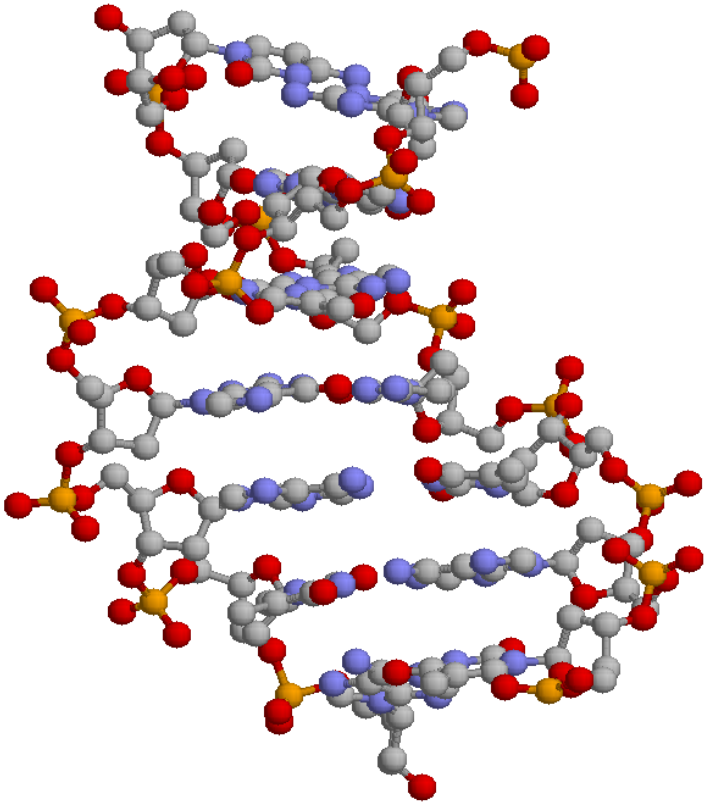
University of Lethbridge

DNA Structure

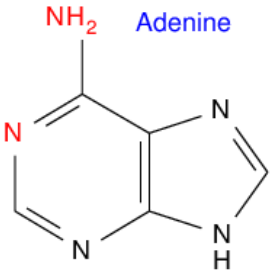
Voet & Voet:

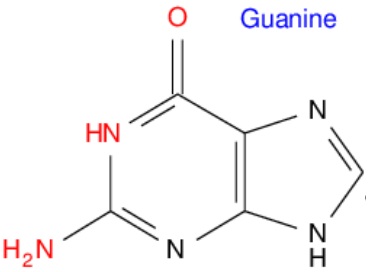
Chapter 29
Pages 1107-1122



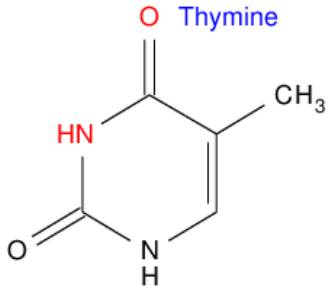
University of Lethbridge

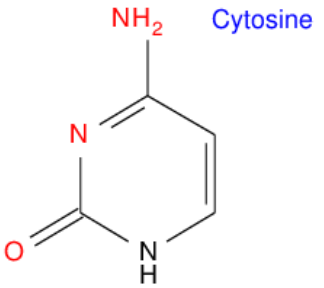
Review





The four DNA bases and their atom names

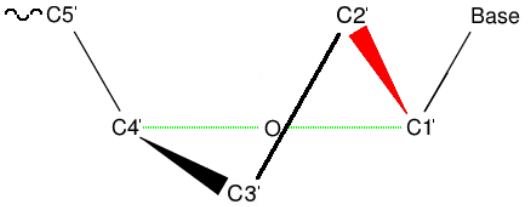




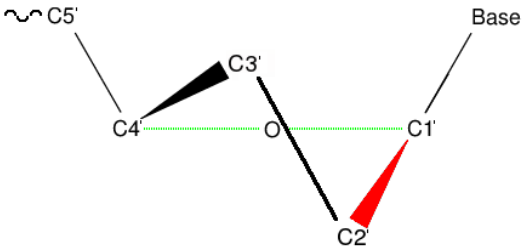
The four common β-D-ribose conformations

- **All B-DNA ribose adopt the C2' endo conformation**
- **All A-DNA ribose adopt the C3' endo conformation**

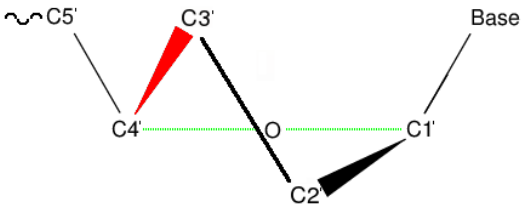
C2' endo (B DNA)



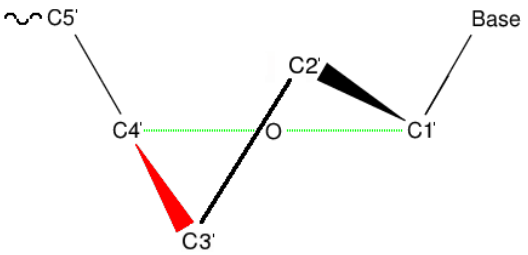
C2' exo



C3' endo (A DNA)



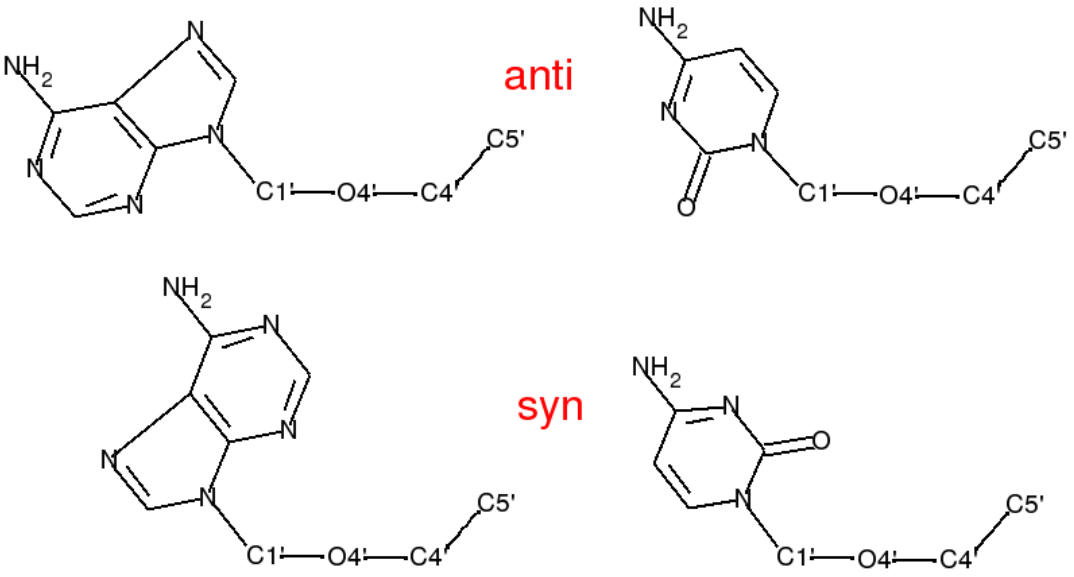
C3' exo



Review – Glycosidic Bond

Glycosidic bond adopts *anti* or *syn* conformations

- ***anti* conformation has the bulky portion of the base furthest from the ring**
- ***anti* is favored and present in both B- and A-DNA**



Adenosine and cytidine in *anti* (top) and *syn* conformation (bottom). The C2' and C3' atoms are not shown.

Note: Many textbooks draw nucleosides with the *syn* conformation to save space

Review – Backbone Conformation

Nucleic acid backbone has six rotatable bonds

Not completely free to rotate due to steric constraints

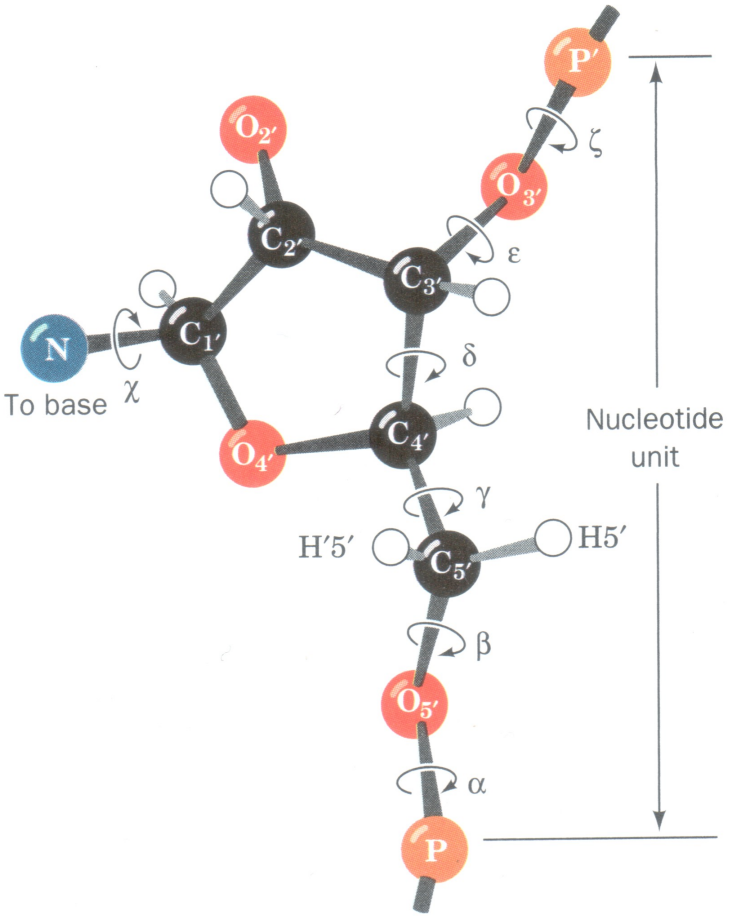
C3'-C4' (δ) torsion angle is restricted by the ring structure

O3'-C3' (ε) & C4'-C5' (γ) torsion angles are restricted by non-hydrogen atoms attached to C3' & C4'

C5'-O5' (α), O5'-P (β) & P-O3' (ζ) are relatively free to rotate

Additional restrictions due to presence of nucleobases

While flexible, the DNA backbone (ss & ds) is far more rigid than the number of rotatable bonds suggest

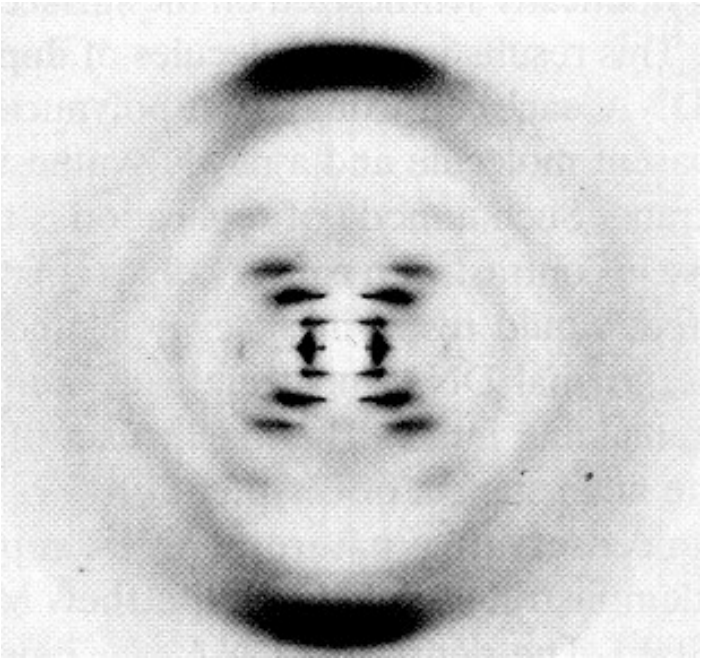


University of Lethbridge

Real DNA Structures

Original double helix structure based upon powder diffraction studies (low resolution) performed by M. Wilkins & R. Franklin

(Late '70s) Nucleic acid synthesis techniques allowed crystallization and structure determination of short double stranded DNA at high resolution



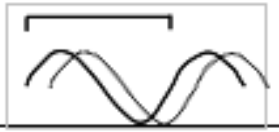
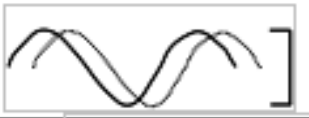
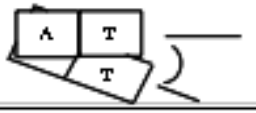
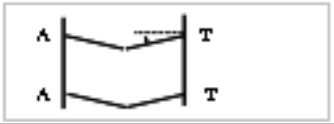
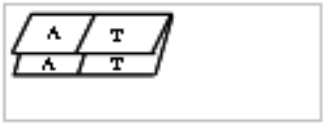
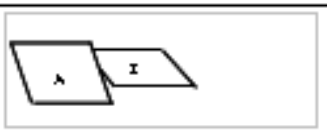
Franklin's X-ray diffraction pattern from crystalline DNA

While average parameters are close to those of B-DNA, the individual residues depart significantly from the average values

University of Lethbridge

Real B-DNA

DNA double helix parameters (Ranges from initial 1.9 Å X-ray structure)

parameter	*definition*	*cartoon*	*B-DNA*
pitch	Length of a turn of double helix		34 Å
diameter	Width of double helix		20 Å
rise	Distance between consecutive stacked DNA bases		3.4 Å
twist	Angle (of rotation) between consecutive base pairs		36 ° (26-43)
tilt	Deviation from planarity of base pairs (parallel to H-bonds)		6 °
roll	Angle between stacked bases (perpendicular to H-bonds)		2 ° (-14 to 17)
Propeller twist	Deviation from planarity of base pairs (perpendicular to H-bonds)		-15 ° (-7 to -30)

University of Lethbridge

B-DNA conformations

Multitude of short dsDNA X-ray and NMR structural studies demonstrate

(1) DNA structure is irregular in a sequence specific manner

(2) Sequence establishes the deformability of dsDNA (eg. most sequences are rigid and only some are readily 'bent')

When considering individual base stacking interactions within nucleic acids:

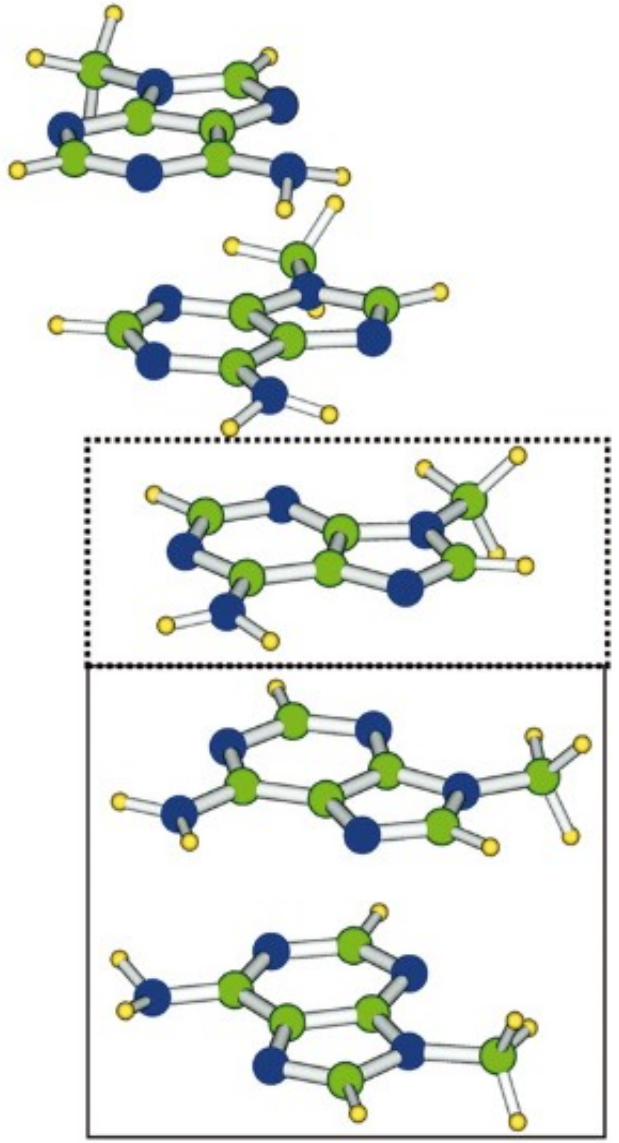
5' – Pur Pyr – 3'	**Relatively easily bent due to minimal base stacking**
5' – Pyr Pur – 3'	**Relatively rigid due to extensive base stacking**
5' – Pyr Pyr – 3', 5'-Pur Pur – 3'	**Relatively rigid due to extensive base stacking (especially ApA)**

Functionally important as many DNA binding proteins induce bends (even > 90°)

Aggregation of Nucleobases (base stacking)

Structural Studies demonstrate purines and pyrimidines form extended stacks in crystals

- **Typically the free nucleobases are partially overlapping (base stacking) in these structures**
- **Crystal structures of chemically similar bases have similar overlapping structures suggesting a degree of specificity**
- **NMR confirms similar structures form in solution**



Aggregating properties are independent of H-bonding

- **Modified bases that have limited H-bonds capacity adopt similar overlapping structures**
- **Nucleobases do not aggregate in non-aqueous solvent where H-bond energies are the greatest (and the hydrophobic effect is weaker)**

Aggregation of Nucleobases II (base stacking)

Physical Chemistry studies also demonstrate purines and pyrimidines form stacks in solution

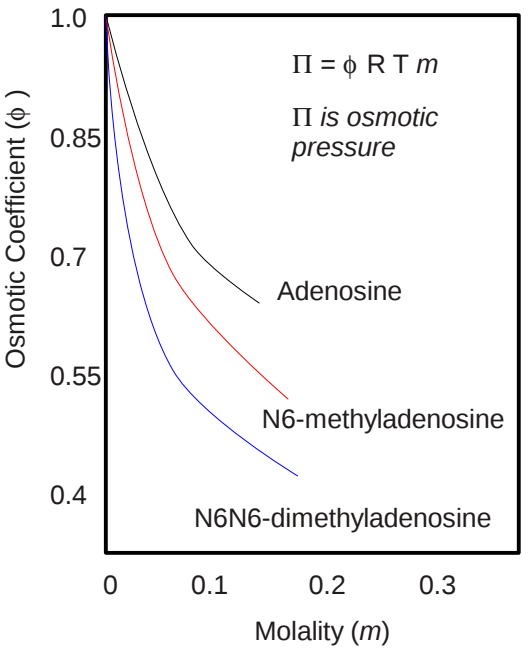
1) Variation of 'osmotic coefficient' with concentration

- **Free nucleobases form base stacks at sub [mM]**
- **Measured as decreasing osmotic pressure with increasing [nucleobase]**

2) Hyperchromic Shift of ssDNA

- **Concentration independent (not due to intermolecular interactions)**
- **Non-cooperative (unlike dsDNA hyperchromic shift) and length independent (even dinucleotides have hyperchromic shift)**

Same Conclusion: Aggregating properties (base stacking) are independent of H-bonding



University of Lethbridge

Hydrophobic Effect & Base Stacking

Base stacking is largely stabilized by the hydrophobic effect

- **BUT … the hydrophobic interactions are qualitatively different from those in proteins**

Thermodynamic analysis of base stacking

- **Nucleoside-5',3'-nucleoside (unstacked) ↔ Nucleoside-5',3'-nucleoside (stacked)**
- **Base stacking is enthalpically driven and entropically opposed**

Contrast: Protein folding is entropically driven and enthalpically opposed

- **Differences in hydrophobic effect are poorly understood though they must relate to structural differences between nucleobases and amino acids**
  - **Apparently due to greater polarity of nucleobases**
  - **Aromatic amino acids (less polar) rarely "stack" within the hydrophobic core**

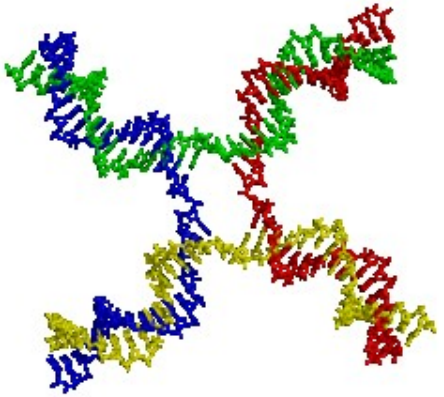
University of Lethbridge

Base Pairing

Free nucleobases can form Watson-Crick AND alternative base pairs in solution

Example: Free A–T nucleobases form 'Hoogsteen base pairs' (next slide) in crystals

- **Hoogsteen geometry is functionally important – Stabilizes tRNA structure, central role in DNA recombination (Holliday Junctions), DNA recognition**



Watson-Crick base pairs are not always the lowest energy geometry for free nucleotides

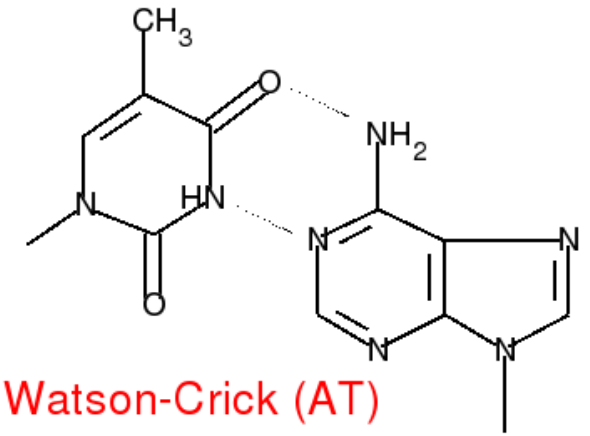
Why does <u>complementary dsDNA</u> exclusively use Watson-Crick base pairing?

- **Steric and electronic factors account for the preference of Watson-Crick base pairs in dsDNA**

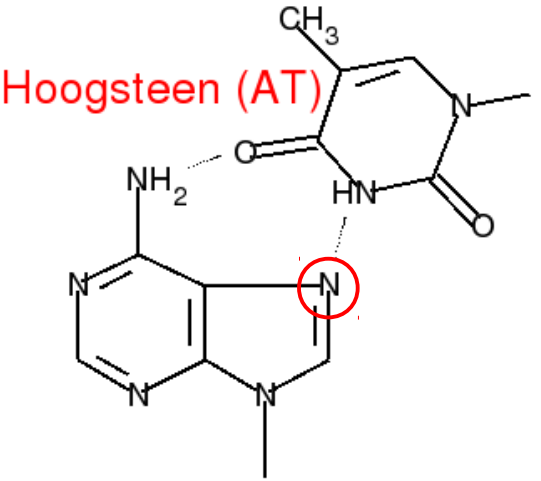
Note: other base pairs do occur in complementary DNA when proteins are bound

'Alternative' Base Pair Geometries (H-bonding)

Other base pair geometries are observed in non-helical DNA/RNA (or in the presence of proteins)



Watson-Crick (AT)

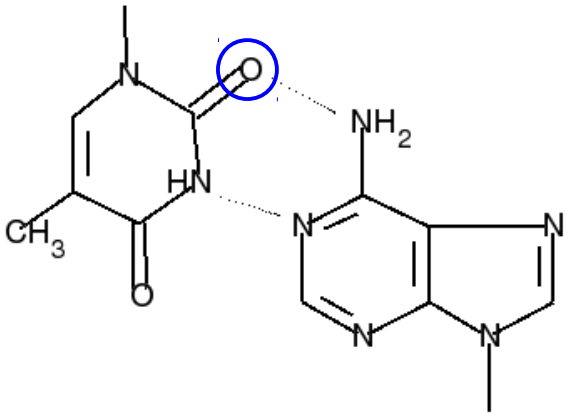


Hoogsteen (AT)

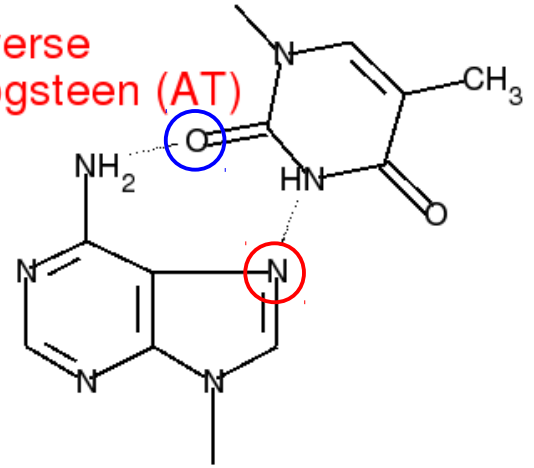
Several common 'alternative' base pairs geometries

Reverse (AT) base pairs involve O2 – N6 H-bonds (blue circles)

Hogsteen base pairs involve the N7 atom (red circles)



Reverse Watson-Crick (AT)



Reverse Hoogsteen (AT)

University of Lethbridge

Preferred Base Pairs

A:T (or A:U) and G:C base pairs are energetically favorable (regardless of geometry)

Infrared spectroscopy studies of base pairing interactions

Base Interaction	Association Constant (M^{-1})
A•A	3.1
U•U	6.1
C•C	28
G•G	10^3-10^4
A•U	10^2
G•C	10^4-10^5

$$B1 + B2 \leftrightarrow B1•B2$$

All other base interactions are negligibly small

An equimolar mixture of A, U, G and C will only form A:U and G:C aggregates

A:G, A:C, U:G and U:C interactions are relatively unstable

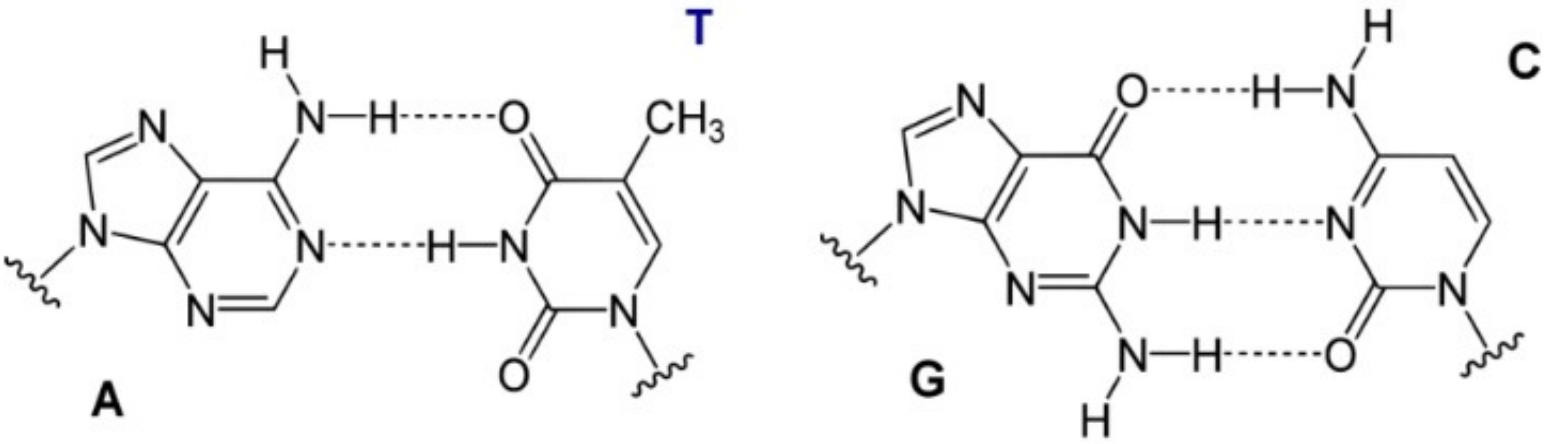
Watson-Crick Base Pair

Watson-Crick Base Pair geometry is favored in dsDNA for two reasons:

(1)* Geometric complementarity within double helix does not disrupt lowest energy backbone conformation

Non Watson-Crick base pair geometry requires higher energy backbone conformations

(2) Electronic complementary of base pairs favours A:T(U) and G:C



University of Lethbridge

'Stable' dsDNA Bending

Early 1980s, gel mobility studies of a 414 bp DNA fragment (Kinetoplastid species) provided first evidence of "stable" bent dsDNA

- **Unusual DNA sequence containing multiple short poly A tracts**
- **Consistently runs as 2x expected M_w in agarose gel electrophoresis**
- **Low resolution structural studies indicate DNA is shorter than linear DNA**
- **(Note: Supercoiled DNA is also shorter but typically runs at less than expected MW)**

Repetitive DNA sequences (containing short poly A tracts) based upon the above fragment also display unexpected electrophoretic mobility

The number and location of short poly A tracts alters the electrophoretic behaviour of dsDNA

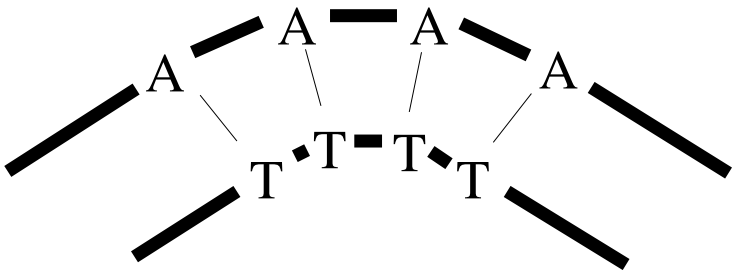
University of Lethbridge

Stable DNA Bending Models

Two models have been proposed to explain how short poly A tracts affect DNA structure. Both models successfully predict most (but not all) stably bent DNA

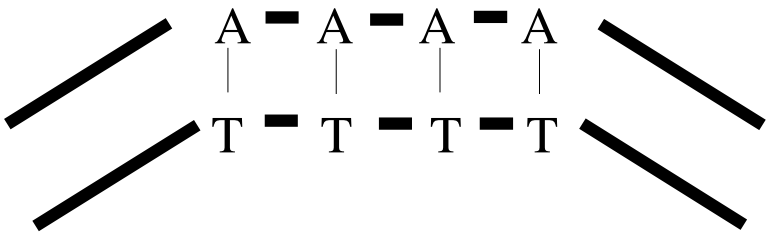
(1) Wedge Model

Bend within a poly A tract is spread across entire poly A tract



(2) Junction Model

Bend occurs at ends of poly A tract



University of Lethbridge

Phasing of DNA bends

Both models for DNA bending share two important features:

(1) A single short polyA tract can only introduce a small bend

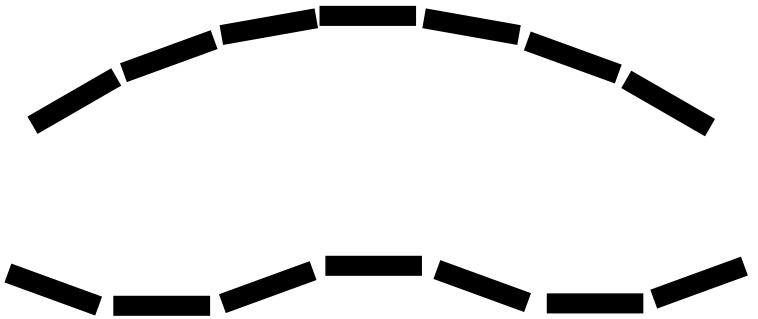
Many short polyA tracts are required to form a detectable stable bend

(2) The polyA tracts must be "in phase" to generate a stable bend

Each of the small bends by the multiple polyA tracts must be in the same direction

Top – All small bends are in the same direction producing a large and stable overall bend

Bottom – Small bends are not in the same direction and there is no stable, overall bend



University of Lethbridge

More on Phasing

B-DNA has an average helical rise of 10 bp/turn

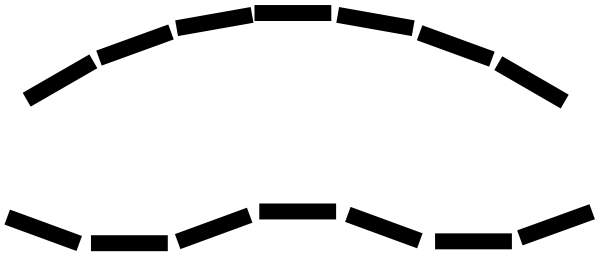
Poly A tracts must occur at intervals separated by 10n (n is an integer) base pairs for each small bends to occur in the same direction

Example

(1) xxxAAAAAxx xxxAAAAAxx xxxAAAAAxx xxxAAAAAxx

(2) xxAAAAAxx AAAAAxxAAA AAxxAAAAAx xAAAAAxxAA

A stable bend is produced by the first sequence as the polyA tract occurs at the same position within each turn of dsDNA



Phase length = 10 bps
- phase is same a helix turn length
- in phase and stably bent

Phase length = 7 bps
- phase not equal to helix turn length
- out of phase and not stably bent

DNA bending and Function

The following biological systems are known to require stably bent DNA conformations

(1) Gene Regulation (primarily in eucaryotes)

Control of promoter access or RNA polymerase binding

(2) Initiation of replication

(3) Recombination

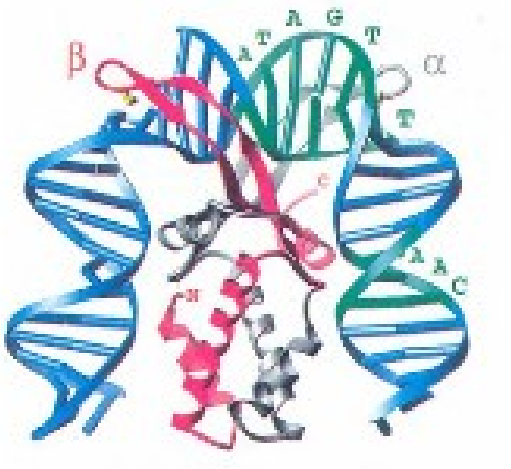
Formation of 'Holliday' junctions (tetraplex)

(4) Repair mechanisms

Excision repair mechanisms

(5) Organization and packing of DNA into higher order structures (*eg.* chromatin)

Histone induced bending

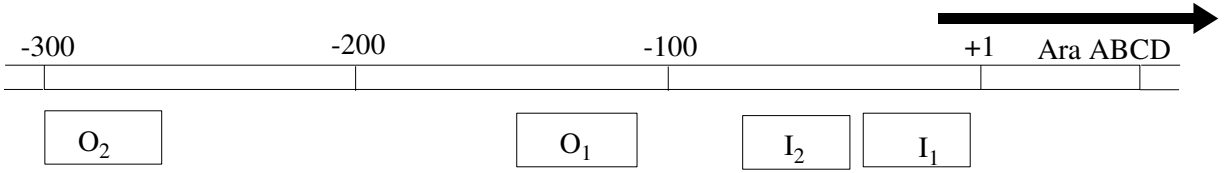


IHF protein induces ~180º bend in dsDNA

University of Lethbridge

Example: Arabinose Operon

Procaryotic operon with an upstream regulatory element (similar to typical eucaryotic case)



Sites I_1, I_2 and O_2 are AraC binding sites and O_1 is a CAP (catabolite activating protein) binding site.

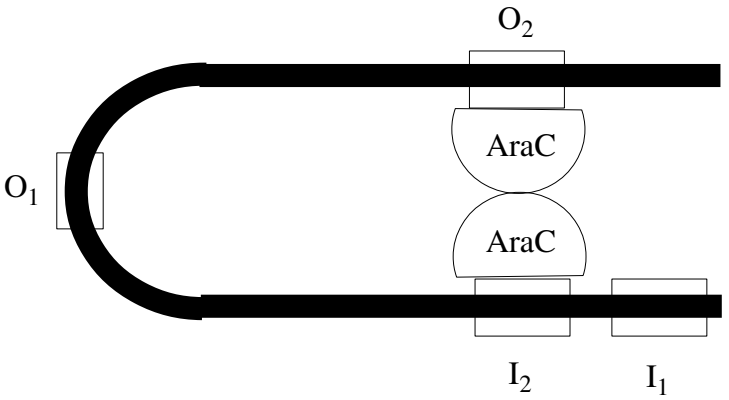
Inserting/deleting multiples of 10 nucleotides between I_2 and O_2 have little or no effect on the regulation of the operon

Inserting/deleting 5,15, ... nucleotides between I_2 and O_2 prevents stable repression

Inserting/deleting 5,15, ... nucleotides between I_1 and I_2 abolishes transcription

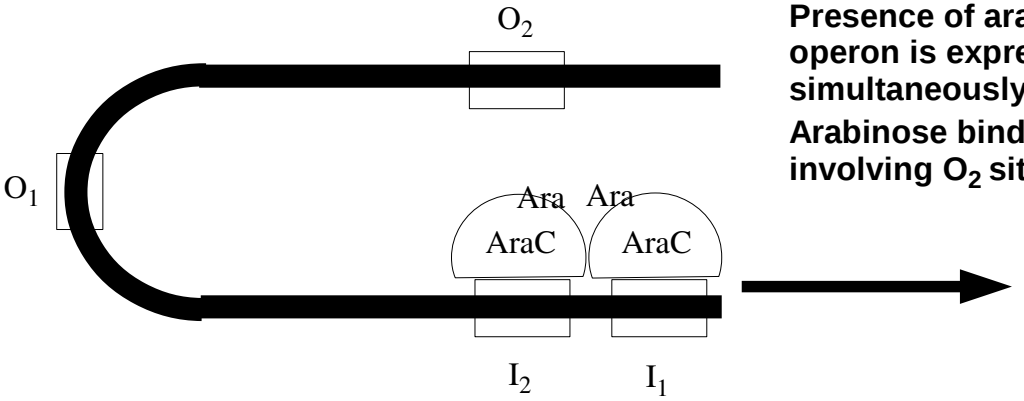
What's going on ??? DNA bending !!!

Arabinose Operon



No arabinose
operon is repressed and the araC dimer binds simultaneously to sites O_2 and I_2

Insertions & Deletion between I_2 and O_2 can affect their phase, preventing required protein-protein contacts



Presence of arabinose
operon is expressed and the araC dimer binds simultaneously to sites I_1 and I_2.
Arabinose binding to araC breaks the interaction involving O_2 site and leads to the binding at site I_1.