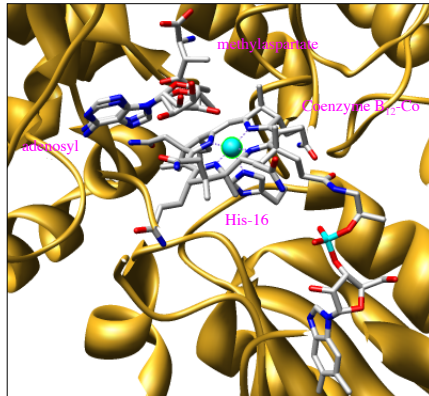
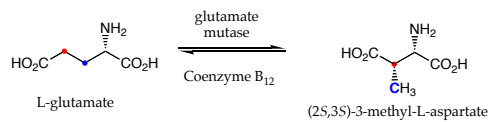


Glutamate mutase

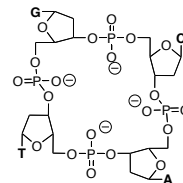


pdb code: 1I9C

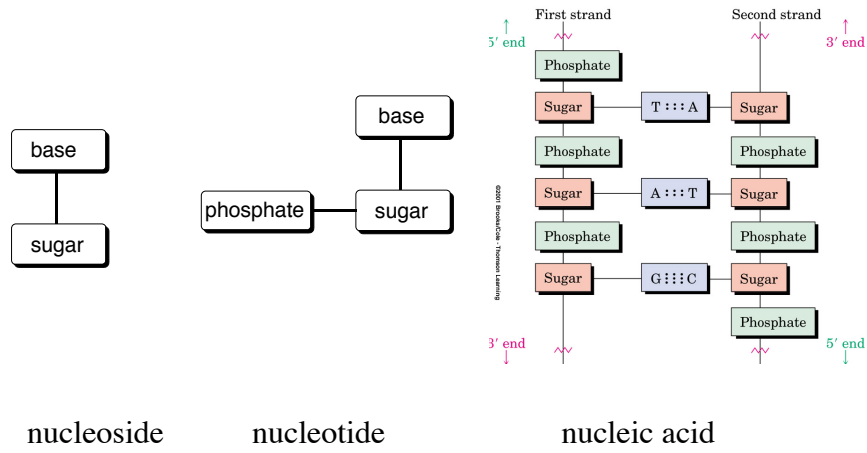
241

Nucleic Acids

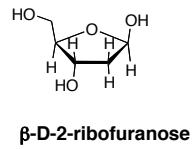
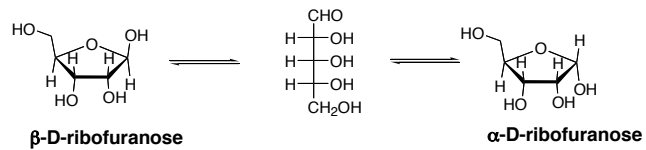
- 1869: Miescher- gelatinous material from cell nuclei of white blood cells containing organophosphorous compounds- nuclein (chromatin)- discovery of nucleic acids
- 1891: Kossel- identified the DNA bases A, T, G and C
identified D-ribose in nucleic acids
- 1889: Altman- purified DNA
- 1901: Ascoli- identified U in RNA
- 1910: DNA and RNA realized to be separate entities. DNA (thymus) RNA (yeast)
- 1929: Levene & Jacobs- identified 2' -deoxyribose in DNA
- 1920's - 1950's: structures of nucleosides and nucleotides - Alexander Todd (Nobel Prize, 1959)
- 1928: Griffith- first to propose that DNA was genetic material; not widely accepted
- 1931: Levine & Bass- first proposed structure. Believed to be part of chromosome physiology, but NOT genetic material
- 1944: Avery, MacLeod & McCarty- Strong evidence that DNA is genetic material
- 1950: Chargaff- careful analysis of DNA from a wide variety of organisms. Content of A,T, C & G varied widely according to the organism, however: A=T and C=G (**Chargaff's Rule**)
- 1952: Hershey & Chase: followed the transfer of ³²P-labelled DNA from T2 bacteriophage to infected bacterial cytoplasm (1969 Nobel Prize)
- 1953: Watson & Crick- structure of DNA (Nobel Prize with M. Wilkens, 1962)



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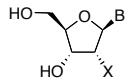
Sugar: D-ribose



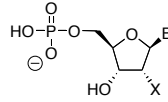
Stereochemistry:

As drawn, the side above the plane of the ring is β
the side below the plane of the ring is α

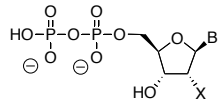
Nucleosides vs Nucleotides



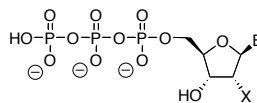
ribonucleoside (X=OH)
deoxyribonucleoside (X=H)



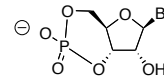
ribonucleotide (X=OH)
deoxyribonucleotide (X=H)



nucleotidediphosphate

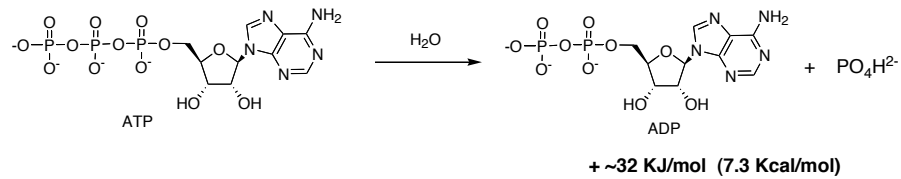


nucleotidetriphosphate



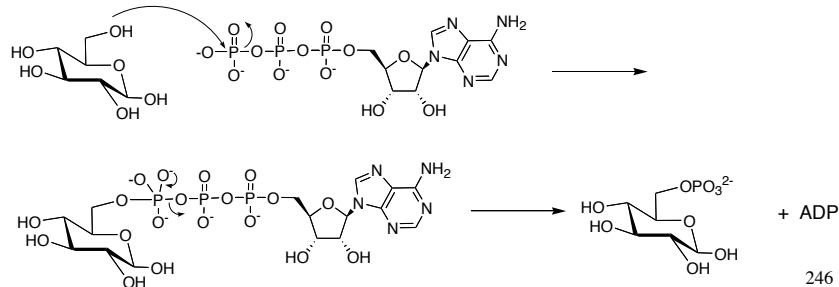
3',5'-cyclic phosphate

245



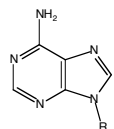
Hydrolysis of ATP is used to drive many biochemical reactions

Phosphoryl transfer reactions:

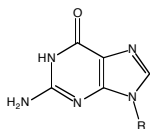


Bases

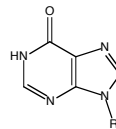
A. Purines



Adenine (R=H)
Adenosine (R=furanose, **A**)

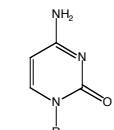


Guanine (R=H)
Guanosine (R=furanose, **G**)

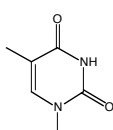


Hypoxanthine (R=H)
Inosine (R=furanose, **I**)

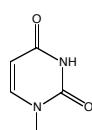
B. Pyrimidines



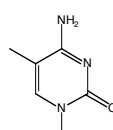
Cytosine (R=H)
Cytidine (R=furanose, **C**)



Thymine (R=H)
Thymidine (R=furanose, **T**)



Uracil (R=H)
Uridine (R=furanose, **U**)



5-Methylcytosine
(R=furanose)

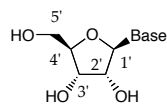
DNA contains A, C, G, T all with 2'-deoxyribose

RNA contains A, C, G, U all with ribose

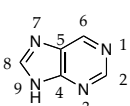
The stereochemistry of the base is β

247

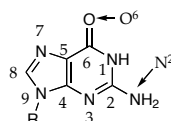
Numbering System



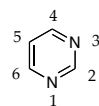
Ribonucleoside



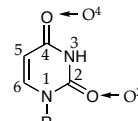
Purine



Guanosine



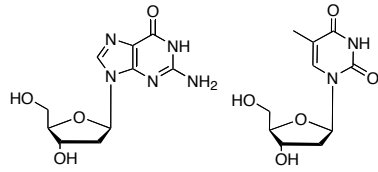
Pyrimidine



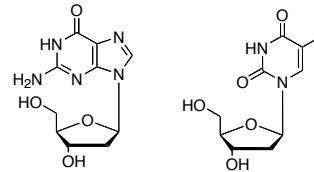
Uridine

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Nucleoside Conformation:

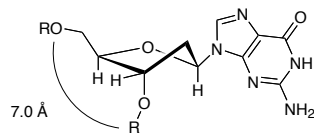
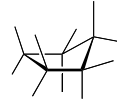


Anti conformation

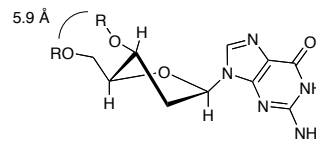


Syn conformation

5-member ring conformation: envelope



**C2' - endo conformation
found in B-form DNA**



**C3' - endo conformation
found in A-form DNA**

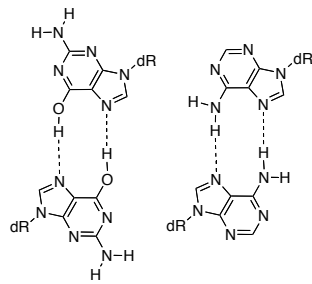
249

Watson & Crick: double helix

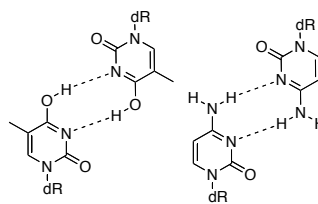
Initial “like-with-like”, parallel helix:

Does not fit with with Chargaff's Rule: $A = T$

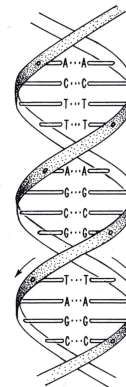
$G = C$



purine - purine



pyrimidine - pyrimidine



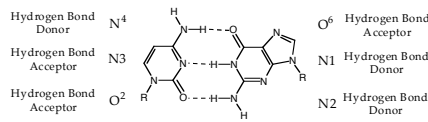
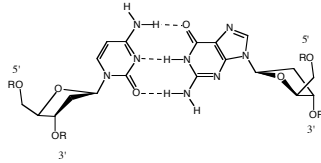
Wrong tautomers !!

Watson, J. D. *The Double Helix*, 1968

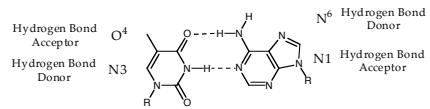
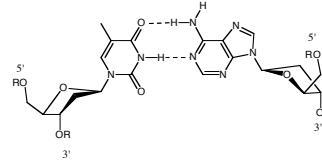
250

Complimentary Base- Pairing in Nucleic Acids: antiparallel double helix

Antiparallel C-G Pair



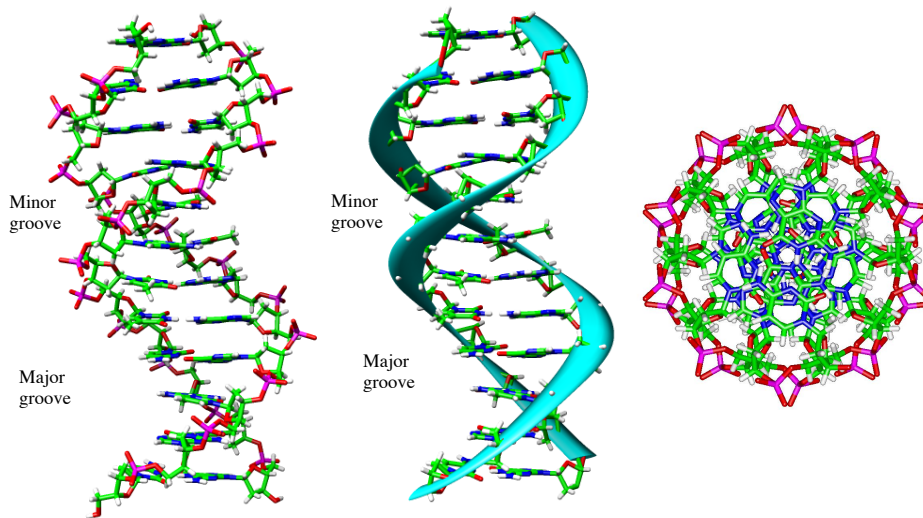
Antiparallel T-A Pair



“Molecular Structure of Nucleic Acids” Watson J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 737-8

251

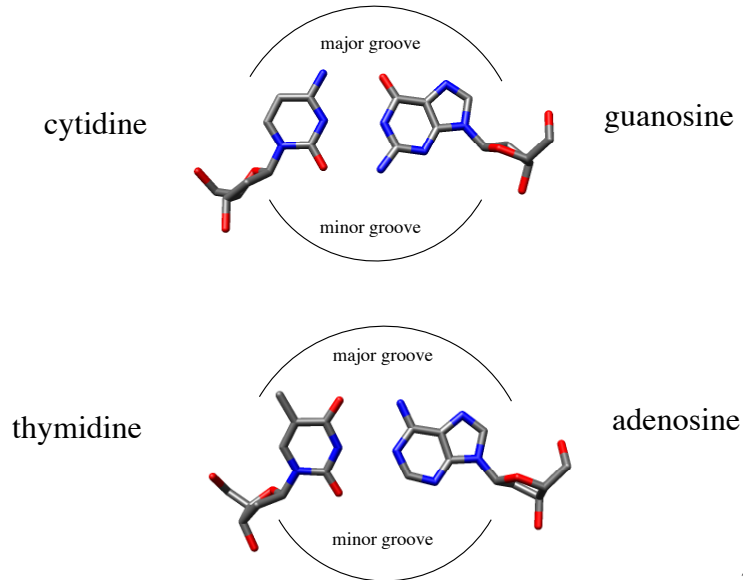
B-DNA



pdb code: 1bna

252

DNA Grooves

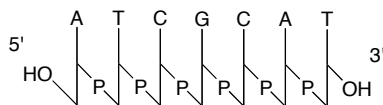


Writing DNA sequences

DNA Sequences are written 5' to 3'

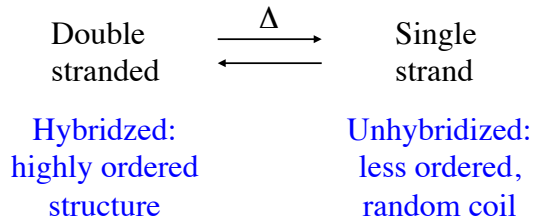
5' -ATCGCAT-3'

5' -d(ATCGCAT)-3'



254

Melting Temperature (T_m): a measure a duplex stability



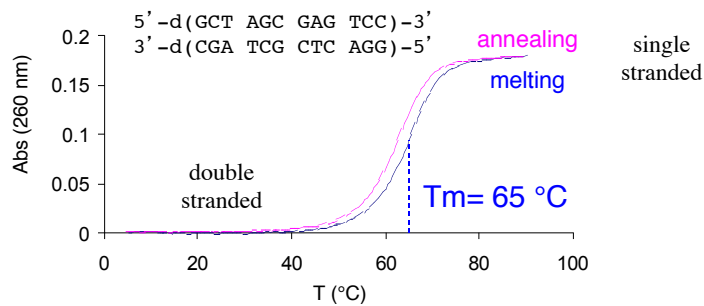
Base stacking causes a decrease in the net UV absorbance when DNA is hybridized (double stranded) versus unhybridized.

Upon thermal denaturation (melting), the UV absorbance increases: hyperchromicity

Blackburn *et al.* Ch. 2.5.1, 11.1.1

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DNA melting curve:



T_m is often taken as a measure of DNA duplex stability

T_m is a measure of ΔG not ΔH

The T_m is dependent upon the length and sequence of the oligonucleotide (CG/AT ratio) and the ionic strength of the medium

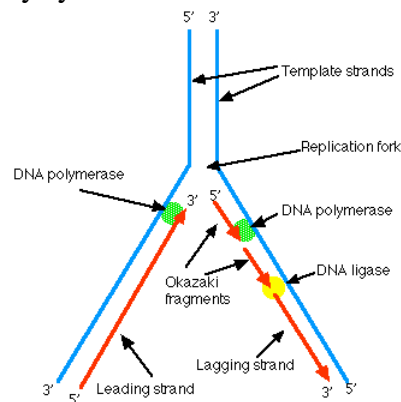
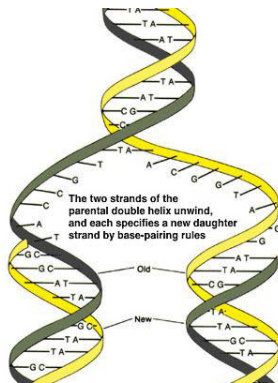
256

DNA processing enzymes:

DNA replication:

Helicase: Unwinds double stranded DNA

DNA polymerase: replicates DNA using each strand as a template for the newly synthesized strand.



"It has not escaped our attention that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."
 Watson & Crick

257

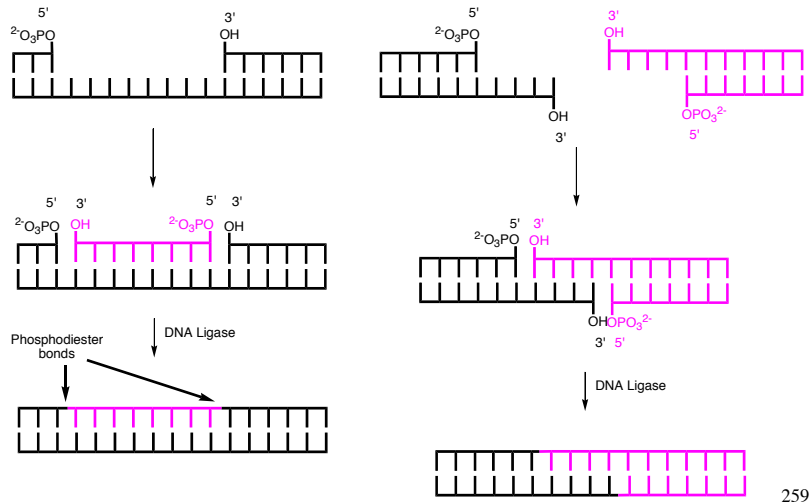
DNA Polymerase: the new strand is replicated from 5' to 3'

DNA polymerase are Mg^{2+} ion dependent

Details regarding the mechanism of recognition of dNTP incorporation for the growing DNA strand are *not* fully understood

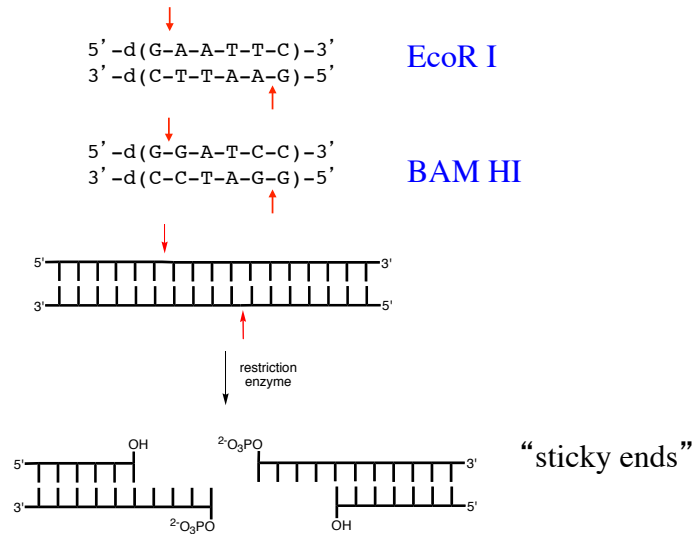
258

DNA Ligase: ATP-dependent enzyme that will join (ligate) two DNA segment by catalyzing the formation of the phosphodiester bond of a terminal 5'-phosphate of one oligonucleotide and the 3'-hydroxyl group of another oligonucleotide.



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Restriction Enzymes (endonucleases): enzymes that will cleave double stranded DNA at specific, known sequences.

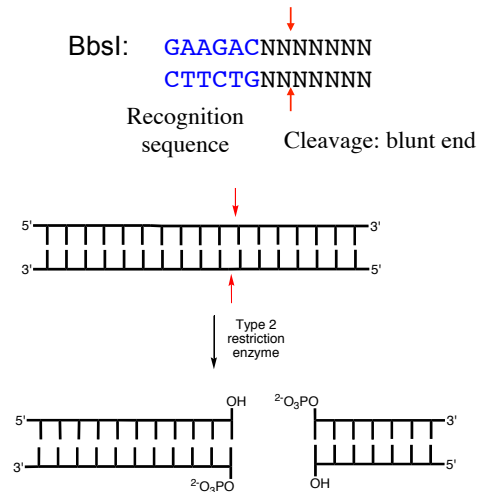


Werner Arber, Daniel Nathans and Hamilton Smith
1978 Nobel Prize in Medicine & Physiology

260

Type I: cleaves at a site very distant from the recognition sequence;
 Mg^{2+} , SAM and ATP dependent.

Type II: cleaves DNA at or near the recognition sequence;
 Mg^{2+} dependent. i.e., EcoR I, BamHI, Bbs I



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Polymerase Chain Reaction (PCR): method of amplifying DNA using DNA polymerase and cycling temperature

Heat stable DNA Polymerases:

Taq: thermophilic bacteria (hot springs)- no proof reading

Pfu: geothermic vent bacteria- proof reading

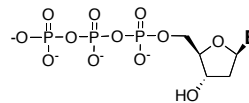
Mg^{2+}

two Primer DNA strands (synthetic, large excess)

one sense primer and one antisense primer

one Template DNA strand

dNTP's



KARY B. MULLIS, 1993 Nobel Prize in Chemistry for his invention of the polymerase chain reaction (PCR) method.

Blackburn *et al.* Ch. 5.2.3, 5.4.2

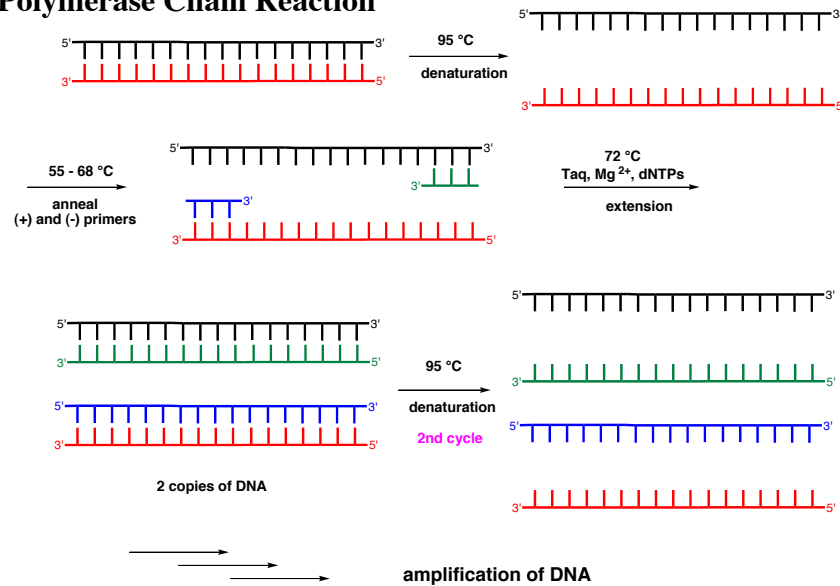
262

A typical PCR temperature cycle

Denaturation:	94 °C	0.5 - 1 min	
Annealing:	55-68 °C	0.5 - 1 min	5 °C below the T_m of the primer
Extension:	72 °C	1 min	+ 1 min per Kb of DNA
# of cycles	25 - 35		
Final extension	72 °C	10 min	

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Polymerase Chain Reaction



For a PCR animation go to: <http://www.blc.arizona.edu/INTERACTIVE/recombinant3.dna/pcr.html>

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\$\$ The Power of Compounded Interest \$\$

$$\begin{aligned}
 &1 \times 2 = 2 \times 2 = 4 \times 2 = 8 \times 2 = 16 \times 2 = 32 \times 2 \\
 &= 64 \times 2 = 128 \times 2 = 256 \times 2 = 512 \times 2 = \\
 &1,024 \times 2 = 2,048 \times 2 = 4,096 \times 2 = 8,192 \times 2 \\
 &= 16,384 \times 2 = 32,768 \times 2 = 65,536 \times 2 = \\
 &131,072 \times 2 = 262,144 \times 2 = 524,288 \times 2 = \\
 &1,048,576
 \end{aligned}$$

In principle, over one million copies per original,
can be obtained after just twenty cycles

265

Oligonucleotide-Based Site-directed mutagenesis

DNA \longrightarrow mRNA \longrightarrow protein

THE STANDARD GENETIC CODE

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

AUG is part of the initiation signal, as well as being the codon for internal methionine.

SYMBOLS FOR AMINO ACIDS

A	Ala	Alanine
B	Asx	Asparagine or aspartic acid
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
Z	Glx	Glutamine or glutamic acid

3' -GAG ATG ACA CCC AAA-5'
 5' -CTC TAC TGT GGG TTT-3'

-Leu Tyr Cys Gly Phe-

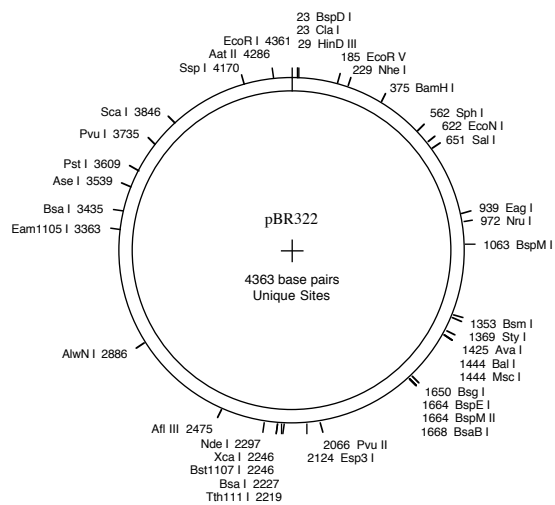


3' -GAG ATG CGA CCC AAA-5'
 5' -CTC TAC GCT GGG TTT-3'

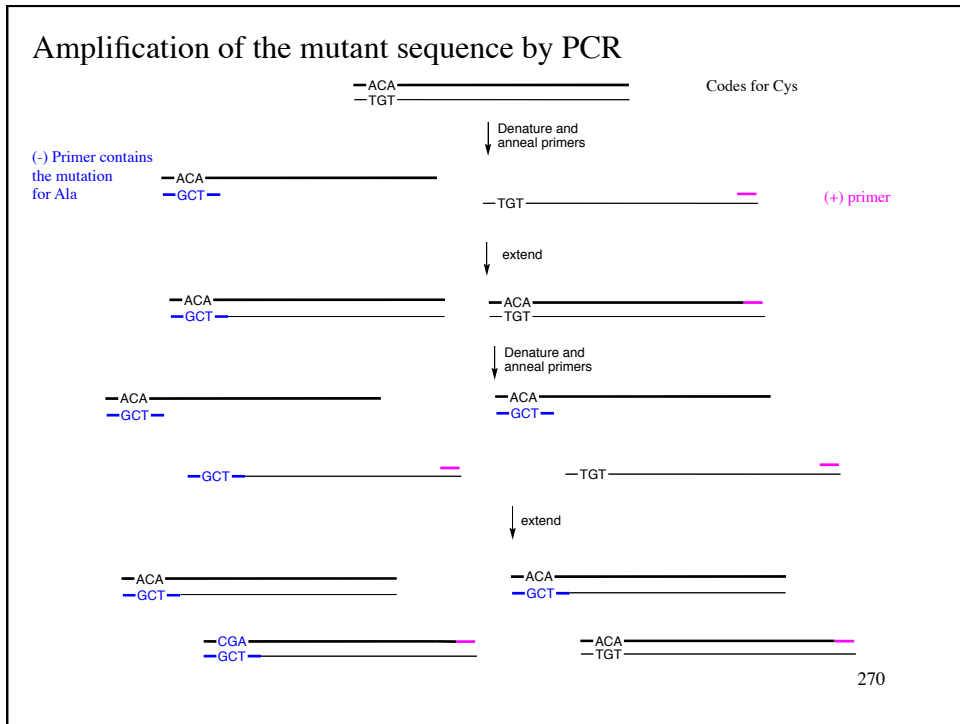
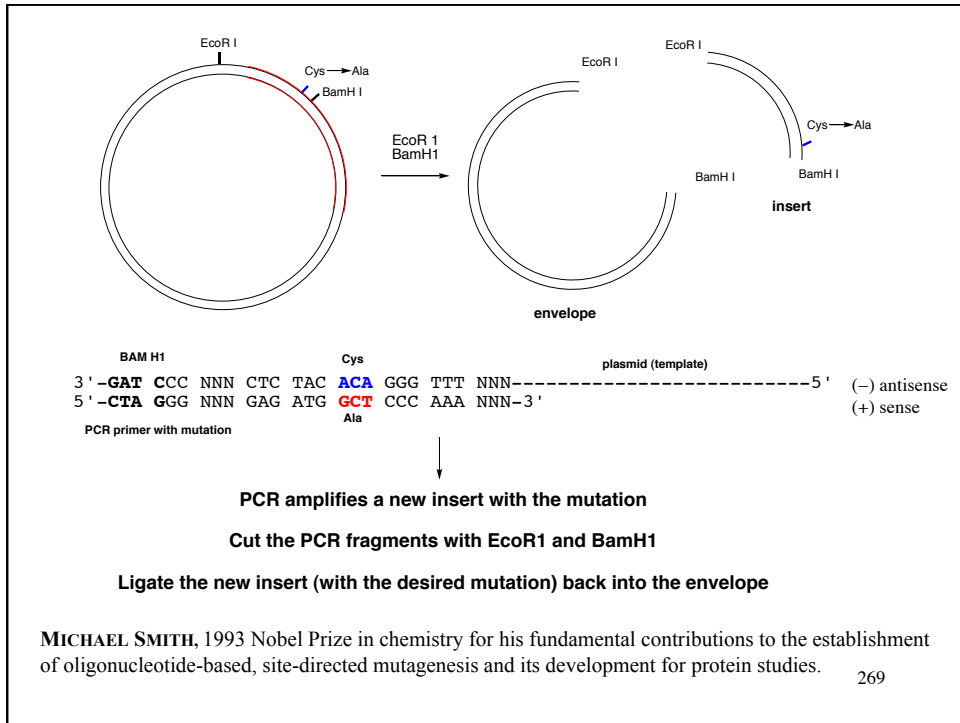
-Leu Tyr Ala Gly Phe-

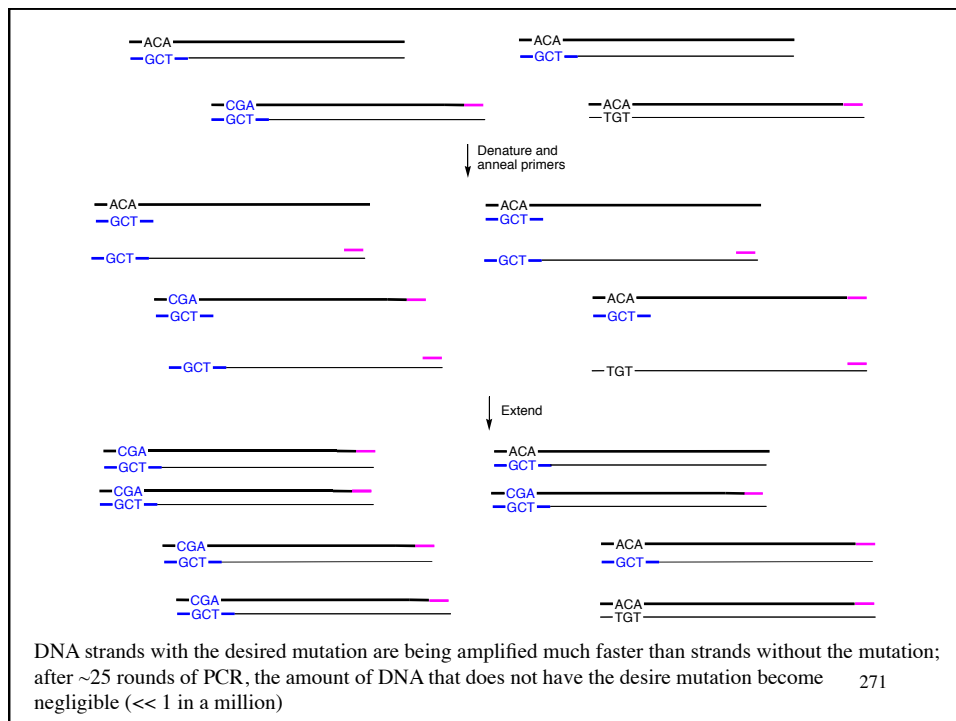
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Restriction Map



268





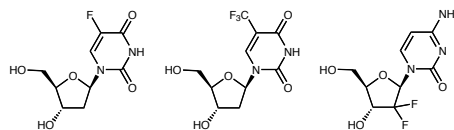
Nucleoside Synthesis:

Blackburn *et al.* Ch. 3.1

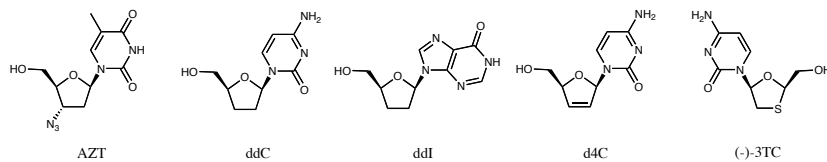
important class of chemotherapeutic agents
(anticancer and antivirals)

important reagents for biotechnology

Anti-Cancer Nucleosides

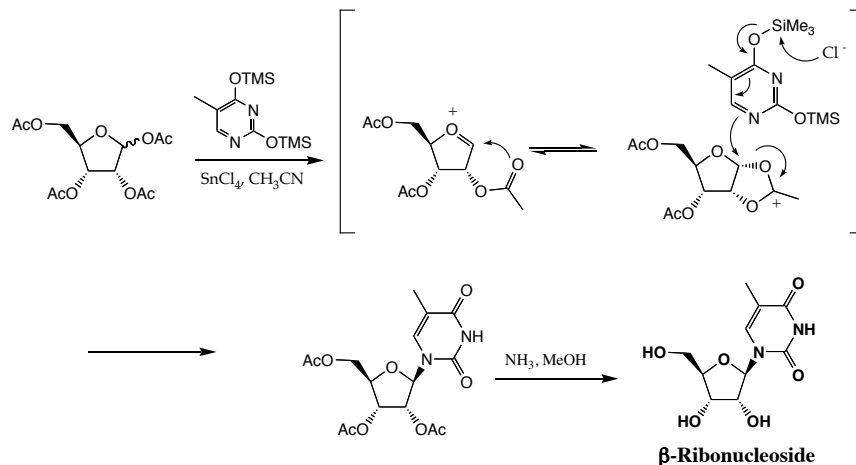


Anti-Viral Nucleosides



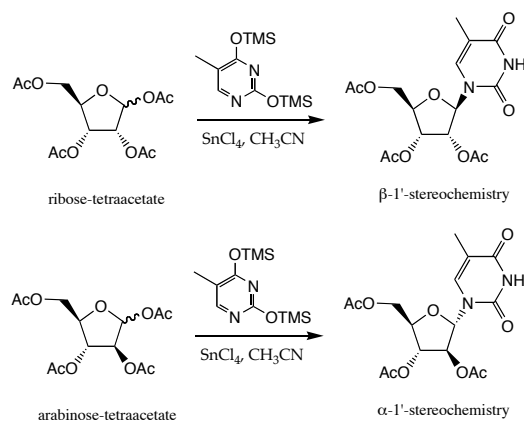
272

Chemical Synthesis of Ribonucleosides via Vorbrüggen Glycosylation



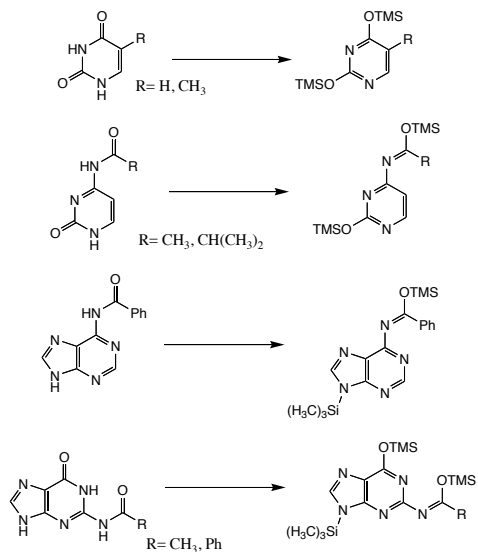
273

The stereochemistry of the C2-ester group of the furanose-tetraester controls the stereochemistry of the glycosylation reaction



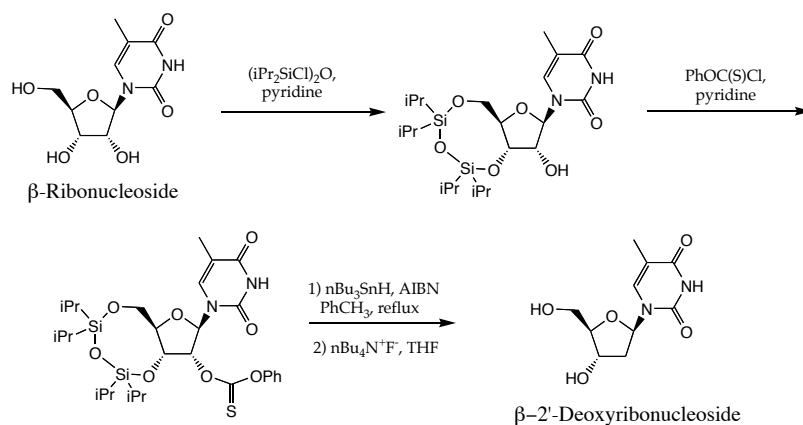
274

Exocyclic amino groups of C, A and G require protecting for the Vorbrüggen Glycosylation reaction



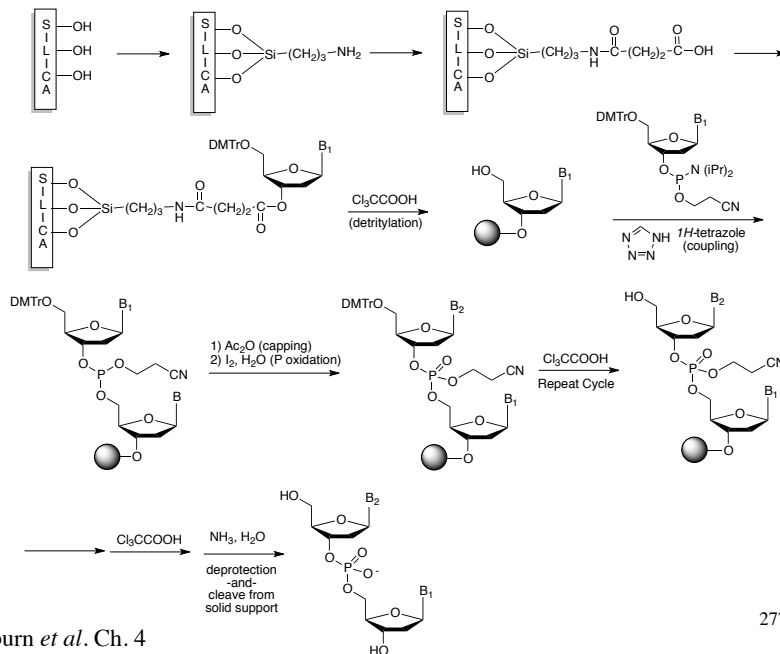
275

Chemical Conversion of Ribonucleoside to 2'-Deoxyribonucleoside:
Free radical deoxygenation of the 2'-hydroxyl group



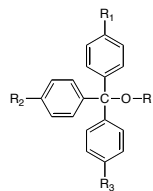
276

Solid-Phase Oligonucleotide Synthesis



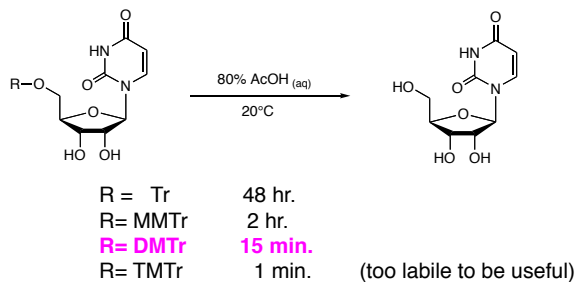
The Protecting Groups for Solid Phase DNA Synthesis:

5' - hydroxyl group

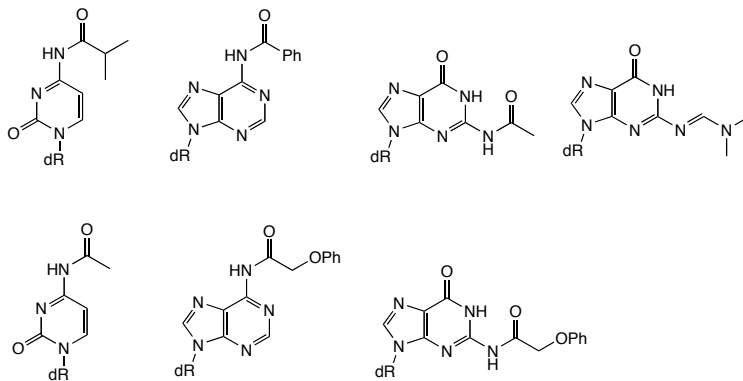


Trityl R ₁ = R ₂ = R ₃ = H	Tr-OR
(p-Methoxyphenyl)diphenylmethyl ether R ₁ = R ₂ = H, R ₃ = OCH ₃	MMTr-OR
4'-methoxytrityl	
Di-(p-methoxyphenyl)phenylmethyl ether R ₁ = H, R ₂ = R ₃ = OCH ₃	DMTr-OR
4',4'-dimethoxytrityl	
Tri-(p-methoxyphenyl)methyl ether R ₁ = R ₂ = R ₃ = OCH ₃	TMTr-OR
4',4',4'-trimethoxytrityl	

Relative rate of hydrolysis:



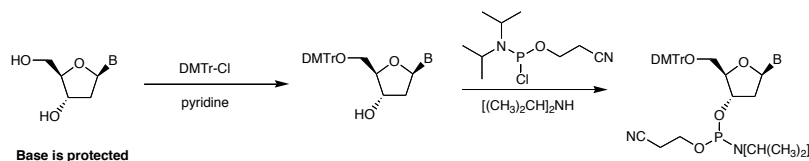
Protecting groups for the exocyclic amino groups



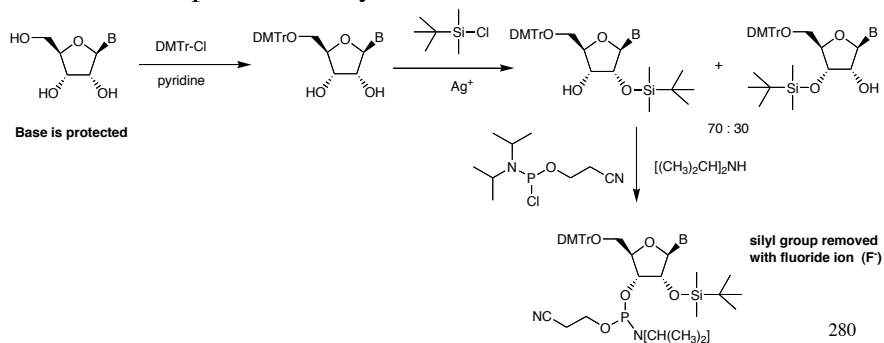
All are removed with $\text{NH}_3/\text{H}_2\text{O}$ at 80°C

279

Phosphoramidite reagents: the monomeric building blocks for solid-phase DNA synthesis



for solid-phase RNA synthesis

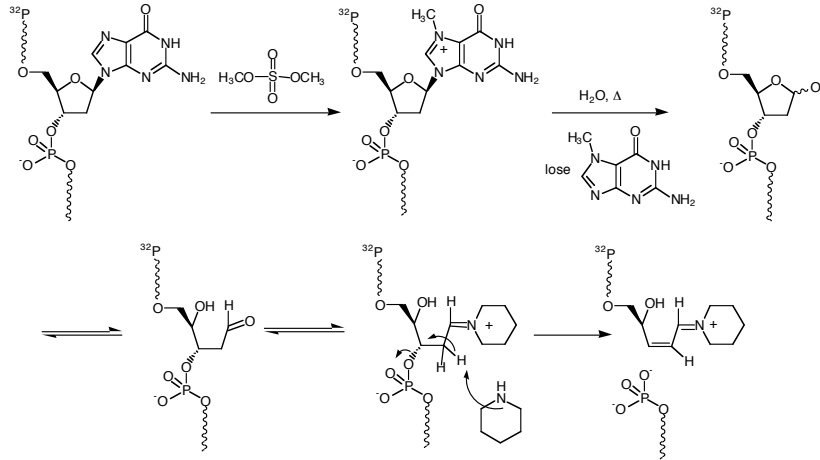


280

DNA Sequencing: Maxam-Gilbert Sequencing Blackburn *et al.* Ch. 5.1
 sequence selective chemical cleavage

Detection: 5'-³²P-labeling

A: Cleavage at the 3'-side of guanosines with a) dimethylsulfate b) Δ, c) piperidine.

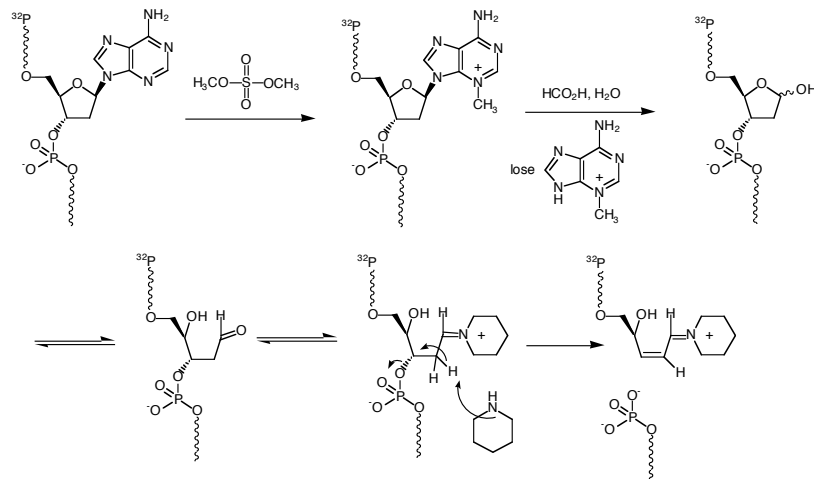


Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, 65, 499-558

281

Maxam-Gilbert Sequencing

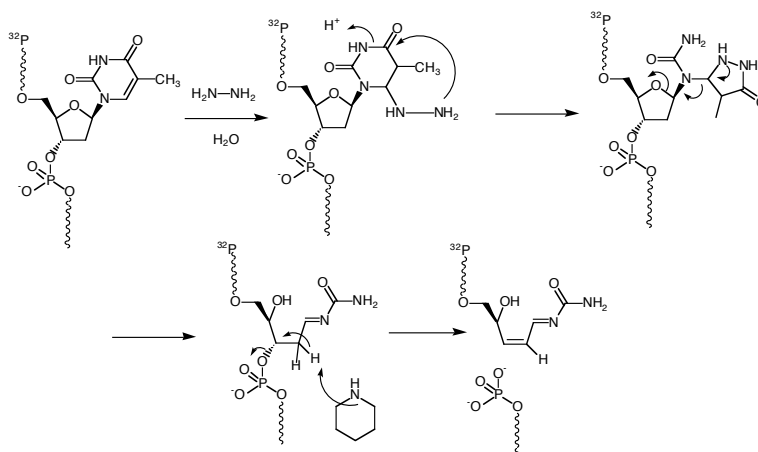
B. Cleavage at the 3'-side of adenosines and guanosines with a) dimethyl sulfate b) HCO₂H, c) piperidine.



282

Maxam-Gilbert Sequencing

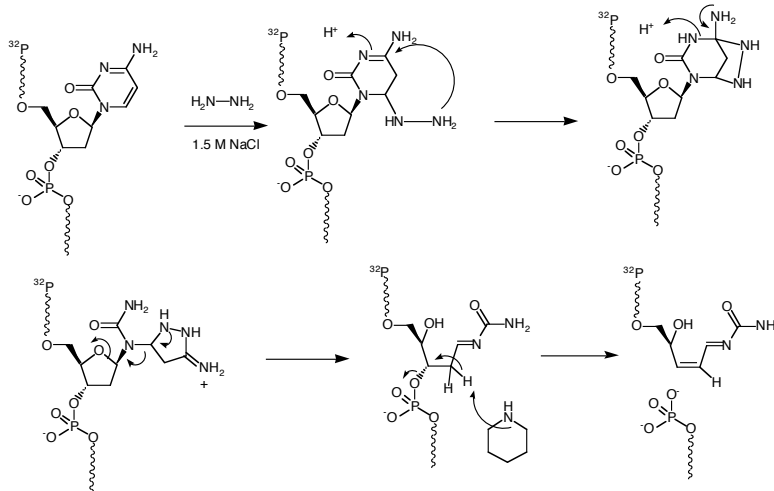
C. Cleavage at the 3'-side of thymidines and cytidines with a) hydrazine, b) Δ , c) piperidine.



283

Maxam-Gilbert Sequencing

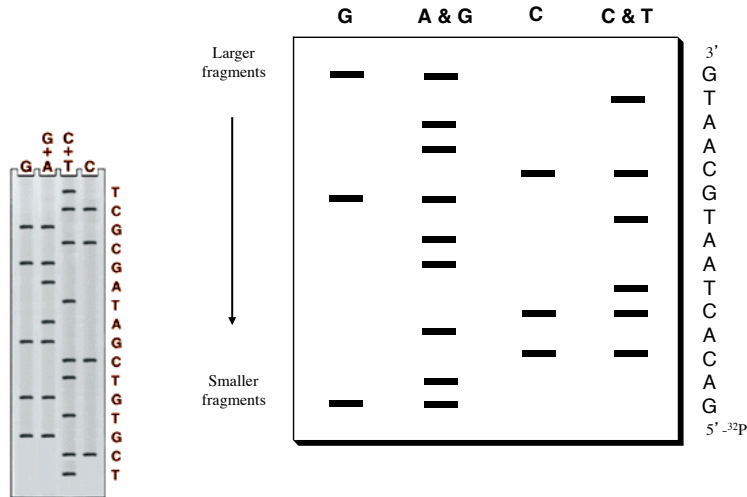
D. Cleavage at the 3'-side of cytidines with a) hydrazine, 1.5 M NaCl, b) Δ , c) piperidine.



284

Maxam-Gilbert Sequencing

Separation and detection of ^{32}P -labeled DNA fragments by polyacrylamide gel electrophoresis (PAGE). DNA fragments separated based upon charge, which is proportional to the length of the DNA fragment.

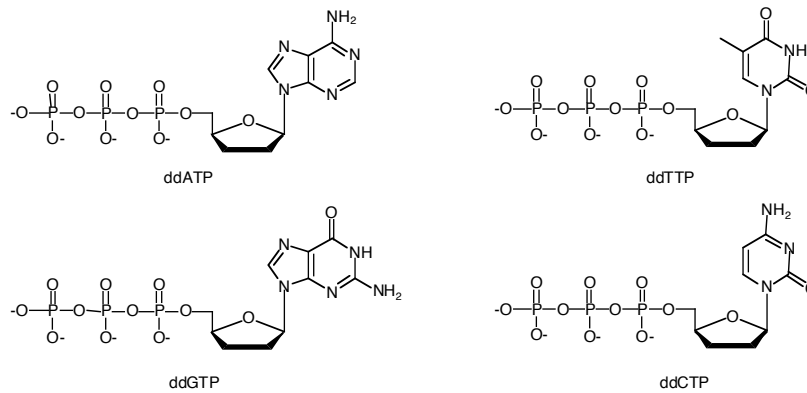


285

Sanger Sequencing

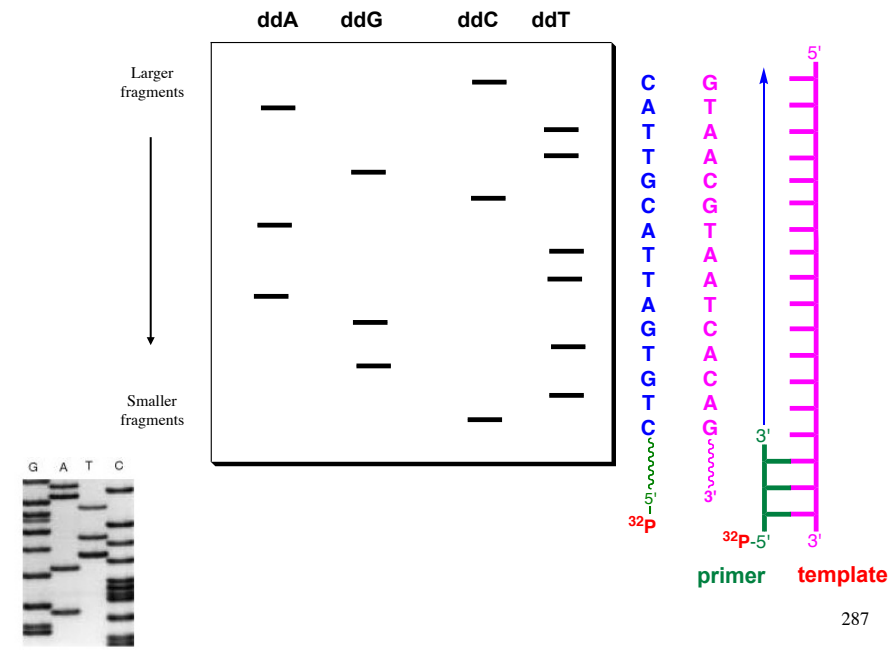
Enzymatic replication of the DNA fragment to be sequenced with DNA polymerase, Mg^{+2} , ddNTP's and ^{32}P -labeled primers.

ddNTP's

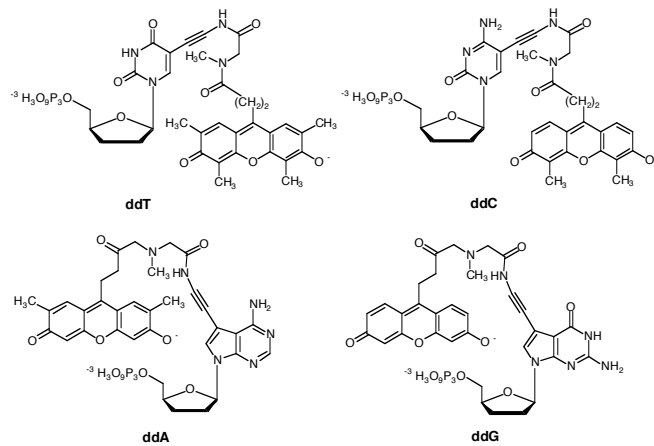


286

Sanger Sequencing



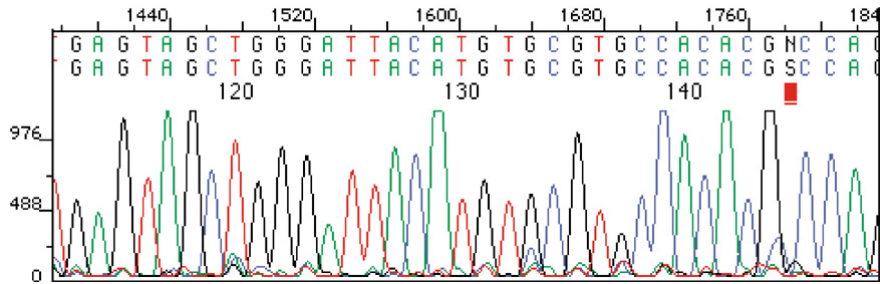
Sanger sequencing with fluorescent ddNTP's



Excitation: ~ 490 nM, Emission: ddT= 526 nm, ddC= 519 nm, ddA= 512 nm, ddG= 505 nm

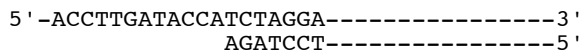
288

Sanger sequencing using fluorescent ddNTP's
 terminated DNA strands are separated by capillary electrophoresis

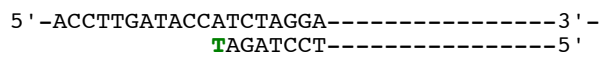


289

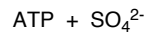
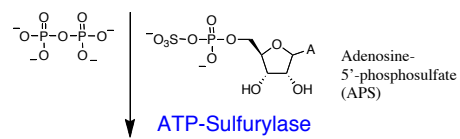
Pyrosequencing



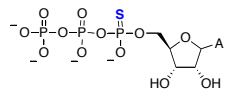
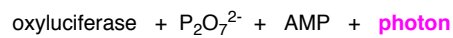
dNTPs are added sequentially one at a time
 dTTP, Mg²⁺, DNA polymerase



Three enzyme system where the incorporation of a complimentary nucleotide opposite a template DNA strand results in the emission of a photon.



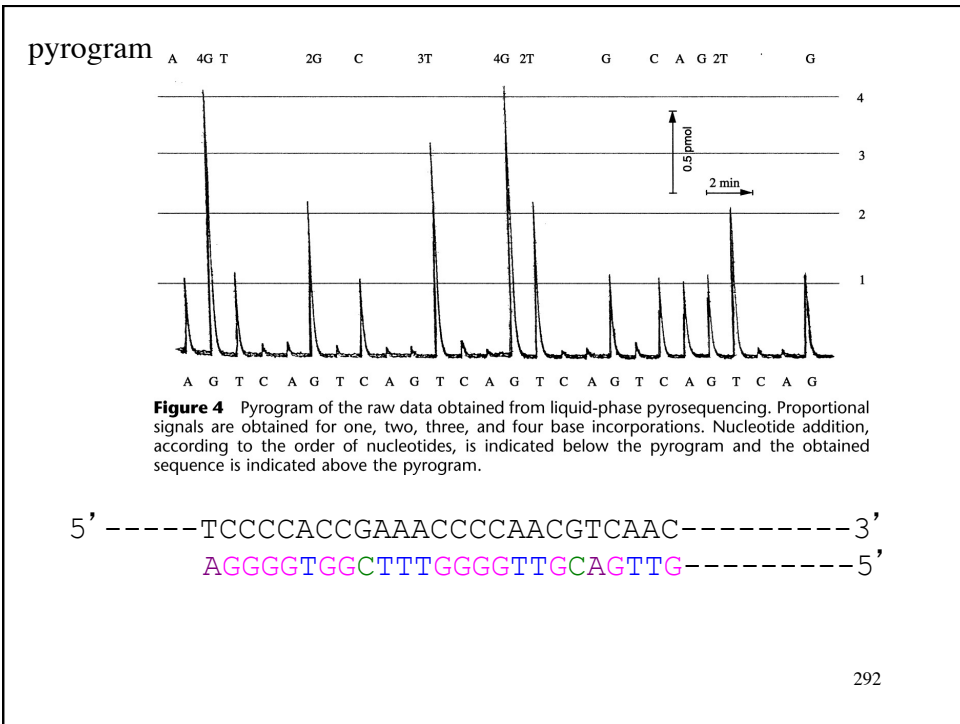
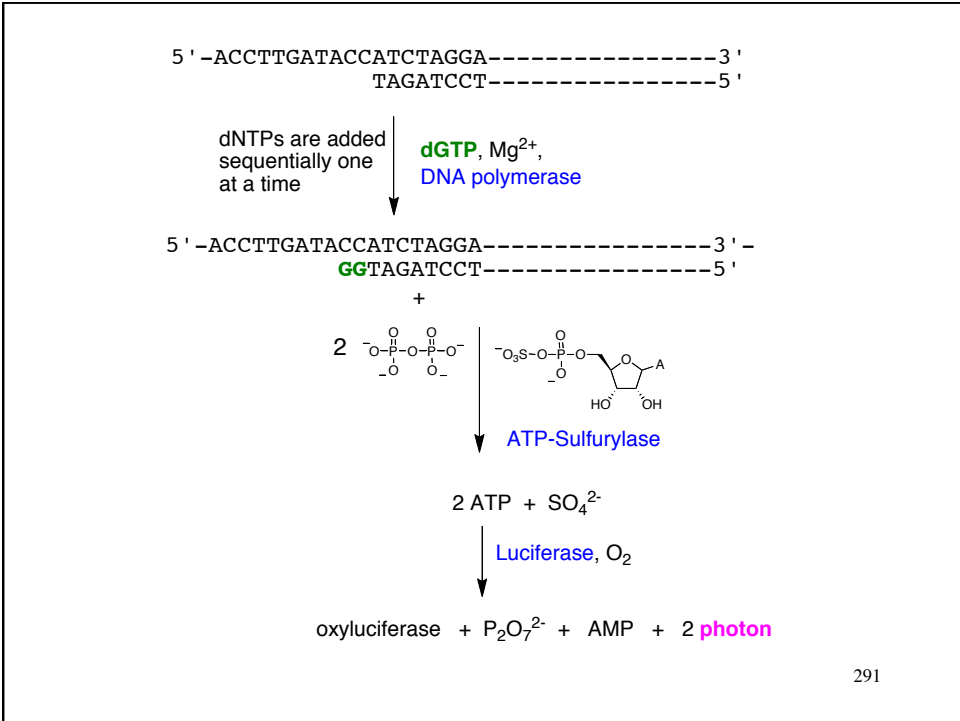
Luciferase, O₂



is used in place of ATP - not a substrate for ATP-sulfurylase & luciferase

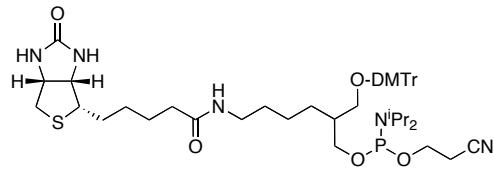
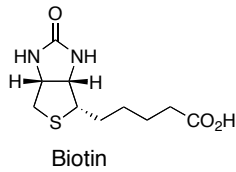
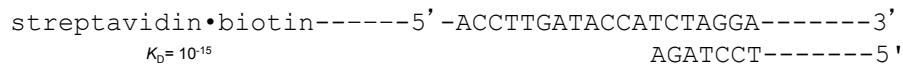
Ronaghi, M., *Genome Res.* **2001**, *11*, 3-11

290



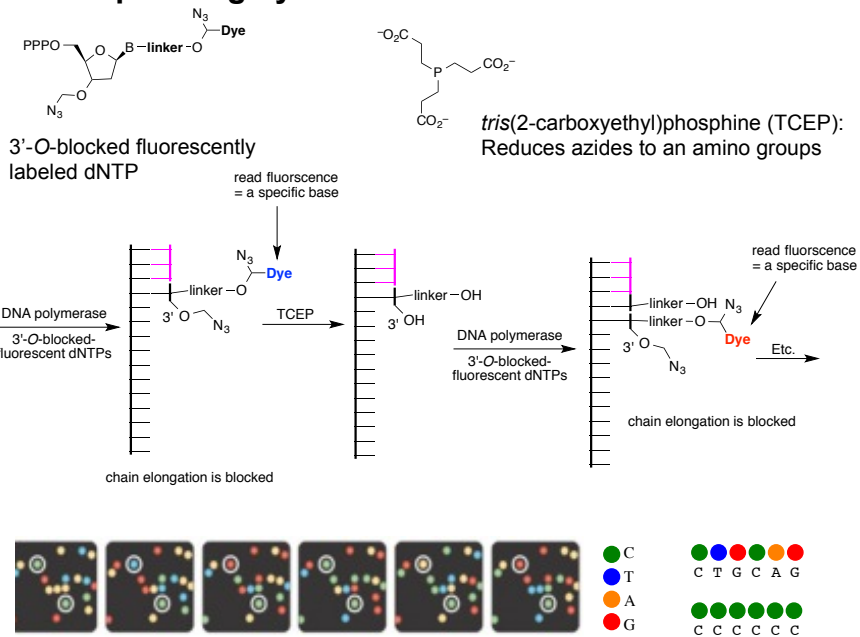
Apyrase is a fourth enzyme that is added that will hydrolyze the dNTP and ATP to the corresponding dNMP and PPi between dNTP additions. The rate of hydrolysis of apyrase is slower than the other three enzymes.

Alternatively, a biotinated template is used which is bound to streptavidin coated beads. The immobilized template DNA is washed between dNTP addition

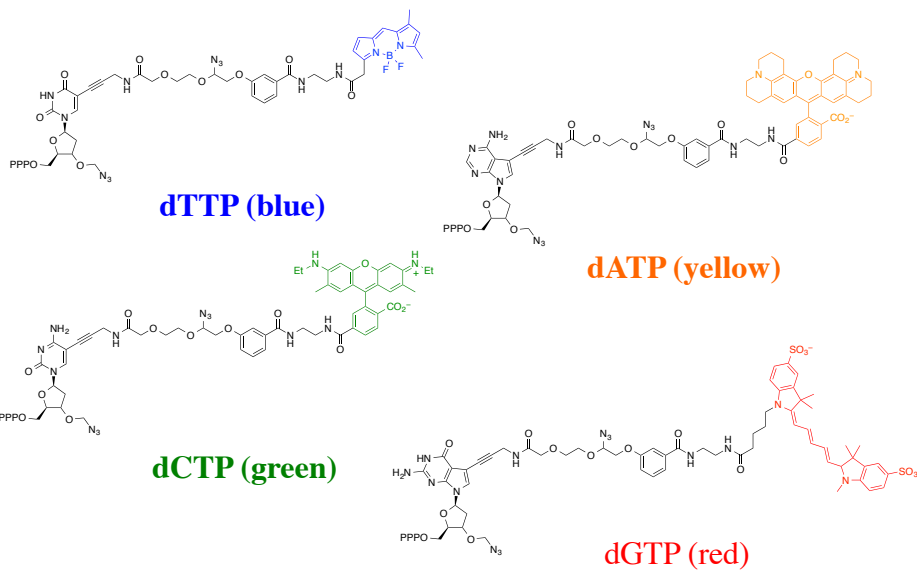


293

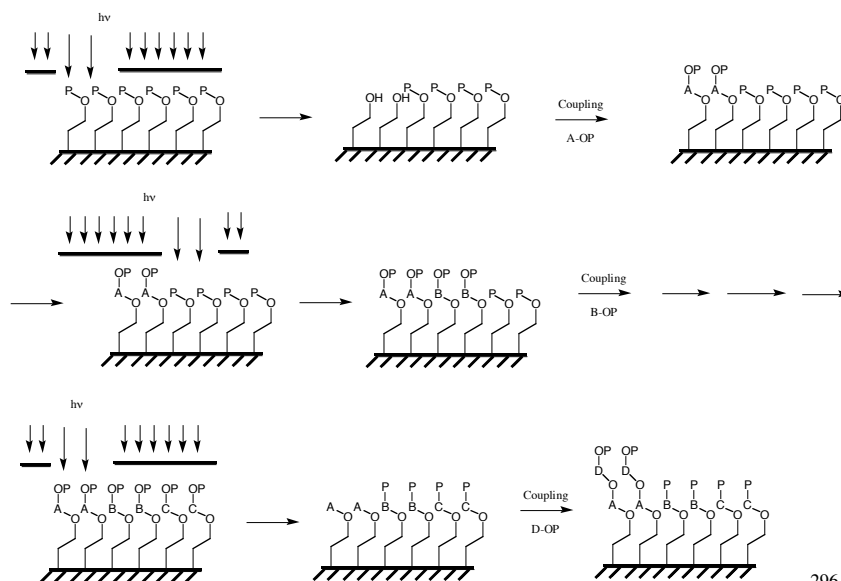
Sequencing by Reversible Chain Termination



Reversible Chain-Terminating, Fluorescent dNTPs

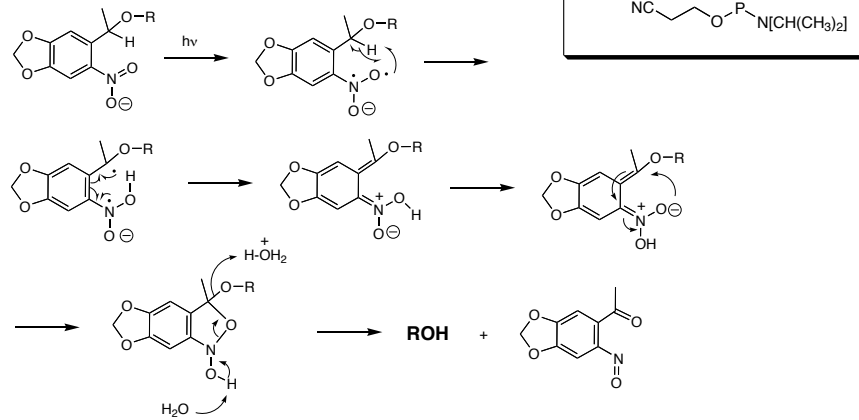


Light-Directed, Spatially Addressable Parallel Synthesis



Photoremovable protecting group: o-nitrobenzyl ether derivatives

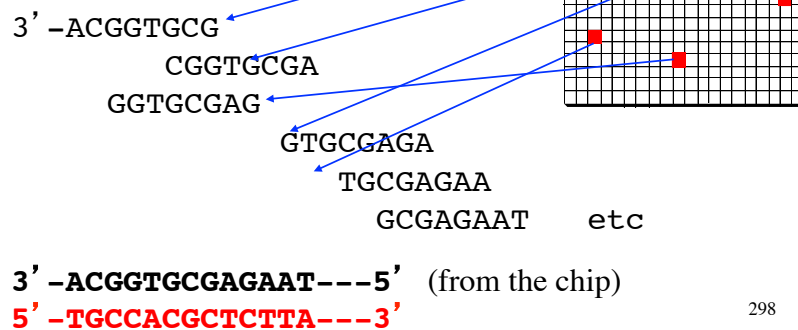
Mechanism: $h\nu$ ($\lambda \sim 350$ nm)



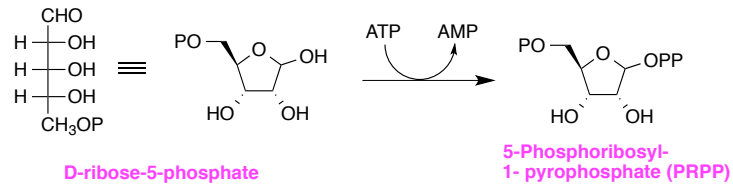
Spatially addressable: 8-mer chip: 65,536 different sequences
12-mer chip: 1,677,216 different sequences

DNA fragment \longrightarrow DNA-fluorophore

Place DNA on the chip, then wash away non-specific hybridization after 1-10 hrs.
Raise temperature and "melt" away partially hybridized sequences.



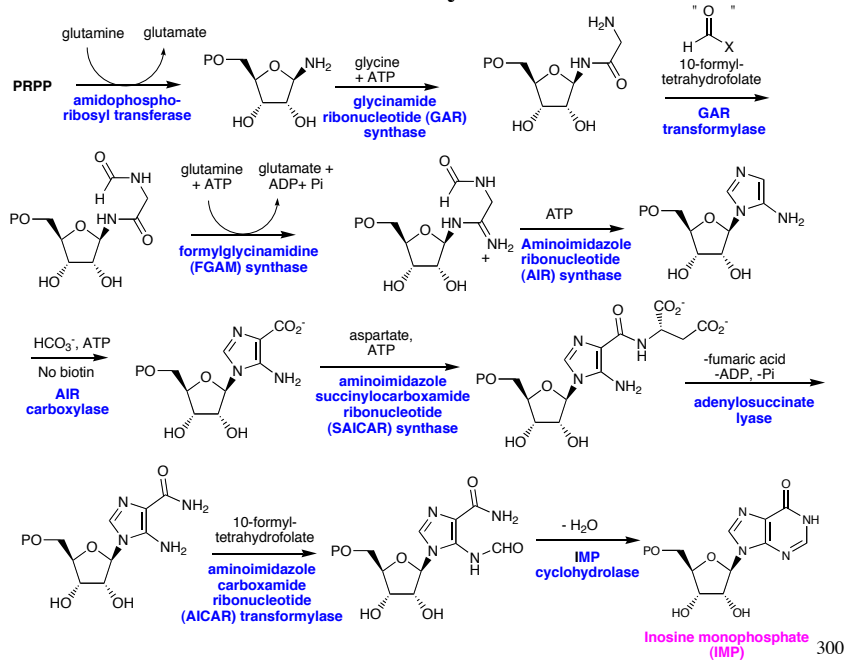
Nucleotide Biosynthesis



Blackburn *et al.* Ch. 3.4, 3.5

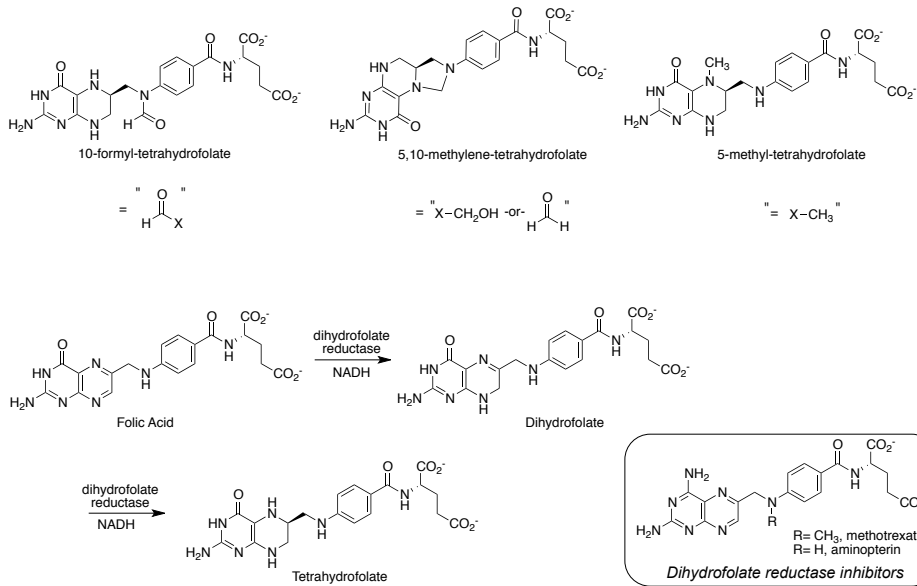
299

Purine Biosynthesis



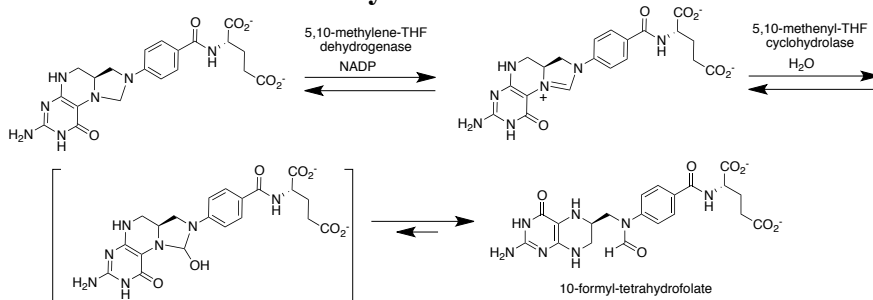
300

Folate derivatives – one-carbon donors

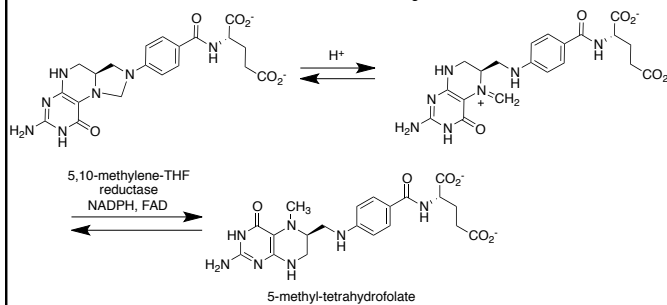


301

5,10-methene-THF to 5-formyl-THF

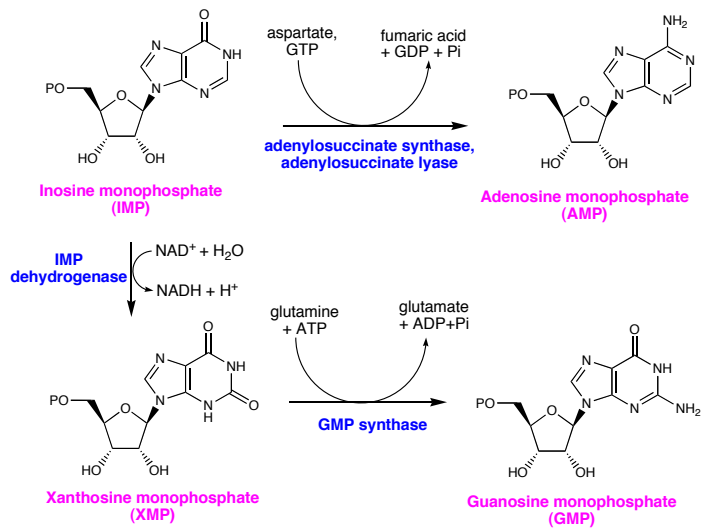


5,10-methene-THF to 5-methyl-THF



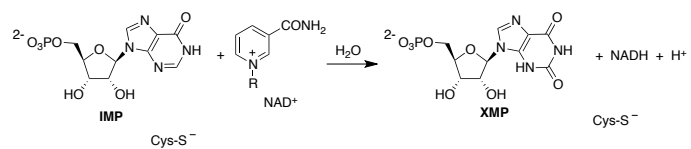
302

Purine Biosynthesis (con' t)



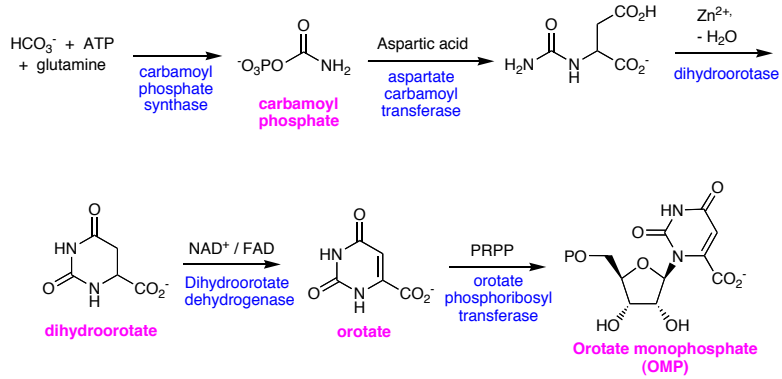
303

IMP Dehydrogenase

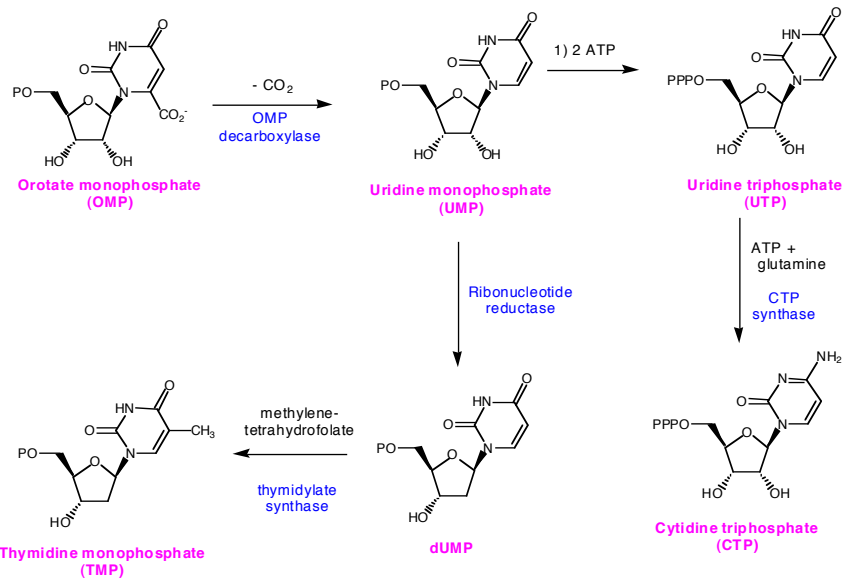


304

Pyrimidine Biosynthesis

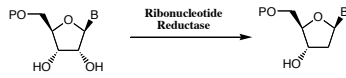


305

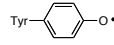
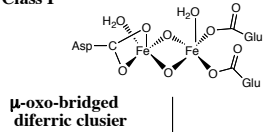


306

Ribonucleotide Reductase



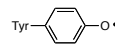
Class I



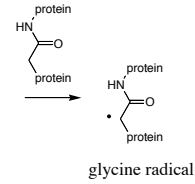
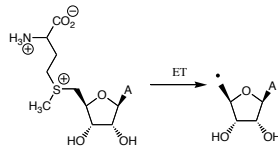
Class II



Class IV

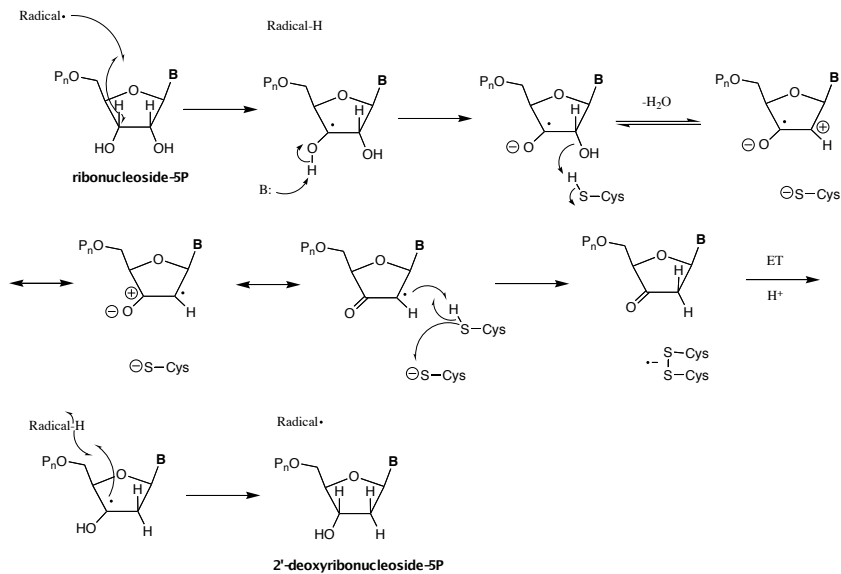


Class III



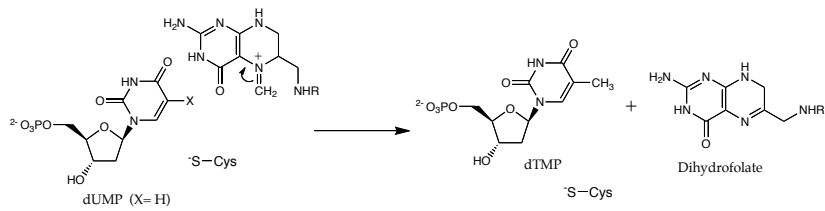
307

Mechanism of Ribonucleoside Reductase



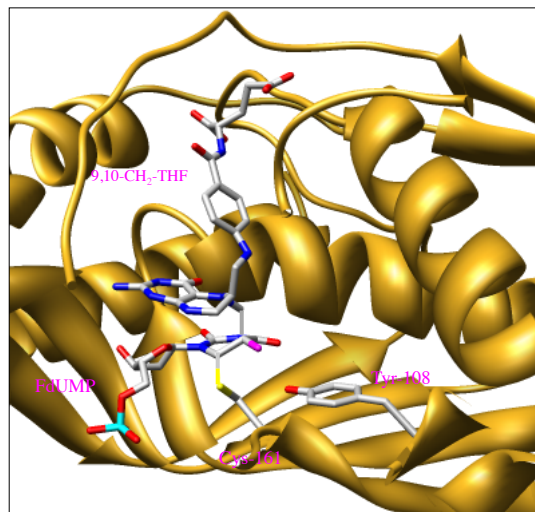
308

Mechanism and Inhibition of Thymidylate Synthase



309

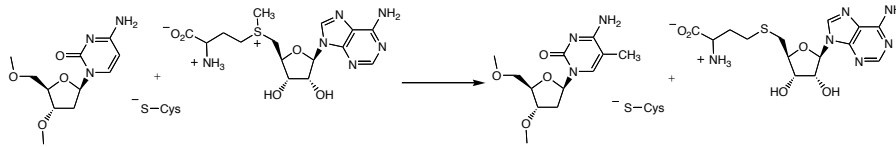
Ternary complex of thymidylate synthase with 5'-fluoro-dUMP and 5,10-dihydrotetrahydrofolate



pdb code: 1B02

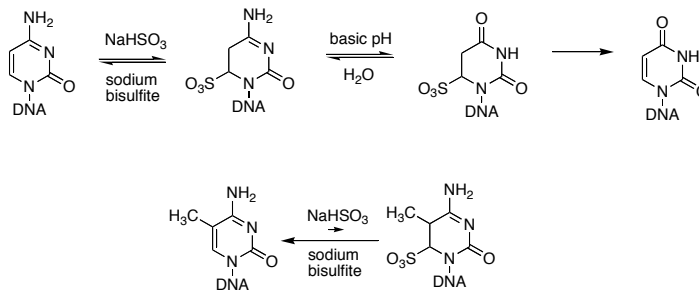
310

DNA Methylation: 5-methyl-2'-deoxycytidine



311

5-Methyl-C (Epigenetic) sequencing



Sodium bisulfite convert C's to U's, but has no effect on 5-methyl-C

312

Antiviral nucleosides:

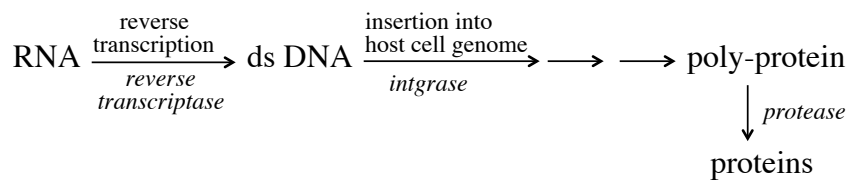
2,3-dideoxynucleosides as reverse transcriptase inhibitors

Protein Biosynthesis



Retrovirus (hepatitis B, HIV, HPV)

ssRNA + proteins (reverse transcriptase, integrase, protease)



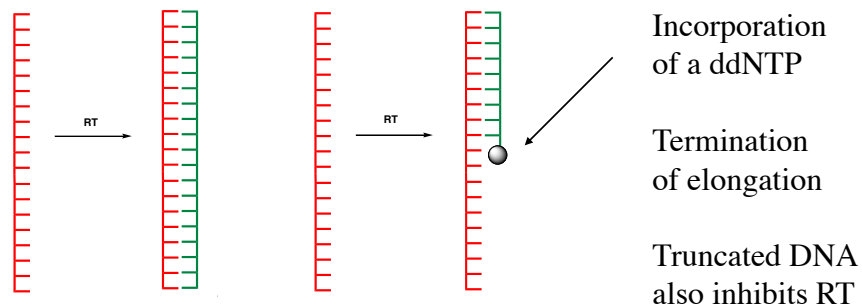
Blackburn *et al.* Ch. 3.7

H. Temin & D. Baltimore
1975 Nobel Prize in Medicine or Physiology

313

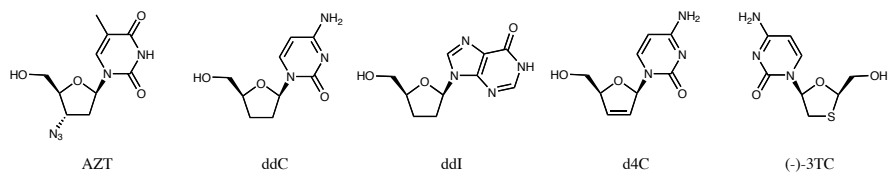
Mechanism of action of 2,3-dideoxynucleosides:

recall Sanger sequencing- termination of a growing DNA chain by enzymatic incorporation of a 2,3-dideoxynucleosides

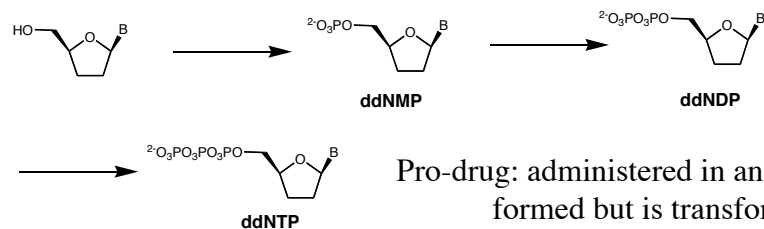


314

Nucleoside-based reverse transcriptase inhibitors



2,3-dideoxynucleoside is enzymatically phosphorylated at the 5-hydroxyl to the ddNTP, which is the active RT inhibitor



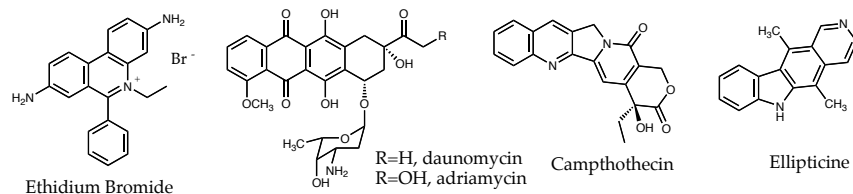
Pro-drug: administered in an inactive form but is transformed *in vivo* into the active drug

315

DNA as a target for therapeutic agents:

Intercalation:

- planar aromatic molecules “slide” between stacked bases
- intercalators span the minor and major grooves of DNA
- driven by hydrophobic interactions
- intercalators stabilize DNA structure
- causes an “unwinding” and elongation of DNA in the area of the intercalation



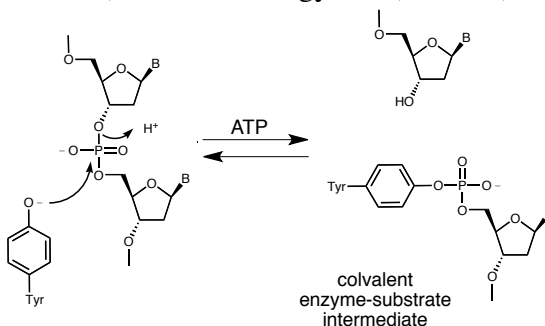
316

Topological relationship of DNA: supercoiling, tangling, knotting for replication (transcription), the supercoiling of DNA must first be "relaxed"

Topoisomerase (mammalian)

DNA gyrase (bacteria)

Mechanism of the DNA cleavage step

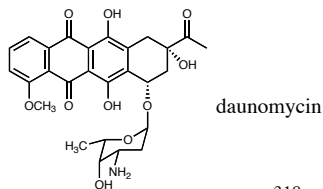
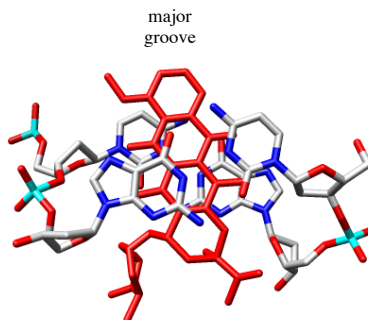
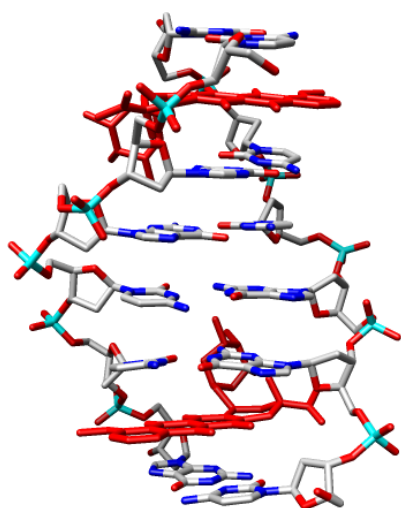


Topo II inhibitors stabilize the covalent DNA•Topo II complex (protein bound double strand cleaved DNA), which causes chromosomal abnormalities and leads to *apoptosis*

Corbett, A.H.; Osheroff, N. *Chem. Res. Toxicol.* 1993, 6, 585-597
 Topo II movies: <http://berger.berkeley.edu/Pages/Teaching.html>

317

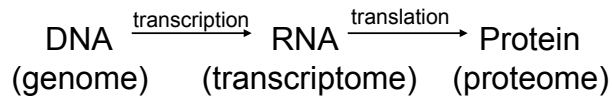
Intercalation



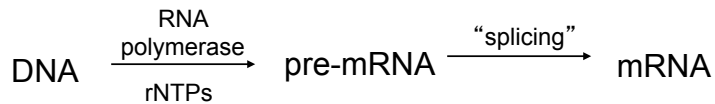
pdb code: 110D

318

Ribosomal Protein Biosynthesis:

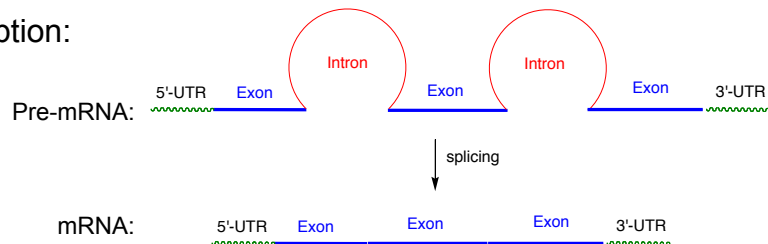


Transcription: only one of the DNA strands is copied (coding or **antisense** strand). Its sequence is converted to the complementary sequence in mRNA (template or **sense** strand), which codes for the amino acid sequence of a protein (or peptide)



319

Transcription:



Translation: mRNAs are transported from the nucleus to the cytoplasm, where they act as the template for protein biosynthesis (ribosomes). A three base segment of mRNA (codon) codes for an amino acid.

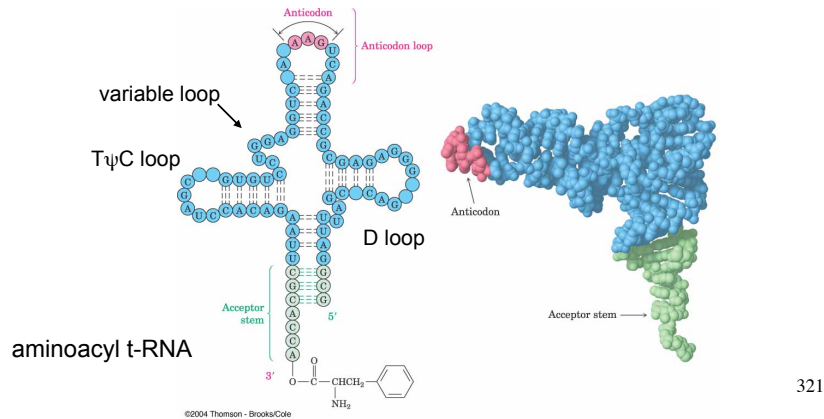
THE STANDARD GENETIC CODE

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

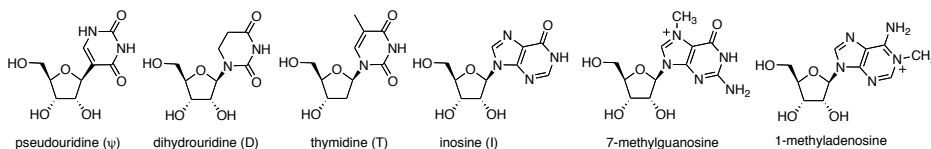
320

AUG is part of the initiation signal, as well as being the codon for internal methionine.

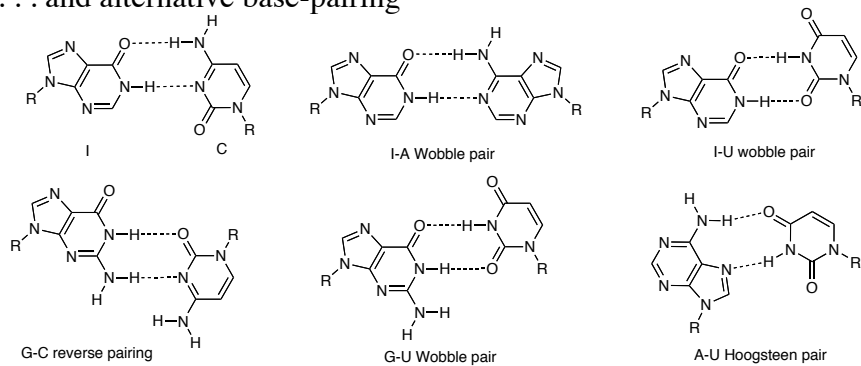
Transfer RNA (tRNA): The “anticodon” region of tRNA is complementary to the mRNA codon sequence. The t-RNA carries an amino acid on the 3’ -terminal hydroxyl (A) (aminoacyl t-RNA) and the ribosome catalyzes amide bond formation. Although single-stranded, there are complementary sequences within tRNA that give it a defined conformation



There are many non-standard bases found in tRNAs

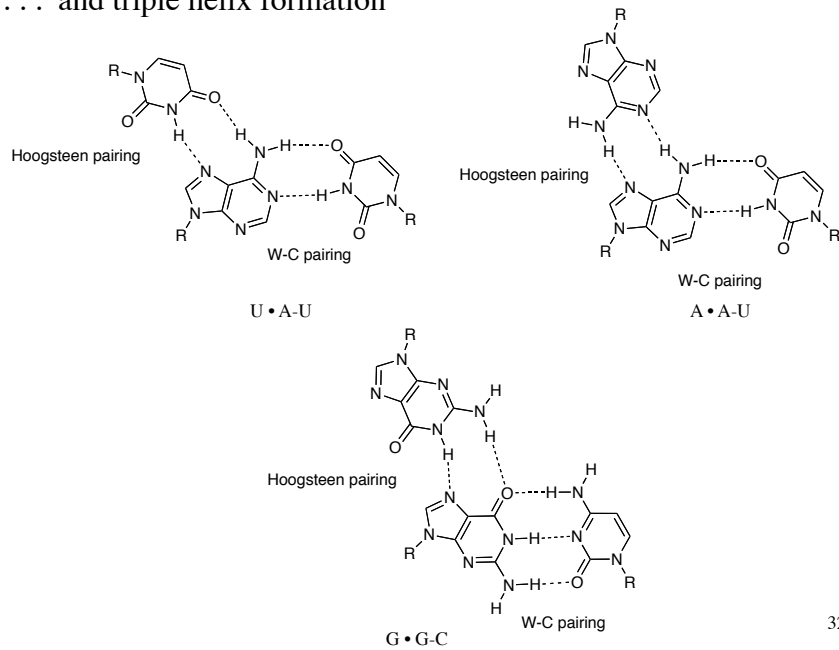


... and alternative base-pairing

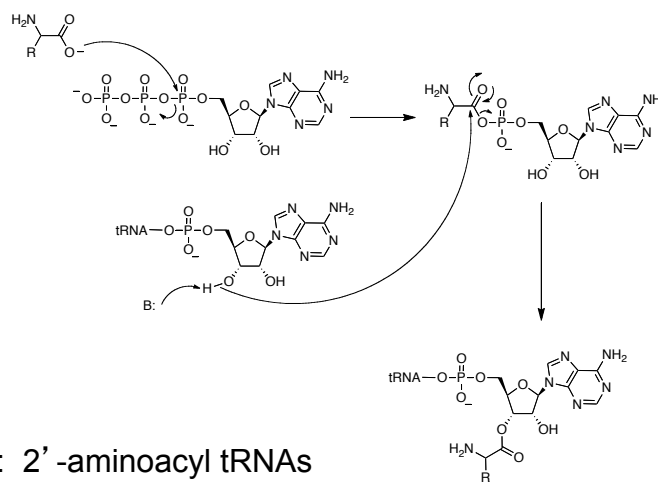


322

... and triple helix formation



tRNA Synthetase: catalyzes the biosynthesis of specific 3'-aminoacyl tRNAs from tRNAs, amino acids, and ATP

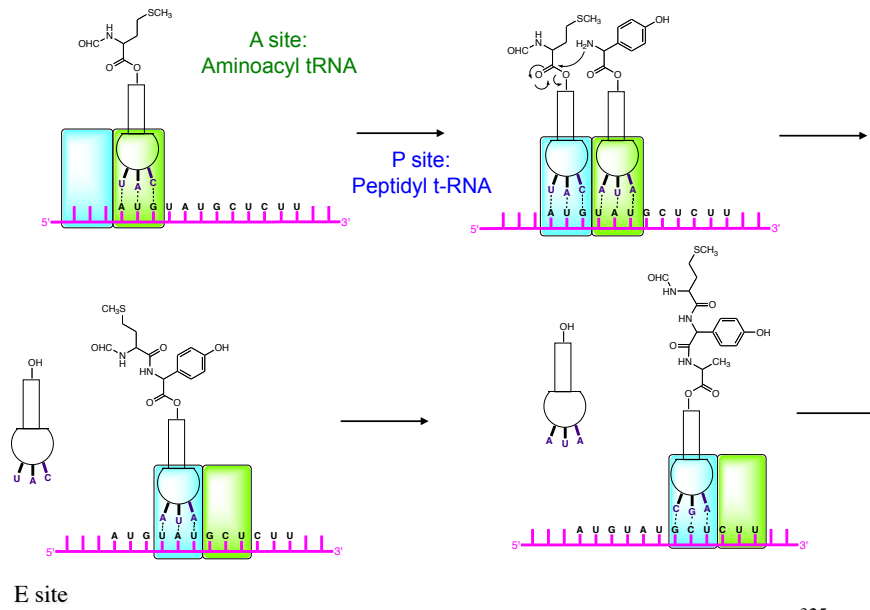


Class I: 2'-aminoacyl tRNAs
Class II: 3'-aminoacyl tRNAs

3'-aminoacyl tRNAs

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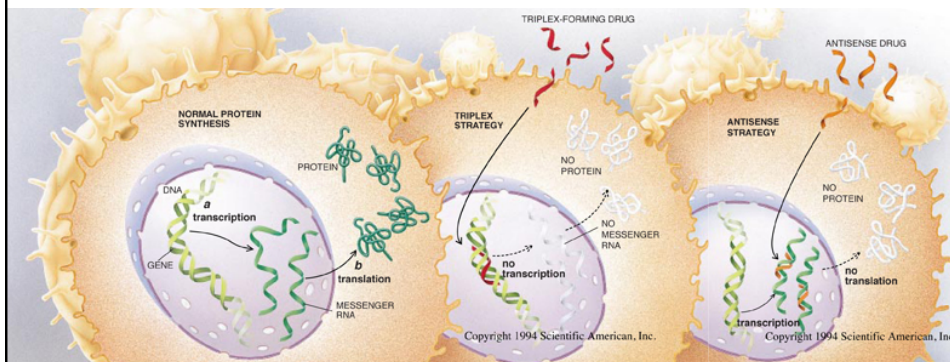
Ribosomal protein synthesis



325

Oligonucleotide-Base Therapies:

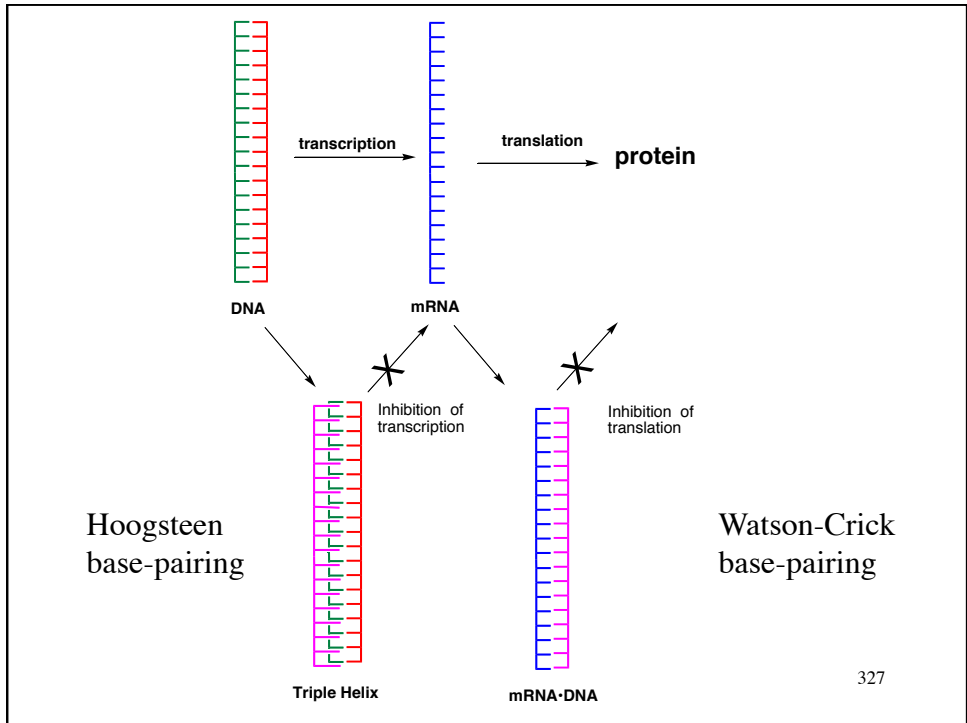
Inhibition of Protein Biosynthesis via the Antisense-Antigen (Triplex) Strategies



Taken from: "The New Genetic Medicines," J. S. Cohen, M. E. Hogan *Scientific American* 1994 (Dec.), pp 75-82.

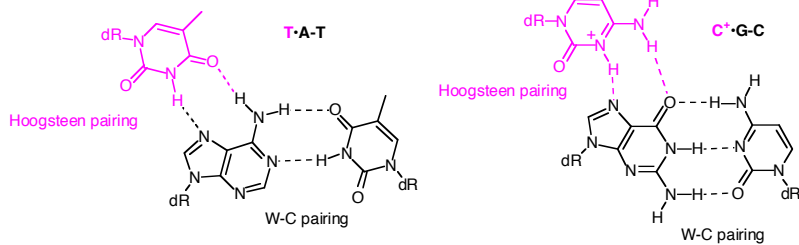
- Potentially highly selective (magic bullet) approach to therapy
- ~15 bases sequence is unique in the human genome

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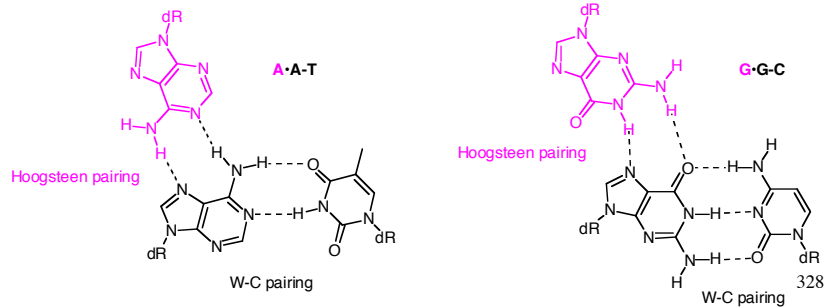


Triple Helix Motifs: third strand binds in the major groove

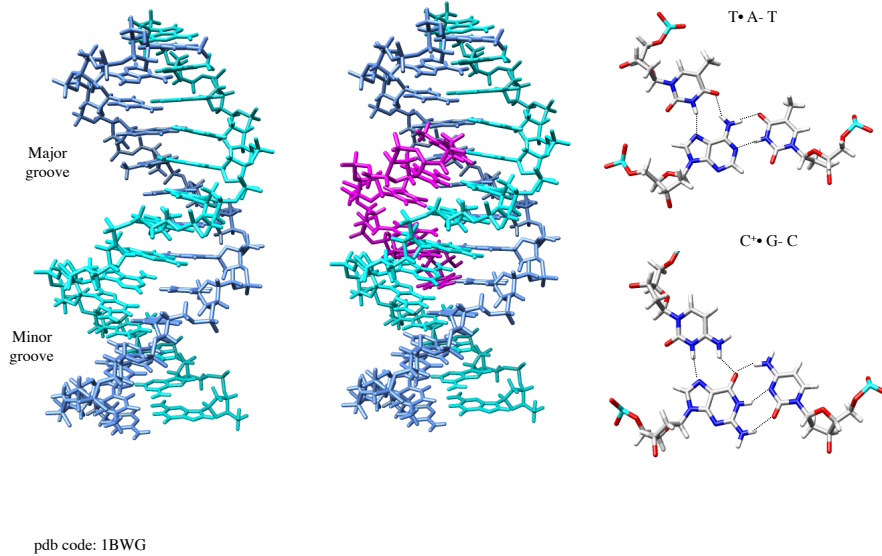
Pyrimidine•Purine-Pyrimidine: third strand runs parallel to the homo-purine strand



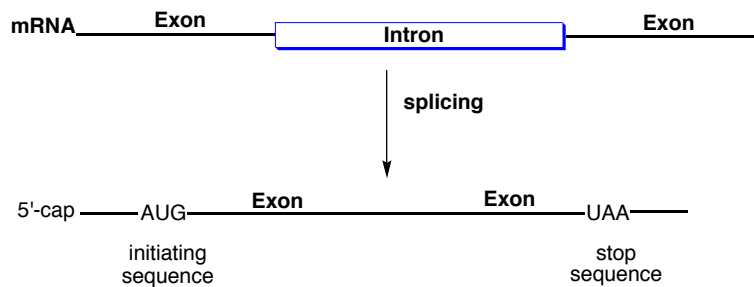
Purine•Purine-Pyrimidine: third strand runs antiparallel to the homo-purine strand



DNA Triple Helix



Antisense Inhibition: inhibits mRNA function



Antisense targeting:

Interon-exon splice junction: interferes with slicing

5' -cap (UTR) region: interferes with binding to the ribosome

Initiation and promoter sequences: protein synthesis is not initiated

Coding sequence: interferes with elongation of the protein

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When an antisense oligonucleotide binds to mRNA, ribonuclease H is up-regulated. The mRNA of the mRNA•DNA hybrid is digested.

Problems with oligonucleotide-based therapies

Transport: DNA does not cross cellular membranes very easily

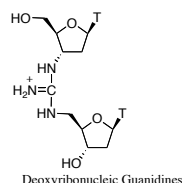
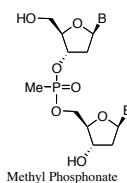
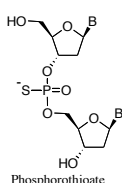
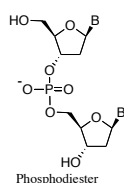
Degradation: DNA is subject to enzymatic digestion by cellular nuclease

Synthetic Oligonucleotides: must maintain affinity and selectivity and impart nuclease resistance

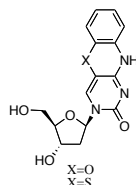
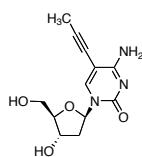
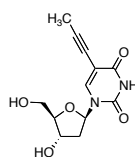
- backbone replacements
- modified bases

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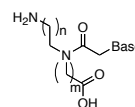
Triple Helix Oligonucleotides



Antisense Nucleosides



Peptide Nucleic Acids (PNAs)

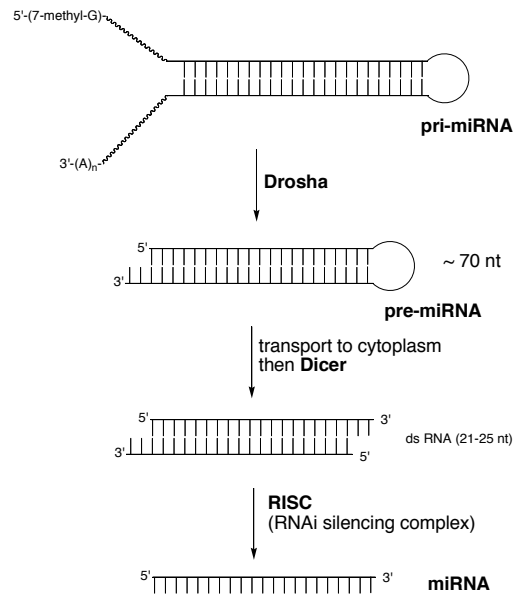


Peptide backbone forms a helical structure.

Base will hybridize with ss or ds DNA or RNA with high affinity

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microRNA (miRNA): genetically encoded (transcribed) but non-translated RNAs (do not code for a protein or peptide)



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Ribonucleases (RNase): enzymes that catalyze the hydrolysis of the phosphodiester bonds of RNA

single strand specific: RNase A

double strand specific: RNase III

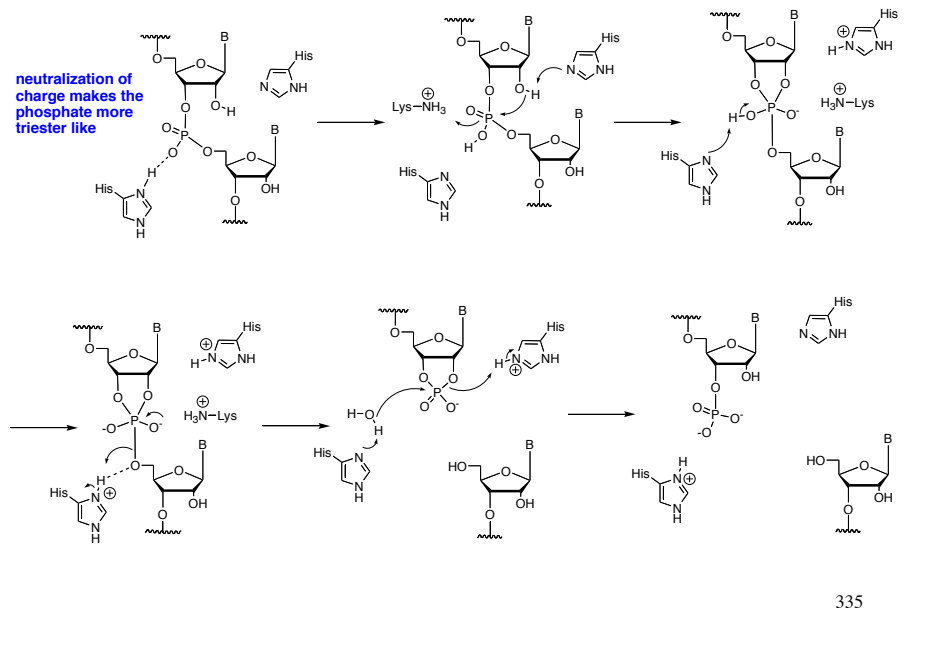
specific for DNA/RNA hybrids: RNase H

endonucleases: RNase A, RNase III

exonuclease: RNase II

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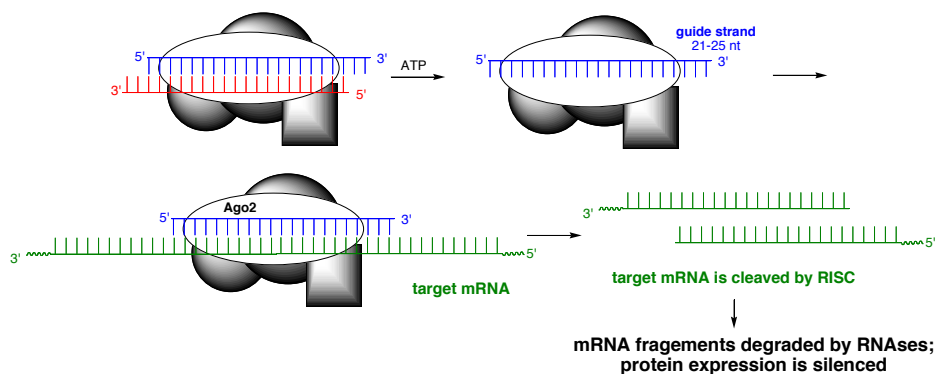
A mechanism for ribonuclease A



Interference RNA (RNAi)

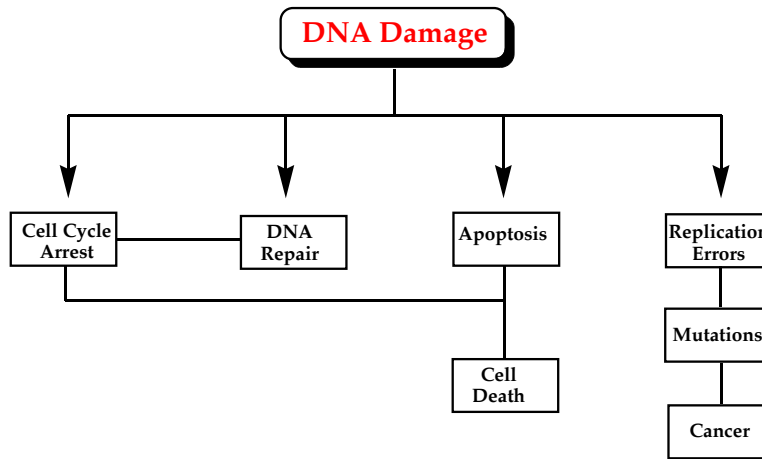
miRNAs (or siRNAs) are important in post-transcriptional regulation of gene expression

RISC (RNAi silencing complex)- multi-protein complex with helicase and ribonuclease activity



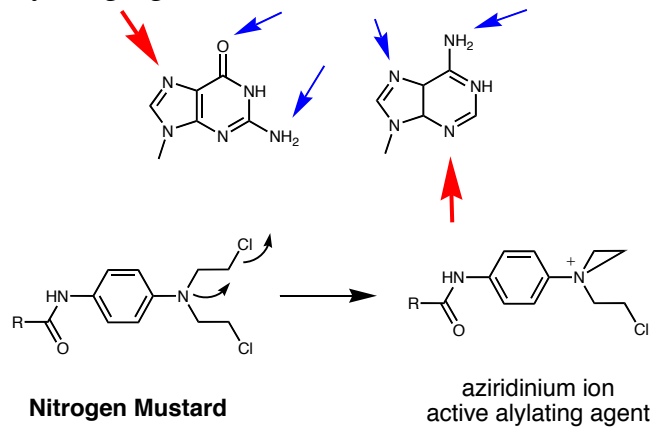
Andrew Fire & Craig Mello, 2006 Nobel Prize in Medicine & Physiology³³⁶
Animation: <http://www.nature.com/focus/rnai/animations/index.html>

Cellular Response to DNA Damage:



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DNA Alkylating Agent



Nitrogen mustards will alkylate adjacent DNA bases causing DNA-DNA cross-links, which is a severe form of DNA damage and can trigger apoptosis or cause mutations (and cancer)

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Other simple DNA alkylating agents:

Alkyl halides (or reactive equivalents): dimethylsulfate
methylene chloride, dichloroethane, SAM (endogenous)

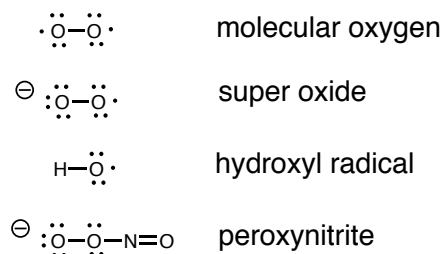
Enals: acrolein (industrial chemical, cigarette smoke),
4-hydroxynon-2-enal, malondialdehyde (endogenous)

Epoxides: Butadiene diepoxide (metabolism of butadiene),
chlorooxirane (metabolism of vinyl chloride), benzo[a]-
pyrene diol epoxide (metabolism of benzo[a]pyrene)

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Free-radical mediated oxidative damage to DNA

Reactive oxygen species (ROS)

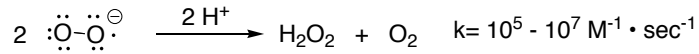
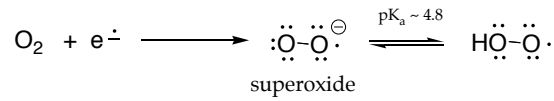


Causes oxidative cleavage of DNA in an O₂ dependent manner

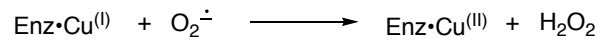
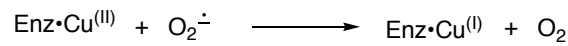
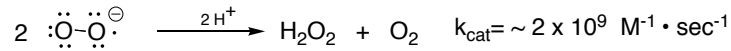
The oxygen paradox: oxygen is necessary for cellular metabolism;
however, oxygen is transformed into highly reactive species
that can damage biomolecules.

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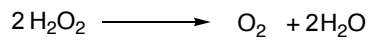
Oxygen metabolism



Superoxide dismutase (SOD): Zn-Cu or Mn-Fe

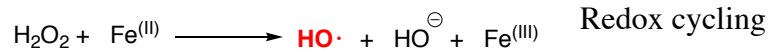
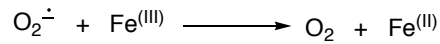


Catalase (heme)



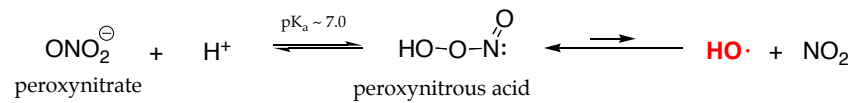
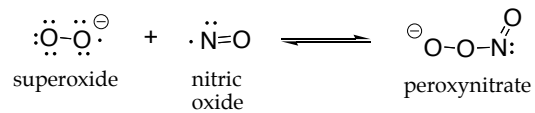
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Fenton reaction



Very little free Fe^(III) in cells

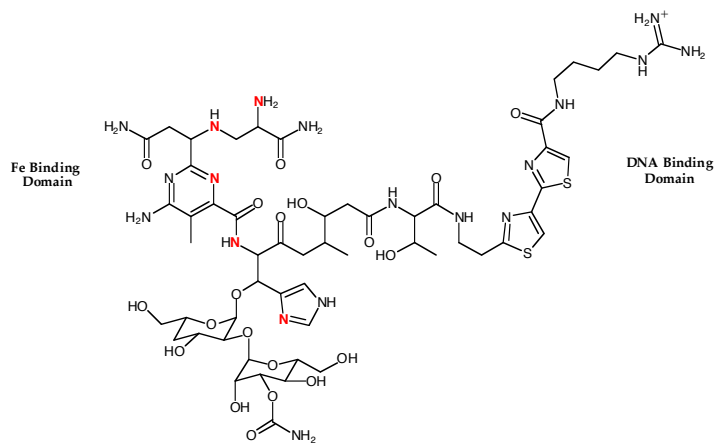
Haber-Weiss reaction



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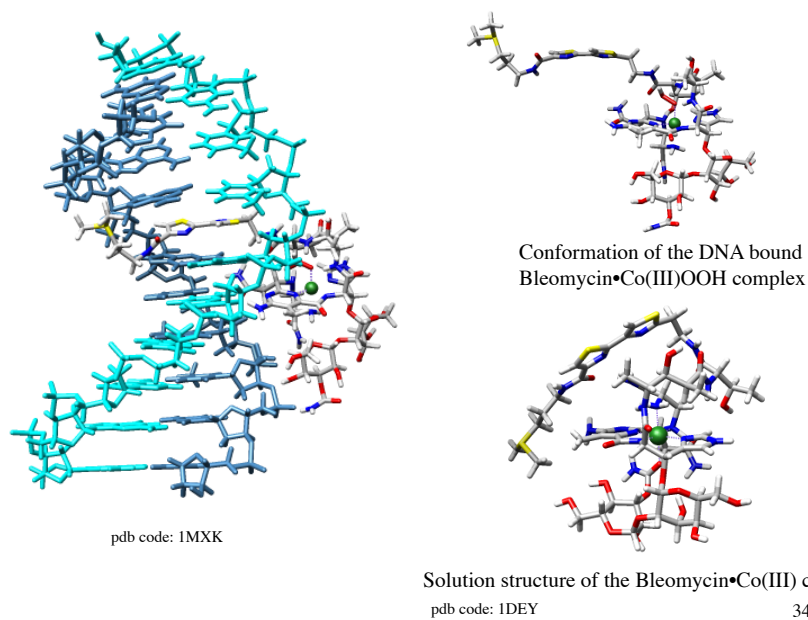
Bleomycin

Binds Fe and activates O_2 giving C4 hydrogen atom abstraction
mechanism of hydrogen atom abstraction may involve a $Fe-O\cdot$, reminiscent of P450



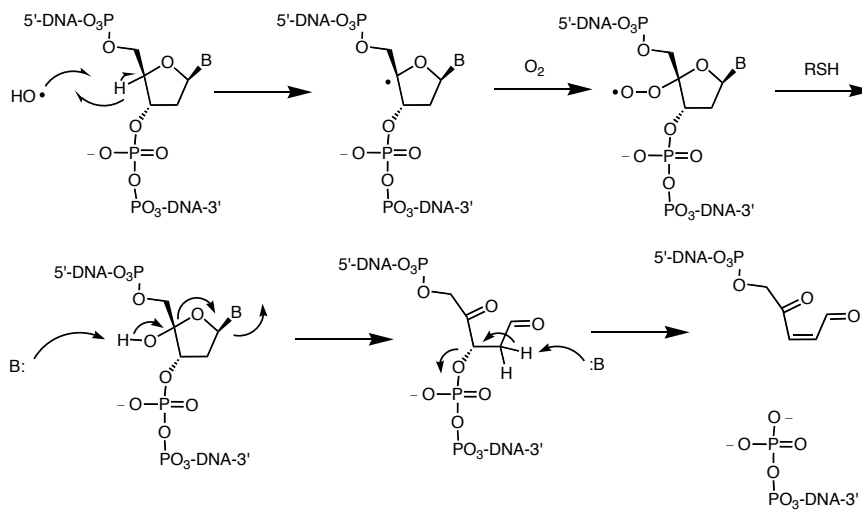
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Bleomycin•Co(III)OOH - DNA complex



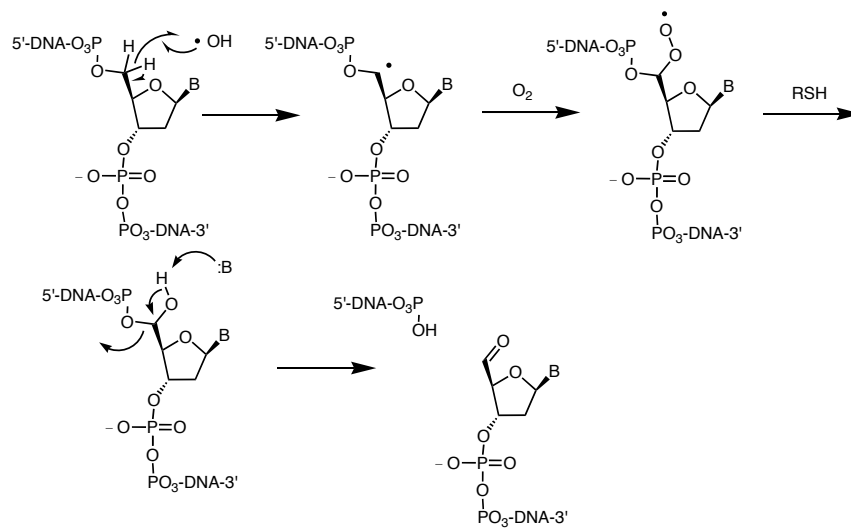
344

Abstraction of the 4'-hydrogen:



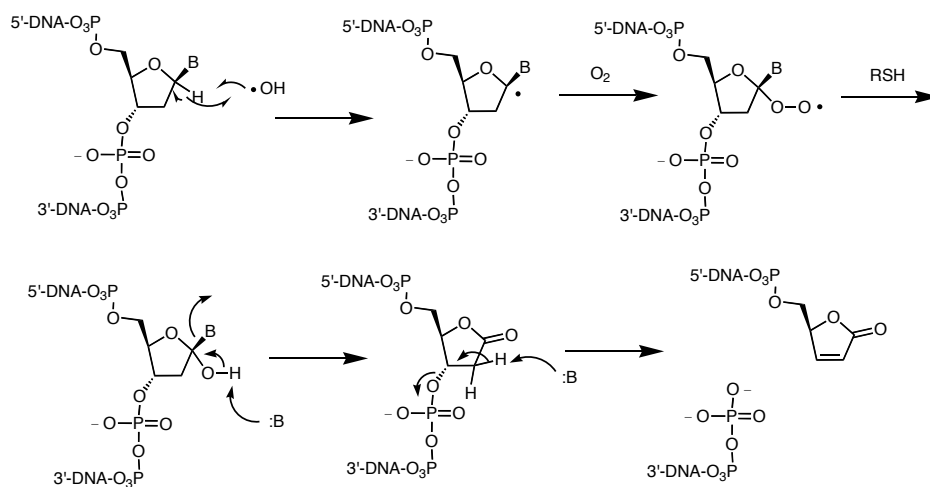
345

Abstraction of the 5'-hydrogen



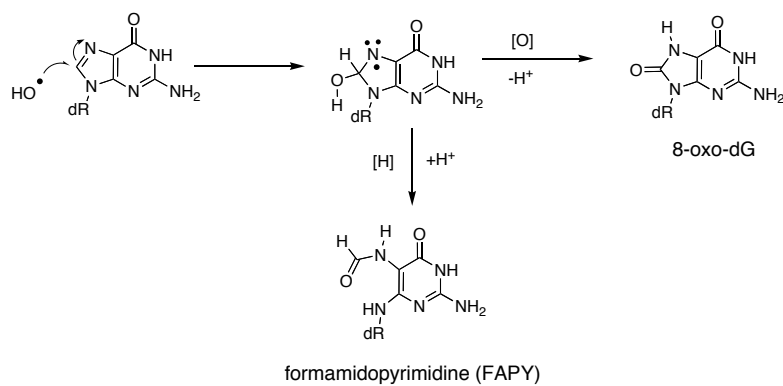
346

Abstraction of the 1'-hydrogen



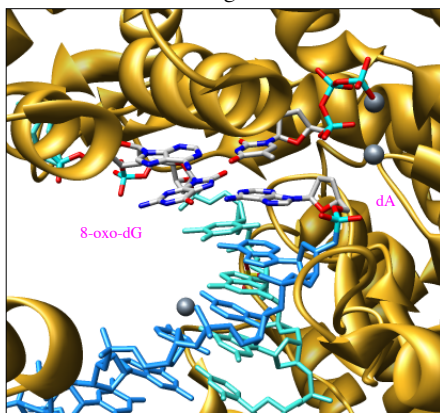
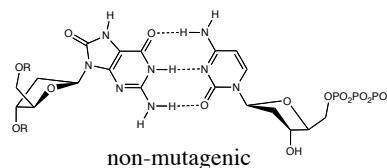
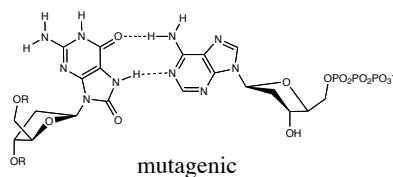
347

Formation of 8-Oxo-2'-deoxyguanosine and Formamidopyrimidine Lesions by the reaction of deoxyguanosine with ROS's

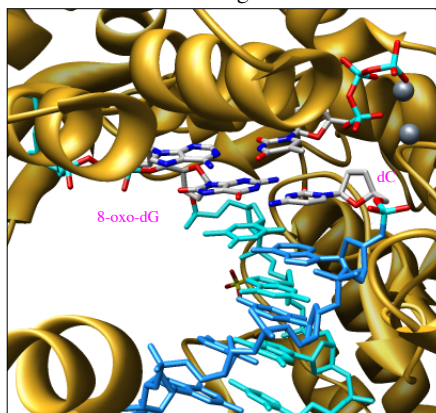


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Alternative base-pairing of 8-oxo-dG during replication with pol T7



pdb code: 1TK8

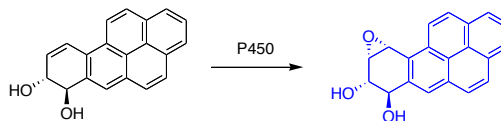
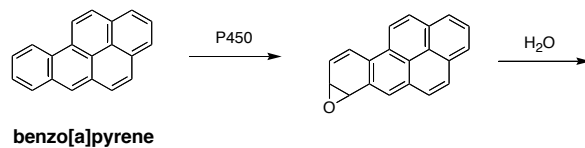


pdb code: 1TKD

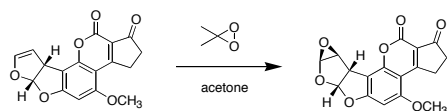
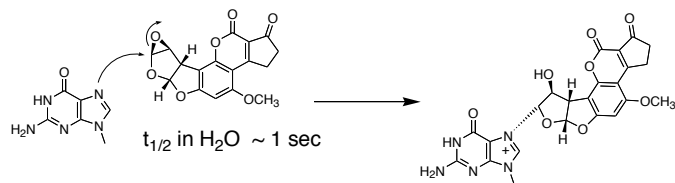
349

DNA as a target for carcinogen:

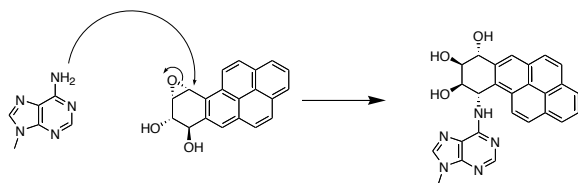
Bioactivation of pro-carcinogens:



350

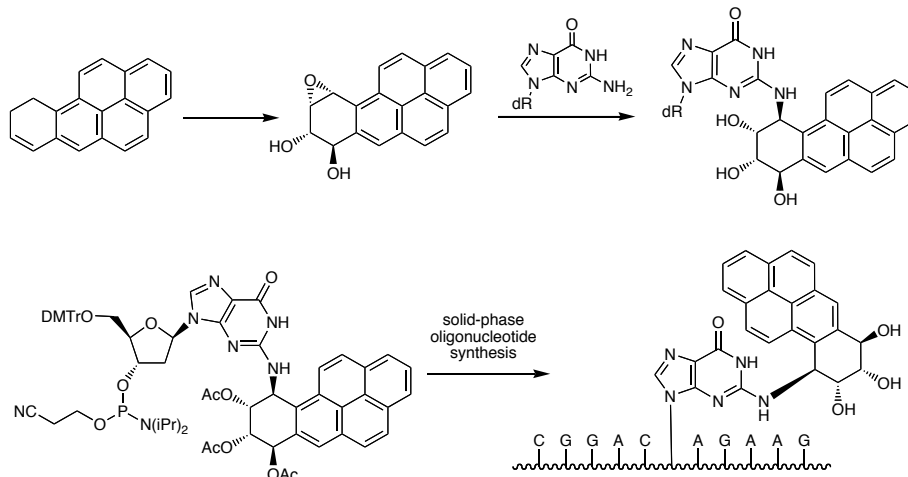


T. M. Harris et al. *J. Am. Chem. Soc.* **1988**, *110*, 7929



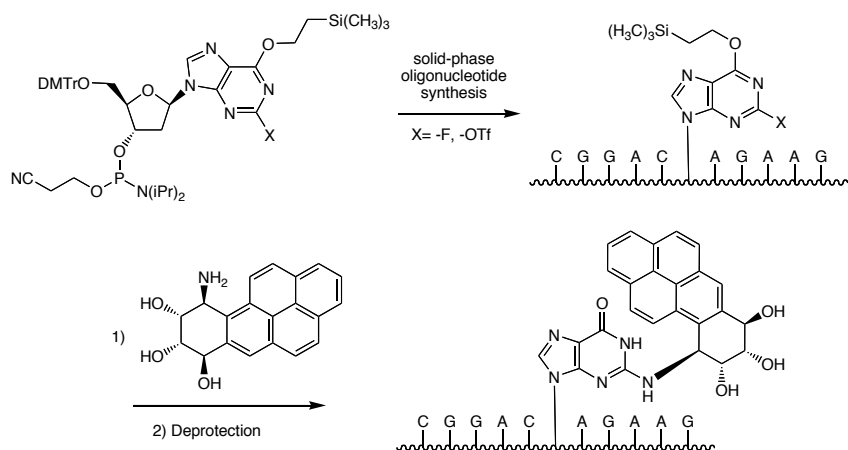
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Adducted Phosphoramidite Approach: Benzo[a]pyrene



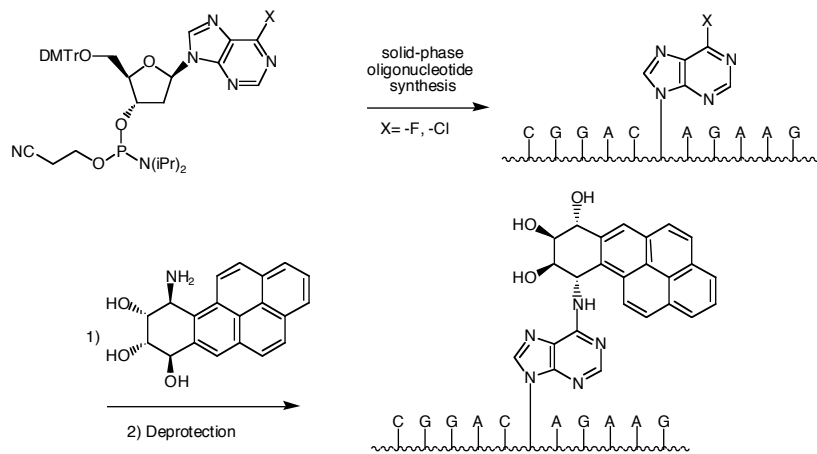
352

Post-Synthetic Modification Strategy for N^2 -2'-Deoxyguanosine Adducts

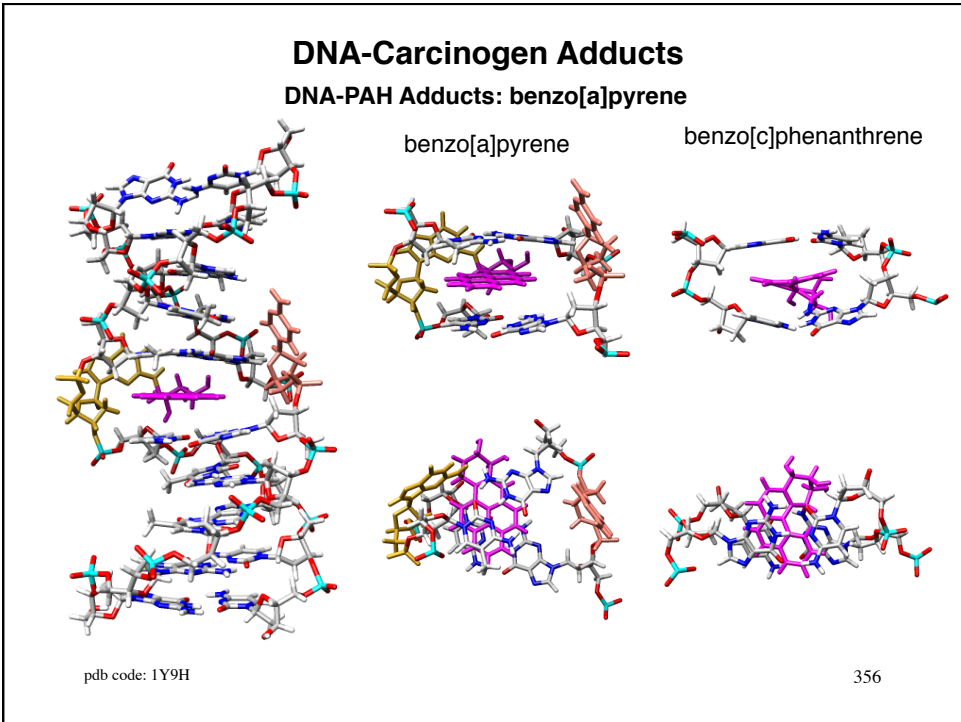
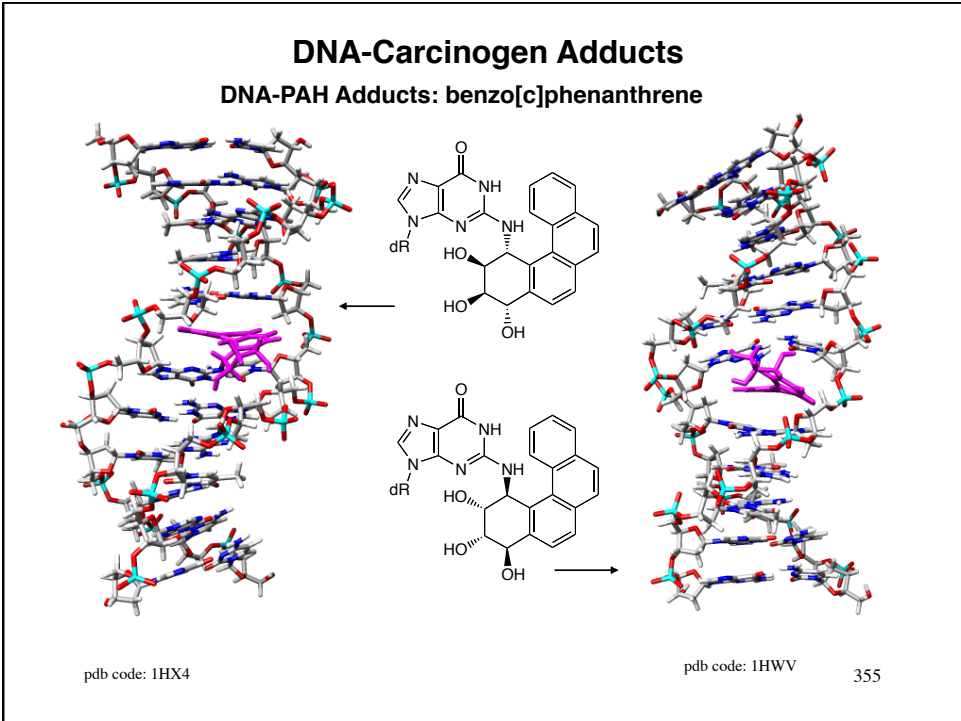


Kim, S. J.; Stone, M. P.; Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* 1992, 114, 5480 353

Post-Oligomerization Strategy for N^6 -2'-Deoxyadenosine Adducts

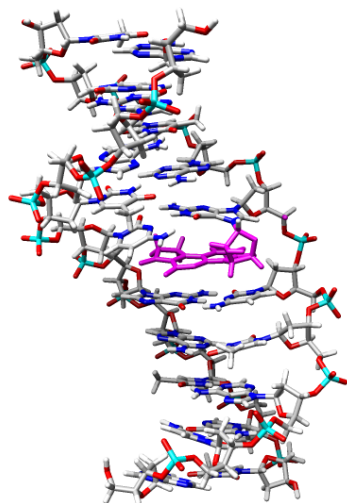


354

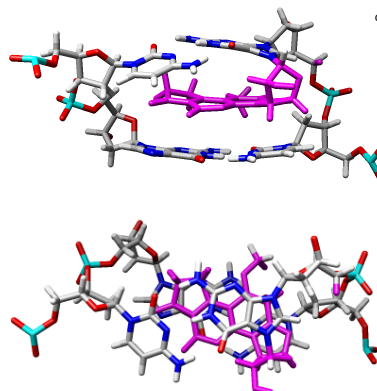
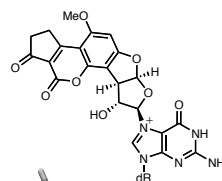


DNA-Carcinogen Adducts

DNA-Aflatoxin Adduct



pdb code: 1MKL



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DNA Repair:

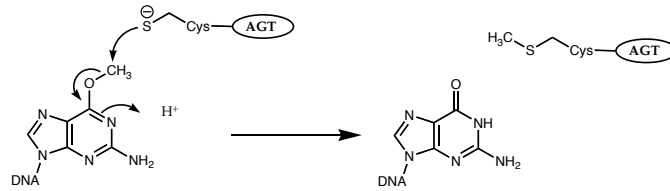
1. *Direct repair*
2. *Base excision repair (BER)*: repair of deglycosylation (lose of the base from the deoxyribose unit) sites, oxidation of the base, or modification by a “small” alkylating agent.
3. *Nucleotide excision repair (NER)*: repair of “bulky” lesions
4. *Mismatch Repair*: repair of mis-paired DNA bases
5. *Recombination*: repair of double strand breaks of DNA

“Chemistry and Biology of DNA Repair” Scharer, O. D. *Angew. Chem. Int. Ed. Engl.* 2003, 42, 2946-2974

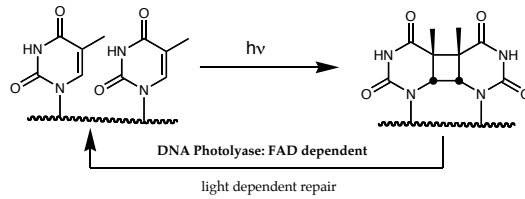
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Direct Repair:

*O*⁶-alkylguanine transferase (AGT): direct reversal by transferring the *O*⁶-alkyl group to an active site cysteine of AGT via an S_N2 reaction.

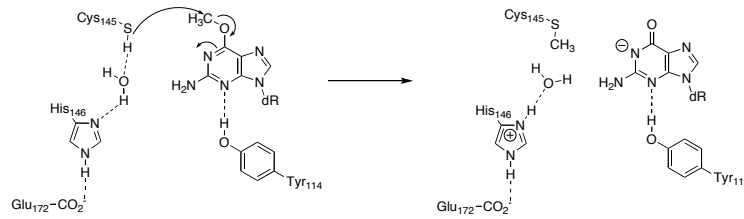


DNA Photolyase (bacterial): direct reversal of pyrimidine-pyrimidine photodimers (UV light induced lesion)

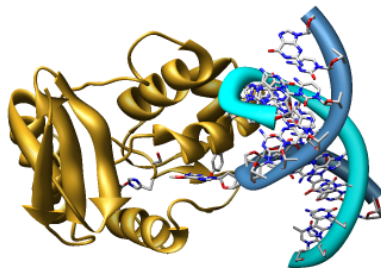


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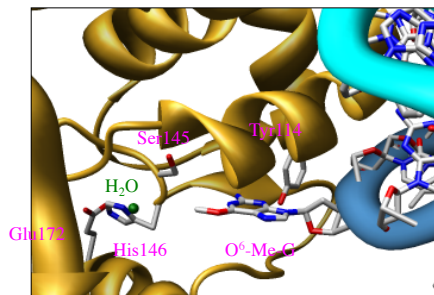
*O*⁶-Alkylguanine transferase (AGT):



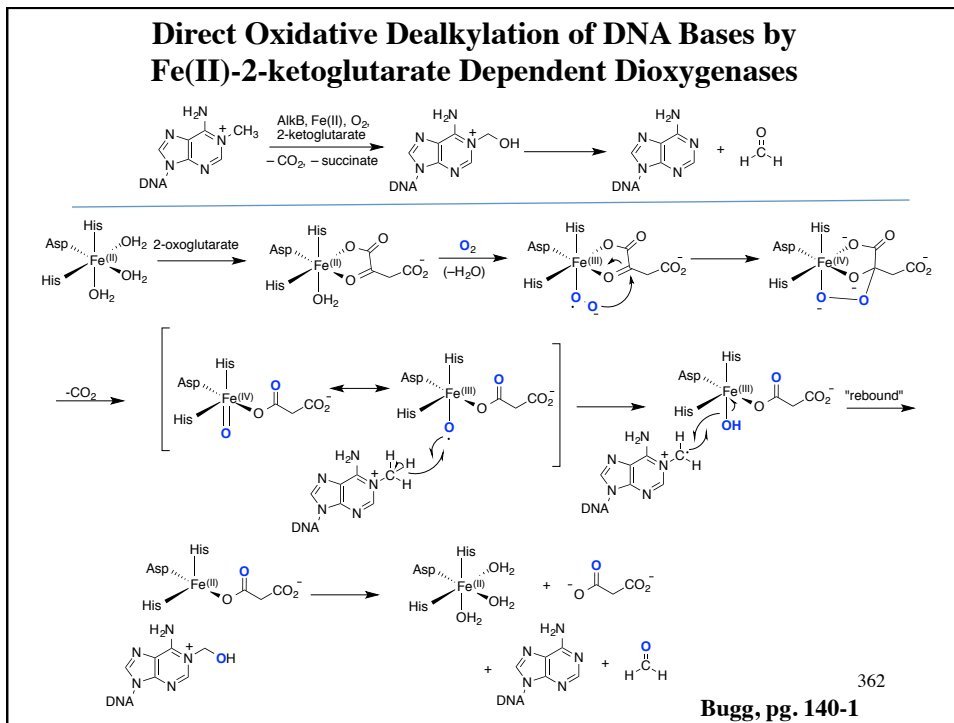
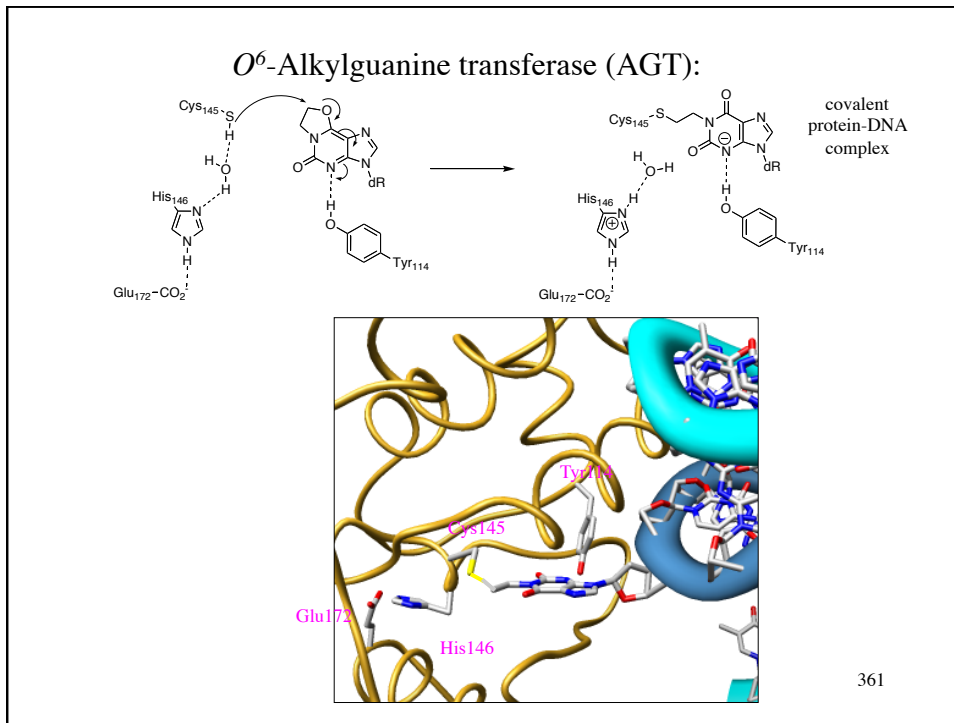
X-ray analysis of the C145S mutant



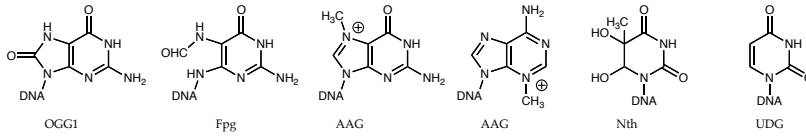
pdb code: 1T38



360

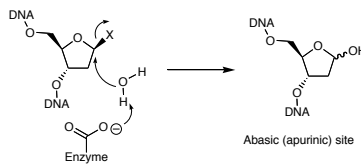


Base-Excision Repair:

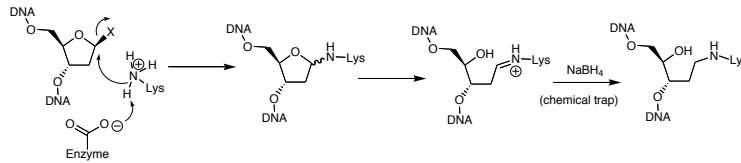


Mechanism of deglycosylation:

DNA glycosylase:



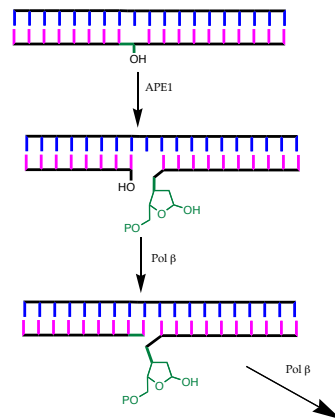
AP lyase: leads to DNA strand scission ala Maxim-Gilbert Chemistry



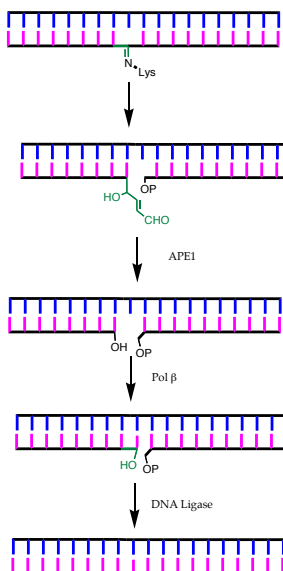
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Base-excision repair:

from DNA glycosylase

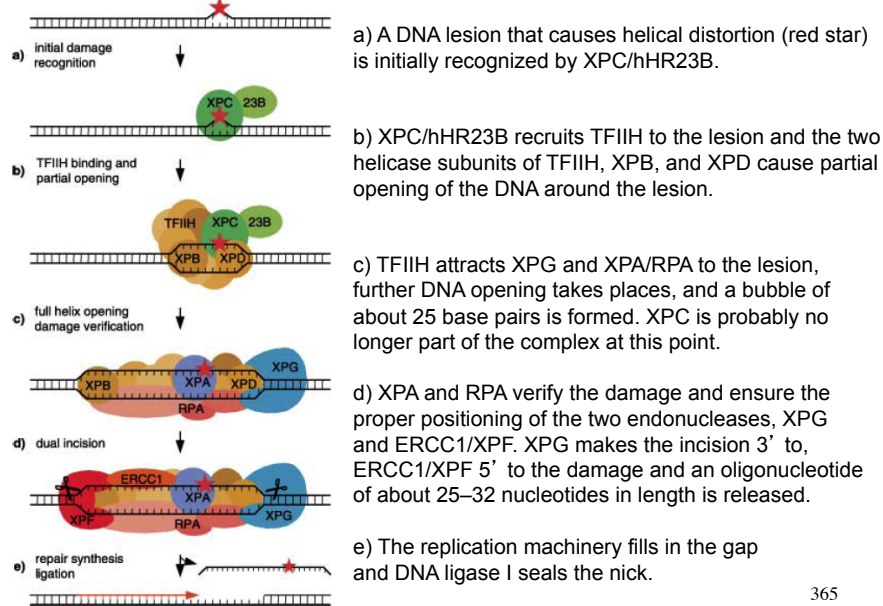


from AP lyase



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Nucleotide Excision Repair (NER): (shamefully pirated from the Schärer review)



DNA Polymerases: Classified by Structural Homology

A (pol I)

<i>E. coli</i> pol I	repair
human pol γ	mitochondrial DNA replication
human pol θ	repair

B (α -like)

human pol α	priming
human pol δ	replication
human pol ζ	lesion bypass
<i>E. coli</i> pol II	repair
T4 DNA polymerase	phage replication

C

<i>E. coli</i> pol III	replication
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D

DNA pol D	replication
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X

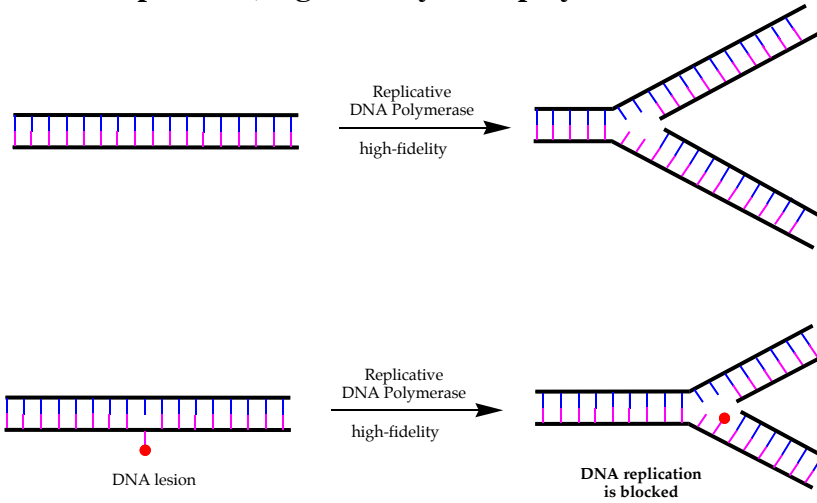
human DNA pol β	repair
human pol λ	repair

Y (Umc/DinB/Rev1p/rad30 superfamily)

<i>E. coli</i> pol IV (Din B)	lesion bypass
<i>E. coli</i> pol V (UmuDC)	SOS induced lesion bypass
human pol η	lesion bypass
human pol κ	lesion bypass
human pol ι	lesion bypass
human Rev1	lesion bypass
Dpo4	archaeobacteria lesion by-pass

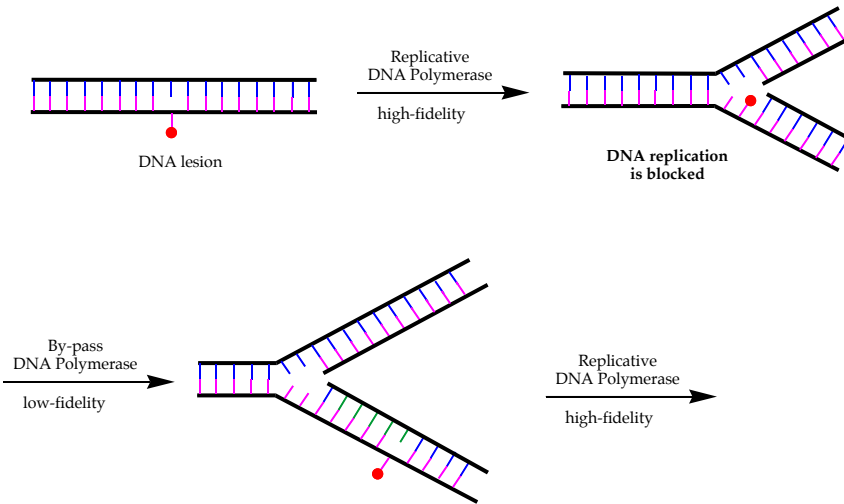
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**DNA Replication:
replicative, high-fidelity DNA polymerases**



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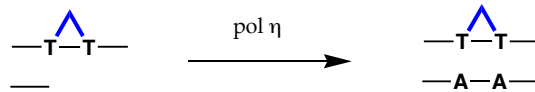
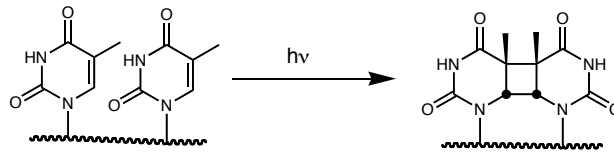
**Trans-lesion Synthesis:
error prone (low fidelity), bypass polymerases (Y family)**



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Xeroderma pigmentosum (XP):

- **genetic predisposition to sunlight induced skin cancer, as well as other abnormalities.**
- **Inefficient repair of sunlight induced DNA lesions (*XP-A-F*)**
- **DNA polymerase η is not expressed (*XP-V*)**



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