Sheet no. **2**

Molecular biology

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Nucleic acid Structure- ch3

-Topics:

0- Nucleic Acid Structure.

1-DNA Size and Fragility.

2- Major and Minor Grooves.

3- DNA Denaturation and Renaturation.

4- Topoisomers

Abbreviations>>bp: **base pairs**

0-Nucleic Acid Structure

- **DNA Structure: Size – Fragility – Structure – Denaturation/Renaturation – Super helicity.**
- **RNA Structure: RNA secondary and tertiary structure – Catalytic RNA.**

1-DNA Size and Fragility DNA in prokaryotic (circular) & eukaryotic(linear).

-DNA size varies from one species to another. It's not the same size in all species. On the other hand, in human, the DNA in each cell is the same size.
-What do I mean by size? It is the length of the DNA, how many base pairs or how many kilo bases.

Distance between base pairs = 0.34nm

If we have DNA with one thousand nucleotides or 1 thousand base pairs, if we multiply the 1 thousand with 0.34nm, we will get the length of the DNA in nanometers.

pBR322 plasmid (number of base pair 4,361 bp) = 1.48µm (the length of the DNA, 4361 bp×0.34nm= 1.48 µm)

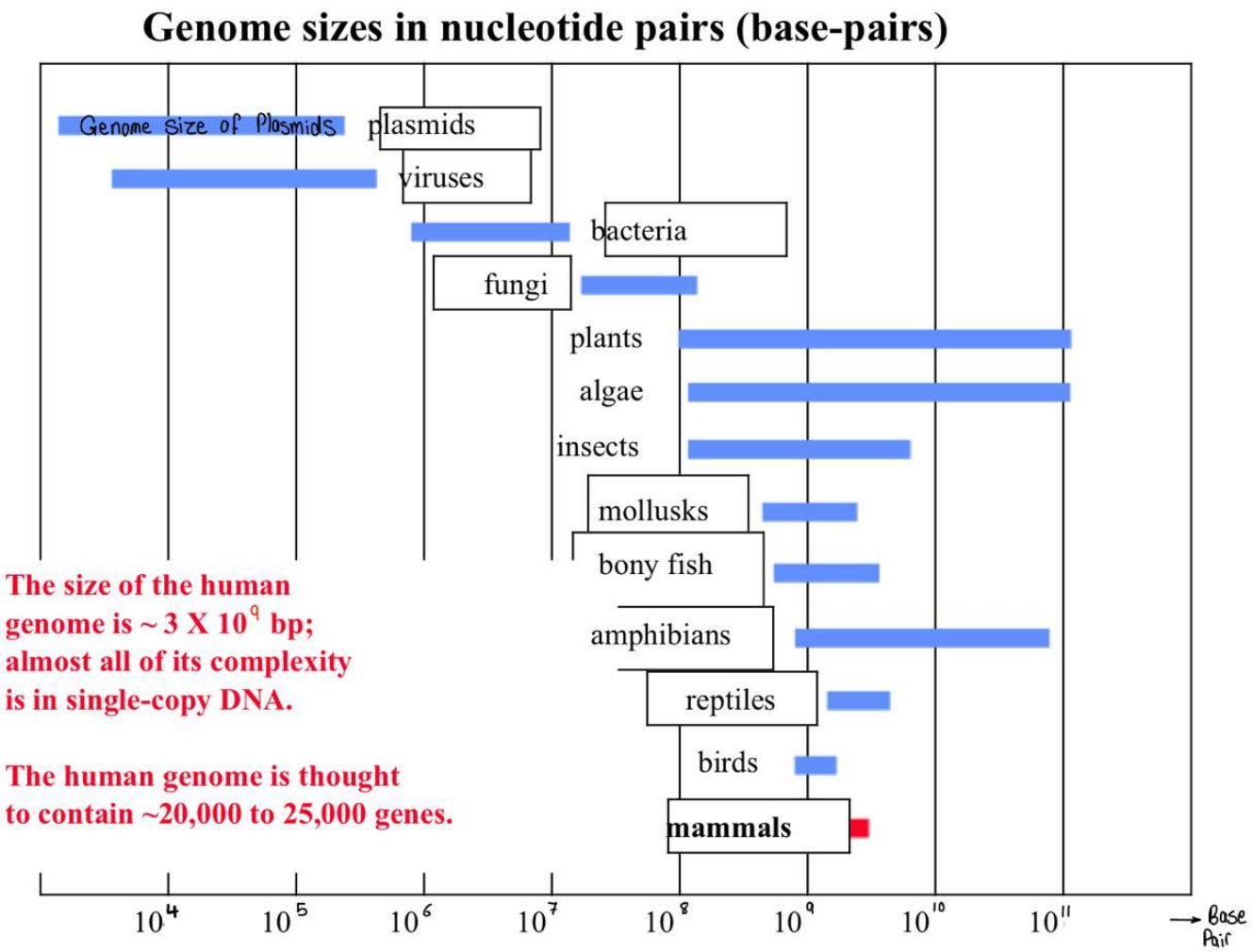
Human Chromosome 1 (245,522,847 bp) = 8.3cm!

We have 46 chromosomes so, we could multiply the total number of base pairs in our cells, which is about 6 billion (bp) multiplied by 0.34nm you could calculate the length in nm of our DNA, which is a very huge number.

Definitions from book:

- the long DNA molecule in each chromosome winds around protein complexes made of basic proteins called **histones**.

-DNA molecules are exposed to breakage by **hydrodynamic shear forces** (microscopic forces that arise when layers in a liquid move at different velocities).



-This is an example to show you how the sizes of genomes or DNA of organisms different from one species to another.

This scale represents the size of DNA.

plasma ranges from 10^4 to 10^5 bp (base pairs) little bit more, this is for the viruses, more for bacteria, and so on.

-As the organism gets more complicated, the base pair increases.

If you notice that the sizes of genomes or DNA in a plant is much higher than bacteria or viruses.

The size for humans or mammals ranges from 10^9 to 10^{10} bp.

The size of the human Genome project is about 3 billion base pairs.

The number of genes that are responsible to produce different proteins in our cells ranges from 20000 to 25000 genes, the percentage of gene in our genome is not more than 2%.

-DNA molecules vary in size. Length of a DNA molecule can be calculated.

Fragility of DNA molecule

DNA molecules could be prepared in the lab, you take a tissue, grind that tissue- to get rid of the membrane -then precipate the DNA with alcohol.

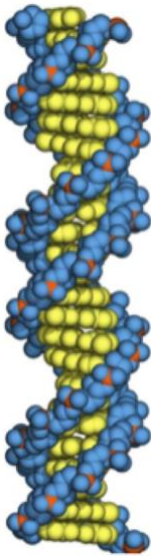
We will get pieces of DNA that precipitate out the solution.

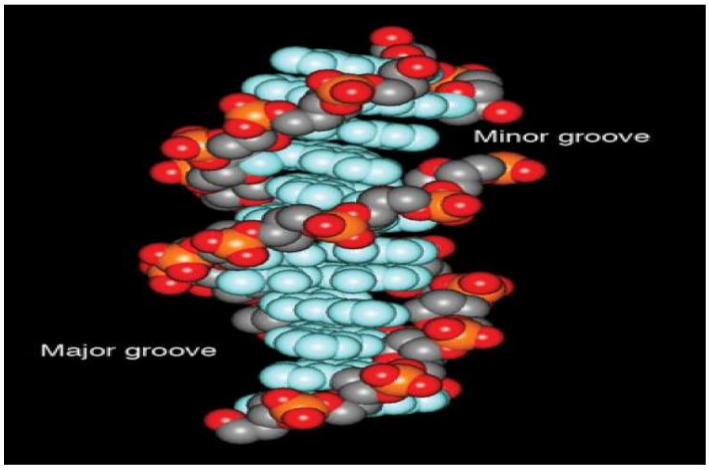
This DNA molecule is fragile when it's exposed to external environmental factors. It will be sheared & cut into pieces.

It's found that the greatest length of DNA that could be obtained from any DNA length from standard isolation procedures is approx. 40,000bp.
Easily shared by ordinary lab practices (pouring, pipetting, mixing), you must be very delicate when you are dealing with DNA samples. Some of the DNA samples are very important to get them to do genetic tests on them.
A lot of genetic diseases nowadays are diagnosed by testing specific genes & the DNA, by purifying the DNA & amplifying the gene that you want to test, so you have to deal & treat the DNA preparation very gently, avoid high temperature, avoid intensive pipetting, avoid strong mixing), all these factors will sheared & cut your DNA in pieces.

2- Major and Minor Grooves.

-Enzymes can recognize patterns in the major and minor groove.



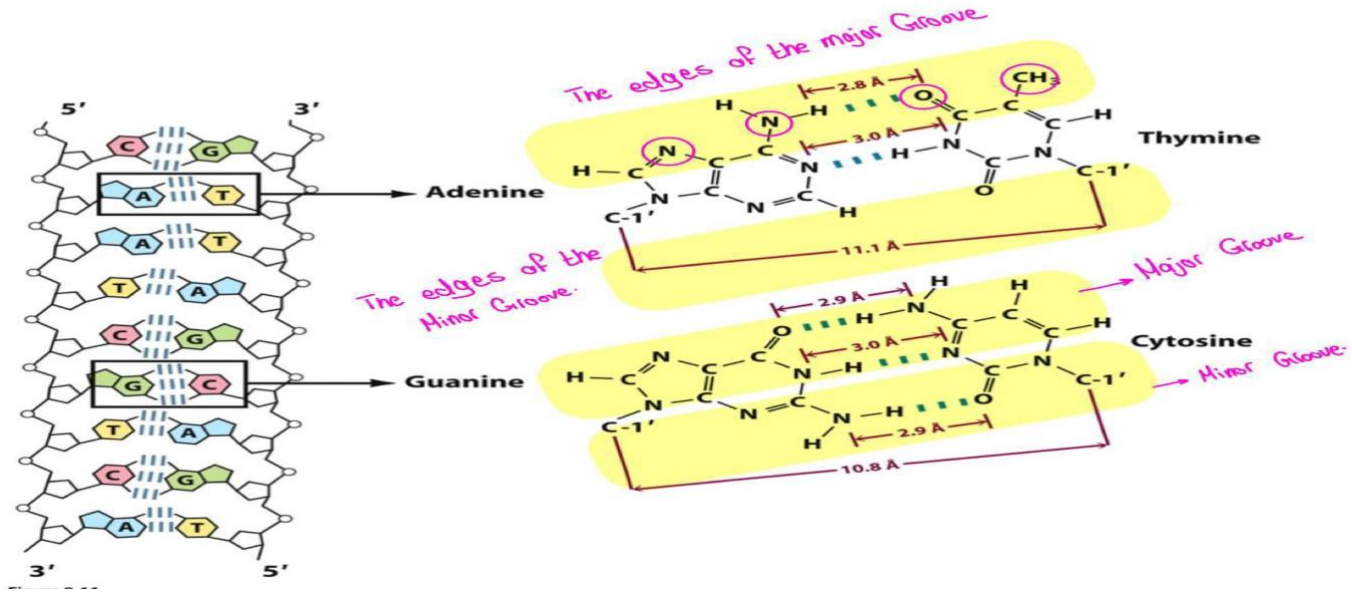


An important phenomenon in the double helical structure model in the DNA, in the image above the blue represents the backbone of the DNA & the yellow represents the base pairing inside the double helical structure.
The Backbone is composed of Phosphate group and sugar, they represent the hydrophilic side of the DNA molecule, while the yellow side represents base pair;Deoxynucleotides complement with each other, and base pairing with each other, with these antiparallel strands of DNA, they are mainly hydrophobic in nature, and they are away from the external hydrophilic region.
Now as you see this structure you see what is called invagination in the double helical structure, wide one & narrow one.
Gaps or invagination in the DNA structure are called major groove & minor groove.
These groove or gaps in the helical structure are very important; because when DNA replicates or transcribes, or any gene when it's expressed it requires many proteins to come and bind to that gene or DNA, the binding of protein that will help to express any gene.
That binding is very specific; they will bind at specific sides.
NOTE:
- These grooves arise because the sugar rings lie closer to one side of the base pair than to the other.

How will those proteins bind specifically? They will come and bind randomly in the major groove & minor groove, while they are in - major & minor- grooves they will start reading the sequence of base pair in those regions.
If those sequences are compatible with their specificity they will bind them, if they are not, they will leave.
Major and minor groove they are the sites in which protein will come, bind, start reading the sequences of deoxynucleotides, they will decide to bind to specific sequence that recognize or not, and start the activity of the gene in terms of replication, transcription or expression.

-proteins MOSTLY bind to major groove.
One student asked the doctor: **Do the proteins that bind to the grooves have a name?**
Doctor answered: **Yes, there are thousand types of proteins and it's impossible to memorize them all, but if you talk about transcription most of the proteins are called transcription factors, that will come and bind to grooves and read the sequence in order to determine or identify the specific sequence of the deoxynucleotides to decide to bind or not.**

-Why does protein bind little to the minor groove compared to the major?
Because the major groove is wider, more proteins will bind on it, the proteins could read and see a lot of bases paired compared to the minor. Because of that, the proteins that are bound to major groove have more information.
How does the protein read the sequence?
The protein will see the atoms at the edges of the major and minor groove, and could identify those edges from where they come, they could identify the nitrogen& oxygen if they are from which deoxynucleotides (A,T,G,C). According to what they see, they will help them to differentiate between these atoms.
- Recognition Patterns in the Major and Minor Grooves; Base sequence recognition by enzymes depends primarily on a unique pattern that G-C or A-T base pairs project it the edge of the major groove.



Explain the image above:

These are the edges of the major group (adenine- thymine) protein when they stand on adenine- thymine in the major groove they could see (N,N,O,CH_3), so they could tell according to what the proteins see at the edges that this base pairing is adenine-thymine, they don't look at the minor groove, because adenine- thymine looks the same as thymine-adenine. [In the major groove A-T isn't asT-A, BUT in minor groove A-T is same as T-A]
While the major groove adenine-thymine is different from thymine- adenine.
There is no difference in the association anywhere in the minor groove, they all give the same result, and this association does not give enough information about the protein, unlike the major groove.

-Guanine -Cytosine we can see (N, O, N)
Nitrogen from guanine \ oxygen acceptor to hydrogen bonding from guanine\ Nitrogen from cytosine.
BUT there is NO difference in the minor groove if we read from guanine-cytosine OR cytosine -guanine, this why we USE the major groove more than the minor groove.
When protein reads it will only read the sequence JUST from the major and know what the deoxynucleotides & if that sequence is comparable to specific recognition sequences of that protein, it will bind to it and will activate that gene.
Student asked:what is the difference between major & minor in protein binding?
Minor groove will NOT give the protein any important information, because it can't identify if it's guanine or cytosine (heterocyclic), BUT if it binds with the major groove,it will differentiate guanine from cytosine.
In the book it's explained by color instead of atoms if you don't understand.

3- DNA Denaturation and Renaturation.

Denaturation: means separation of two strands from each other.
Renaturation: means reannealing or retailing or re base pairing between the two strands.
Renaturation or Reannealing (book's definition(modified)):is the process by which solution that has denatured DNA is treated.
Why Denaturation and Renaturation are important?
BECAUSE, in **DNA replication** the 2 strands must be separated from each other.Also, **TRANSCRIPTION** one of the strands will be used as template, the 2 strands must be separate from each other.
What **causes** Denaturation and Renaturation?
Inside the cell there are **enzymes responsible for denaturation**, these enzymes are called **helicases,** and there are proteins which are important **to prevent or reassociate of these strands**, in order for the protein to do their function in **replicating DNA or transcribing DNA to RNA.**

Denaturation and Renaturation could be studied in the lab by **spectrophotometry**.

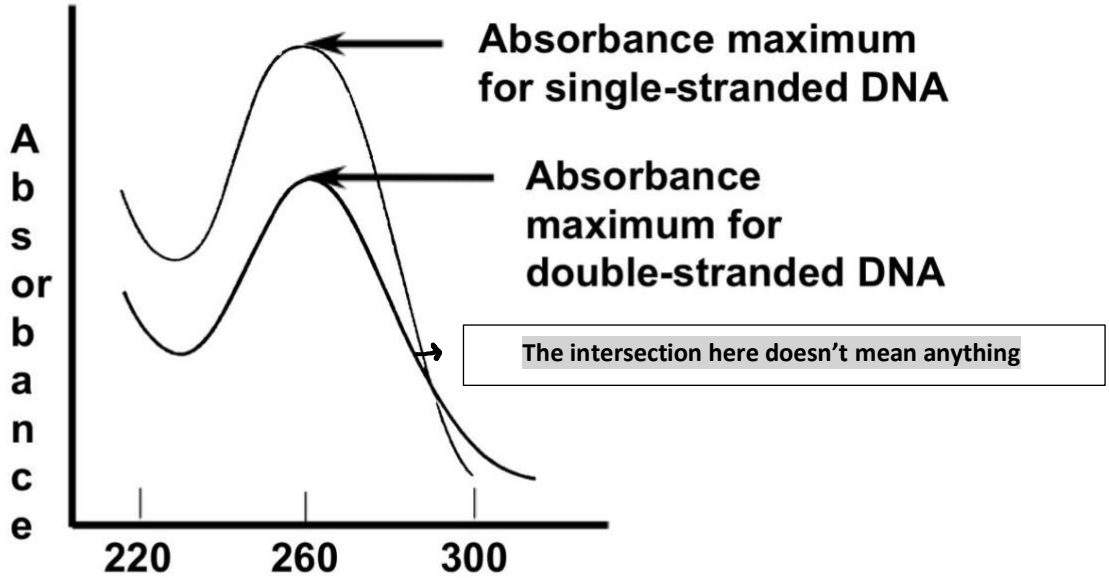
What is spectrophotometry?

Use an instrument called spectrophotometer; it's an instrument that measures the amount of light which are absorbed by any solution at a specific concentration.

It's an instrument that measures the **optical density** or **the light which are absorbed** by a specific solution at any concentration.

It was found that DNA solutions will absorb light at maximally at **wavelength 260 nm**, it's true if **the DNA is completely renatured or double stranded**, if that DNA is Denatured and you measure the absorption of these solution, **it will absorb more when it's single stranded than when it's double stranded.**

NOTE: the mass-to-length ratio of DNA before heating is twice that of DNA after heating.



The absorbance at 260 nm of a DNA solution increases when the double helix is melted into single strands.

Why does the single strand absorb more than double?

The structure that absorbs the light in the single stranded or double stranded, **deoxynucleotides (adenine- thymine- Guanine -Cytosine).**

In the double stranded they are less exposed to light when it is passed through the DNA solution.

While when they are single stranded, they are more exposed, **the surface area of exposure is more than the surface area in the double strand** (the helical structure is hidden).

These phenomena of absorbing light in single strand of DNA more than the double stranded is **called Hyperchromicity**.

Hyperchromicity: higher light absorption by single stranded than double stranded DNA.

These phenomena will help us to understand and to know about denaturation and renaturation of DNA molecules.

Note>> every substance in nature has constant physical properties.

DNA (single or double) absorbs at maximally 260nm, it may absorb a little beyond or less than that.
Proteins have fixed or constant physical properties of light absorption at 280nm, every protein, material or molecule in solution has its distinct value of light absorption according to its structure.
Note>> light absorption does not to do anything with H bonding, it depends on how much deoxynucleotides exposed to light passage.
General rule ((Any single strand of DNA will absorb more light than double stranded in the same molecule)), we can't compare the single strand with double strand with different size, (WE COMPARE IF THEY HAVE SAME SIZE).

Can long strands of DNA unwind? DNA solutions drop in viscosity when heated this observation was interpreted to mean the Double helical structure collapses into single strands – (Denaturation)

Viscosity : an intermediate state between liquid and solid form

So why the drop in viscosity?

When DNA is heated there is an increase in the KE (kinetic energy)of atoms leading to the increase of space between atoms increasing the fluidity and lowering viscosity and finally leading to denaturation

unwinding process in vitro was to expose DNA to Physical and chemical agents that would disrupt the weak **non-covalent** bonds.

Denaturation can be recognized by U.V. 260 absorbance.

- for a 50µg/ml solution

– Double stranded DNA A260=1.00
– Single stranded DNA A260=1.37
– Free Nucleotides A260=1.60

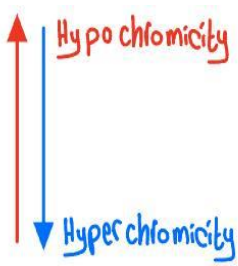
A 260: The absorbance of λ =260 nm

You could calculate the concentration of any DNA solution, it was found that optical density of one at 260 -wavelength- of double stranded DNA the concentration of DNA 50µg/ml, and every DNA solution that had a concentration 50µg/ml, if it double stranded it will absorb 1, if it in a single stranded it will absorb 1.37 & if it is free nucleotides, it will absorb 1.6.

The free nucleotides absorb more light.

So, there is a relation between concentration and light absorp

The higher the concentration of the solution, the greater its al



A 260: The absorbance of λ =260 nm

*Because Purine and Pyrimidine bases absorb 260 nm U.V Light

Hyperchromic effect: the increase in A 260 when double strand

Hypochromic effect the decrease in A 260 when DNA is renatured.

To sum up:

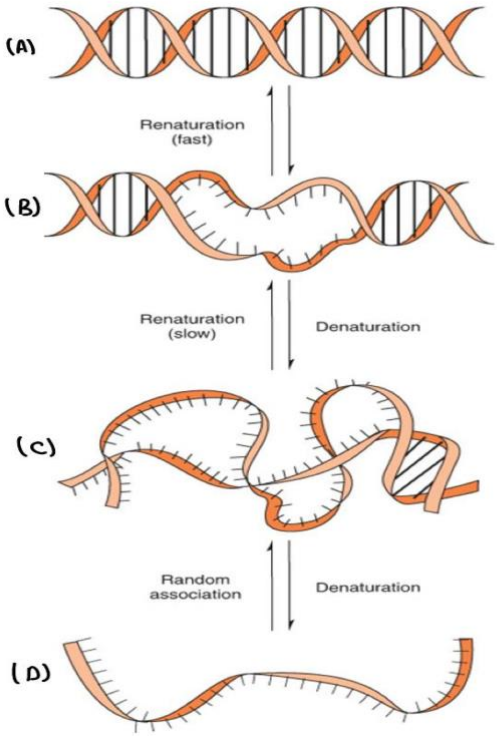
Hyperchromicity->single strand->denaturation->increase A260

Hypochromicity->double strand->renaturation->decrease A260

Question:

arrange (a), (b), (c), (d) DNA molecules
From the least to the highest light absorption?

- answer: (d)> (C)> (b)> (a)



-Double strands are more ordered.

Factors that affect the U.V light absorbance:
1.the more ordered the structure, the less light that is absorbed.

2.The higher the concentration of the solution, the greater its absorption.

In vivo(living cell), there are enzymes that cause denaturation. However, the renaturation (reannealing)will take place naturally (occurs spontaneously), after a process of replication or transcription is ended.

The Hyperchromic (light absorption) will help us to follow the denaturation of DNA or renaturation(reannealing), this **is the basic principle of PCR mechanism.**
In vivo: research done on a living cell
In vitro: in laboratory or test tube.

PCR (Polymerase Chain Reaction) is a method used to make copies of a DNA sample, is used to amplify any piece of DNA or gene to be tested for diagnosis of genetic diseases and infection.

*Note: PCR technique will be discussed in coming lectures.

Follow the denaturation by light absorption.

(a) Suppose there is a Double helix DNA in a specific Temperature, then you raise the Temperature or add chemicals that break hydrogen bonds between the two strands.
Denaturation: breaking hydrogen bonds and separating the two strands from each other to get single stranded DNA molecule

(b) As you can see, there are some regions that will have hydrogen bonds broken, while others are still impacted.

(C) raising the Temperature or adding chemicals, will increase hydrogen bonds breaking, and more single stranded regions will appear.

(d) At the beginning, denaturation occurs slowly, the hydrogen bonds breaking would finally faster, till occur single stranded DNA which will have the whole molecule converted to two between the (a, b, c, absorption light higher)

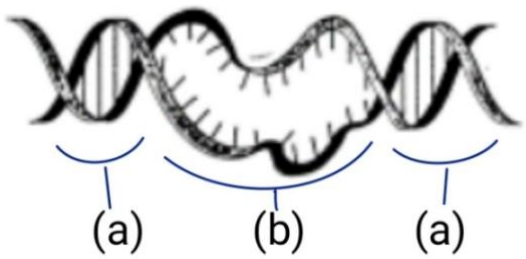
Denaturation agents:

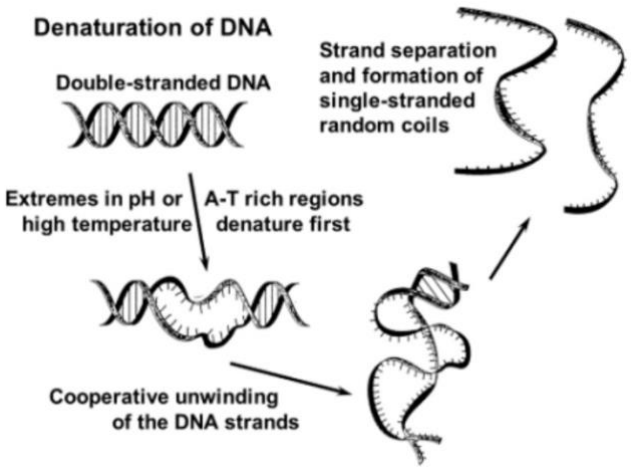
1. Heat
2. alkali solutions, solutions with high PH e.g.: NaOH alkali solutions are slightly different from bases
3. Formamide (HCONH2)
4. Urea (NH2CONH2)
5. Size of the DNA, & the deoxynucleotides the DNA contain.

Denaturation: breaking the hydrogen bond and separating the two strands from each other, the heat and alkali solution will not break the phosphor diester bonds nor N - glycosidic bonds only hydrogen bond

Renaturation: when removing the denaturation factors and decreasing the temperature, the single strand starts looking for its complementary strand, and once the nucleated region of base pairing appears the rest regions would fast renature back to double stranded DNA molecule.

-LESS SIZE, FASTER RENATURATION.





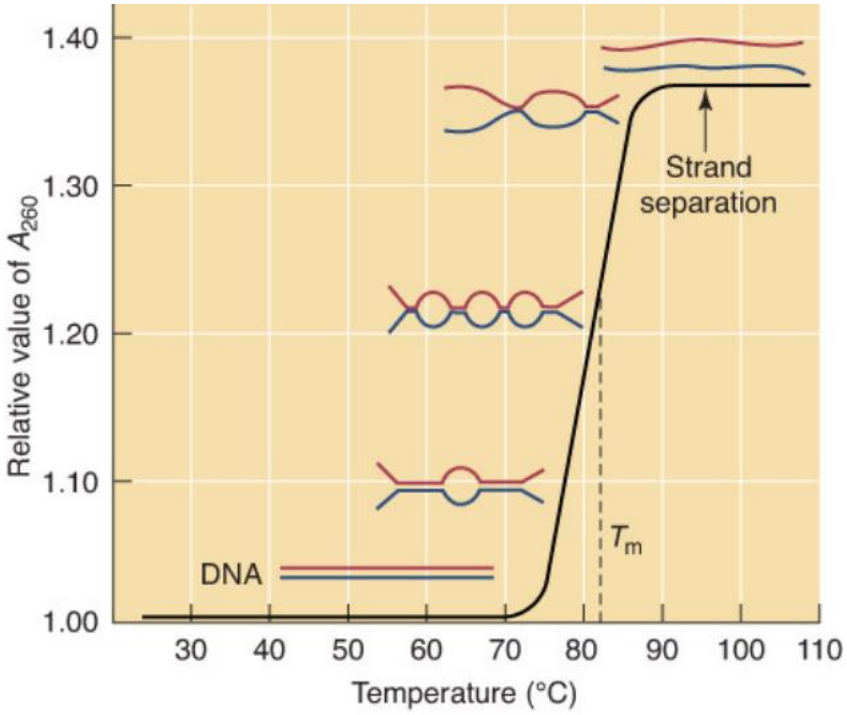
(B)rich in A=T and poor in C≡G (more renaturation,less hydrogen bonds)

(A)region is rich in C≡G and poor in A=T (less renaturation, more hydrogen bonds)

The denaturation starts at (b) before (a); because it requires more energy to disrupt the three hydrogen bonds in a C≡G base pair than to disrupt the two hydrogen bonds in an A=T base pair.

Slowly heated DNA solution in 0.15 M NaCl

Figure 03.04:



DNA melting curve. A melting curve of DNA showing Tm (the melting temperature) and possible molecular conformations for various degrees of melting.

Y axis represents the absorption of 260 nm light wave
X axis represents temperature (Temp).

- DNA molecule is double stranded (30-70) °C

• Increasing the Temp 5° for example above 70°c, increases the absorbance.

• Once the heat breaks the hydrogen bonds the DNA starts to denatured partially.

• The continuous increasing of Temp will form more bubble regions, which are (single stranded/ denatured) regions, due to hydrogen bonds breaking.

• At 88° C all of the DNA has denatured except some regions, which are rich in C≡G that require more heat to disrupt teir bonds and convert this molecule completely into single stranded.

• When the molecule is completely denatured the increasing of Temp doesn't increase the absorbance (A260 is constant now)

• The maximum A260 is about 37% higher than the starting value

Recap:

✓ single stranded DNA absorbs more light than double stranded DNA.

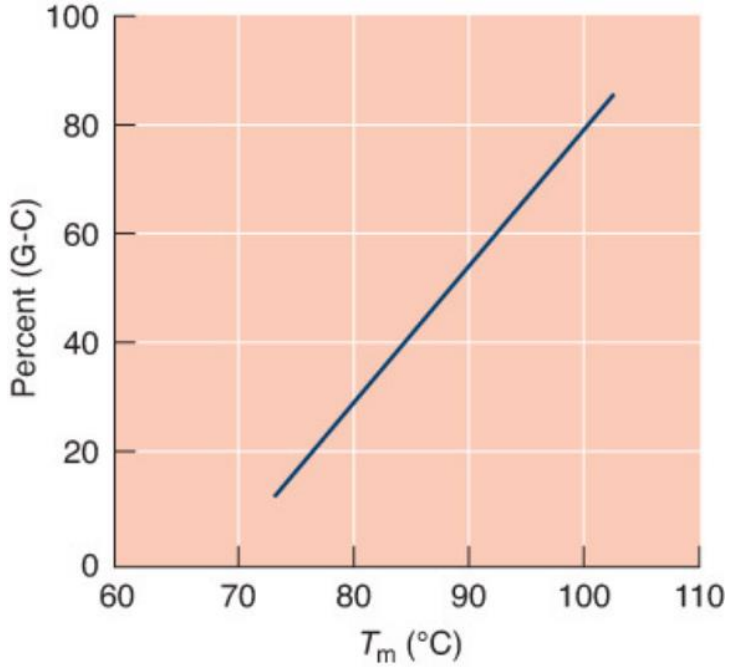
✓ Heat breaks hydrogen bonds.

✓ C≡G, A=T.

In vivo, the DNA molecule doesn't reach the complete denaturation, it denaturates partially then renaturates.

Melting temperature (Tm):

A convenient parameter to characterize a melting transition is the <u>temperature</u>



<u>at which the rise in A260 is half-complete.</u> When the DNA molecule is half denatured.

Figure 03.05:

Effect of G-C content on DNA melting temperature.

* Tm increases will be increasing percentage of G-C.

* Hydrogen bonds stabilize double stranded DNA by lowering denaturation.

– Tm increases with increasing G-C content

Forces that affecting the stability of the DNA double helix

• hydrophobic interactions - stabilize

_ hydrophobic inside and hydrophilic outside,

_are formed by the nitrogen bases; because nitrogen bases are hydrophobic while the backbone is hydrophilic.

-They are occurred far away from backbone.

• stacking interactions - stabilize

_ This force is produced from the position of nitrogen bases closely over each other.

_Distrupted by thermal energy.

_relatively weak but additive van der Waals forces

– Base stacking is maintained by van der Waals forces

– Base stacking helps stabilize hydrogen bonds between base pairs

• hydrogen bonding – stabilize

_hydrogen bonds are formed between the complementary nitrogen bases.

_They are rapidly reformed and disrupted by heat.

_ relatively weak but additive and facilitates stacking; the single hydrogen bonds are weak, however the total force interaction of millions hydrogen bonds is strong enough to facitilate the stacking force and stabilize the DNA structure.

• electrostatic interactions - **destabilize**

- contributed primarily by the negative charge of phosphates
- affect intrastrand and interstrand interactions
- repulsion can be neutralized with positive charges

(e.g., positively charged(Na+,K+,Mg+2 ions or proteins such as histone (which has positive charge at physiological pH, and it will bind chromosomes to each other, and it will neutralize the negative charge)

Ionic strength influences DNA structure

_ In the absence of salt (NaCl) DNA strands repel (تتنافر)each other through the negatively charged phosphates

_ As salt is added Na+ shields the phosphates from each other , this shielding occurs near the physiological salt concentration of about 0.2 M.

_ Tm rises with NaCl concentration and the stability of DNA structure.

_ In presence of a denaturing agent such as urea or formamide, which can form hydrogen bond with DNA bases. Hydrogen bonds between bp have very low energies and so are easily broken. Desaturating agents shift the equilibrium by forming hydrogen bonds with an unpaired base on one strand and thereby **prevent** the base form re-forming hydrogen bonds with the complementary unpaired base.

_Denaturing agents lower Tm by disrupting hydrogen bonds between base pairs
_ Supports the role of hydrogen bonds in double stranded DNA structure.

Alkali denatures DNA strands without breaking phosphodiester bonds
• High temperature can break phosphodiester backbone
– Degradation can be avoided by using a base (NaOH) for denaturing DNA
– Acid causes depurination.

_ Depurination: cleavage of purine groups from polynucleotide chain by using acids.

-Van der waals forces help indirectly to form double helical structure.

The DNA molecule is in a dynamic state

2 observations help us to reach this fact:

1. When Formaldehyde can react with the NH2 groups of the bases and thus eliminate their ability to hydrogen bond. When formaldehyde is added to double-stranded DNA, the DNA slowly and irreversibly denatures.

2.when double-stranded DNA is dissolved in tritiated water ([3H]H2O). There is a rapid exchange between the hydrogen-bonded protons of the bases and the tritium ions in the water.

_ Evidence that DNA bases continually un-pair and pair called (DNA breathing)

– Transient melting occurs more often in A-T rich regions

• A-T base pairs with 2 hydrogen bonds
• G-C base pairs with 3 hydrogen bonds

-DNA structure has pairing regions and non-pairing regions.

……

DNA renaturation or re-anealing is a critical tool in molecular genetics

_ DNA renaturation or reannealing: the process in which asolution of denatured DNA can be treated in such a way that native DNA re-forms.

Two requirements must be met:

– Salt concentration high enough to eliminate electrostatic repulsion.

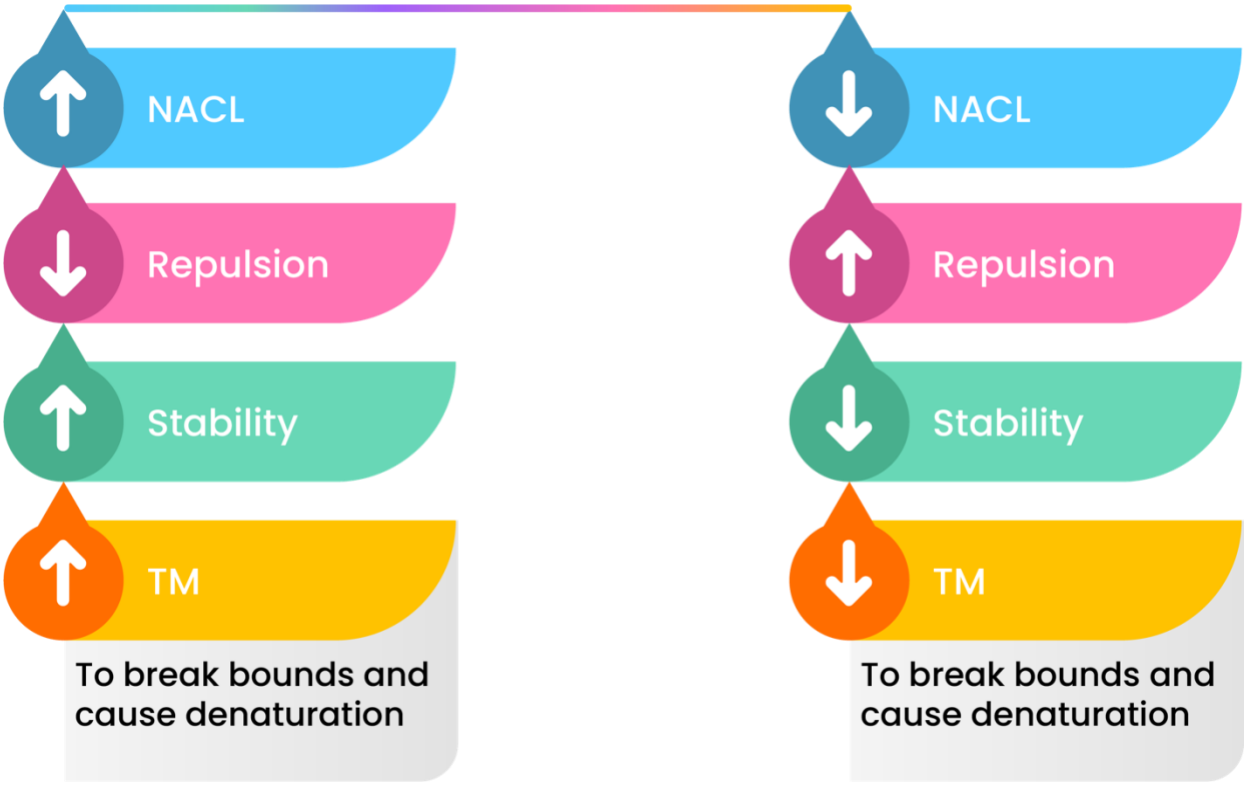
• Usually 0.15 to 0.5 M

– Temperature high enough to disrupt random hydrogen bonds (false base pairing) but low enough to allow for stable inter-strand base pairing (correct pairing).

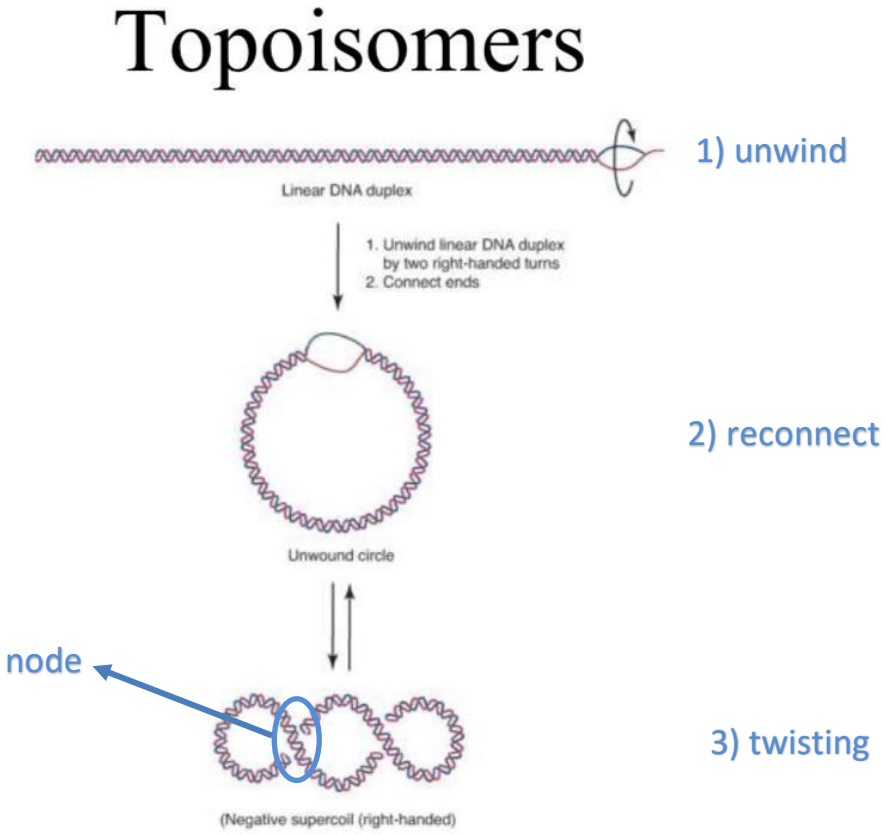
• 20˚-25˚ C below the Tm

-It might be missing base pairing in renaturation

_Renaturation is a slow process compared with denaturation. the precise collision between complementary strands such that base pairs are formed at the correct positions. Because renaturation is a result only of random motion, it is a concentration-dependent process.



4- Topoisomers.



• Topoisomers: same sequence but different forms of DNA.

when we talk about isomers like isomers of glucose which means different structure of the same molecule, different stereoisomers of the same molecule depend on asymmetric of carbon atoms, the same thing here. DNA has different forms e.g. in mammals is double strand liner DNA and in bacteria is circular double strand DNA, each type could have different forms of different topoisomers, when you talk about bacteria or plasmid DNA double helical structure (if you talk on B form) there are about 10 pieces per turn, if you unwounded this DNA molecule, and then re-connect the two ends you still have this molecule with same number of nucleotides but in different form (topoisomer) this is one form of isomers of DNA. Now, if you twist the molecule it will go into another type of isomers called supercoiled. (see the picture above)

Supercoiling has two types, it could be positive or negative supercoiled, depending on the direction of the coiling. This happen during DNA replication and transcription, for example in DNA replication two strands will be open from each other and more nodes will form and the structure will take supercoiled form.

Definitions:

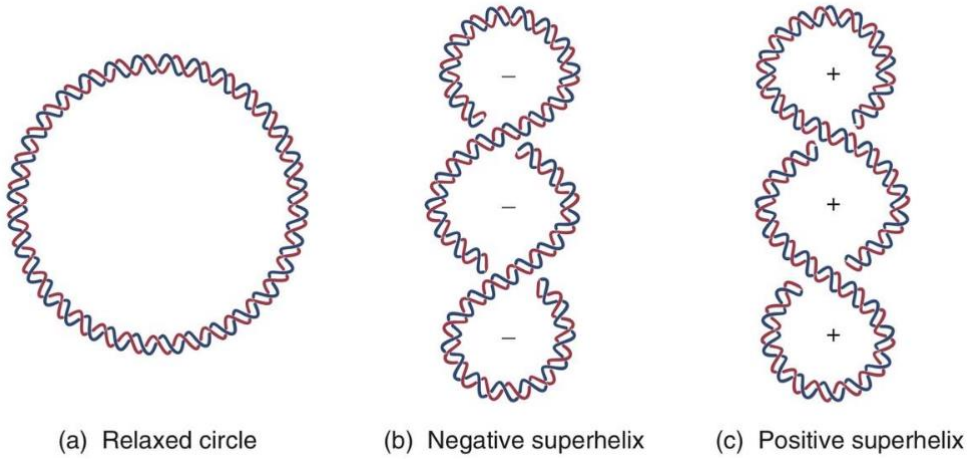
nodes: crossover points (overlapping between the two double strands)

writhe: circular DNA molecule with nodes

what's the difference between overwound and underwound?

Overwound: reduce distances between turns → not relaxed

Underwound: twisting the molecule in opposite direction → relaxed



- Underwinding can also introduced into preexisting circle by an enzyme called **DNA gyrase**.
- A-T rich regions (sequences) are more likely to be unpaired (because they have two hydrogen bonds), so they are playing important roles in processes such as DNA replication and transcription.

TEST YOUR KNOWLEDGE

1)What is the concentration of DNA that we use if we have free nucleotides that absorbed 32 unit of 260nm U.V:

A)10 microgram/ml

B)1microgram/ml

C)1gram/L

D)100nm/ml

E)0.1milligram/ml

2)The importance of grooves of DNA is included by its importance in:

A) DNA replication

B) DNA transcription

C)Denaturation

D)A+B

E) All of the above

3)One application of denaturation and renaturation is:

A) Genetic testing

B) PCR

C)Find treatment for COVID 19

D)A+B

E) All of the above

4)What is not wrong about proteins that bind to DNA:

A) They distinguish different atoms

B) They distinguish the same atoms

C)They mostly bind to major groove.

D)They always bind to minor groove

E)A+C

5)Proteins bind mostly with DNA by:

A)major groove

B)minor groove

C)phosphate group

D) nitrogenous bases

E)A+C

6)Which of these causes' denaturation:

A)High temperature

B)Low PH C)Helicase

D)A+C E)A+B+C

7)If we have a sample of DNA with a length of 68nm ,how many nucleotides we should find in it:

A)200

B)20

C)400

D)40

E)100

8)In the T-A rich region in a DNA , what is of the following is true:

A)We need higher PH to denature it in comparison with G-C rich regions

B)We need lower PH to denature it in comparison with G-C rich regions

C)We need higher PH to renature it in comparison with G-C rich regions D)We need lower heat to denature it in comparison with G-C rich regions

E)B+D

9)What determine the speed of renaturation:

A)Size of DNA

B)A+D

C)Acid concentration

D)DNA Concentration

E)None of the above

10)which of these has more complex DNA:

A)Bacteria

B)Archea

C)Viruse

D)Human

E)Parasites

Answers: 1) C

2) E

3) E

4) E

5) E

6) D

7) C

8) E

9) B

10) D

-MOLECULAR BIOLOGY TEAM:

1- ABDELRAHMAN HASASNEH.

2-ABDELRAHMAN WA'EL.

3- IBRAHEM ALSHAWABKAH.

4-HEBA MEGDADI.

5-FATIMA KAAKO.

6-SHAHD MOHAMMED .

7-LAILA ORAIKAT.

8-MERA ABU HUSSEIN.

9-MOMEN ALKATEEB.

10-OMAR MOHAMMED.

11-MUSAB HUSSEIN.

12-MOHAMMED ZURIQAT.

13-MOHAMMED NOFAL.

14-YAHIA JAROSHA.

15-SARA OMAR.

16-AHMAD MILHEM.

17- ABDULLAH KHWALDEH.

18-MALAK ALBUSTANJI

V2: ***we modified many mistakes that were found in V1. Because of that, the highlighted sentences indicate the modified sentences.***