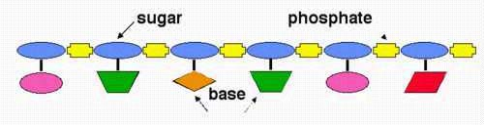
The Central Dogma of Biology

DNA → RNA → Protein

Biological length scale

Chemical bond	1 Å	(10^{-10} m)
Amino acid	10 Å	(10^{-9} m)
Globular protein	100 Å	(10^{-8} m)
Virus	1000 Å	(10^{-7} m)
Cell nucleus	1 μm	(10^{-6} m)
Bacterial cell	5 μm	(10^{-5} m)
Chromosome DNA	10 cm	(10^{-1} m)

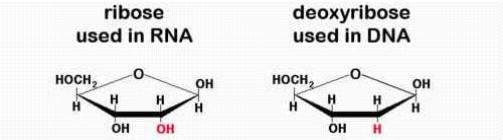


Nucleic Acids and Nucleic Acid Structure

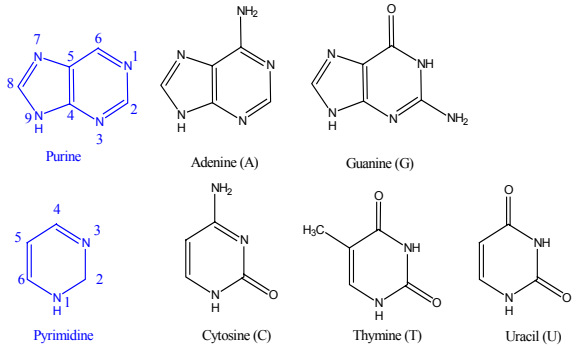
The nucleic acids are made up of polymers of four different nucleotide residues each.

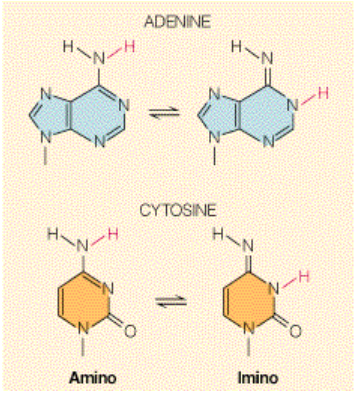
RNA uses AMP, CMP, GMP, and UMP
DNA use the deoxy forms:
dAMP, dCMP, dGMP, and dTMP.

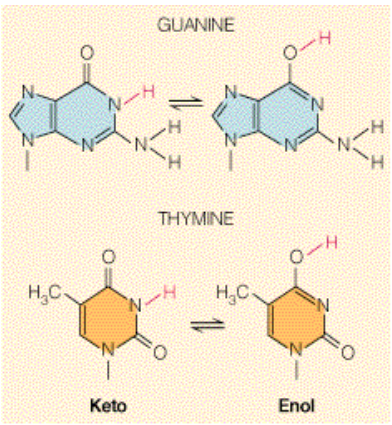
The two nucleic polymers differ by both the 2' functional group (-OH or -H) and the use of *either* uridine or thymine as the fourth base.

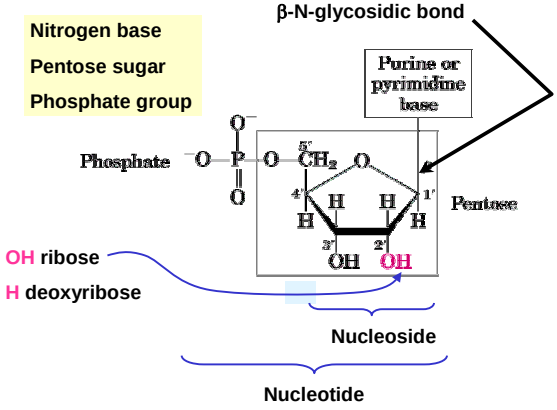


Base families



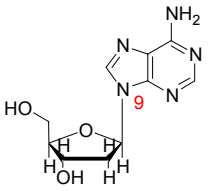






Example:

Nucleoside

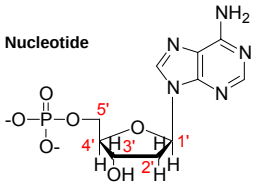


2'-deoxyadenosine

Nucleobase

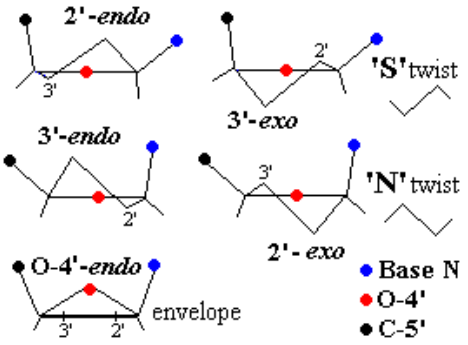
Adenine

Nucleotide



2'-deoxyadenosine-5'-monophosphate

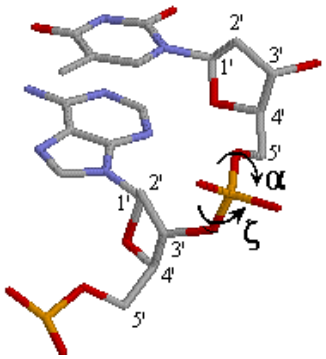
Conformational considerations

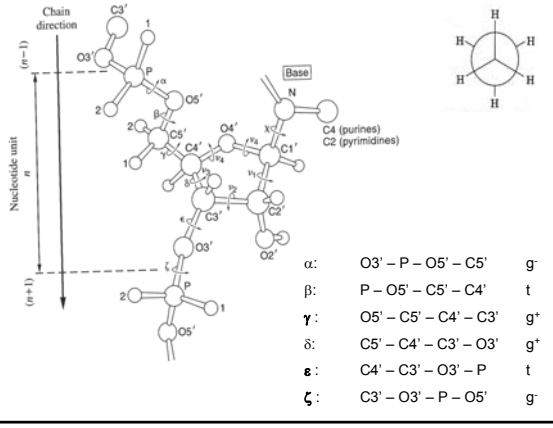


Torsion at the phosphodiester bonds

- **Phosphate is tetrahedral like carbon, so favoured conformations occur at ±*g* (gauche, ±60°) or *t* (extended, 180°).**

α (bond to 5'-O) and ζ (bond to previous 3'-O).



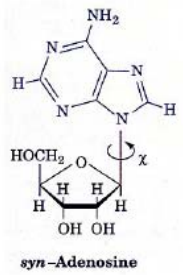


α:	O3' – P – O5' – C5'	g⁻
β:	P – O5' – C5' – C4'	t
γ:	O5' – C5' – C4' – C3'	g⁺
δ:	C5' – C4' – C3' – O3'	g⁺
ε:	C4' – C3' – O3' – P	t
ζ:	C3' – O3' – P – O5'	g⁻

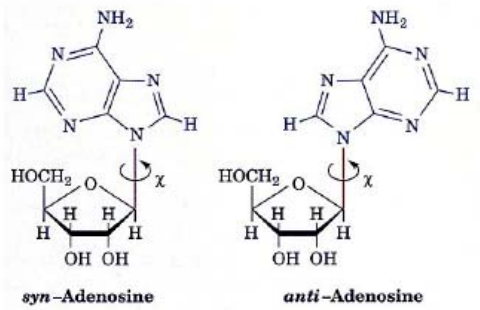
Rotation about the C-1' bond allows two orientations:

anti- **extends the base and pentose rings in opposite directions. For pyrimidines, this means that O-2 faces away from the pentose.**

syn- **orients the base and the pentose in the same direction.**

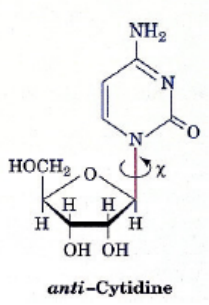


syn–Adenosine



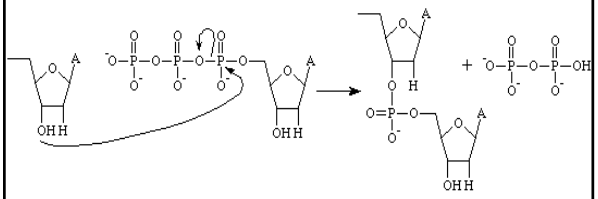
Free purine nucleosides, in particular guanosine, favour the *syn*- orientation, but adopt the common *anti*- orientation within most DNA and RNA helices.

Pyrimidines adopt *anti*-orientation almost exclusively, because of steric interference between O-2 and C-5' in the *syn*- orientation,



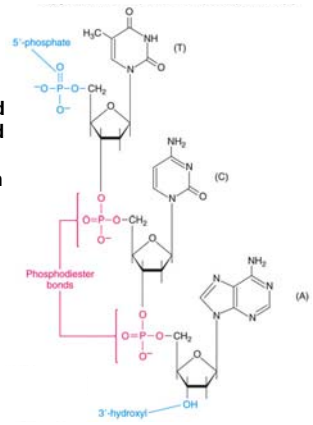
anti–Cytidine

In synthesizing a nucleic acid polymer from individual monomers, the 3'-hydroxyl group of one nucleotide attacks the α-phosphate of a nucleotide triphosphate, displacing a pyrophosphate with the cleavage of an anhydride bond:

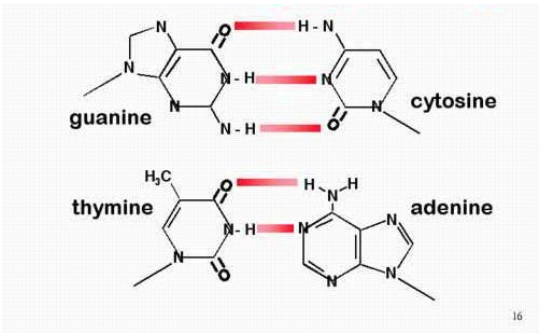


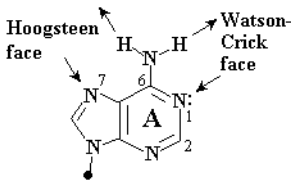
- **a polymer made by linking nucleotides via phosphodiester bonds.**
- **these bonds are synthesized by the attack of an alcohol residue from ribose on the α-phosphate to release a diphosphate residue (which is subsequently hydrolyzed to phosphate)**

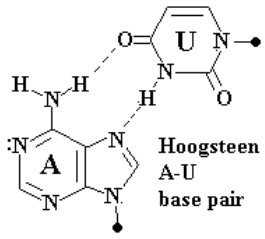
- **Nucleotides are linked by phosphodiester bond**
- **Strand has a direction (5'→3')**

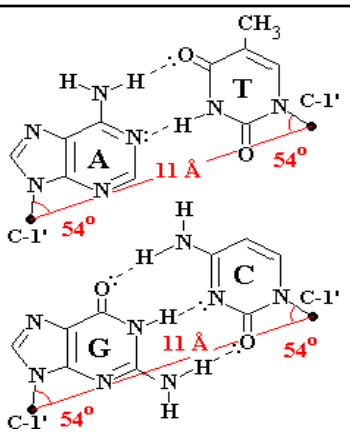


Watson Crick Base pairing





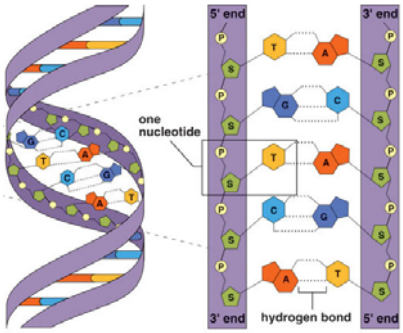




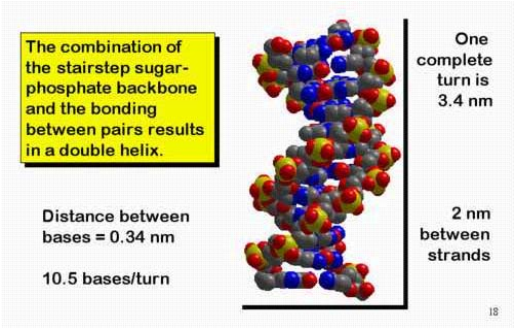
Features of the Watson Crick pairing

1. **The permitted hydrogen bonds are: adenine with thymine (2 bonds); and, cytosine with guanine (3 bonds).**
2. **The dimensions of the 2 permitted base-pairs are similar, i.e. the C1'-C1' distance is nearly identical in both cases.**
3. **The beta-glycosidic bond is attached on the same edge of the base pair.**
4. **Although some of the atoms in the purine and pyrimidine bases are involved in hydrogen bonds, there is still potential for further hydrogen bonding. This potential is particularly important for sequence specific protein binding.**
5. **The Watson-Crick base-pair is a planar structure.**

DNA is double-stranded, with *complementary base pairing*.



The Double Helix



The original Watson and Crick model for the double helix, B-DNA, is one of several conformations.

B-DNA - believed to be the predominate form under physiological conditions. It has 10.5 bases per turn.

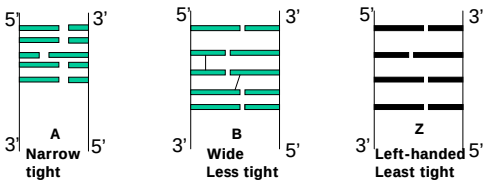
A-DNA - formed when B-DNA is chemically treated. It has 11 bases per turn.

Z-DNA - left handed helix with 12 bases per turn. It may play a role in regulation of gene expression.

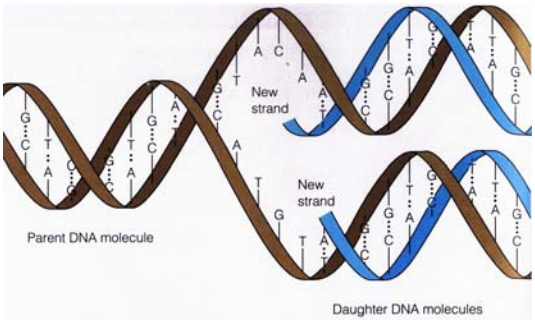
	A-DNA	B-DNA	Z-DNA
PITCH	2.8 nm	3.4 nm	4.5 nm
bp / repeat	11	10	12
TWIST / bp	33.6°	35.9°	30°
bp TILT	19°	4.1°	7°

Types of DNA Double Helix

- Type A: major conformation of RNA, minor conformation of DNA;
- Type B: major conformation of DNA;
- Type Z: minor conformation of DNA



DNA Duplication



RNA Structural Elements

- RNA always exists as single-stranded molecules.
- It does not take on an extended, secondary structure like DNA.
- The strands tend to fold into a uniform, periodic pattern.
- Several structural elements are observed.

RNA Structural Elements

Hairpin turns

Loops in the chain that bring together complementary base pairs. If long enough, a double helix region is observed.

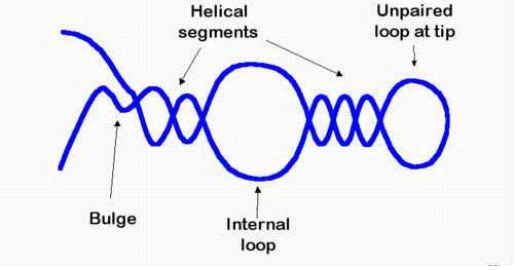
Right-handed double helix

Result from intrastrand folding.

Internal loops and bulges

Relatively common in RNA molecules. These are structural features that disrupt the formation of continuous double helix regions.

RNA Structural Elements



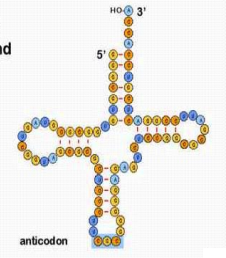
RNA: RNA is mostly an adaptor molecule, used to translate between information in DNA and protein machinery/structure.

Both RNA and proteins are involved in catalysis.

- **mRNA - a transcription of the information in the DNA. Used to carry the information in temporary format from the DNA archive to the protein synthetic machinery**
- **rRNA - This RNA forms the core of a large molecular machine, the ribosome, which is used to make proteins. The ribosome consists of 3-4 RNA molecules and many proteins (~100 in eukaryotes). Both RNA and proteins are involved in catalysis**
- **tRNA - an adaptor molecule. Classic "clover-leaf" shape (secondary structure) folds up into a 3D L-shape (tertiary structure). The proper amino acid is attached to the CCA end by an enzyme specific for the amino acid and for the tRNA. One arm, the anticodon loop, recognizes the code for an amino acid on the mRNA.**

tRNA Structure

All tRNA have a common 2º and 3º structure.



The smallest type of RNA. It consists of 74-93 nucleotides.

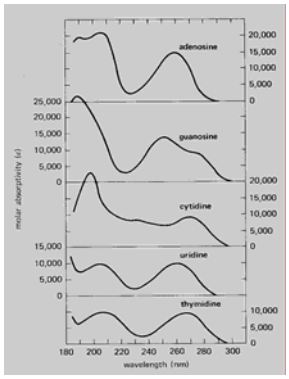
These molecules are the carriers of the 20 amino acids with at least one tRNA for each.

They often contain several unusual purines or pyrimidine bases -modifications of the basic four.

Comparison of structures

	DNA	RNA
Sugar	Deoxyribose	Ribose
Bases	Adenine, guanine, thymine, cytosine	Adenine, guanine, uracil, cytosine
Strands	Double stranded with base pairing	Single stranded
Helix	Yes	No

ABSORPTION SPECTRA



$\pi \rightarrow \pi^*$ transition of aromatic ring of base

The position and shape of the absorption band changes when hydrogen bonding interactions are present

Stability of DNA

Forces that determine DNA structure

1) Electrostatic (repulsion)
2) Hydrophobic "forces"
3) H-bonds
4) Stacking interactions

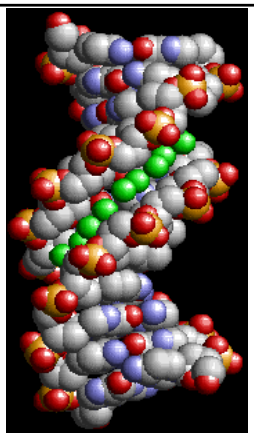
- **base pairs are found in the interior of the helix**
- **charged and hydrophilic sugar-phosphate backbone are on the exterior**
- **phosphates from the 2 strands are also kept away from each other (as much as possible) to reduce the electrostatic repulsion**
- **somewhat neutralized by divalent cations, like Mg^{2+}, and polyamines**

Stability of DNA

Stacking interactions stabilize the double helix about as much as the base-pair H-bonds

- **stacking interactions are not sufficient to overcome the electrostatic repulsion of the phosphates**
- **the H-bonding guarantees the complementarity of the strands**

Hydrogen bonds due to the formation of a water spine in the minor groove.



Stability of DNA

The two polynucleotide chains of double-helical DNA can be separated under certain conditions, most usually by raising the temperature.

Terms that describe the change from dsDNA to ssDNA are: melting, denaturation, strand separation.

Terms that describe the change from ssDNA to dsDNA are annealing, renaturation, and, in certain contexts, hybridization.

DNA denaturation

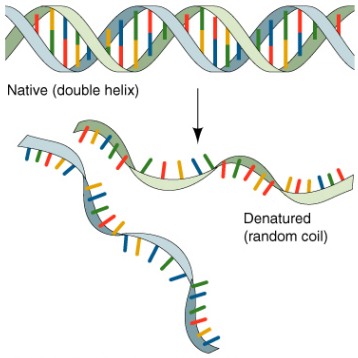
DNA can be denatured under extreme conditions of temperature or pH.

Conditions disrupt the hydrogen bonding and hydrophobic interactions between the bases, and result in separation of the strands.

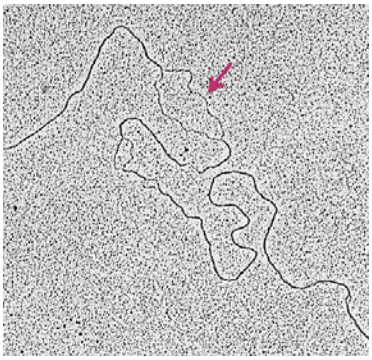
Denatured DNA is less viscous than native double-helical DNA, and the bases exhibit greater UV absorption.

The transition from double-stranded DNA (dsDNA) to single-stranded random coil DNA (ssDNA) is called a helix-coil transition.

DNA denaturation



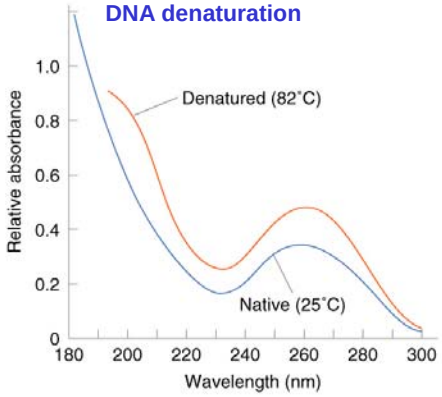
DNA denaturation



DNA denaturation

- The denaturation of double stranded DNA is easily followed spectroscopically
- The purine and pyrimidine bases in DNA absorb UV light maximally at a wavelength of approximately 260 nm.
- In double-stranded DNA, however, the absorption is decreased due to base-stacking interactions.
- When DNA is denatured, these interactions are disrupted and an increase in absorbance is seen.
- This change is called the hyperchromic effect.
- The extent of the effect can be monitored as a function of temperature.

DNA denaturation



DNA renaturation

If the temperature is rapidly decreased, the change in viscosity /absorption is not fully reversed, and the change occurs over a much broader range of temperatures.

Subsequent heating and cooling cycles will appear similar to this, indicating an irreversible change.

The overall rate of renaturation is determined by:
1) the concentration of DNA
2) the average length of the DNA segments
3) the complexity of the DNA — how many different sequences there are in the mix

DNA renaturation

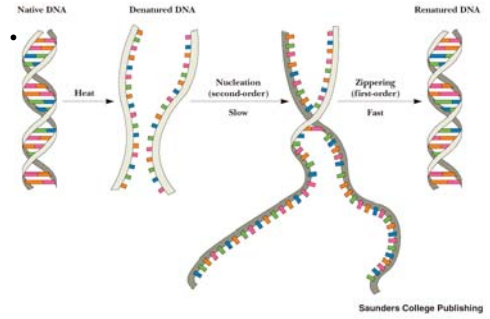
Under certain conditions, DNA can be renatured — the complementary strands are brought back together and the correct H-bonding pattern is reproduced. This occurs efficiently at the annealing temperature:

Annealing temperature = T_m - 25°C

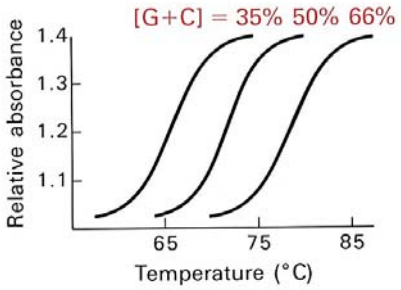
There are two steps in renaturation:
1) nucleation — the 2 strands find a region of complementarity and form a short double helix.
This is a second order reaction and is rate limiting.

2) zippering — in either direction from the paired region of complementarity, the double helix is elongated. This is a rapid first order reaction.

Structural Changes in DNA Melting



The melting temperature is defined as the temperature at the midpoint in denaturation curve.



Influences on T_m:

1. GC content: the higher the GC%, the higher the T_m (G-C base pairs have 3 H-bonds and are thus stronger than A-T base pairs)

2. Salt: the higher the [salt], the higher the T_m (ions shield charges and thus lessen repulsion between phosphates)

3. Low (<2.3) or high (>11.5) pH decrease T_m (ionization of the bases)

4. Organic compounds that destabilize the double helix by competing as H-bond partners or by disrupting the water clathrate shell around the bases

Renaturation (or Annealing)

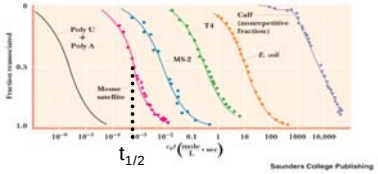
- Renaturation refers to the process of DNA strands associate into a double helix;
- Renaturation can be analyzed quantitatively: C is the amount of single stranded DNA remaining, C_0 is the initial single stranded DNA.

$$-\frac{dC}{dt} = k_2 C^2, \qquad where \quad k_2 \; is \quad the \quad second \quad order \quad rate \quad constant.$$

$$\Rightarrow \frac{C}{C_0} = \frac{1}{1 + k_2 C_0 t} \qquad This \; gives \quad the \quad C_0 t \quad plot.$$

$$\Rightarrow C_0 t_{1/2} = \frac{1}{k_2} \qquad where \quad t_{1/2} \; is \quad the \quad time \quad when \quad half \quad DNA \quad is \quad renatured.$$

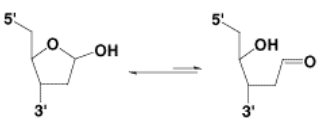
C_0t Plot



$t_{1/2}$

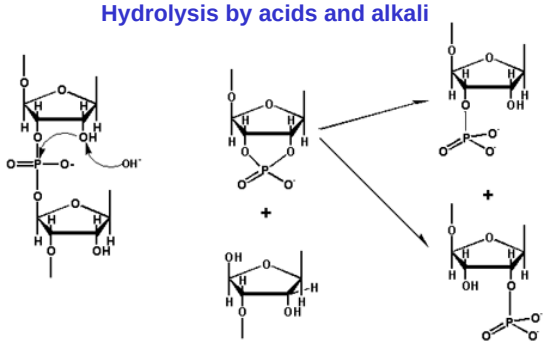
Hydrolysis by acids and alkali

DNA is generally quite stable - resists attack in acid and alkali solutions.
In mild acid solutions - at pH 4 - the β-glycosidic bonds to the purine bases are hydrolyzed.
Protonation of purine bases (N7 of guanine, N3 of adenine) occurs at this pH.
Protonated purines are good leaving groups



the depurinated sugar can easily isomerize into the open-chain form and in this form the depurinated (or apurinic) DNA is susceptible to cleavage by hydroxyl ions.

Hydrolysis by acids and alkali



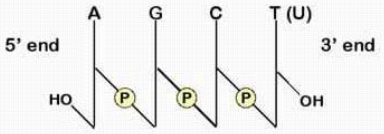
Hydrolysis by acids and alkali

RNA is very unstable in alkali solutions due to hydrolysis of the phophodiester backbone. The 2'OH group in ribonucleotides renders RNA molecules susceptible to strand cleavage in alkali solutions.

Enzymatic hydrolysis of RNA

There are many enzymes that cleave RNA - ribonucleases.

Representation of an oligonucleotide showing the 3',5'-phosphodiester bonds.

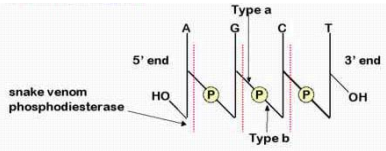


A very abbreviated representation is to simply list the base sequence starting from the 5' end - AGCT

Nucleases

- **Exonucleases** - catalyze the removal of terminal nucleotides, 3' and 5' types.
- **Endonucleases** - catalyze removal of internal phosphodiester bonds.
  - Type a - act on the 3' hydroxyl group of a nucleotide with the phosphorous group.
  - Type b - act on the 5' hydroxyl group of a nucleotide with the phosphorous group.

Endonucleases



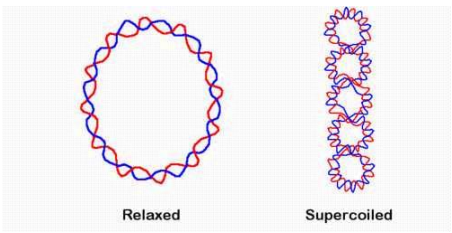
While these enzymes are useful for cutting DNA and RNA into manageable sizes, they are not very specific.

Enzyme	Substrate	Type	Specificity
Rattlesnake venom phosphodiesterase	DNA,RNA	exo(a)	3' end, no base specificity.
Spleen phosphodiesterase	DNA,RNA	exo(b)	5' end, no base specificity.
Pancreatic ribonuclease A	RNA	endo(b)	3' side preference for pyrimidines.
Spleen deoxyribonuclease II	DNA	endo(b)	Internal ester bonds. No base specificity

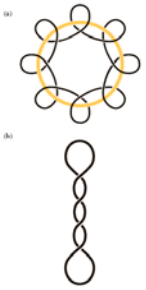
Tertiary Structure of DNA

Studies of native, intact DNA have revealed two distinct forms - linear and circular.

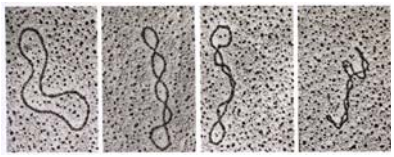
The circular form is the result of covalent joining of the two ends of a linear double helix.



- Supercoils refer to the DNA structure in which **double-stranded circular** DNA twists around each other. Supercoiled DNA contrasts relaxed DNA;
- In DNA replication, the two strands of DNA have to be separated, which leads either to overwinding of surrounding regions of DNA or to supercoiling;
- The degree of supercoils can be quantitatively described.



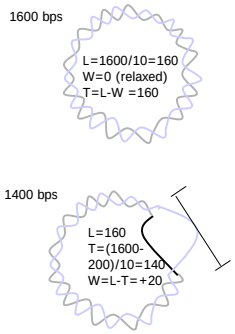
- The linking number of DNA, a topological property, determines the degree of supercoiling;
- The linking number defines the number of times a strand of DNA winds in the right-handed direction around the helix axis when the axis is constrained to lie in a plane;
- If both strands are covalently intact, the linking number cannot change;
- For instance, in a circular DNA of 5400 basepairs, the linking number is 5400/10=540, where 10 is the basepair per turn for type B DNA.

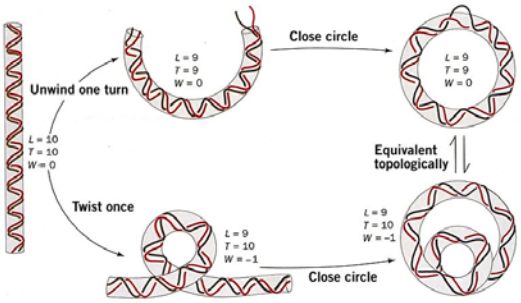


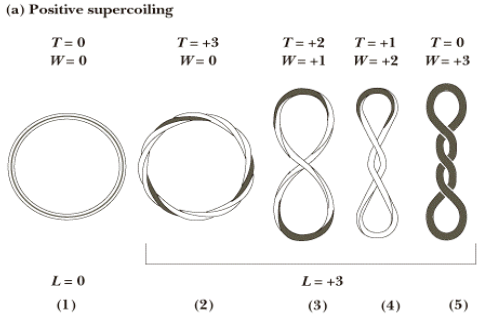
- Twist is a measure of the helical winding of the DNA strands around each other. Given that DNA prefers to form B-type helix, the preferred twist = **number of basepair/10**;
- Writhe is a measure of the coiling of the axis of the double helix. A right-handed coil is assigned a negative number (negative supercoiling) and a left-handed coil is assigned a positive number (positive supercoiling).
- Topology theory tells us that the sum of T and W equals to linking number:

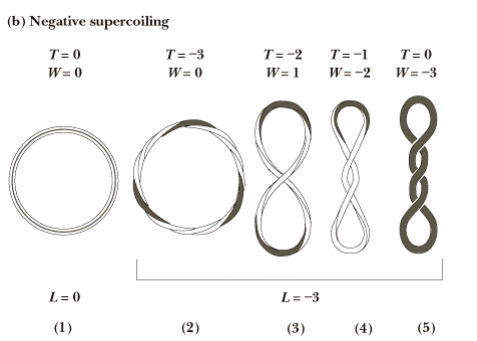
  L=T+W
- For example, in the circular DNA of 5400 basepairs, the linking number is 5400/10=540
  - If no supercoiling, then W=0, T=L=540;
  - If positive supercoiling, W=+20, T=L-W=520;

- A relaxed circular, double stranded DNA (1600 bps) is in a solution where conditions favor 10 bps per turn. What are the L, T, and W?
- During replication, part of this DNA unwinds (200 bps) while the rest of the DNA still favor 10 bps per turn. What are the new L, T, and W?

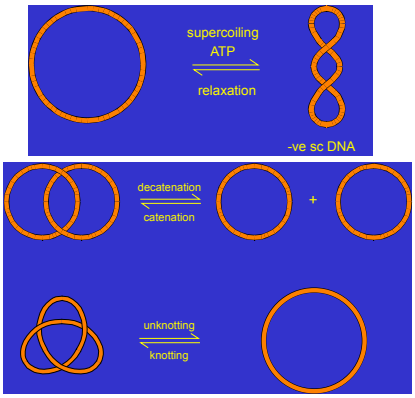




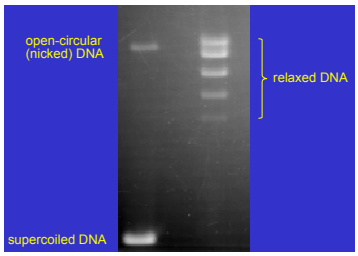




DNA gyrase

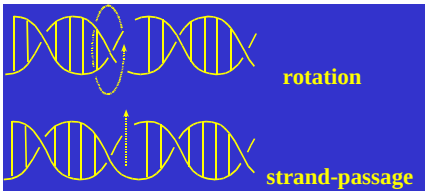


Agarose gel electrophoresis



DNA topoisomerases

- Change linking number of DNA
- How?
- Must break backbone of DNA
- Break one strand
  - Allow free end to rotate
- Break both strands
  - Allow one end to rotate with respect to the other

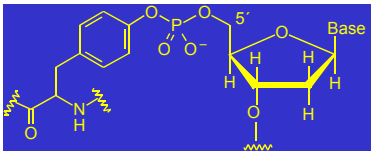


Classification of topoisomerases

- Type IA
  - Cleaves one strand
  - 5′-phosphotyrosine linkage
  - ΔLk = ± 1
- Type IB
  - Cleaves one strand
  - 3′-phosphotyrosine linkage
  - ΔLk = ± 1
- Type II
  - Cleaves both strands (4 bp stagger)
  - 5′-phosphotyrosine
  - ΔLk = ± 2
  - ATP-dependent

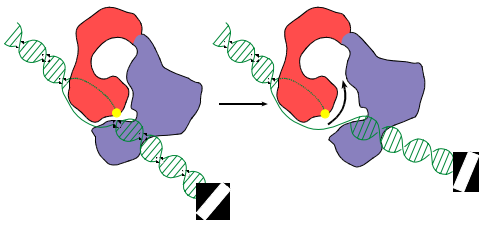
Covalent intermediate

- Active-site tyrosine attacks DNA phosphate
- Forms transient phosphotyrosine



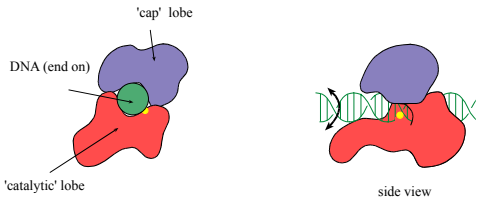
Type IA mechanism

- Type IA
  - Monomeric (97 kDa; *E. coli*)
  - Only binds to -ve supercoils
  - Works by strand-passage (decatenation)



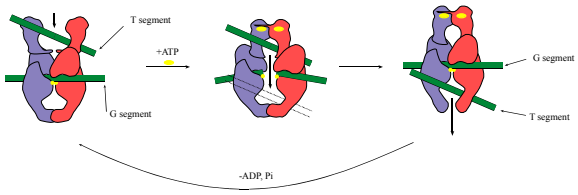
Type IB mechanism

- Type IB
  - Approx 90 kDa
  - Monomeric
  - Binds to +ve and -ve supercoils
  - no DNA distortion

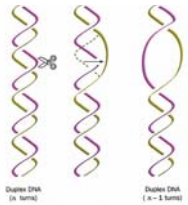


Type II mechanism

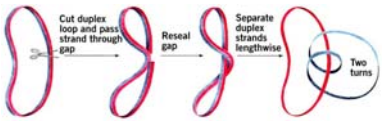
- Type II
  - Dimeric (approx. 170-190 kDa)
  - Tetrameric in bacteria
  - Interacts with 2 DNA segments
  - ATP-dependent clamp



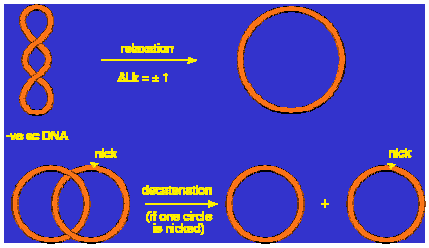
Topoisomerase I – single strand cuts



Topoisomerase II – double strand cuts

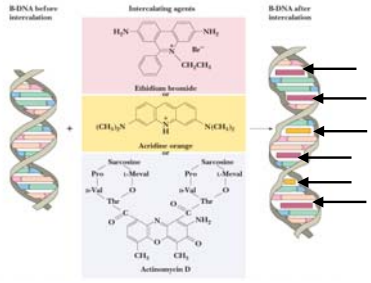


E. coli topo I reactions



Intercalating Agents Distort the Double Helix

Several hydrophobic molecules containing flat aromatic and fused heterocyclic rings can insert between the stacked base pairs of DNA. These molecules are called intercalating agents.



Intercalators and groove binders