DNA STRUCTURE

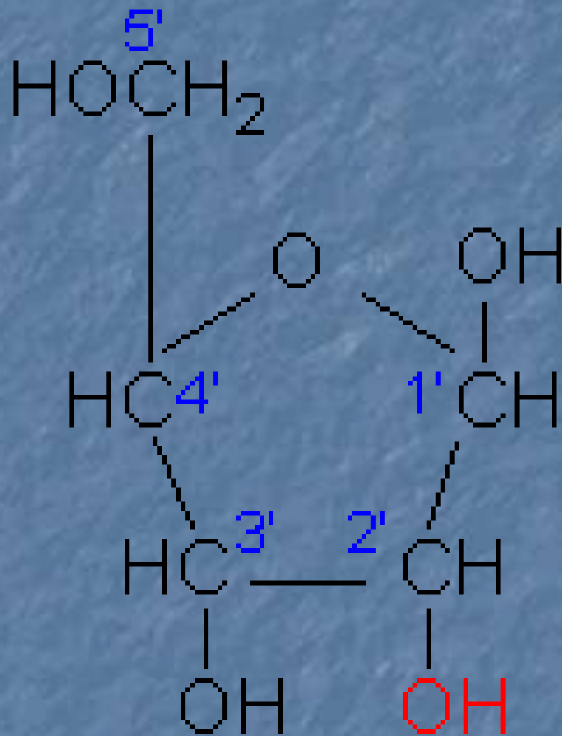
NUCLEIC ACIDS

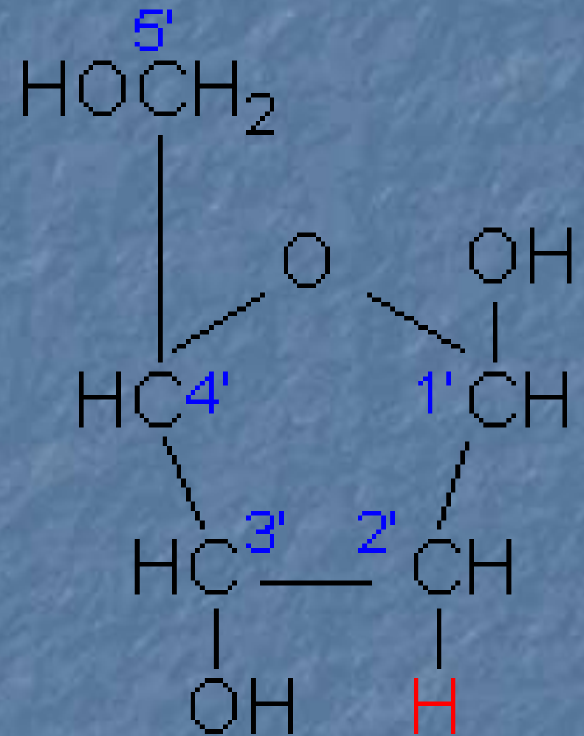
- Nucleic acids are polymers
- Monomer---nucleotides
  - Nitrogenous bases
    - Purines
    - Pyrimidines
  - Sugar
    - Ribose
    - Deoxyribose

  } Nucleosides

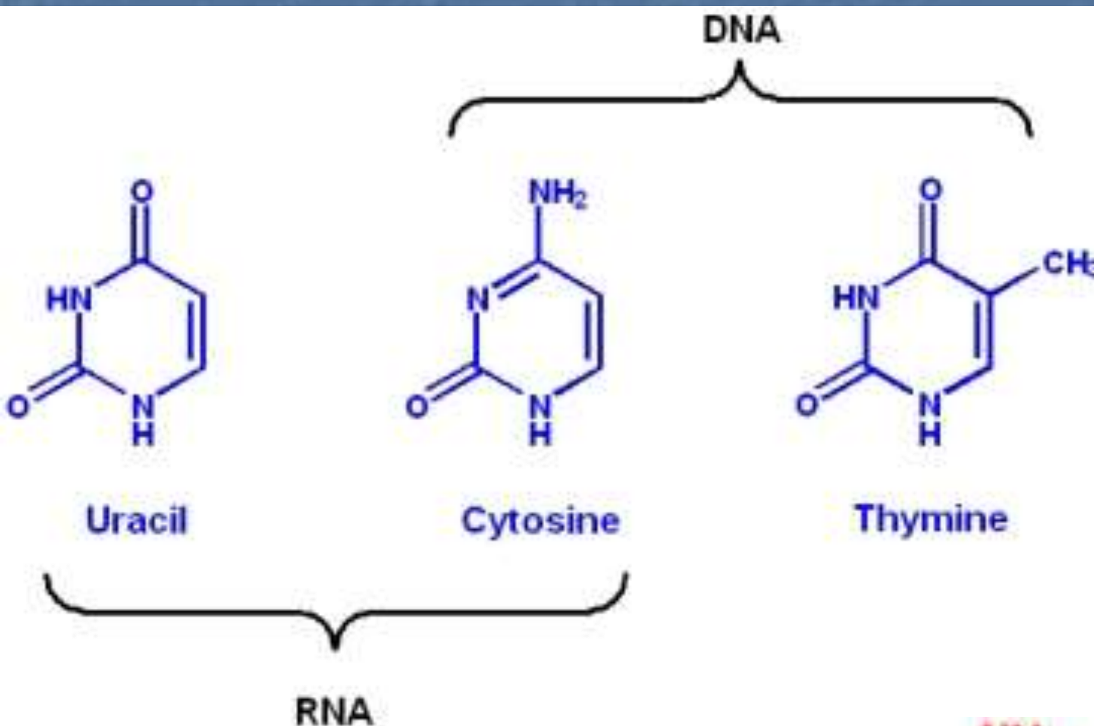
  - Phosphates
    - +nucleoside=nucleotide

The Sugars



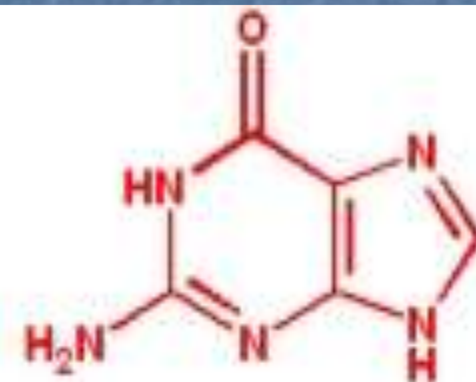


The Bases



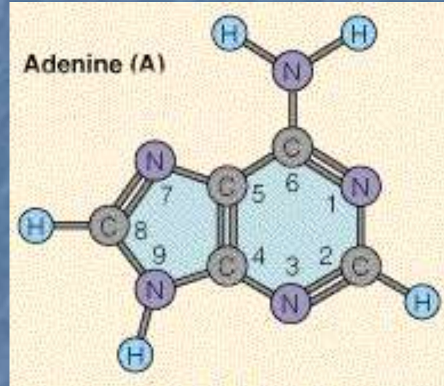
PYRIMIDINES

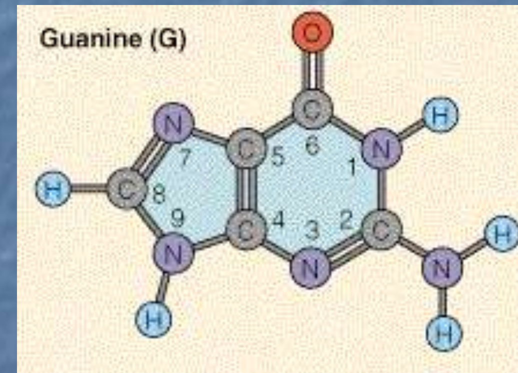
PURINES



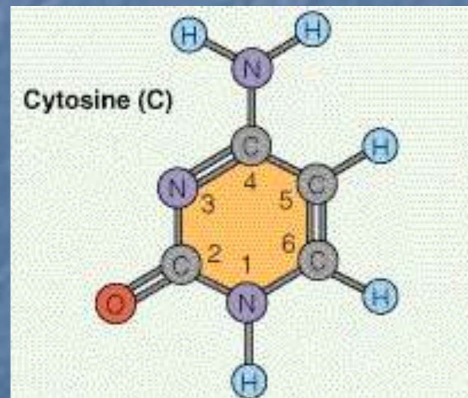
Bases of DNA (and RNA)

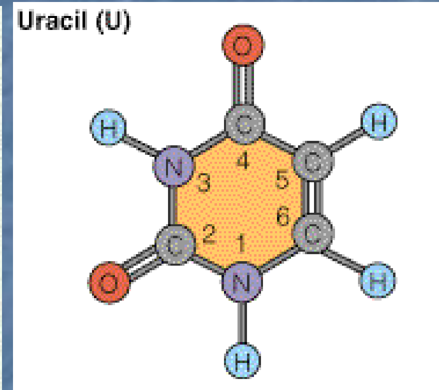
Purines:

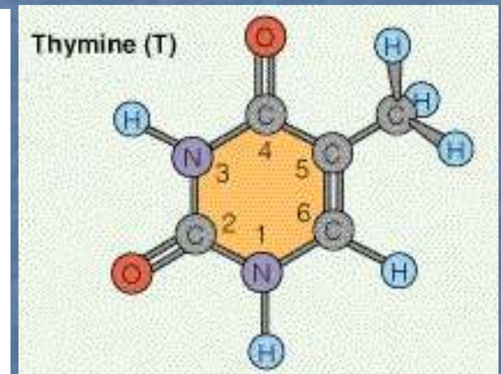




Pyrimidines:





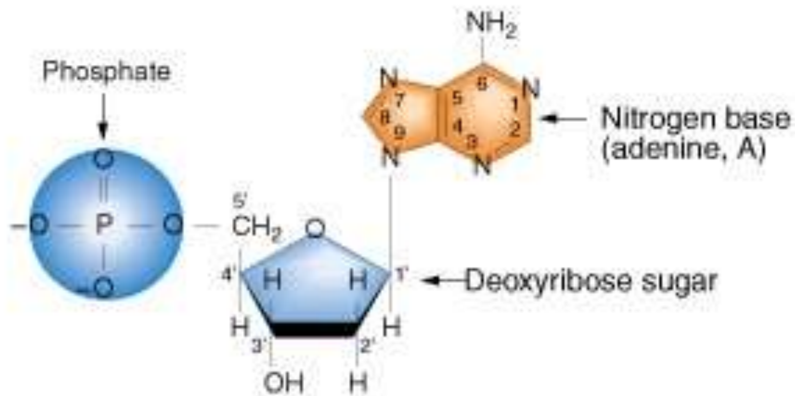


RNA only

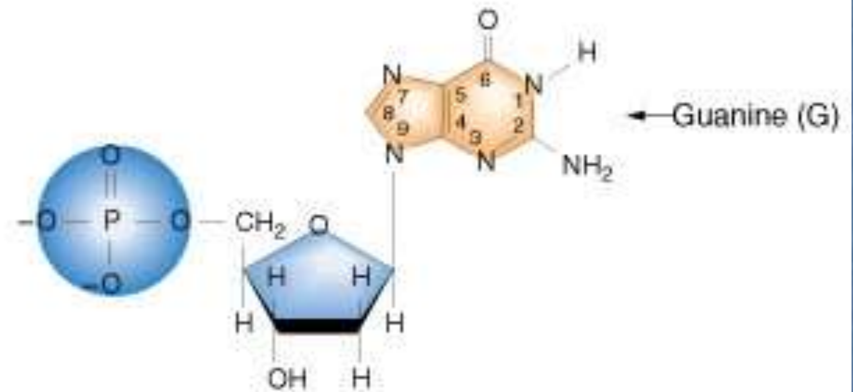
DNA only

Nucleotides and Nucleosides

Purine nucleotides

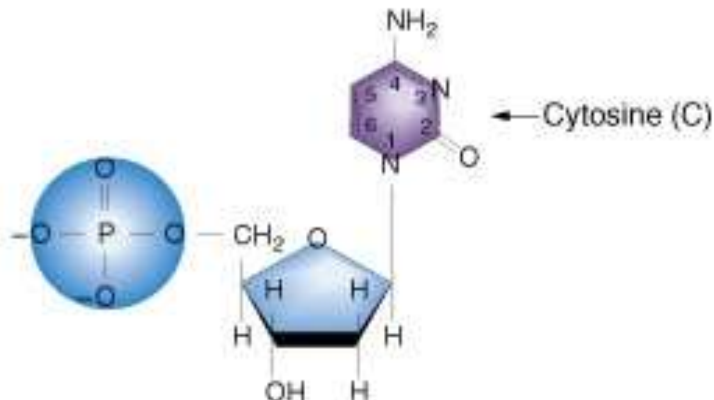


Deoxyadenosine 5'-phosphate (dAMP)

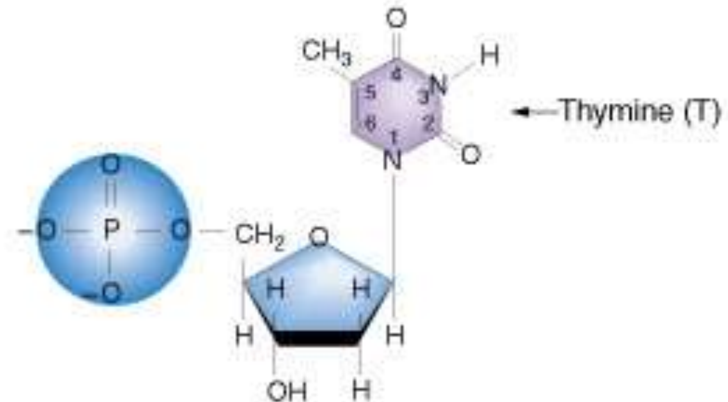


Deoxyguanosine 5'-phosphate (dGMP)

Pyrimidine nucleotides



Deoxycytidine 5'-phosphate (dCMP)



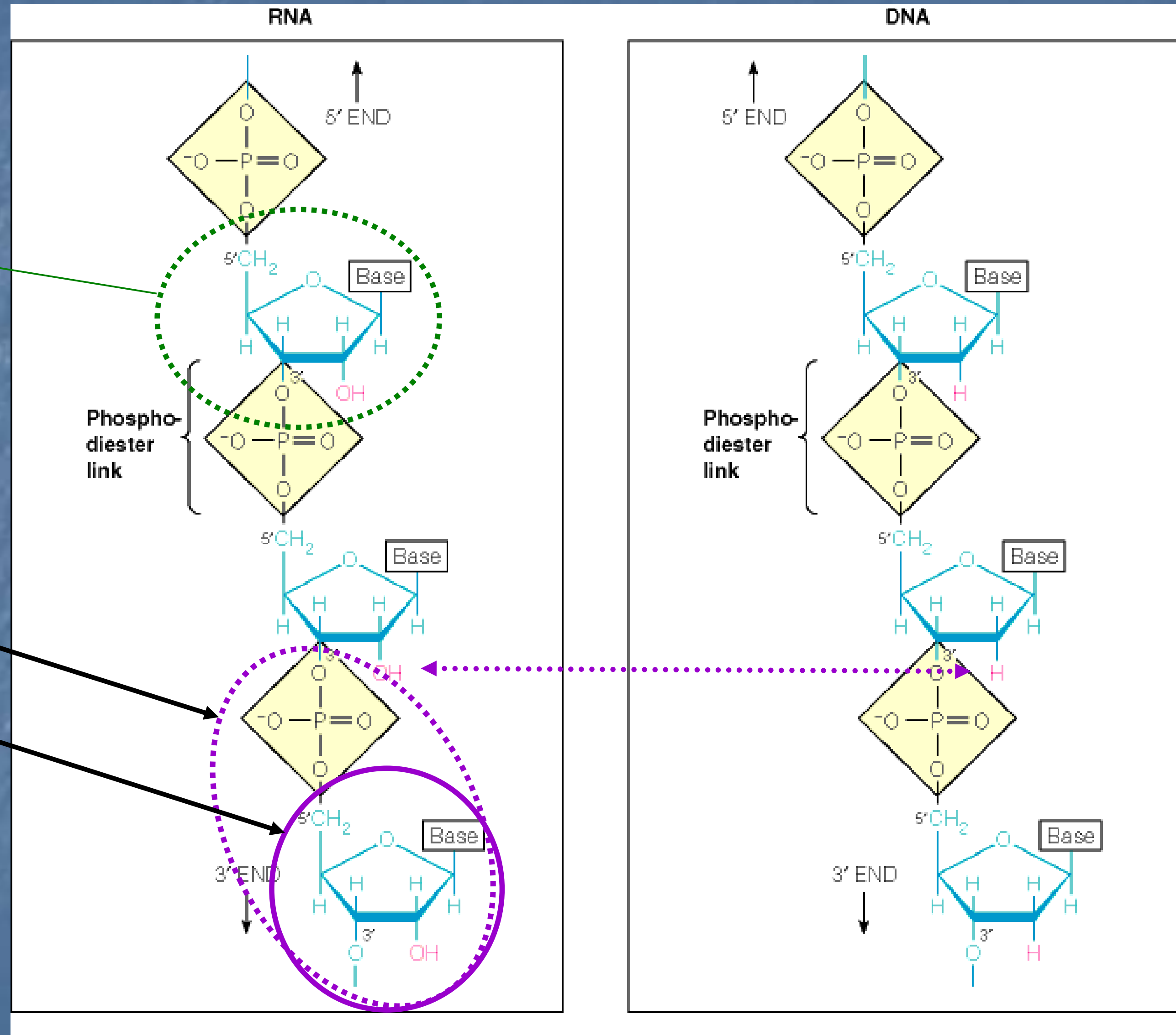
Deoxythymidine 5'-phosphate (dTMP)

Chemical Structure of DNA and RNA

The C is named 1’-5’

Nucleotide

Nucleoside

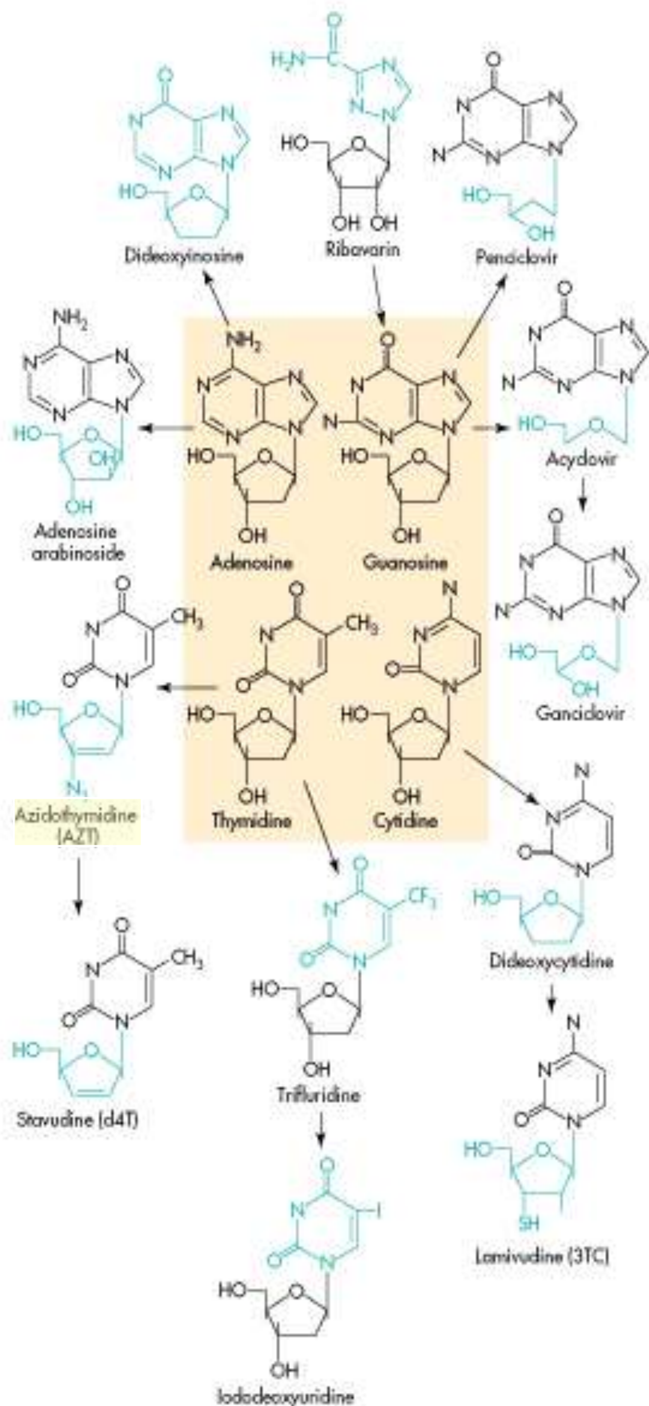


Resume

Nucleotides and Nucleosides

BASE	NUCLEOSIDE	DEOXYNUCLEOSIDE
Adenine	Adenosine	2-deoxyadenosine
Guanine	Guanosine	2-deoxyguanosine
Cytosine	Cytodine	2-deoxycytodine
Uracil	Uridine	Not usually found
Thymine	Not usually found	2-deoxythymidine

Nucleotides are nucleosides + phosphate

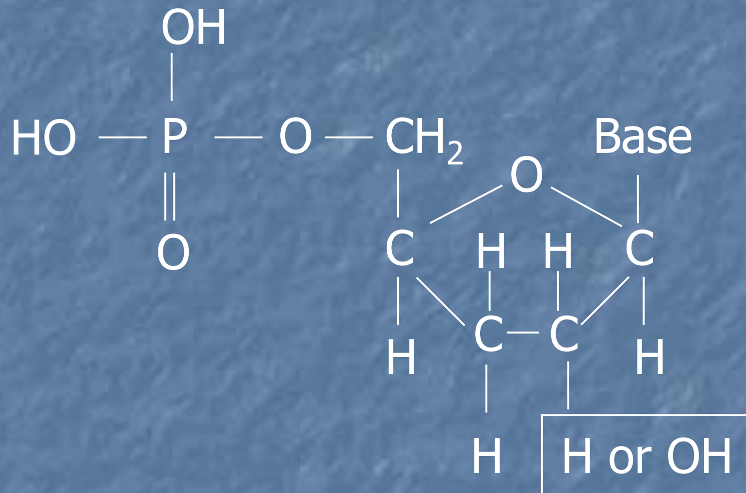


Nucleotide Analogs as Drugs

Nucleic Acids

make up 13-34% of the dry weight in bacteria
deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)

Nucleotide: a building block



Nucleoside: base + sugar

Sugar:
- RNA – ribose (OH)
- DNA – deoxyribose (H)

Bases:
- adenine (A), cytosine (C), guanine (G), thymine (T)
- RNA uses uracil (U) instead of thymine

- certain nucleotides serve as a storage of energy and reducing power

e.g. **ATP -> ADP -> AMP**

hydrolysis (energy is released)

DNA Stabilization– Complementary Base Pairing

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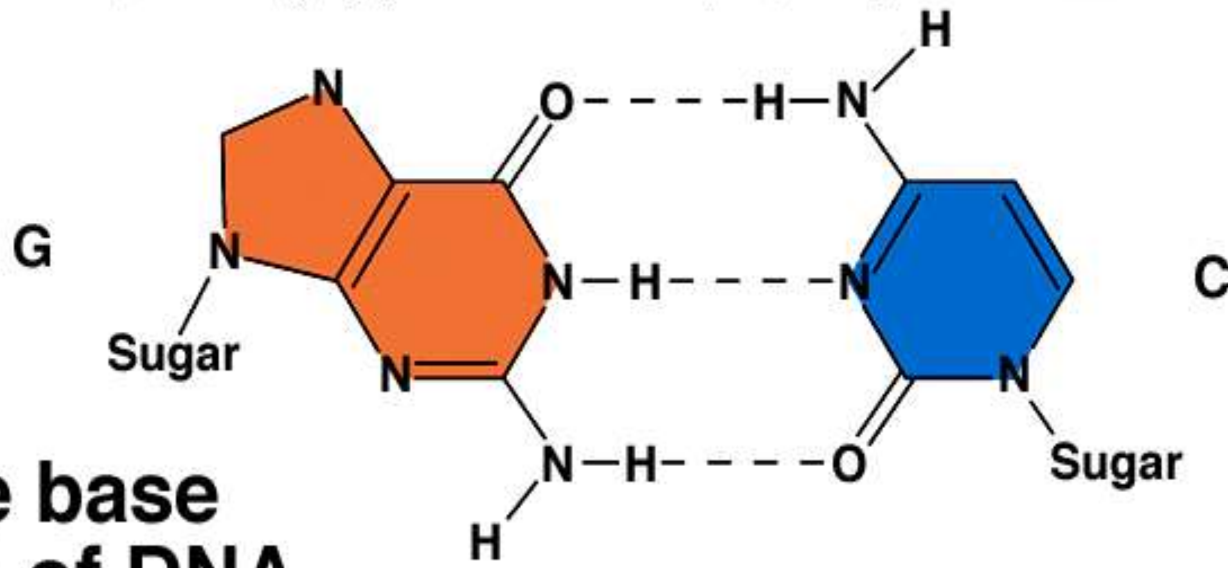
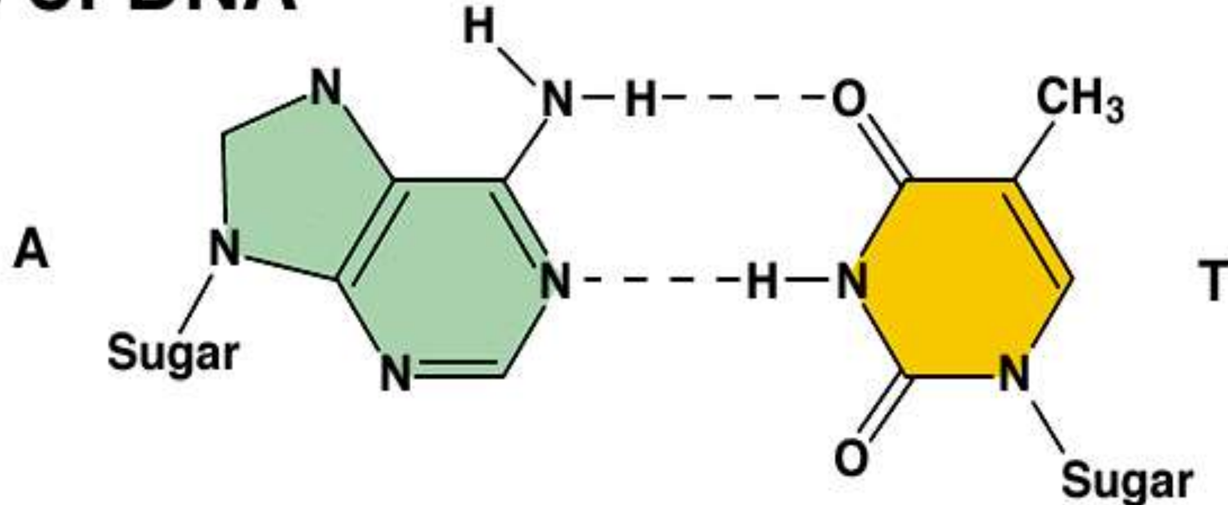
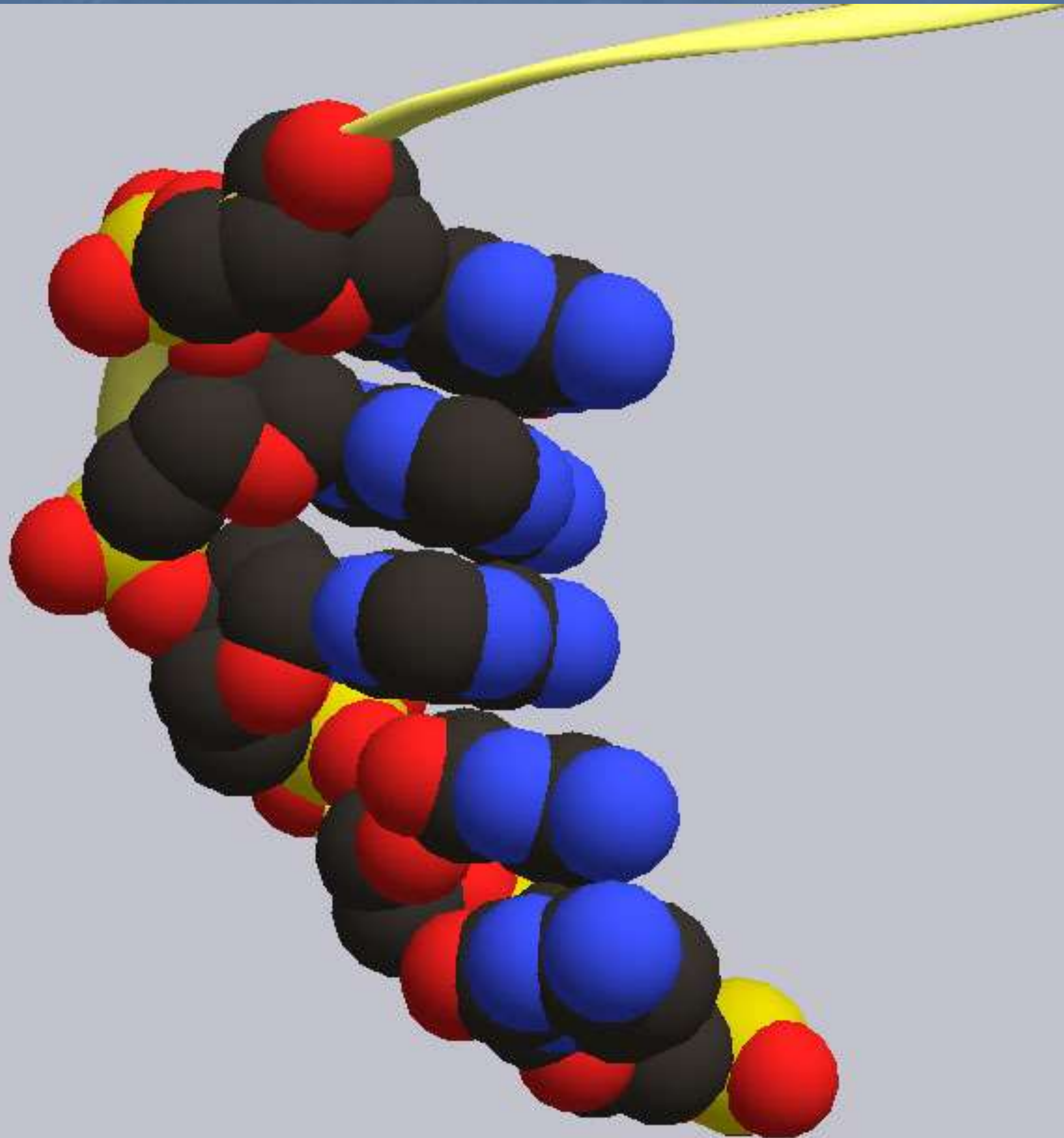
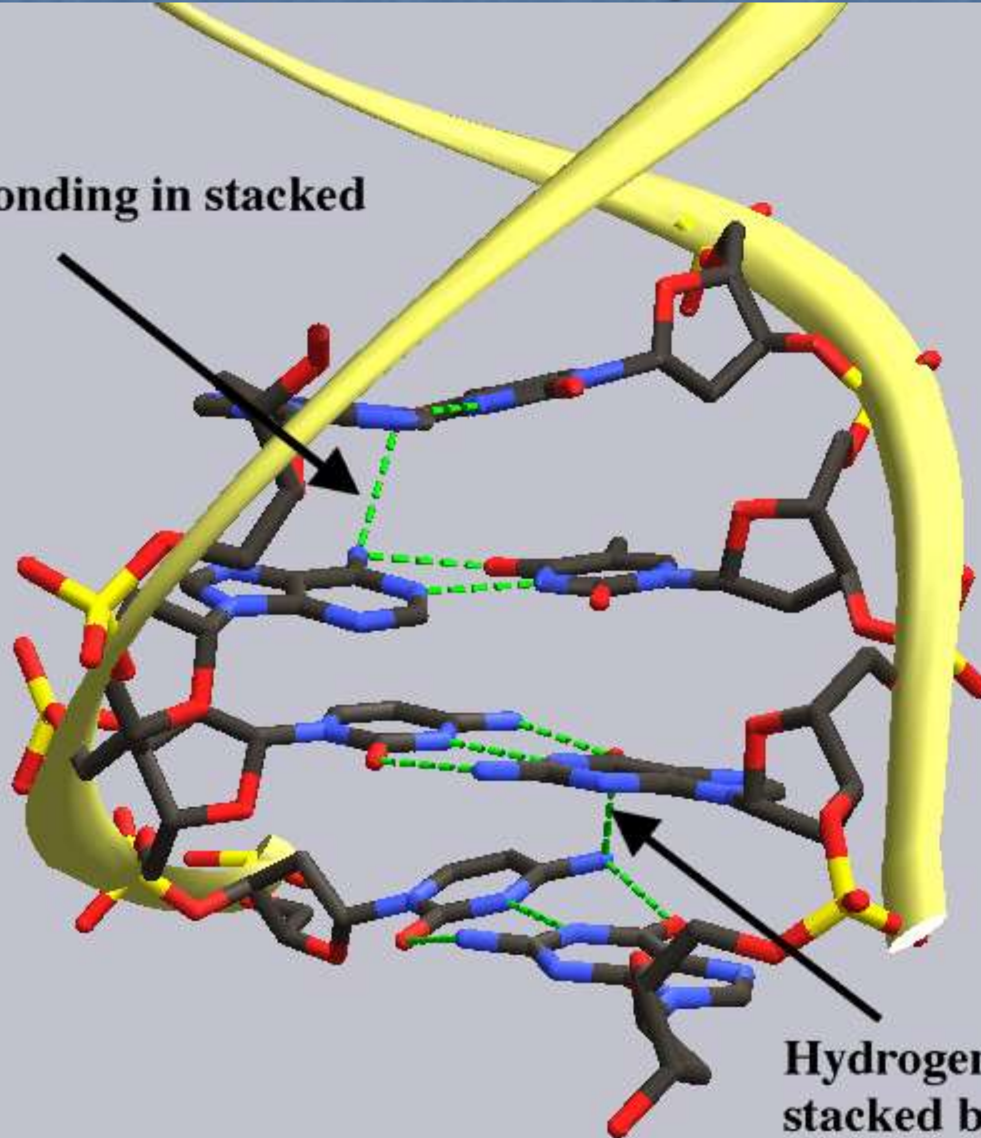




DNA Stabilization-Base Stacking



DNA Stabilization--H-bonding between DNA base pair stacks



Advantages to Double Helix

- Stability---protects bases from attack by H_2O soluble compounds and H_2O itself.
- Provides easy mechanism for replication

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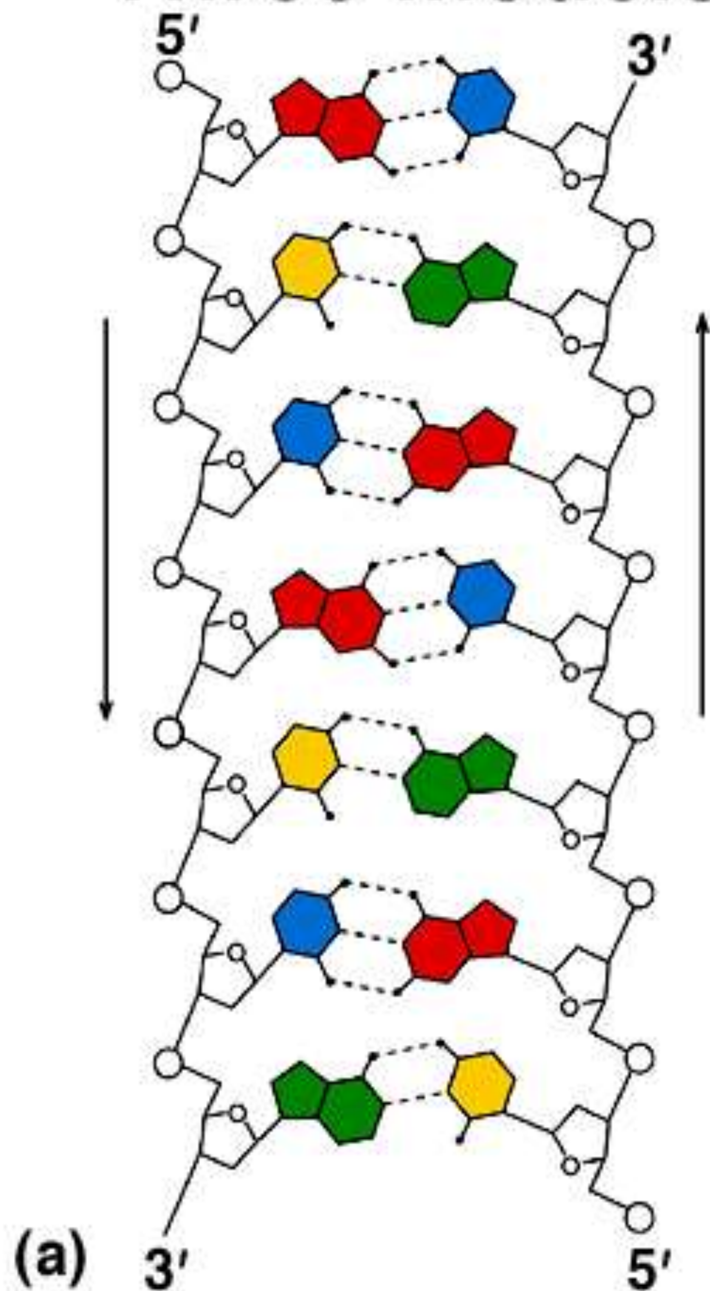
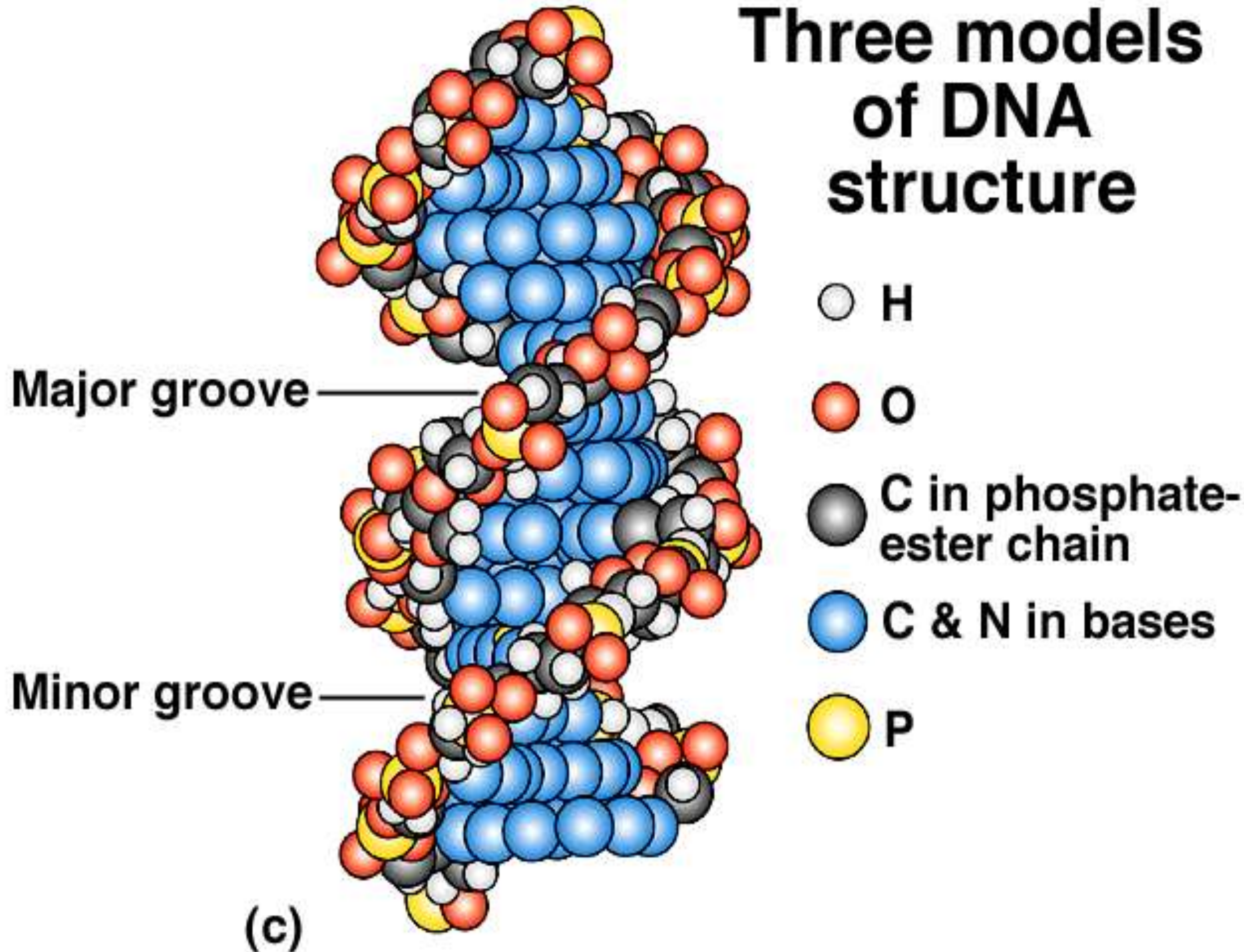
Three models of DNA structure



5′ 3′
0.34nm
(3.4Å)
3.4nm
(34Å)
Major groove
Minor groove
5′ 3′
2nm
(20Å)
(b)

Physical Structure (cont'd)



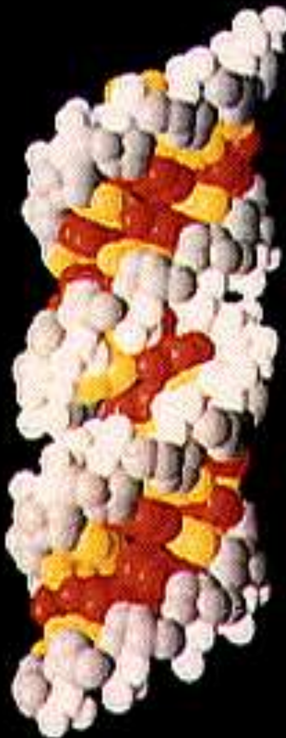
G-C Content

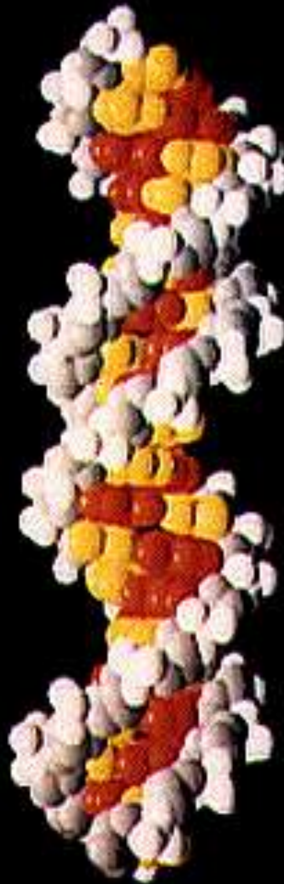
- A=T, G=C, but AT≠GC
- Generally GC~50%, but extremely variable
- EX.
  - Slime mold~22%
  - Mycobacterium~73%
- Distribution of GC is not uniform in genomes

CONSEQUENCES OF GC CONTENT

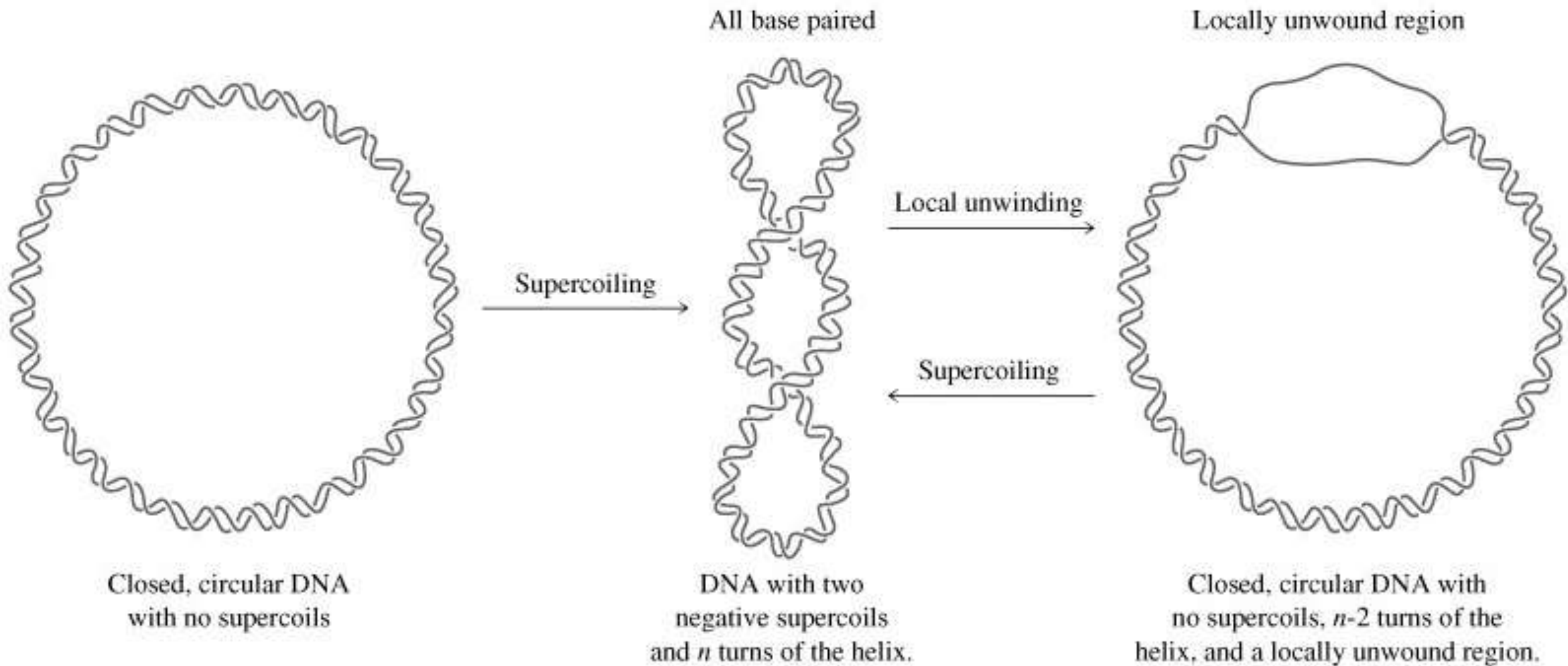
- GC slightly denser
  - ∴Higher GC DNA moves further in a gradient
- Higher # of base pairs=more stable DNA, i.e. the strands don’t separate as easily.

FORMS OF DNA

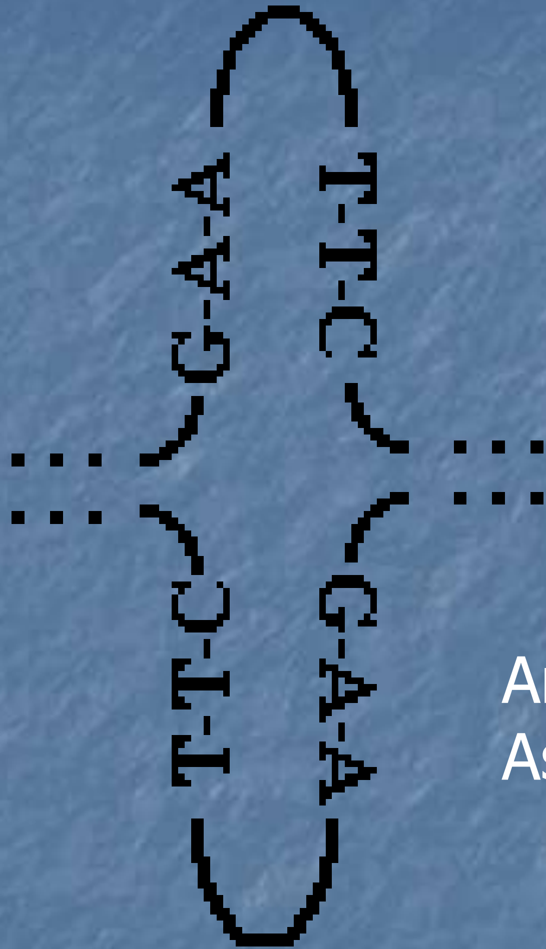




Supercoiling



Cruciform Structures

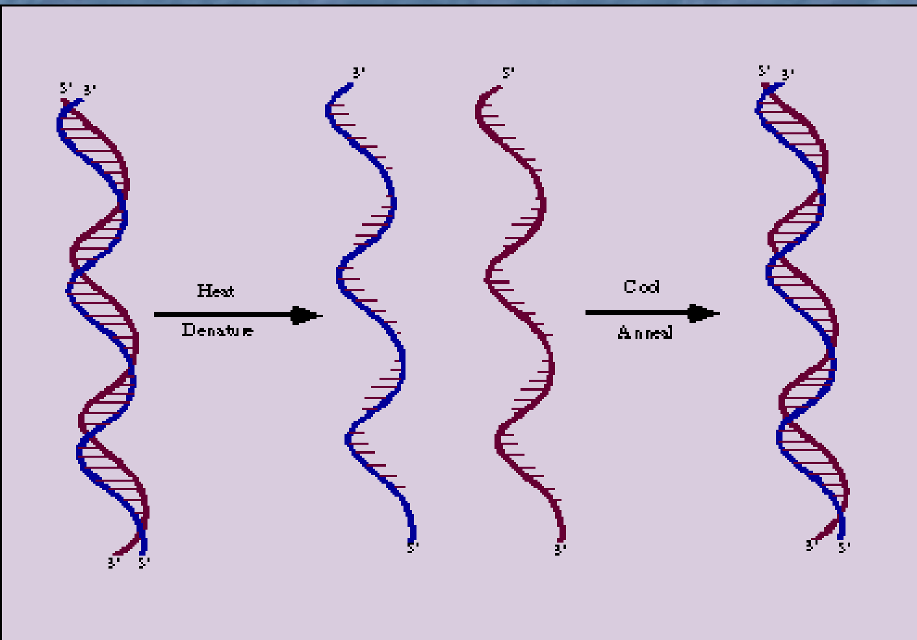


Another adaptation to supercoiling
Associated with palindromes

DNA is Dynamic

- Like proteins, DNA has 3° structure
- Why so many deviations from normal conformation?
  - Effects on transcription (gene expression)
  - Enhances responsiveness
  - May also serve in packaging
- NOTE: most cellular DNA exists as protein containing supercoils

Denaturation of DNA



The T at which ½ the DNA sample is denatured is called the melting temperature (T_m)

- Denaturation by heating.
- How observed?
  - A_{260}
  - For dsDNA,
    A_{260}=1.0 for 50 µg/ml
  - For ssDNA and RNA
    A_{260}=1.0 for 38 µg/ml
  - For ss oligos
    A_{260}=1.0 for 33 µg/ml
  - Hyperchromic shift

Importance of T_m

- Critical importance in any technique that relies on complementary base pairing
  - Designing PCR primers
  - Southern blots
  - Northern blots
  - Colony hybridization

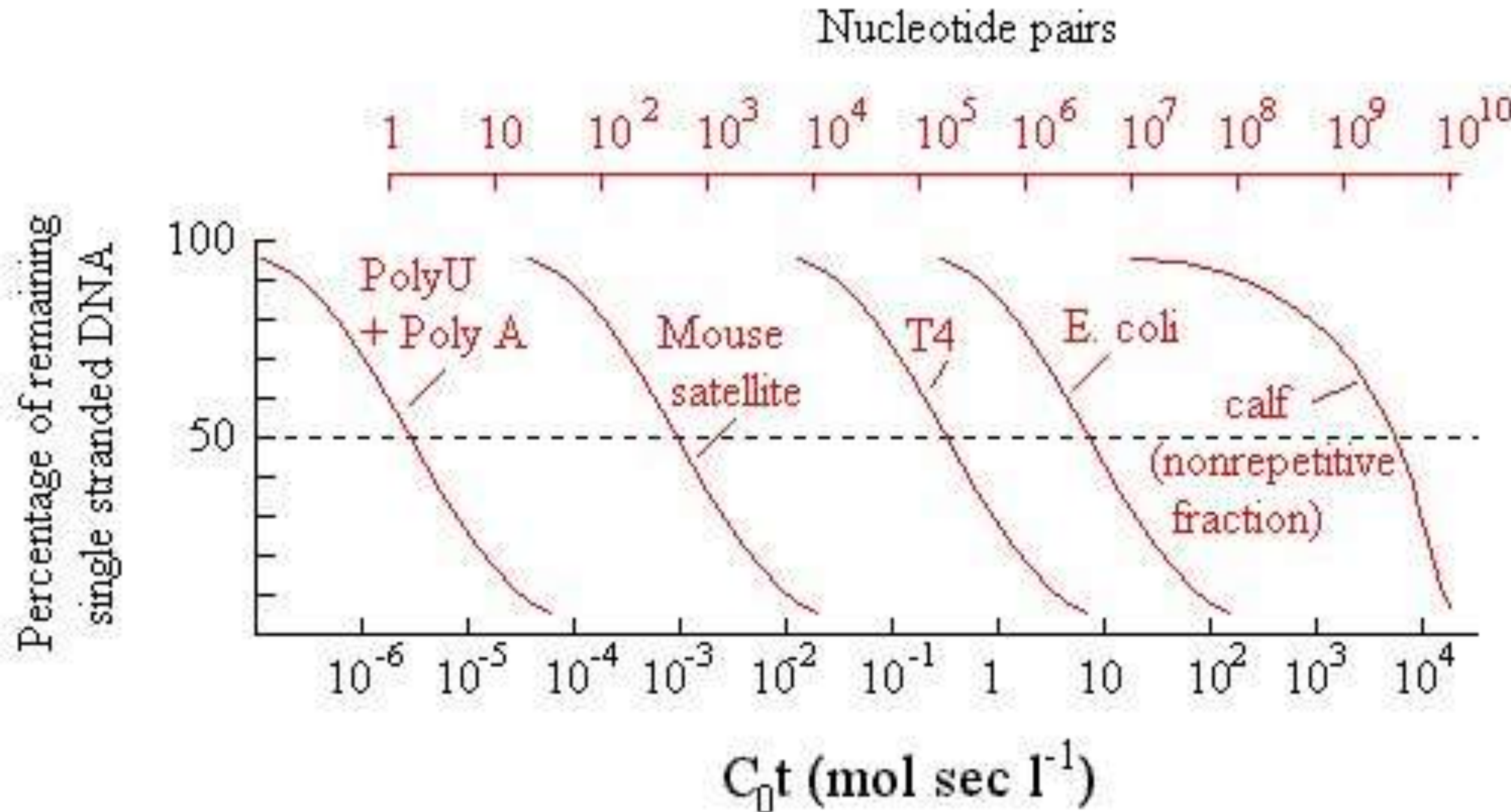
Factors Affecting T_m

- G-C content of sample
- Presence of intercalating agents (anything that disrupts H-bonds or base stacking)
- Salt concentration
- pH
- Length

Renaturation

- Strands can be induced to renature (anneal) under proper conditions. Factors to consider:
  - Temperature
  - Salt concentration
  - DNA concentration
  - Time

$C_o t$ Curves



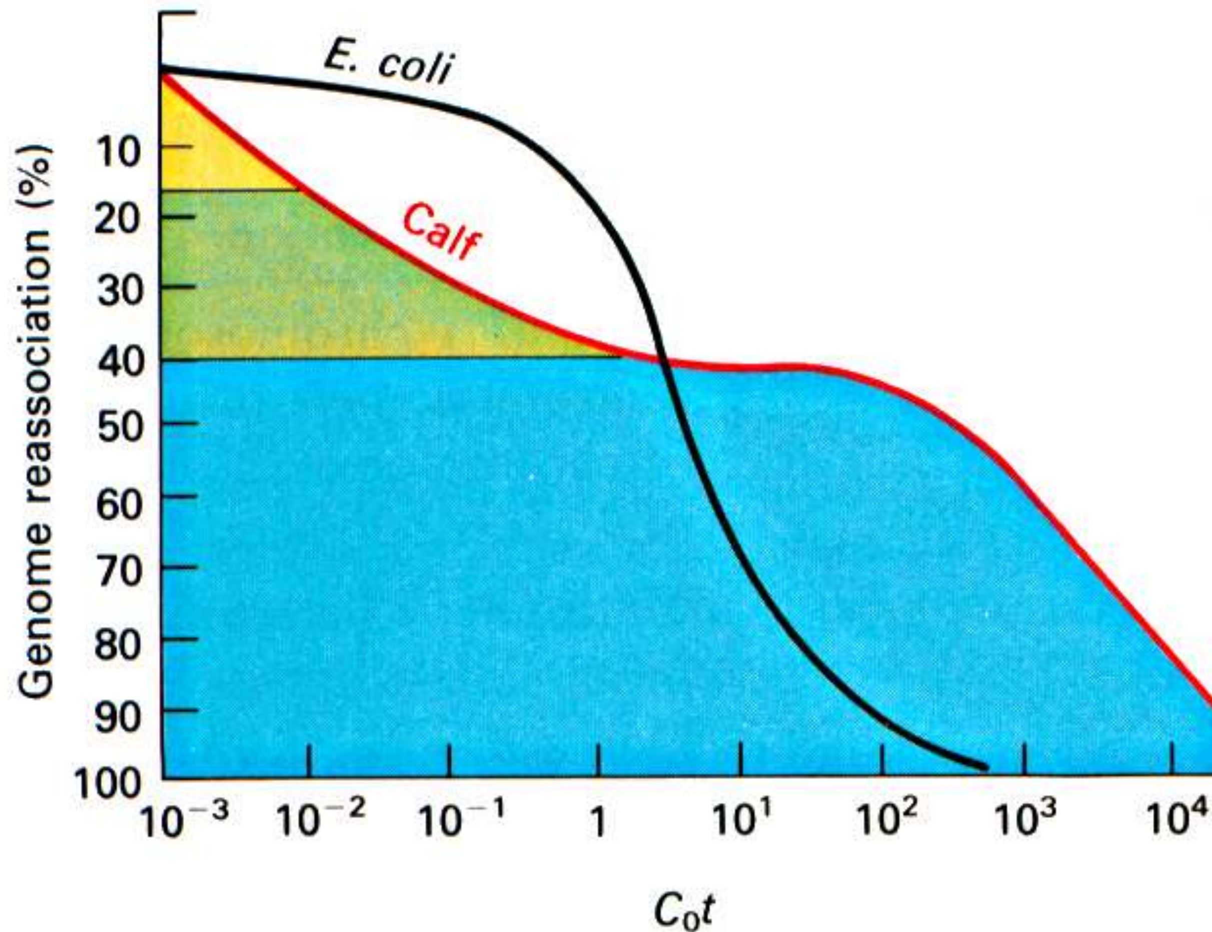
What Do C_ot Curves Reveal?

- Complexity of DNA sample
- Reveals important info about the physical structure of DNA
- Can be used to determine T_m for techniques that complementary base pairing.

Complexity of DNA- Factors Repetitive Sequences

- Single Copy Genes
- Highly repetitive (hundreds to millions)
  - Randomly dispersed or in tandem repeats
  - Satellite DNA
    - Microsatellite repeats
    - Miniisatellite repeats
- Middle repetitive (10- hundreds)
  - Clustered
  - Dispersed
- Slightly repetitive (2-10 copies)

Renaturation curves of *E. coli* and calf DNA



Highly repetitive sequences

Middle repetitive sequences

Unique sequences

RNA

- Types
  - mRNA
  - tRNA
  - rRNA
- It's still an RNA world
  - snRNA
  - siRNA
  - Ribozymes

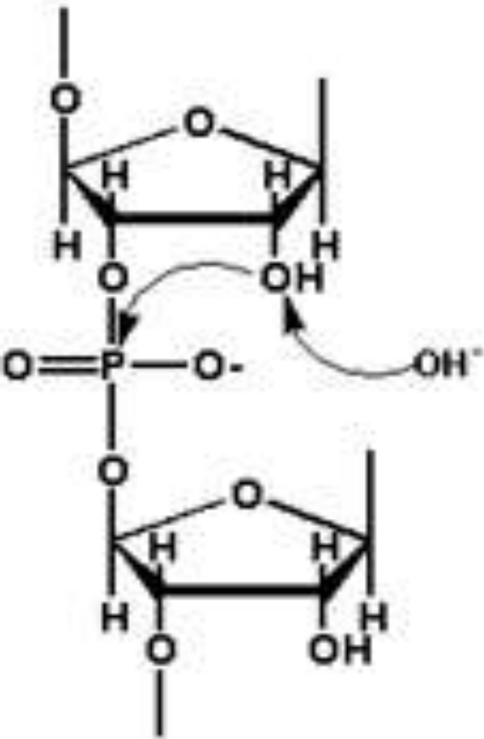
Behavior in Acids

- Dilute or mild acidic conditions
- Intermediate conditions. EX. 1N HCl @ 100ºC for 15m : Depurination
- Harsher treatment-EX. 2-6N HCl, higher temps: Depyrimidination.
- NOTE: some phosphodiester bond cleavage observed during depurination, much more during depyrimidination

Behavior in Bases

- N-glycosidic bonds stable in mild alkaline conditions
- DNA melts
- Phosphodiester linkages in DNA and RNA show very different behavior in weak bases (EX 0.3 N KOH @37°C ~1 hr.)

RNA Hydrolysis in Alkaline Solutions

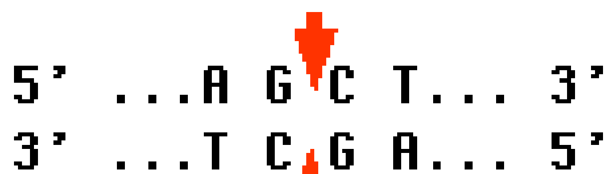


Hydrolysis by Enzymes

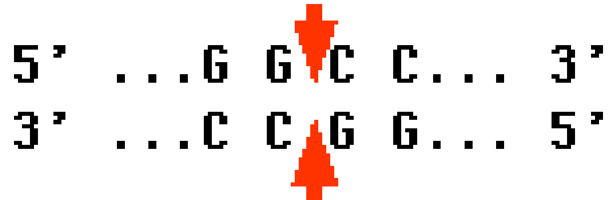
- Nuclease—catalyzes hydrolysis of phosphodiester backbone
  - Exonucleases
  - Endonucleases
    - General. Ex DNAse I
    - Specific Ex. Restriction endonucleases
- Ribozymes

Restriction Enzymes

AluI



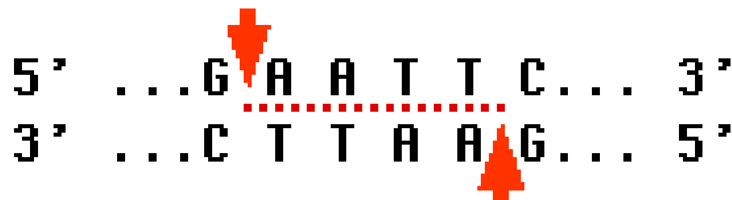
HaeIII



BamHI

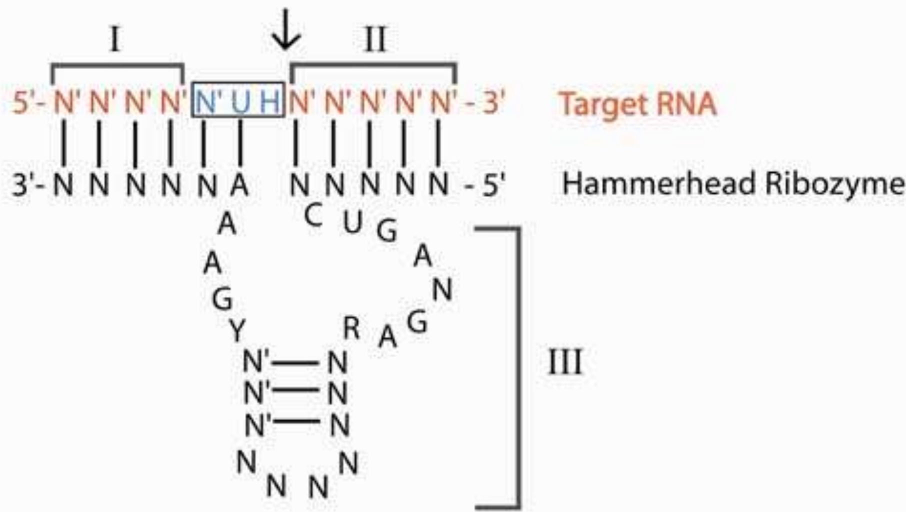
HindIII

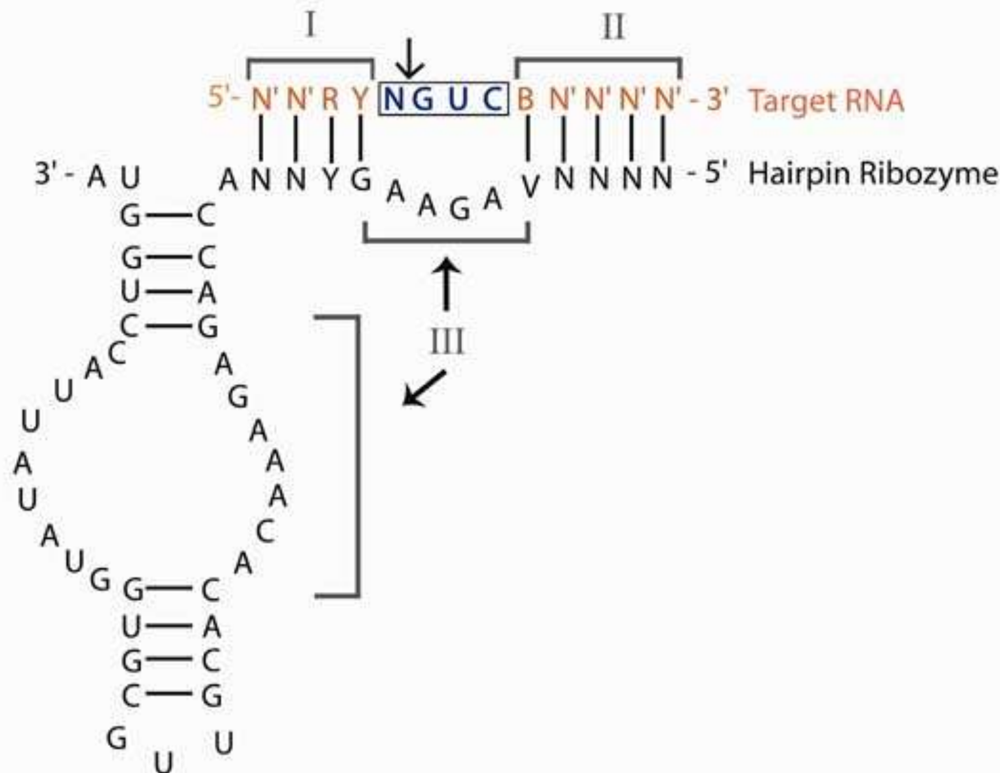
EcoRI



AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce “sticky” ends





RIBOZYMES

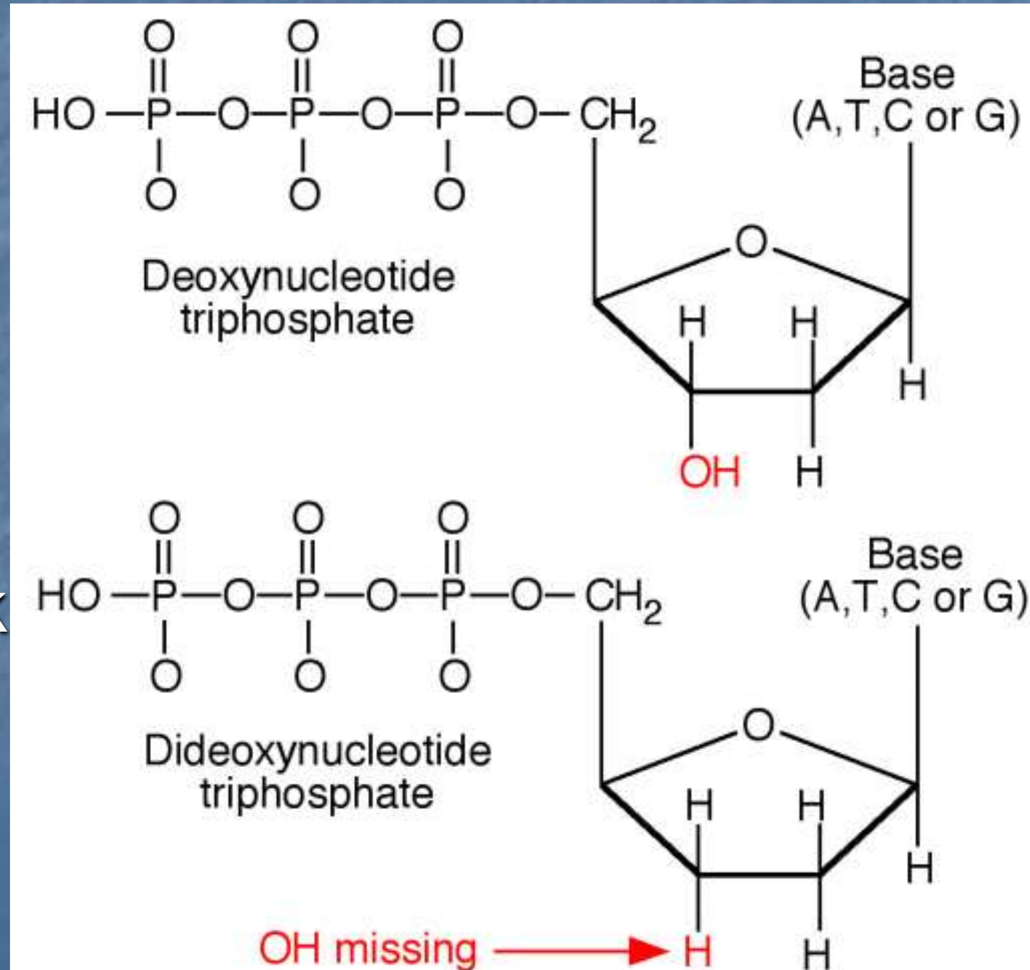
- Catalytic RNA
- Can work alone or with proteins
- Therapeutic applications?

SEQUENCING

- Purpose—determine nucleotide sequence of DNA
- Two main methods
  - Maxam & Gilbert, using chemical sequencing
  - Sanger, using *dideoxynucleotides*

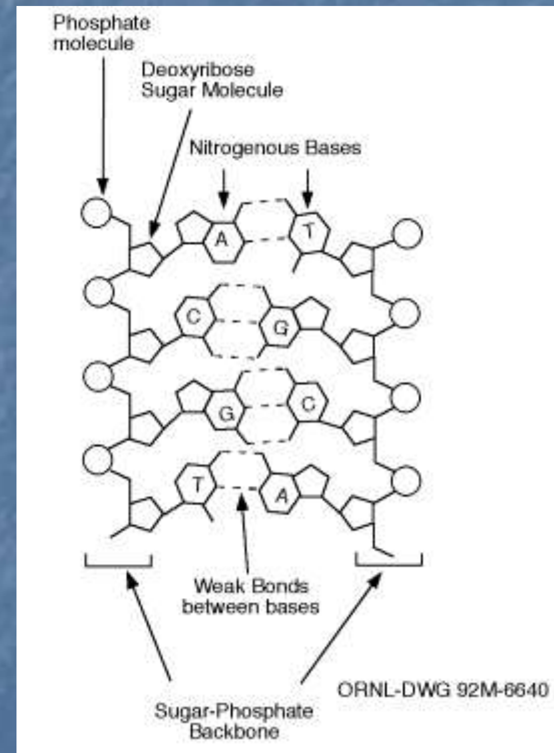
The Sanger Technique

- Uses dideoxynucleotides (dideoxyadenine, dideoxyguanine, etc)
- These are molecules that resemble normal nucleotides but lack the normal -OH group.



- Because they lack the -OH (which allows nucleotides to join a growing DNA strand), replication stops.

Normally, this would be where another phosphate Is attached, but with no -OH group, a bond can not form and replication stops



The Sanger Method Requires

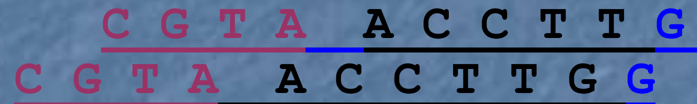
- Multiple copies of single stranded template DNA
- A suitable primer (a small piece of DNA that can pair with the template DNA to act as a starting point for replication)
- DNA polymerase (an enzyme that copies DNA, adding new nucleotides to the 3’ end of the template
- A ‘pool’ of normal nucleotides
- A small proportion of dideoxynucleotides labeled in some way (radioactively or with fluorescent dyes)

- The template DNA pieces are replicated, incorporating normal nucleotides, but occasionally and at random dideoxy (DD) nucleotides are taken up.
- This stops replication on that piece of DNA
- The result is a mix of DNA lengths, each ending with a particular labeled DDnucleotide.
- Because the different lengths ‘travel’ at different rates during electrophoresis, their order can be determined.

Termination during Replication

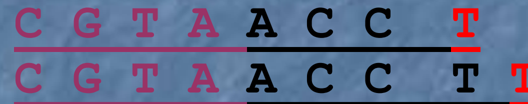
DNA SEQUENCE 3′	G	C	A	T	T	G	G	G	A	A	C	C
PRIMER 5′	C	G	T	A								
NO OF BASES	1	2	3	4	5	6	7	8	9	10	11	12

G terminated



A terminated

Tterminated



C terminated

Sanger ddNTP Chain Termination Sequencing

Template 3' .... GCATTGGGAACC .... 5'

Prime 5' .... CGTA 3'