**Lecture 35: DNA Stability/Protein-DNA Interactions, DNA Modification Enzymes**

**Key Terms:**

* **DNA Stability**
* **DNA-Protein Interactions**
* **Restriction endonuclease & DNA ligase**

**1. Forces Stabilizing Nucleic Acid Structures.**

Double stranded DNA & RNA can be *reversibly* denatured ("melting"). Cooperative transition from double stranded helix → single stranded random coils; the change in absorbance of the bases at λ=260 nm can be used to monitor this transition. The absorbance (A260) increases when the DNA melts (**hyperchromatic effect**).



**Comparison of Dominate Forces in DNA and Protein Stability:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Energetic Term** | **Protein Stability**  | **dsDNA stability** | **Molecular Description of Energetic Terms in DNA stability.** |
| ΔH | Hydrogen Bonds | **+** | **+ +** |  |
| Electrostatic interactions | **0** | **- -** |  |
| Van der Waals | **++** | **+ + + + + +**  |  |
| ΔS | Conformational Entropy | **- - - - -** **- -** | **- - - - -** **- -** |  |
| Hydrophobic Effect | **+ + + +** | **+** |  |

**DNA Stability:** The two DNA molecules on the left are mixed under conditions that promote formation of double stranded DNA. Draw any forms of double stranded (duplex) DNA that would form on the right.

2. DNA-Protein Interactions

**3’-A-C-G-T-5’**

 **3’-A-T-G-C-A-G-A-C-G-T-G-5’**

 **1 2 3 4 5 6 7 8 9 10 11**

**3’-A-T-G-C-A-G-A-C-G-T-G-5’**

 **1 2 3 4 5 6 7 8 9 10 11**

Forces and functional groups involved in recognition.

1. Electrostatic bonding to the backbone.

1. side chains of Lys and Arg to phosphates.
2. Release of metal ions (e.g. K+) favors binding ( large increase in ∆S of ions).

2. Van der Waals: Stacking (and intercalation) of Phe, Trp, and Tyr side chains. More prevalent in single stranded (ss) nucleic acid.

3. Hydrophobic interaction with the 5-methyl of T.

4. Non- Watson-Crick Hydrogen bonding to the polar edges of the bases and to sugars.

a) Side chains of Arg, Asn, Gln, etc.

b) Protein main chain, C=O and NH groups.

c) "Bridging H2O" can also participate.

**Protein-DNA Binding:** The following curves show the dissociation constants (KD) for DNA binding for three different proteins (A, B, or C) as a function of salt concentration and DNA composition. Based on these binding constants, which groups on the nucleic acid are recognized by which protein?

**Protein A:** Binding independent of salt ∴ not phosphate.

 Binding same for U and C ∴ not recognizing base.

**Protein B:** Binding very dependent on salt.

**Protein C:** Binding independent of salt ∴ not phosphate.

 Binding changes between rU and rC.

3. DNA Modifying Enzymes – ‘Cut and Paste’

**B. Restriction Endonuclease:** [*endo* - cut within, *nuclease* - cleave nucleic acid]. Used by bacteria to degrade invading viral DNA. Named after bacterial species the particular enzyme was isolated from.

1. Enzyme binds to specific **recognition sequence**s with near absolute specificity and high affinity (KD = 10-10 M).

2. Enzymes usually bind in *major* groove, forming *both* specific and non-specific interactions.

3. Homodimeric enzymes have 180 degree rotational symmetry. Because of the symmetry in the enzyme, the DNA sequence also symmetrical. The sequence is the same on the top and bottom strands (referred to as palindromic sequences).

4. Require Mg2+ for cleavage, usually cleave both strands at the same position. Generating a 3’OH.

Example restriction enzyme cleavage sequences are shown on the right.

**Enzyme Recognition Products**

 **Sequence**

**HindIII(A^AGCTT):-A-A-G-C-T-T- -A A-G-C-T-T-**

 **-T-T-C-G-A-A- -T-T-C-G-A A-**

**BamHI(G^GATCC): -G-G-A-T-C-C- -G G-A-T-C-C-**

 **-C-C-T-A-G-G- -C-C-T-A-G G-**

**BglII(C^GATCG): -C-G-A-T-C-G- -C G-A-T-C-G-**

 **-G-C-T-A-G-C- -G-C-T-A-G C-**

**EcoRV(GAT^ATC): -G-A-T-A-T-C- -G-A-T A-T-C-**

 **-C-T-A-T-A-G- -C-T-A T-A-G-**

**HaeIII (GG^CC): -G-G-C-C -G-G C-C-**

 **-C-C-G-G -C-C G-G-**

**Example: EcoR1: G^AATTC**

|  |  |
| --- | --- |
| a) Non-specific interactions with DNA phosphates. phos | ---G-A-A-T-T-C------C-T-T-A-A-G------G A-A-T-T-C------C-T-T-A-A G------G A-A-T-T-C------C-T-T-A-A G--- |
|  b) Specific hydrogen bonds with donor and acceptors at the edge of bases in the major groove: |
|  |  |

**C. DNA Ligase** – Uses ATP to join 5’phosphate to 3’-OH, provided the two groups are in close proximity. Fragments created by the same restriction enzyme can always be joined to each other.

*Sticky end ligation:*

 -G A-A-T-T-C- -G A-A-T-T-C- --G**-**A-A-T-T-C--

 -C-T-T-A-A G- -C-T-T-A-A G- --C-T-T-A-A**-**G—

*Blunt end ligation:*

 -G-A-T A-T-C- -G-A-T A-T-C- -G-A-T**-**A-T-C-

 -C-T-A T-A-G- -C-T-A T-A-G- -C-T-A**-**T-A-G-