CBCS 3rd Sem(M)

Unit 4: Nucleic Acid

STRUCTURE: PURINES AND PYRIMIDINES, NUCLEOSIDES, NUCLEIC ACIDS, COT CURVES: BASE PAIRING, DENATURATION AND RENATURATION OF DNA; TYPES OF DNA AND RNA, COMPLEMENTARITY OF DNA, HYPO-HYPERCHROMATICITY OF DNA.

By-
Dr. Luna Phukan

Structure of Nucleic Acids

DNA and RNA have great chemical similarities. In their primary structures both are linear polymers (multiple chemical units) composed of monomers (single chemical units), called nucleotides. Cellular RNAs range in length from less than one hundred to many thousands of nucleotides. Cellular DNA molecules can be as long as several hundred million nucleotides. These large DNA units in association with proteins can be stained with dyes and visualized in the light microscope as chromosomes.

Polymerization of Nucleotides Forms Nucleic Acids

and RNA each consists of only four different nucleotides. All nucleotides have a common structure: a phosphate group linked by a phosphoester bond to a pentose (a five-carbon sugar molecule) that in turn is linked to an organic base . In RNA, the pentose is ribose; in DNA, it is deoxyribose . The only other difference in the nucleotides of DNA and RNA is that one of the four organic bases differs between the two polymers. The bases adenine, guanine, and cytosine are found in both DNA and RNA; thymine is found only in DNA, and uracil is found only in RNA. The bases are often abbreviated A, G, C, T, and U, respectively. For convenience the single letters are also used when long sequences of nucleotides are written out.

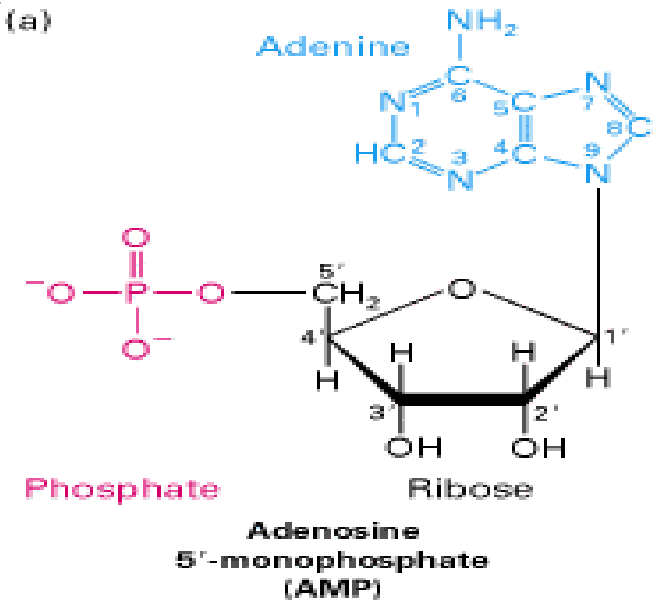


Fig : All Nucleotides have a common structure. (a) Chemical structure of adenosine 5′-monophosphate (AMP), a nucleotide that is present in RNA. All nucleotides are composed of a phosphate moiety, containing up to three phosphate groups, linked to the

The base components of nucleic acids are heterocyclic compounds with the rings containing nitrogen and carbon. Adenine and guanine are purines, which contain a pair of fused rings; cytosine, thymine, and uracil are pyrimidines, which contain a single ring . The acidic character of nucleotides is due to the presence of phosphate, which dissociates at the pH found inside cells, freeing hydrogen ions and leaving the phosphate negatively charged . Because these charges attract proteins, most nucleic acids in cells are associated with proteins. In nucleotides, the 1′ carbon atom of the sugar (ribose or deoxyribose) is attached to the nitrogen at position 9 of a purine (N9) or at position 1 of a pyrimidine (N1).

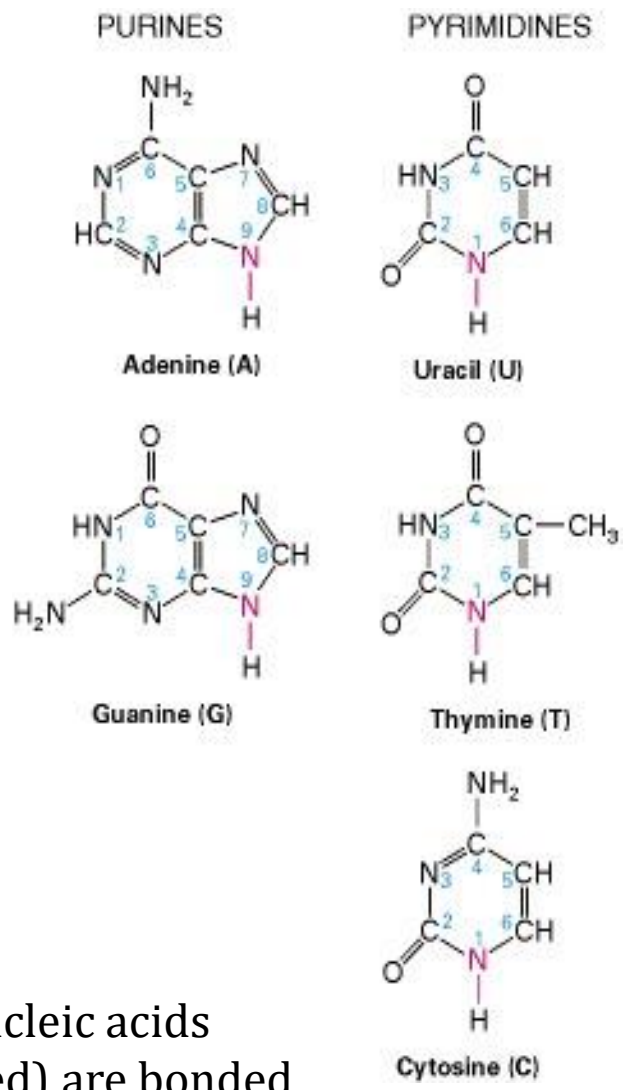
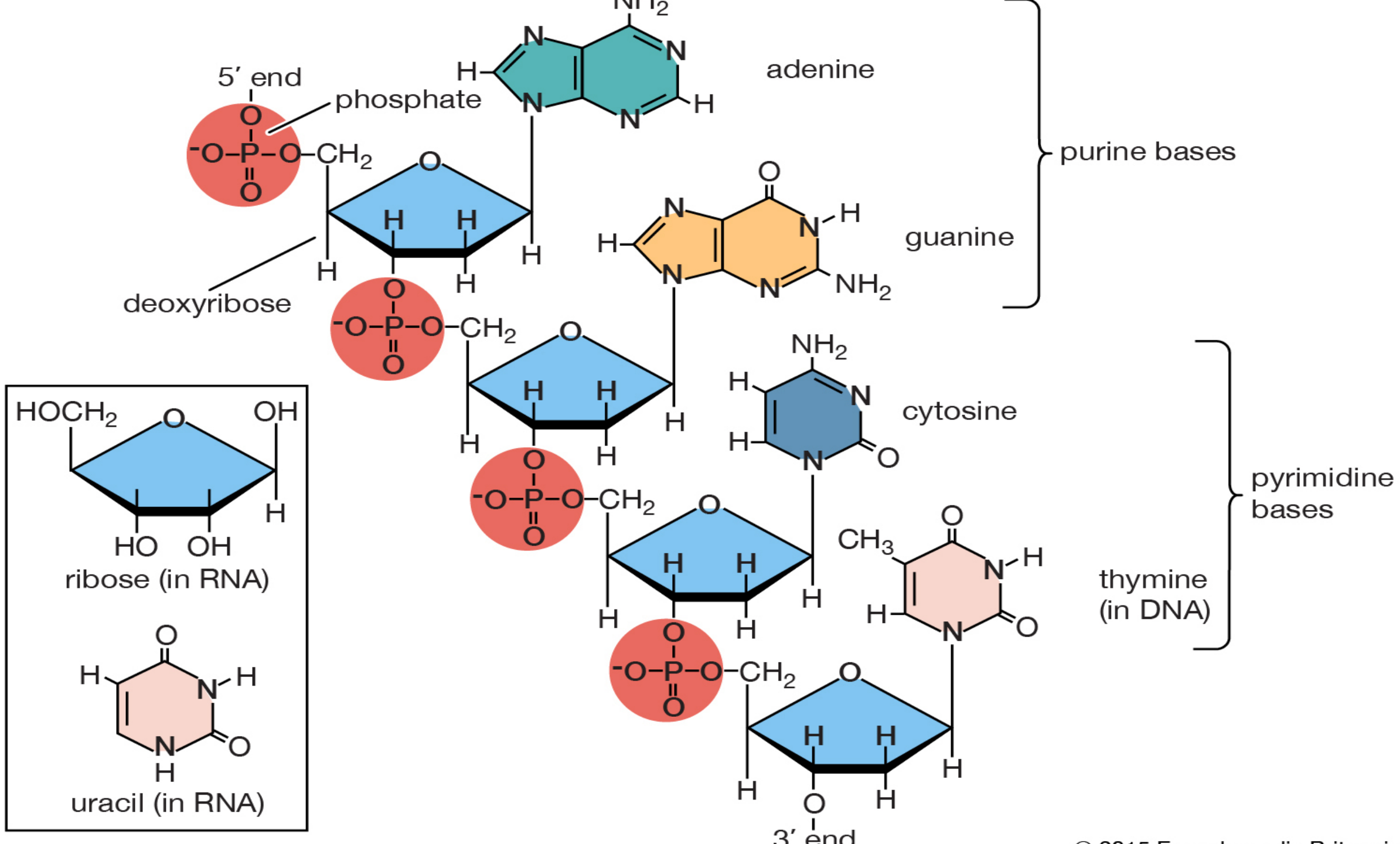
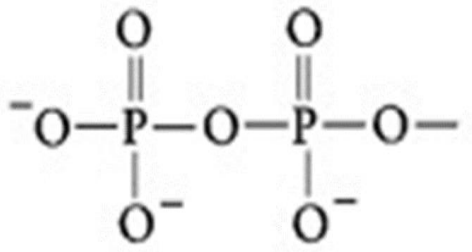


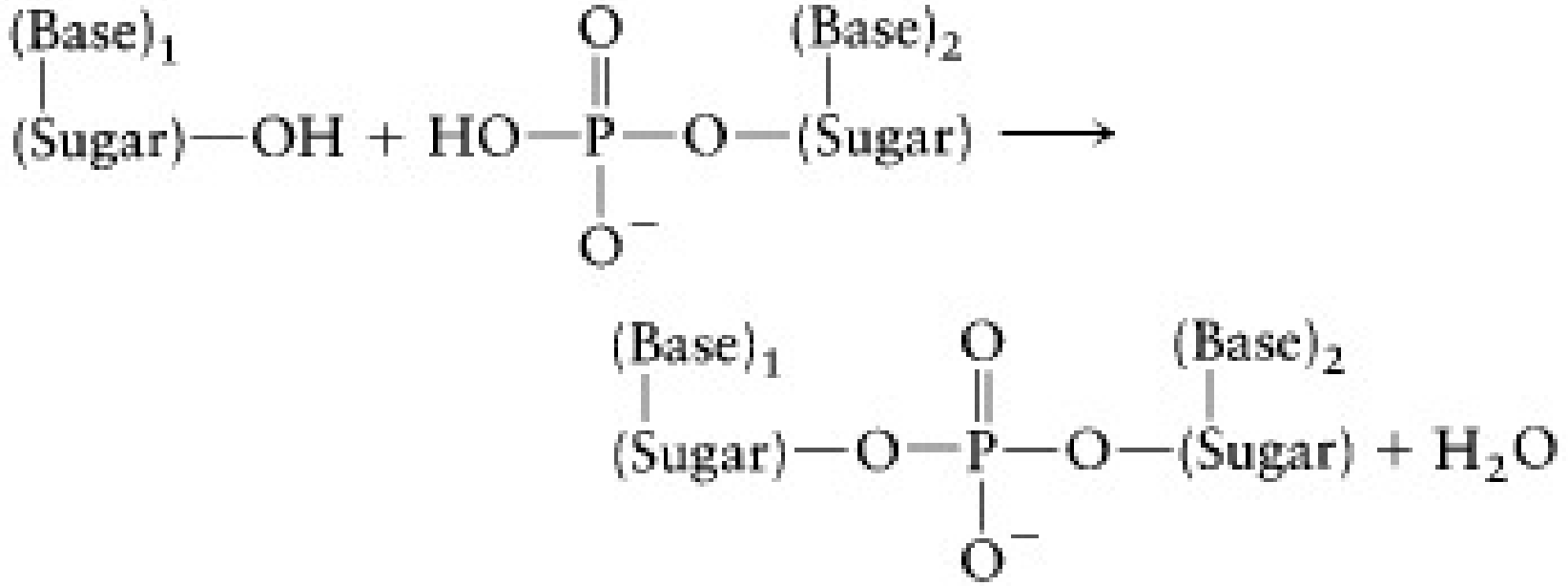
Fig:The chemical structures of the principal bases in nucleic acids. In nucleic acids and nucleotides, nitrogen 9 of purines and nitrogen 1 of pyrimidines (red) are bonded to the 1′ carbon of ribose or deoxyribose.


5′ end
phosphate
deoxyribose
adenine
guanine
cytosine
thymine
(in DNA)
purine bases
pyrimidine
bases
3′ end
ribose (in RNA)
uracil (in RNA)

Cells and extracellular fluids in organisms contain small concentrations of nucleosides, combinations of a base and a sugar without a phosphate. Nucleotides are nucleosides that have one, two, or three phosphate groups esterified at the 5′ hydroxyl. Nucleoside monophosphates have a single esterified phosphate (see Figure), diphosphates contain a prophosphate group

When nucleotides polymerize to form nucleic acids, the hydroxyl group attached to the 3′ carbon of a sugar of one nucleotide forms an ester bond to the phosphate of another nucleotide, eliminating a molecule of water:

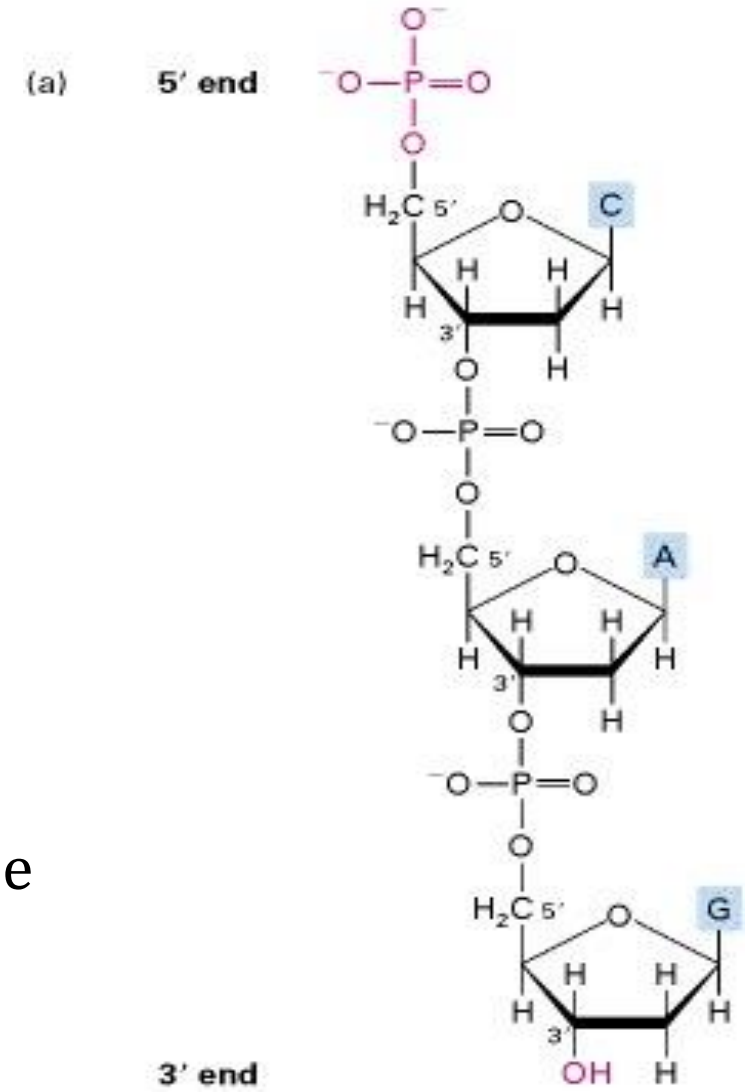




This condensation reaction is similar to that in which a peptide bond is formed between two amino acids (Chapter 3). Thus a single nucleic acid strand is a phosphate-pentose polymer (a polyester) with purine and pyrimidine bases as side groups. The links between the nucleotides are called phosphodiester bonds.

Like a polypeptide, a nucleic acid strand has an end-to-end chemical orientation: the 5′ end has a free hydroxyl or phosphate group on the 5′ carbon of its terminal sugar; the 3′ end has a free hydroxyl group on the 3′ carbon of its terminal sugar (Figure). This directionality, plus the fact that synthesis proceeds 5′ to 3′, has given rise to the convention that polynucleotide sequences are written and read in the 5′ → 3′ direction (from left to right); for example, the sequence AUG is assumed to be (5′)AUG(3′). (Although, strictly speaking, the letters A, G, C, T, and U stand for bases, they are also often used in diagrams to represent the whole nucleotides containing these bases.) The 5′ → 3′ directionality of a nucleic acid strand is an extremely important property of the molecule.

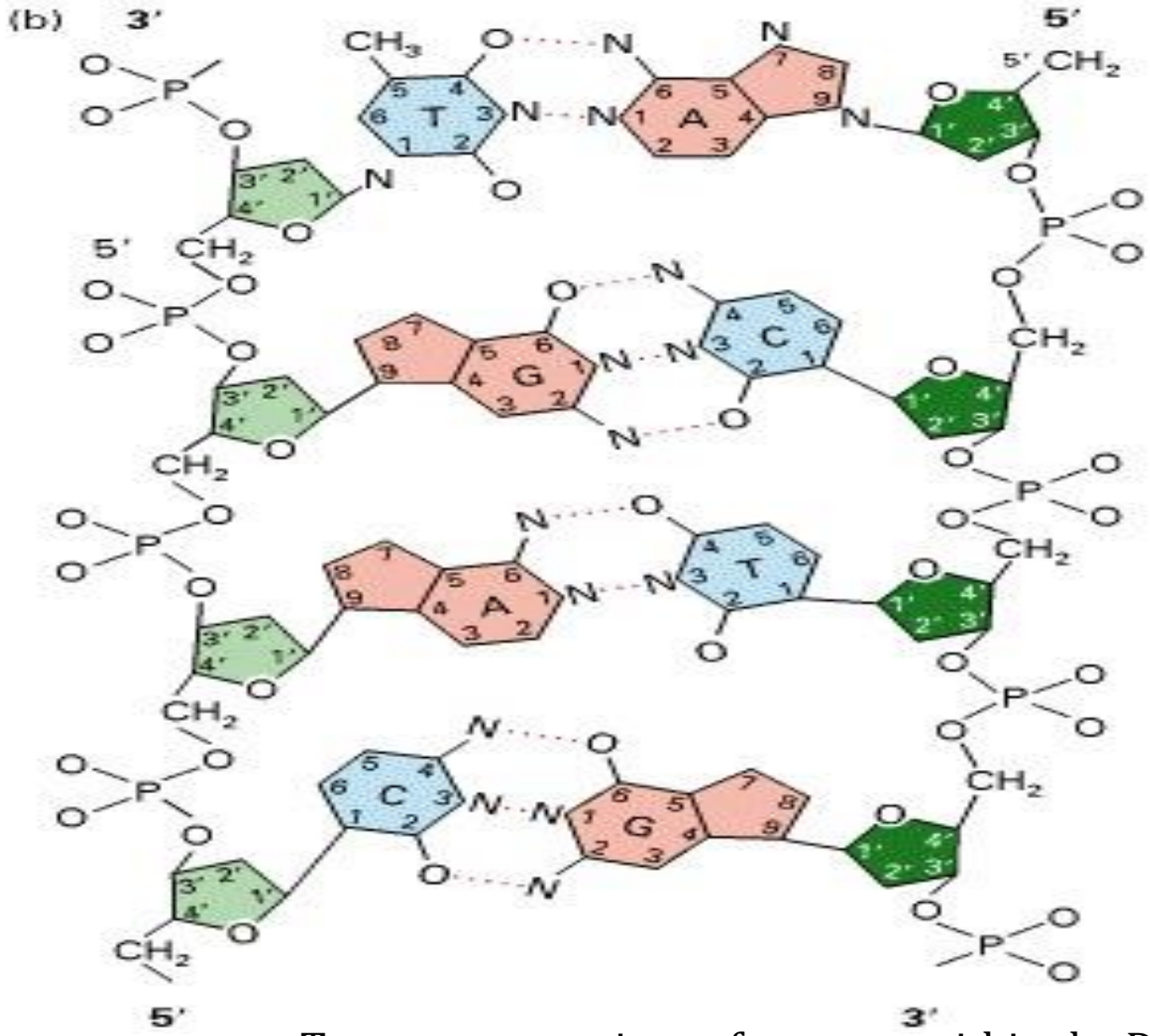
Fig: Alternative ways of representing nucleic acid chains, in this case a single strand of DNA containing only three bases: cytosine (C), adenine (A), and guanine (G). (a) Chemical structure of the trinucleotide CAG. Note the free hydroxyl group at the 3′

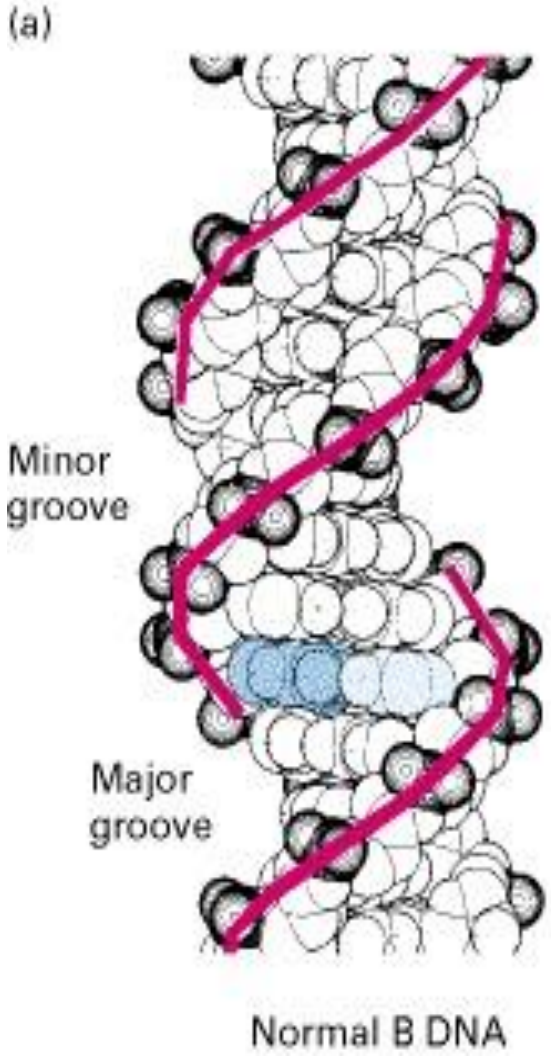


The linear sequence of nucleotides linked by phosphodiester bonds constitutes the primary structure of nucleic acids. As we discuss in the next section, polynucleotides can twist and fold into three-dimensional conformations stabilized by noncovalent bonds; in this respect, they are similar to polypeptides. Although the primary structures of DNA and RNA are generally similar, their conformations are quite different. Unlike RNA, which commonly exists as a single polynucleotide chain, or strand, DNA contains two intertwined polynucleotide strands. This structural difference is critical to the different functions of the two types of nucleic acids.

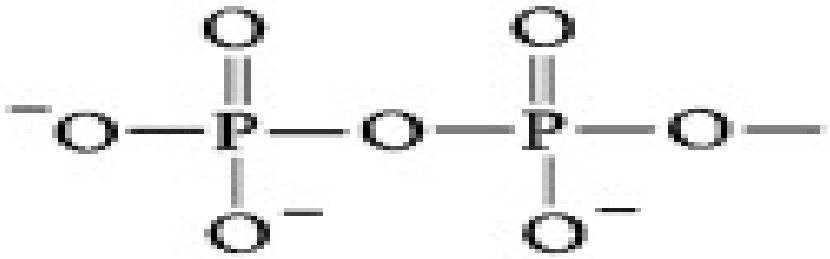
Native DNA Is a Double Helix of Complementary Antiparallel Chains

DNA consists of two associated polynucleotide strands that wind together through space to form a structure often described as a double helix. The two sugar-phosphate backbones are on the outside of the double helix, and the bases project into the interior. The adjoining bases in each strand stack on top of one another in parallel planes (Figure 4-4a). The orientation of the two strands is antiparallel; that is, their $5' \rightarrow 3'$ directions are opposite. The strands are held in precise register by a regular base-pairing between the two strands: A is paired with T through two hydrogen bonds; G is paired with C through three hydrogen bonds (Figure 4-4b). This base-pair complementarity is a consequence of the size, shape, and chemical composition of the bases. The presence of thousands of such hydrogen bonds in a DNA molecule contributes greatly to the stability of the double helix. Hydrophobic and van der Waals interactions between the stacked adjacent base pairs also contribute to the stability of the DNA structure.





Two representations of contacts within the DNA double helix



Cells and extracellular fluids in organisms contain small concentrations of nucleosides, combinations of a base and a sugar without a phosphate. Nucleotides are nucleosides that have one, two, or three phosphate groups esterified at the 5′ hydroxyl. Nucleoside monophosphates have a single esterified phosphate (see Figure 4-1a), diphosphates contain a prophosphate group

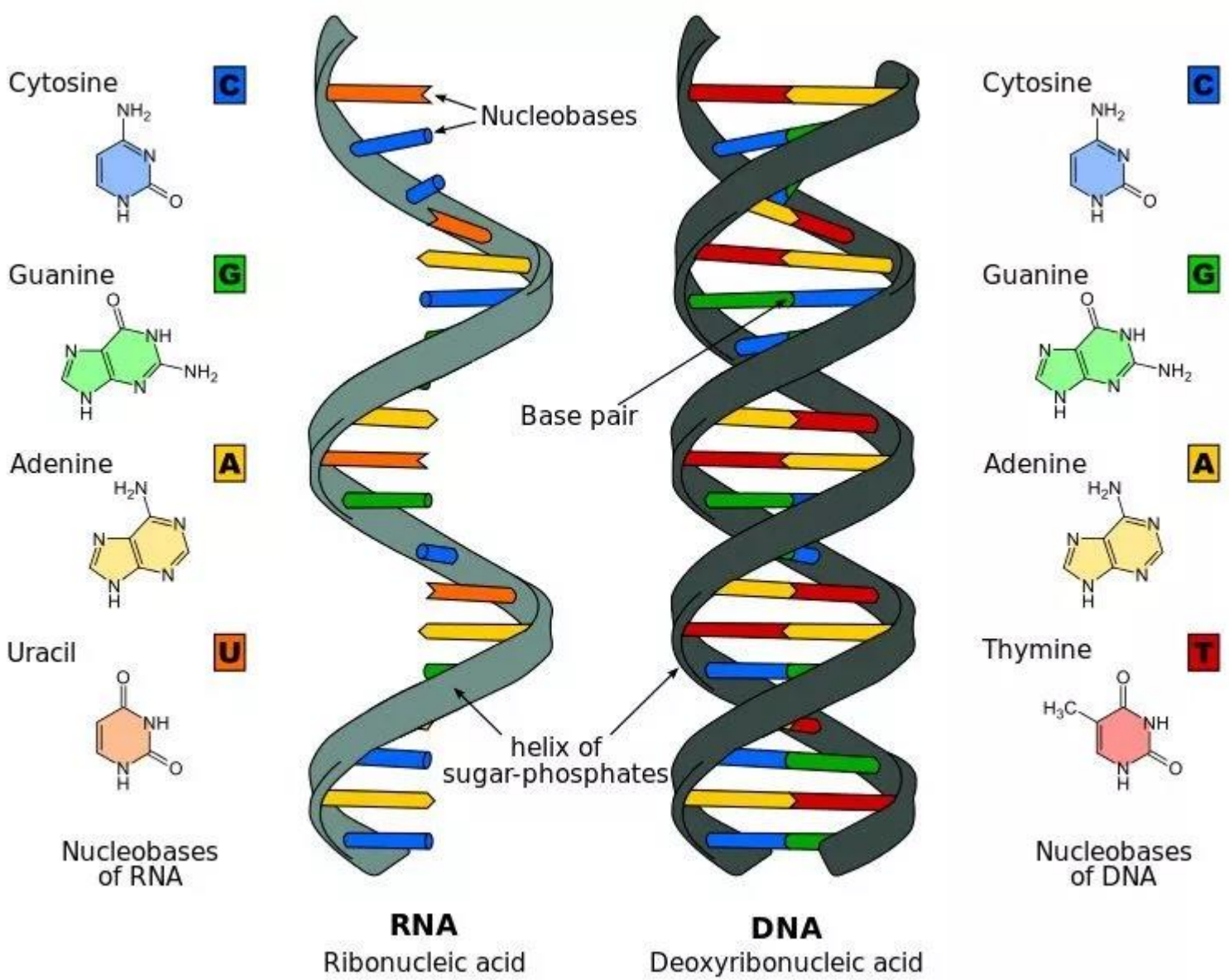
HISTORY

Nuclein were discovered by Friedrich Miescher in 1869. In the early 1880s Albrecht Kossel further purified the substance and discovered its highly acidic properties. He later also identified the nucleobases.

In 1889 Richard Altmann creates the term nucleic acid.

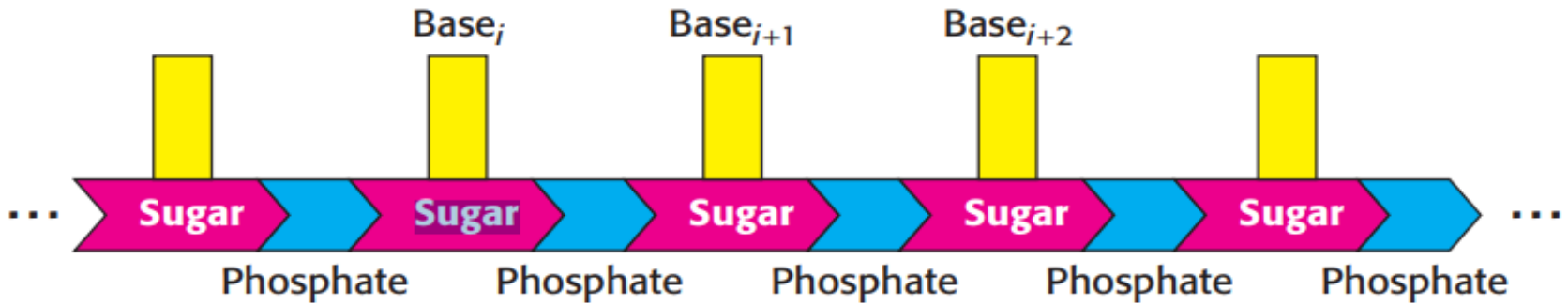
In 1938 Astbury and Bell published the first X-ray diffraction pattern of DNA.

In 1953 Watson and Crick determined the structure of DNA


Cytosine
C
Guanine
G
Adenine
A
Uracil
U
Nucleobases of RNA
Nucleobases
Base pair
helix of sugar-phosphates
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
Cytosine
C
Guanine
G
Adenine
A
Thymine
T
Nucleobases of DNA

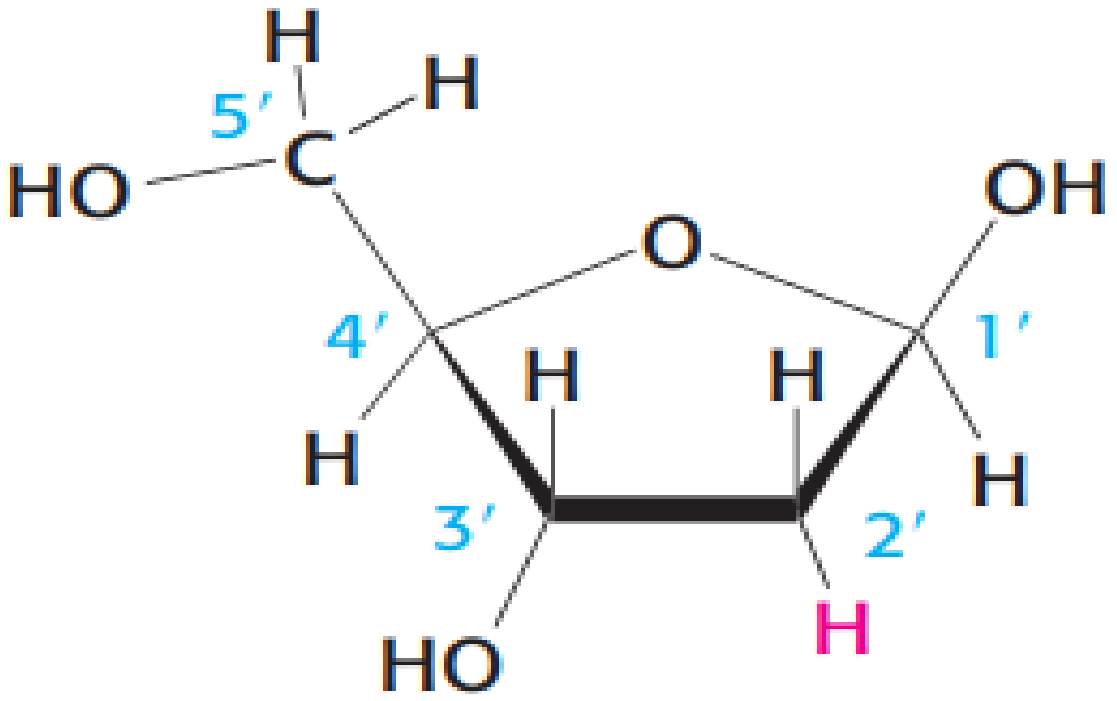
The nucleic acids DNA and RNA are well suited to function as the carriers of genetic information by virtue of their covalent structures. These macromolecules are linear polymers built up from similar units connected end to end (Figure 1). Each monomer unit within the polymer is a nucleotide. A single nucleotide unit consists of three components: a sugar, a phosphate, and one of four bases.

The sequence of bases in the polymer uniquely characterizes a nucleic acid and constitutes a form of linear information.

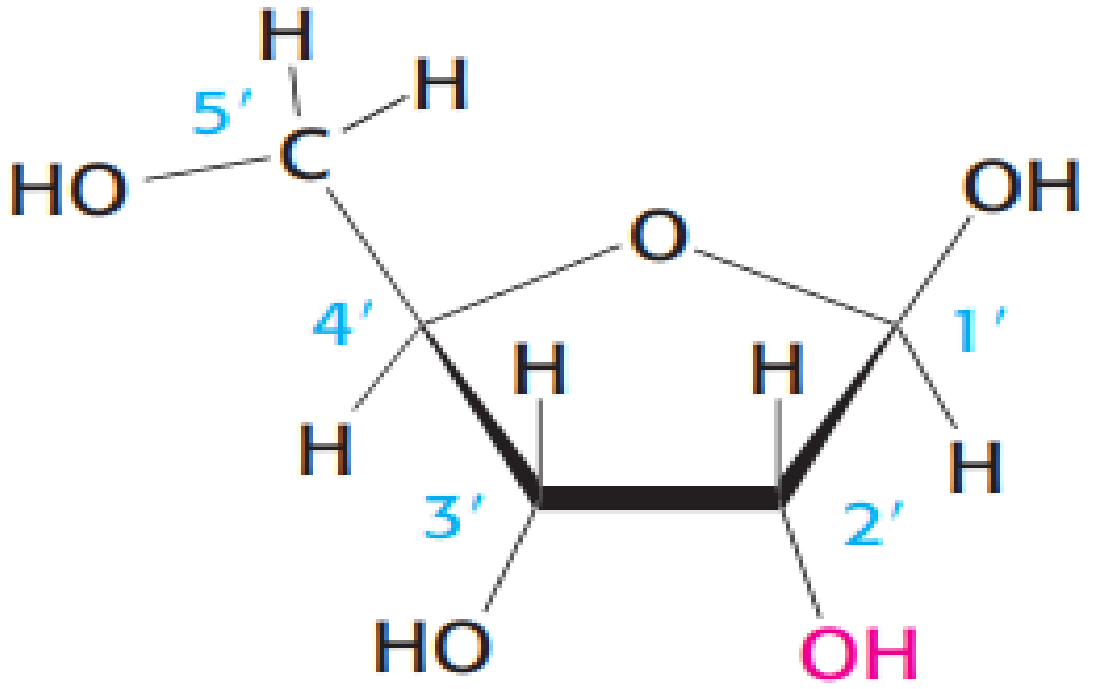


DNA and RNA are differ sugar component of the bases:

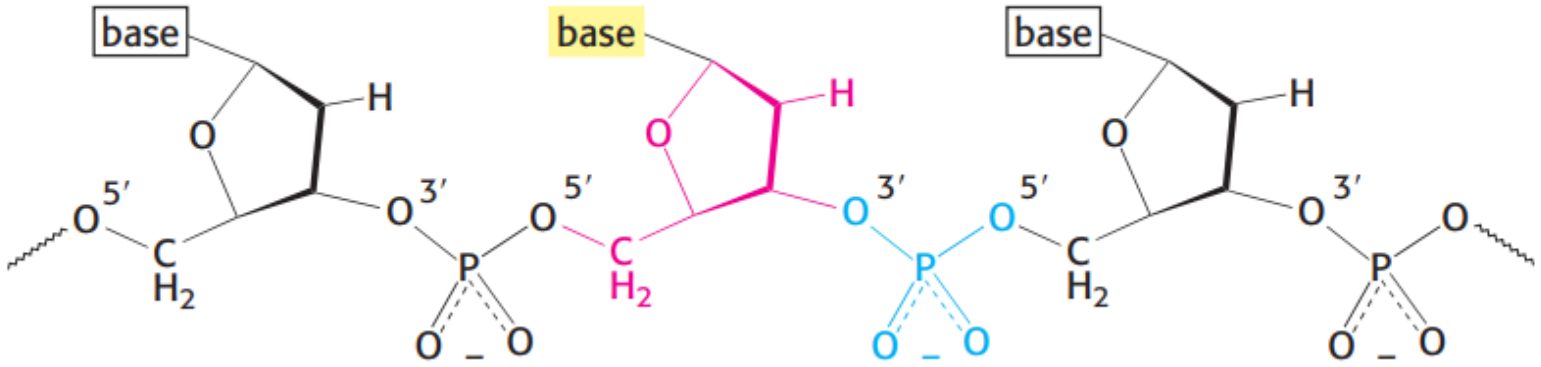
The sugar in deoxyribonucleic acid (DNA) is deoxyribose. The prefix deoxy indicates that the 29-carbon atom of the sugar lacks the oxygen atom that is linked to the 29-carbon atom of ribose, as shown in Figure. Note that sugar carbons are numbered with primes to differentiate them from atoms in the bases. The sugars in both nucleic acids are linked to one another by phosphodiester bridges. Specifically, the 39-hydroxyl (39-OH) group of the sugar moiety of one nucleotide is esterified to a phosphate group, which is, in turn, joined to the 59-hydroxyl group of the adjacent sugar. The chain of sugars linked by phosphodiester bridges is referred to as the backbone of the nucleic acid . Whereas the backbone is constant in a nucleic acid, the bases vary from one monomer to the next. Two of the bases of



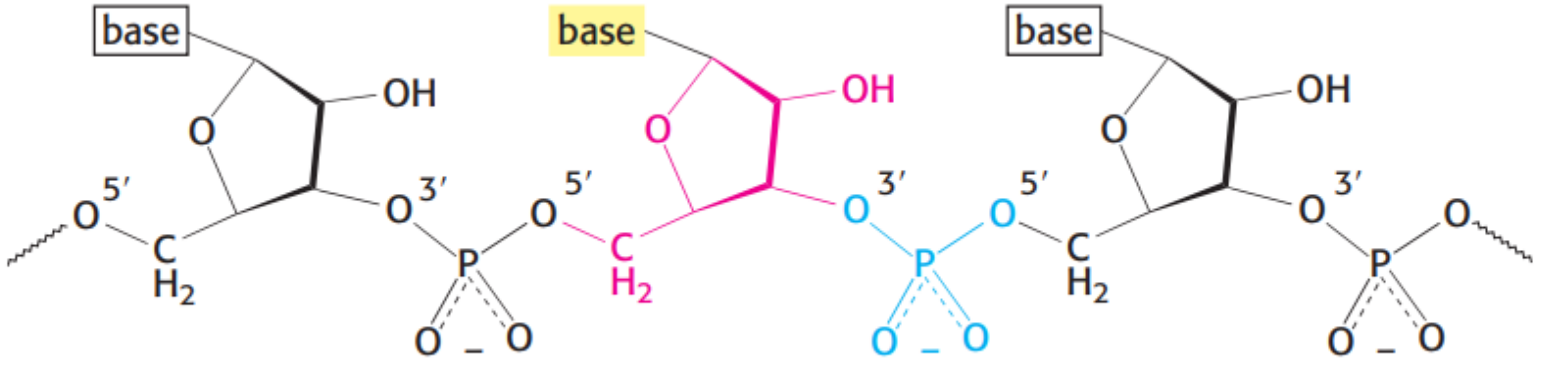
Deoxyribose



Ribose



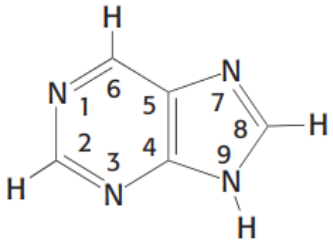
DNA



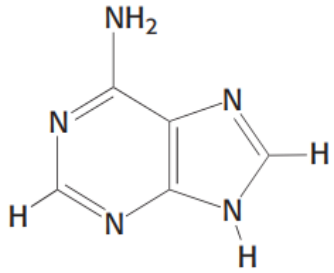
RNA

PURINES and PYRIMIDINS

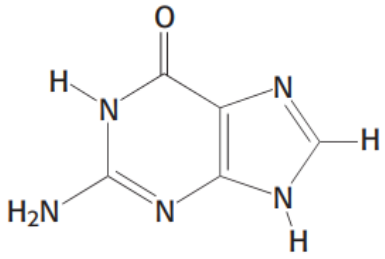
PURINES



Purine

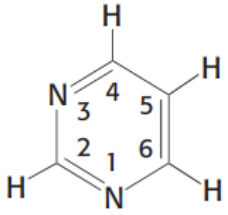


Adenine

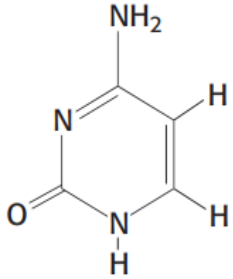


Guanine

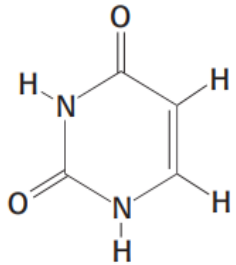
PYRIMIDINES



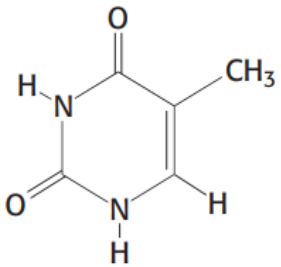
Pyrimidine



Cytosine



Uracil



Thymine

DNA are derivatives of purine—adenine (A) and guanine (G)—and two of pyrimidine—cytosine (C) and thymine (T), as shown in Figure 5..Ribonucleic acid (RNA), like DNA, is a long unbranched polymer consisting of nucleotides joined by 39-to-59 phosphodiester linkages . The covalent structure of RNA differs from that of DNA in two respects.

First, the sugar units in RNA are riboses rather than deoxyriboses. Ribose contains a 29-hydroxyl group not present in deoxyribose.

Second, one of the four major bases in RNA is uracil (U) instead of thymine (T).

Each phosphodiester bridge has a negative charge. This negative charge repels nucleophilic species such as hydroxide ions; consequently ,phosphodiester linkages are much less susceptible to hydrolytic attack

than are other esters such as carboxylic acid esters. This resistance is crucial for maintaining the integrity of information stored in nucleic acids. The absence of the 29-hydroxyl group in DNA further increases its resistance tohydrolysis. The greater stability of DNA probably accounts for its use rather than RNA as the hereditary material in all modern cells and in many viruses.

NUCLEOSIDE and NUCLEOTIDE

The building blocks of nucleic acids and the precursors of these building blocks play many other roles throughout the cell—for instance, as energy currency and as molecular signals. Consequently, it is important to be familiar with the nomenclature of nucleotides and their precursors. A unit consisting of a base bonded to a sugar is referred to as a nucleoside. The four nucleoside units in RNA are called adenosine, guanosine, cytidine, and uridine, whereas those in DNA are called deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine.

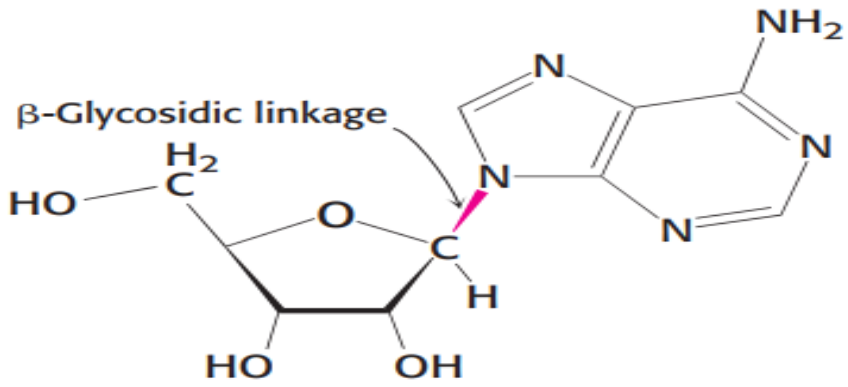
NUCLEOSIDE

A unit consisting of a base bonded to a sugar is referred to as a nucleoside. The four

nucleoside units in RNA are called adenosine, guanosine, cytidine, and uridine, whereas those in DNA are called deoxyadenosine, deoxyguanosine,deoxycytidine, and thymidine.

In each case, N-9 of a purine or N-1 of apyrimidine is attached to C-19 of the sugar by an N-glycosidic linkage(Figure). The base lies above the plane of sugar when the structure is

written in the standard orientation; that is, the configuration of theN-glycosidic linkage is b



NUCLEOTIDE

A nucleotide is a nucleoside joined to one or more phosphoryl groups by

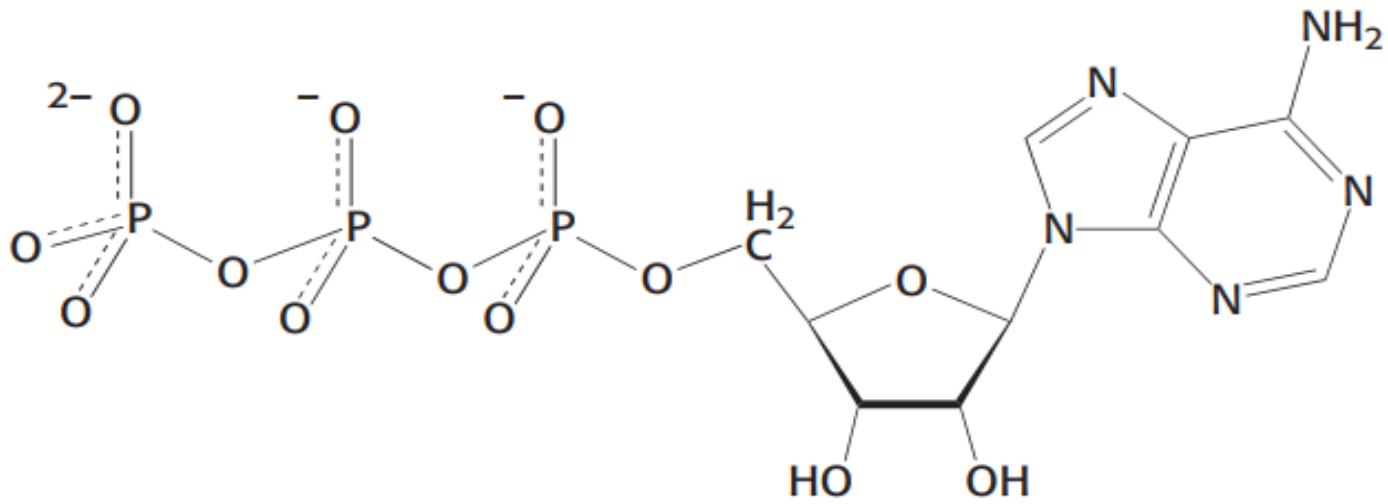
an ester linkage. Nucleotide triphosphates, nucleosides joined to three phosphoryl groups, are the monomers—the building blocks—that are linked to form

RNA and DNA. The four nucleotide units that link to form DNA are nucleotide monophosphates called deoxyadenylate, deoxyguanylate, deoxycytidylate, and thymidylate. Note that thymidylate contains deoxyribose; by

convention, the prefix deoxy is not added because thymine-containingnucleotides are only rarely found in RNA. Similarly, the most common

nucleotides that link to form RNA are nucleotide monophosphates

adenylate, guanylate, cytidylate and uridylate.



5′-ATP

Another means of denoting a nucleotide is the base name with the suffi “ate”. This nomenclature does not describe the number of phosphorylgroups or the site of attachment to carbon of the ribose. A more precise nomenclature is also commonly used. A compound formed by the attachment of a phosphoryl group to C-59 of a nucleoside sugar (the most common site of phosphate esterification) is called a nucleoside 59-phosphate or a

59-nucleotide. In this naming system for nucleotides, the number of phosphoryl groups and the attachment site are designated. Look, for example at adenosine 59-triphosphate

This nucleotide is tremendously important because, in addition to being a building block for RNA, it is the most commonly used energy currency. The energy released from cleavage of the triphosphate group is used to power many cellular processes. Another nucleotide is deoxyguanosine 39-monophosphate (39-dGMP;). This nucleotide differs from ATP in that itcontains guanine rather than adenine, contains deoxyribose rather than ribose (indicated by the prefix “d”), contains one rather than three phosphoryl groups, and has the phosphoryl group esterified to the hydroxyl group in the 39 rather than the 59 position.

NUCLEIC ACID COT CURVE

The X-axis is a log-scale of the product of the initial concentration of DNA (in moles/liter) multiplied by length of time the reaction proceeded (in seconds). The designation for this value is Cot and is called the "Cot" value. The curve itself is called "Cot" curve.

History

It was first developed and utilized by Roy Britten and his colleagues at the Carnegie Institution of Washington in the 1960s. Of particular note, it was through C0t analysis that the redundant (repetitive) nature of eukaryotic genomes was first discovered However, it wasn't until the breakthrough DNA reassociation kinetics experiments of Britten and his colleagues that it was shown that not all DNA coded for genes. In fact, their experiments demonstrated that the majority of eukaryotic genomic DNA is composed of repetitive, non-coding elements.

Cot analysis

C0t analysis, a technique based on the principles of DNA reassociation kinetics, is a biochemical technique that measures how much repetitive DNA is in a DNA sample such as a genome.It is used to study genome structure and organization and has also been used to simplify the sequencing of genomes that contain large amounts of repetitive sequence

Procedure

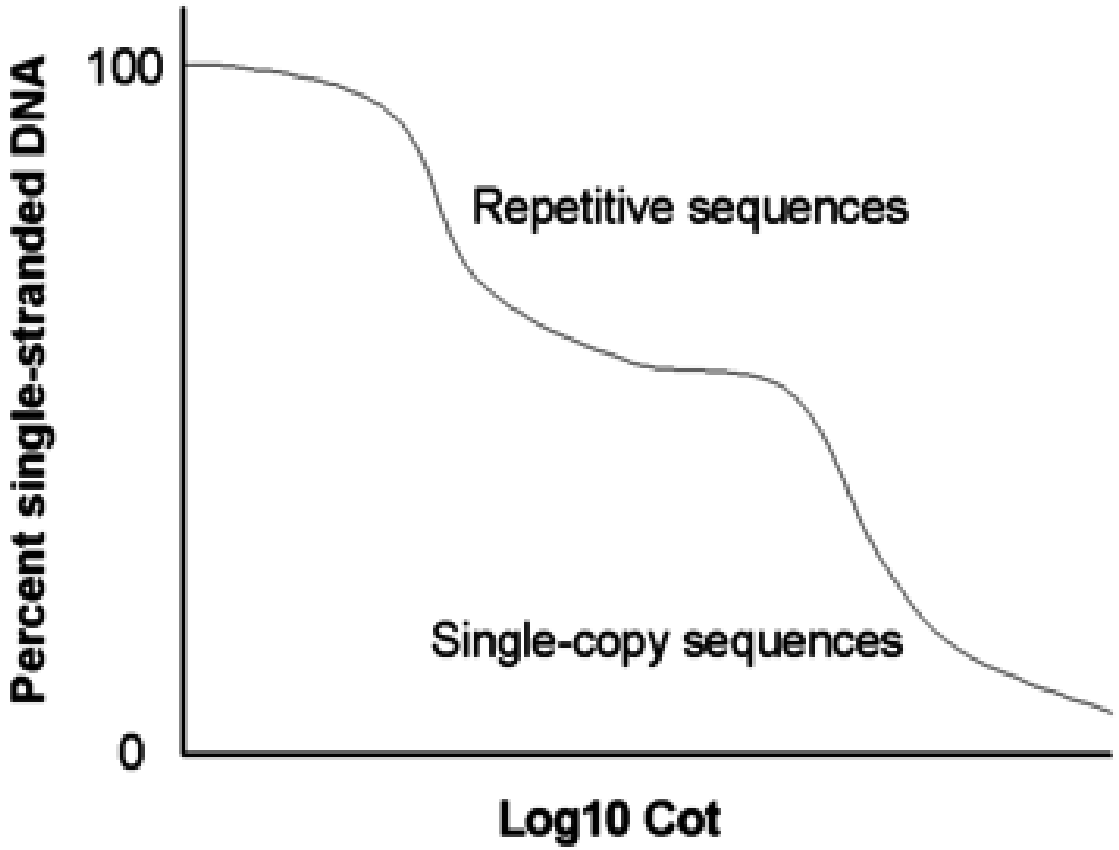
The procedure involves heating a sample of genomic DNA until it denatures into the single stranded-form and then slowly cooling it, so the strands can pair back together. While the sample is cooling, measurements are taken of how much of the DNA is base paired at each temperature.

The amount of single and double-stranded DNA is measured by rapidly diluting the sample, which slows reassociation, and then binding the DNA to a hydroxylapatite column. The column is first washed with a low concentration of sodium phosphate buffer, which elutes the single-stranded DNA, and then with high concentrations of phosphate, which elutes the double stranded DNA. The amount of DNA in these two solutions is then measured using a spectrophotometer.

Analysis

Since a sequence of single-stranded DNA needs to find its complementary strand to reform a double helix, common sequences renature more rapidly than rare sequences. Indeed, the rate at which a sequence will reassociate is proportional to the number of copies of that sequence in the DNA sample. A sample with a highly-repetitive sequence will renature rapidly, while complex sequences will renature slowly.

However, instead of simply measuring the percentage of double-stranded DNA versus time, the amount of renaturation is measured relative to a C0t value. The C0t value is the product of C0 (the initial concentration of DNA), t (time in seconds), and a constant that depends on the concentration of cations in the buffer. Repetitive DNA will renature at low C0t values, while complex and unique DNA sequences will renature at high C0t values. The fast renaturation of the repetitive DNA is because of the availability of numerous complementary sequences.



Repetitive DNA sequences renature at lower C0t values than single-copy sequences.

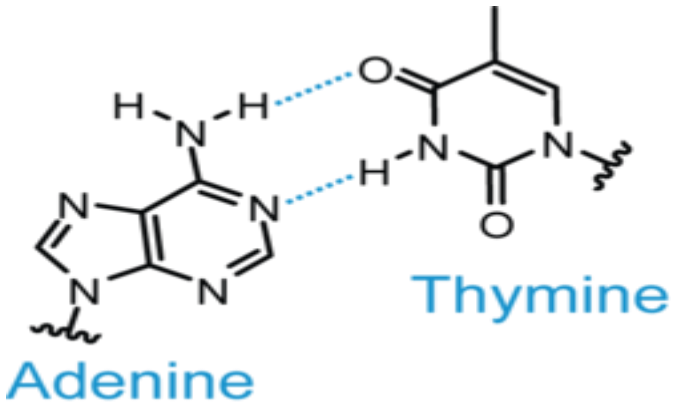
Application to genome sequencing

C0t filtration is a technique that uses the principles of DNA renaturation kinetics to separate the repetitive DNA sequences that dominate many eukaryotic genomes from "gene-rich" single/low-copy sequences.[2] This allows DNA sequencing to concentrate on the parts of the genome that are most informative and interesting, which will speed up the discovery of new genes and make the process more efficient.

BASE PAIRING

A base pair (bp) is a fundamental unit of double-stranded nucleic acids consisting of two nucleobases bound to each other by hydrogen bonds. They form the building blocks of the DNA double helix and contribute to the folded structure of both DNA and RNA. Dictated by specific hydrogen bonding patterns, "Watson–Crick" base pairs (guanine–cytosine and adenine–thymine) allow the DNA helix to maintain a regular helical structure that is subtly dependent on its nucleotide sequence.The complementary nature of this based-paired structure provides a redundant copy of the genetic information encoded within each strand of DNA.

. The regular structure and data redundancy provided by the DNA double helix make DNA well suited to the storage of genetic information, while base-pairing between DNA and incoming nucleotides provides the mechanism through which DNA polymerase replicates DNA and RNA polymerase transcribes DNA into RNA. Many DNA-binding proteins can recognize specific base-pairing patterns that identify particular regulatory regions of genes



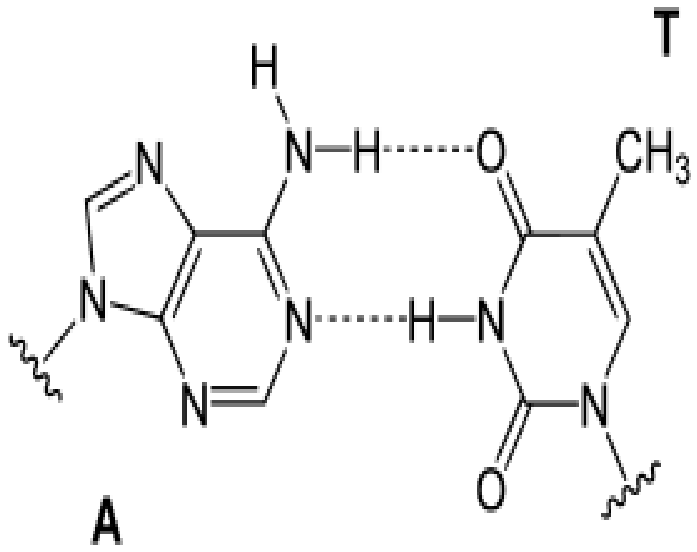
Depiction of the adenine–thymine Watson–Crick base pair

Intramolecular base pairs can occur within single-stranded nucleic acids. This is particularly important in RNA molecules (e.g., transfer RNA), where Watson–Crick base pairs (guanine–cytosine and adenine–uracil) permit the formation of short double-stranded helices, and a wide variety of non-Watson–Crick interactions (e.g., G–U or A–A) allow RNAs to fold into a vast range of specific three-dimensional structures. In addition, base-pairing between transfer RNA (tRNA) and messenger RNA (mRNA) forms the basis for the molecular recognition events that result in the nucleotide sequence of mRNA becoming translated into the amino acid sequence of proteins via the genetic code.

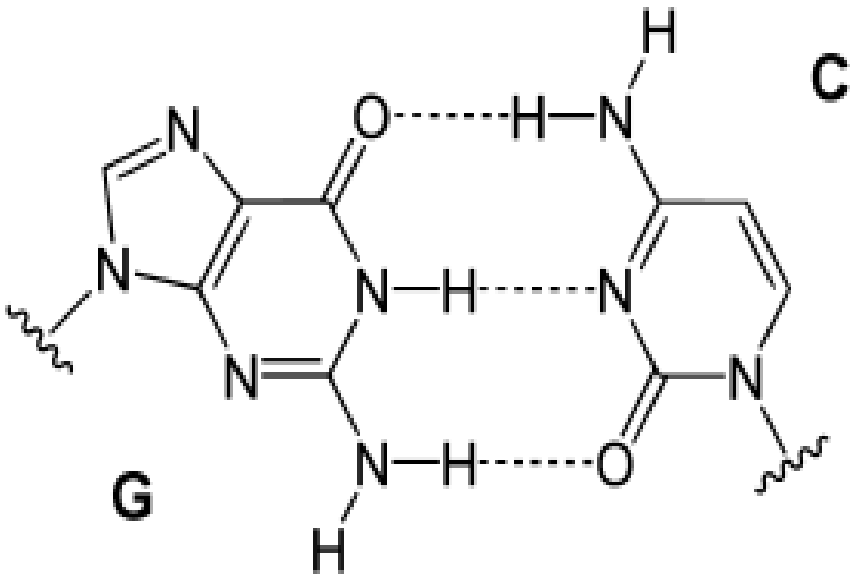
The size of an individual gene or an organism's entire genome is often measured in base pairs because DNA is usually double-stranded. Hence, the number of total base pairs is equal to the number of nucleotides in one of the strands (with the exception of non-coding single-stranded regions of telomeres). The haploid human genome (23 chromosomes) is estimated to be about 3.2 billion bases long and to contain 20,000–25,000 distinct protein-coding genes.[3][4][5] A kilobase (kb) is a unit of measurement in molecular biology equal to 1000 base pairs of DNA or RNA.[6] The total number of DNA base pairs on Earth is estimated at 5.0×1037 with a weight of 50 billion tonnes.[7] In comparison, the total mass of the biosphere has been estimated to be as much as 4 TtC (trillion tons of carbon).[8

Hydrogen bonding and stability

Hydrogen bonding is the chemical interaction that underlies the base-pairing rules described above. Appropriate geometrical correspondence of hydrogen bond donors and acceptors allows only the "right" pairs to form stably. DNA with high GC-content is more stable than DNA with low GC-content. But, contrary to popular belief, the hydrogen bonds do not stabilize the DNA significantly; stabilization is mainly due to stacking interactions.



A.T base pair with two hydrogen bonds.



a G.C base pair with three hydrogen bonds.

Non-covalent hydrogen bonds between the bases are shown as dashed lines. The wiggly lines stand for the connection to the pentose sugar and point in the direction of the minor groove.

The bigger nucleobases, adenine and guanine, are members of a class of double-ringed chemical structures called purines; the smaller nucleobases, cytosine and thymine (and uracil), are members of a class of single-ringed chemical structures called pyrimidines. Purines are complementary only with pyrimidines: pyrimidine-pyrimidine pairings are energetically unfavorable because the molecules are too far apart for hydrogen bonding to be established; purine-purine pairings are energetically unfavorable because the molecules are too close, leading to overlap repulsion. Purine-pyrimidine base-pairing of AT or GC or UA (in RNA) results in proper duplex structure. The only other purine-pyrimidine pairings would be AC and GT and UG (in RNA); these pairings are mismatches because the patterns of hydrogen donors and acceptors do not correspond. The GU pairing, with two hydrogen bonds, does occur fairly often in RNA (see wobble base pair).

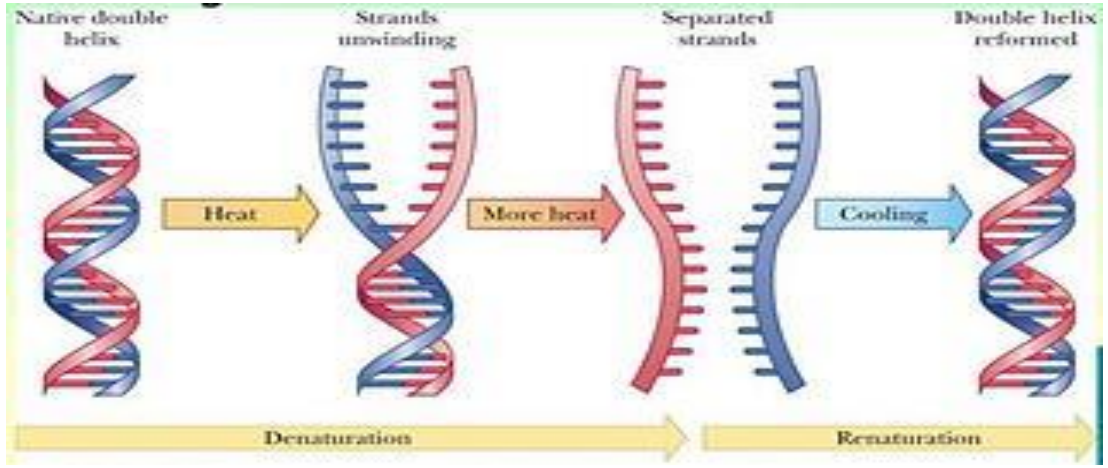
Base analogs and intercalators

Chemical analogs of nucleotides can take the place of proper nucleotides and establish non-canonical base-pairing, leading to errors (mostly point mutations) in DNA replication and DNA transcription. This is due to their isosteric chemistry. One common mutagenic base analog is 5-bromouracil, which resembles thymine but can base-pair to guanine in its enol form.

Other chemicals, known as DNA intercalators, fit into the gap between adjacent bases on a single strand and induce frameshift mutations by "masquerading" as a base, causing the DNA replication machinery to skip or insert additional nucleotides at the intercalated site. Most intercalators are large polyaromatic compounds and are known or suspected carcinogens. Examples include ethidium bromide and acridine.

DENATURATION OF DNA

DNA denaturation is a process of separating dsDNA into single strands, which are favorable to DNA hybridization. Even though the denaturation is a key reaction that determines the success of DNA hybridization based bioassays, no systematic characterization of denaturation method for dsDNA has been attempted thus far.



Nucleic acids (including RNA and DNA) are nucleotide polymers synthesized by polymerase enzymes during either transcription or DNA replication. Following 5'-3' synthesis of the backbone, individual nitrogenous bases are capable of interacting with one another via hydrogen bonding, thus allowing for the formation of higher-order structures. Nucleic acid denaturation occurs when hydrogen bonding between nucleotides is disrupted, and results in the separation of previously annealed strands. For example, denaturation of DNA due to high temperatures results in the disruption of Watson and Crick base pairs and the separation of the double stranded helix into two single strands. Nucleic acid strands are capable of re-annealling when "normal" conditions are restored, but if restoration occurs too quickly, the nucleic acid strands may re-anneal imperfectly resulting in the improper pairing of bases.

Biologically-induced denaturation

The non-covalent interactions between antiparallel strands in DNA can be broken in order to "open" the double helix when biologically important mechanisms such as DNA replication, transcription, DNA repair or protein binding are set to occur.The area of partially separated DNA is known as the denaturation bubble, which can be more specifically defined as the opening of a DNA double helix through the coordinated separation of base pairs.

Denaturation due to chemical agents

With polymerase chain reaction (PCR) being among the most popular contexts in which DNA denaturation is desired, heating is the most frequent method of denaturation.

Other than denaturation by heat, nucleic acids can undergo the denaturation process through various chemical agents such as formamide, guanidine, sodium salicylate, dimethyl sulfoxide (DMSO), propylene glycol, and urea.

These chemical denaturing agents lower the melting temperature (Tm) by competing for hydrogen bond donors and acceptors with pre-existing nitrogenous base pairs. Some agents are even able to induce denaturation at room temperature. For example, alkaline agents (e.g. NaOH) have been shown to denature DNA by changing pH and removing hydrogen-bond contributing protons.These denaturants have been employed to make Denaturing Gradient Gel Electrophoresis gel (DGGE), which promotes denaturation of nucleic acids in order to eliminate the influence of nucleic acid shape on their electrophoretic mobility.

Applications

Many laboratory techniques rely on the ability of nucleic acid strands to separate. By understanding the properties of nucleic acid denaturation, the following methods were created:

PCR

Southern blot

Northern blot

DNA Sequencing

Denaturants

Chemical Acidic nucleic acid denaturants include: Acetic acid , HCl , Nitric Acid

Basic nucleic acid denaturants include NaOH

Other nucleic acid denaturants include: DMSO Formamide Guanidine Sodium salicylate Propylene glycol Urea

Physical

Thermal denaturation

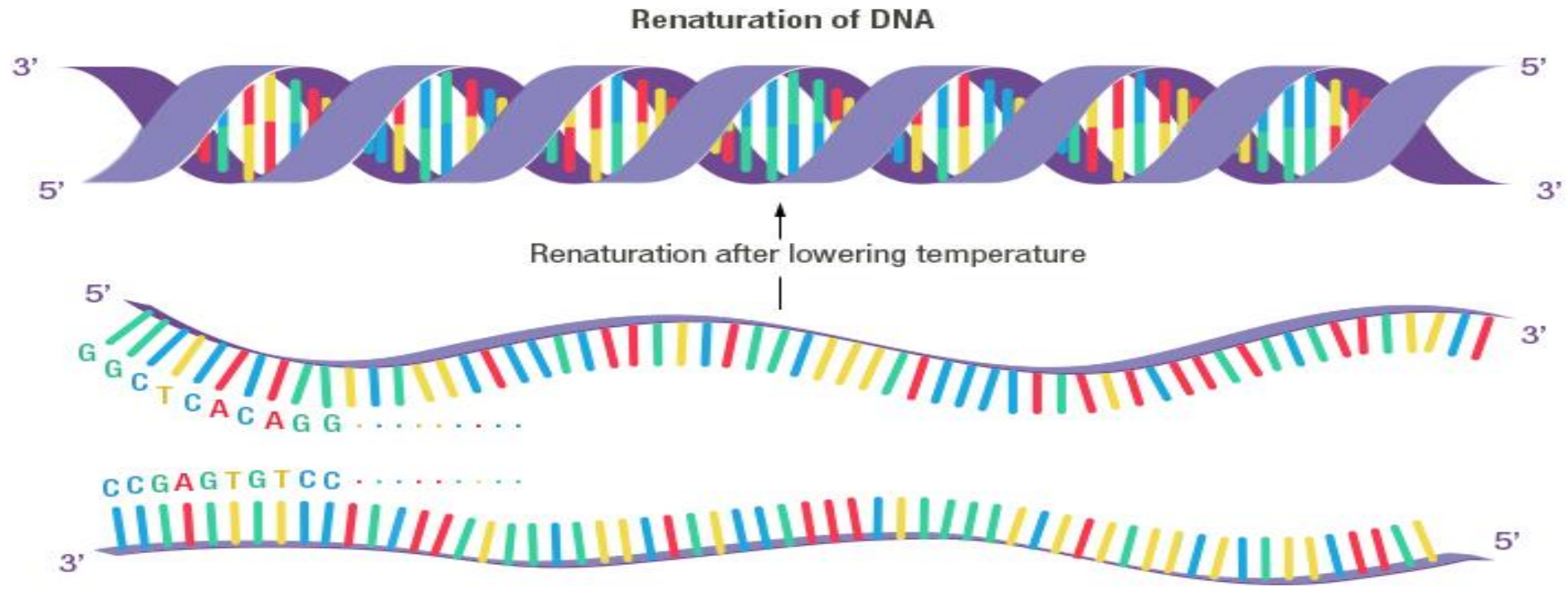
Beads mill

Probe sonication

Radiation

RENATURATION OF DNA

Renaturation in molecular biology refers to the reconstruction of a protein or nucleic acid (such as DNA) to their original form especially after denaturation. ... For instance, a heat-denatured DNA can revert to its original form by cooling slowly the two strands and then reform into its original double-stranded helix.



The DNA denaturation process is reversible under controlled conditions of pH and ionic strength. If the temperature is slowly decreased in the solution where the DNA had been denatured, the DNA chains will spontaneously reanneal and the original double helix structure is restored. This process can be followed in a spectrophotometer at 260 nm and the temperature/absorbency relationship can be described by a curve that is the opposite of the denaturation curveshown in Fig.. The DNA renaturation resulting from slow cooling is called reannealing. When the complementary strands meet, they completely reconstitute the double helix.

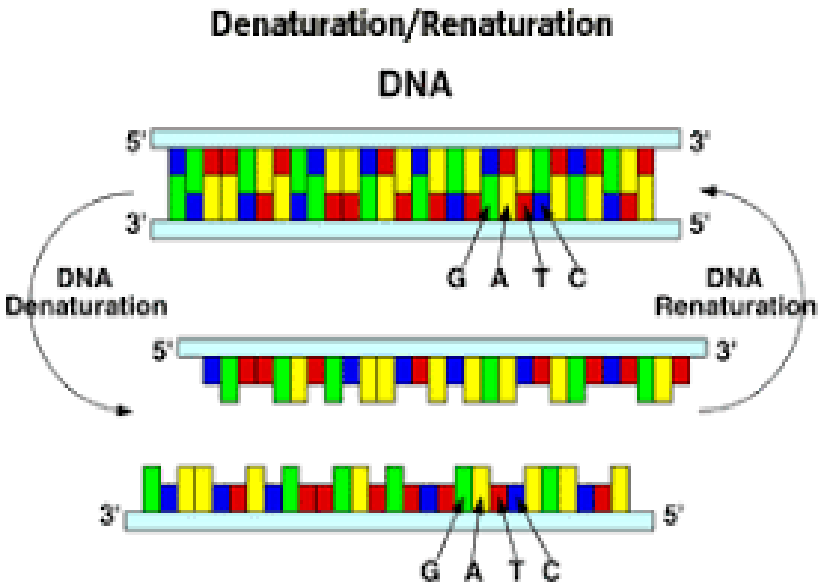
The first model that attempted to describe the thermodynamics of the denaturation bubble was introduced in 1966 and called the Poland-Scheraga Model. This model describes the denaturation of DNA strands as a function of temperature. As the temperature increases, the hydrogen bonds between the Watson and Crick base pairs are increasingly disturbed and "denatured loops" begin to form However, the Poland-Scheraga Model is now considered elementary because it fails to account for the confounding implications of DNA sequence, chemical composition, stiffness and torsion Recent thermodynamic studies have inferred that the lifetime of a singular denaturation bubble ranges from 1 microsecond to 1 millisecond.This information is based on established timescales of DNA replication and transcription Currently, biophysical and biochemical research studies are being performed to more fully elucidate the thermodynamic details of the denaturation bubble.

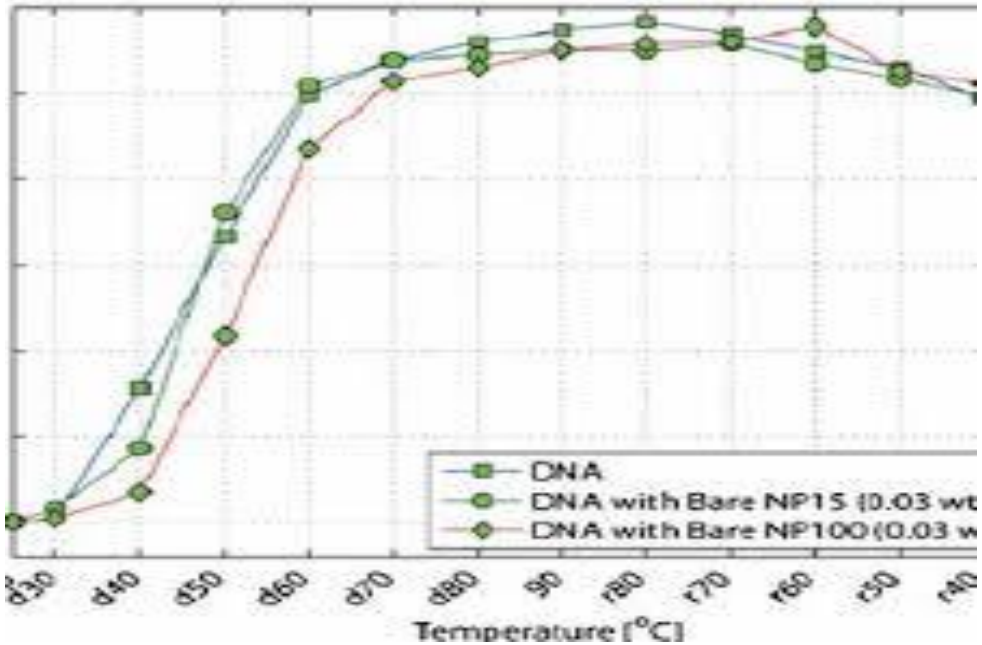
The rate of renaturation depends on the structure of DNA. When a given DNA has segments with the same sequence (repetitive sequences), the annealing time is shorter because the chance that one chain meets a complementary one is greater. In contrast, DNA sections with unique sequences require a longer time to find its complementary strand to reform the double helix.

The repeated segments allow for a faster reassociation of DNA after denaturation. Another portion of DNA has moderately repetitive pieces, composed of hundreds to thousands of copies. These anneal at lower rate. Finally, a third fraction of the DNA that in mammals comprises about 60% of total DNA, corresponds to sequences only found in one to three copies (single copy DNA) that anneal very slowly. In bacteria, almost all the DNA exists as single copy DNA.

DNA renaturation

- J. Marmur (Harvard, 1960) – first described
  - Slowly cool heat-denatured DNA
  - or drop temperature quickly to ~25°C below T_m & incubate awhile
  - Complementary single-stranded DNAs can reassociate or reanneal
- Renaturation very useful
  - genome complexity: variety & copy number
  - hybridization: molecular identification





TYPES OF DNA

There are three different DNA types:

A-DNA: It is a right-handed double helix similar to the B-DNA form. ...

B-DNA: This is the most common DNA conformation and is a right-handed helix. ...

Z-DNA: Z-DNA is a left-handed DNA where the double helix winds to the left in a zig-zag pattern

Why do different forms of DNA exist?

There is simply not enough room for the DNA to be stretched out in a perfect, linear B-DNA conformation. In nearly all cells, from simple bacteria through complex eukaryotes, the DNA must be compacted by more than a thousand fold in order even to fit inside the cell or nucleus Refined resolution of the structure of DNA, based on X-ray crystallography of short synthetic pieces of DNA, has shown that there is a considerable variance of the helical structure of DNA, based on the sequence. For example, a 200-bp piece of DNA can run as if it were more than 1000 bp on an acrylamide gel if it has the right sequence. The double helix is not the same uniform structure.

B-form DNA

B-DNA is the Watson–Crick form of the double helix that most people are familiar with. They proposed two strands of DNA — each in a right-hand helix — wound around the same axis. The two strands are held together by H-bonding between the bases (in anti-conformation). The two strands of the duplex are antiparallel and plectonemically coiled. The nucleotides arrayed in a 5′ to 3′ orientation on one strand align with complementary nucleotides in the 3′ to 5′ orientation of the opposite strand.

Bases fit in the double helical model if pyrimidine on one strand is always paired with purine on the other. From Chargaff's rules, the two strands will pair A with T and G with C. This pairs a keto base with an amino base, a purine with a pyrimidine. Two H-bonds can form between A and T, and three can form between G and C.

These are the complementary base pairs. The base-pairing scheme immediately suggests a way to replicate and copy the genetic information. 34 nm between bp, 3.4 nm per turn, about 10 bp per turn 9 nm (about 2.0 nm or 20 Angstroms) in diameter.

34o helix pitch; -6o base-pair tilt; 36o twist angle

A-form DNA

The major difference between A-form and B-form nucleic acid is in the confirmation of the deoxyribose sugar ring. It is in the C2′ endoconformation for B-form, whereas it is in the C3′ endoconformation in A-form.

A second major difference between A-form and B-form nucleic acid is the placement of base-pairs within the duplex. In B-form, the base-pairs are almost centered over the helical axis but in A-form, they are displaced away from the central axis and closer to the major groove. The result is a ribbon-like helix with a more open cylindrical core in A-form.

Right-handed helix 11 bp per turn; 0.26 nm axial rise; 28o helix pitch; 20o base-pair tilt 33o twist angle; 2.3nm helix diameter

Z-form DNA

Z-DNA is a radically different duplex structure, with the two strands coiling in left-handed helices and a pronounced zig-zag (hence the name) pattern in the phosphodiester backbone.

Z-DNA can form when the DNA is in an alternating purine-pyrimidine sequence such as GCGCGC, and indeed the G and C nucleotides are in different conformations, leading to the zig-zag pattern.
The big difference is at the G nucleotide.

It has the sugar in the C3′ endoconformation (like A-form nucleic acid, and in contrast to B-form DNA) and the guanine base is in the synconformation.
This places the guanine back over the sugar ring, in contrast to the usual anticonformation seen in A- and B-form nucleic acid

The base in the anticonformation places it in the position where it can readily form H-bonds with the complementary base on the opposite strand.

The duplex in Z-DNA has to accommodate the distortion of this G nucleotide in the synconformation. The cytosine in the adjacent nucleotide of Z-DNA is in the "normal" C2′ endo, anticonformation.

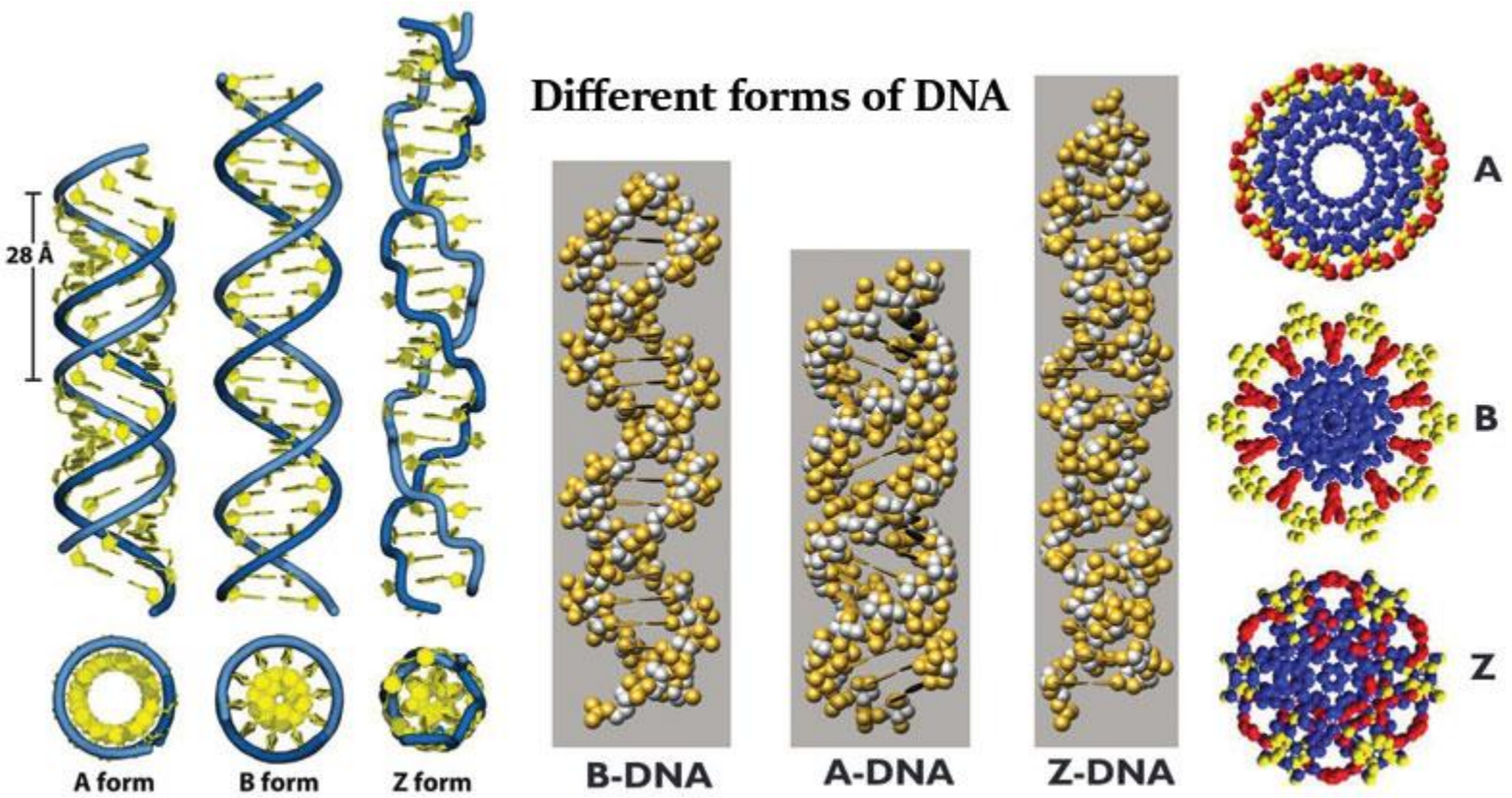
Discovered by Rich, Nordheim &Wang in 1984.

It has antiparallel strands as B-DNA.

It is long and thin as compared to B-DNA.

12 bp per turn; 0.45 nm axial rise; 45o helix pitch; 7o base-pair tilt

-30o twist angle; 1.8 nm helix diameter


Different forms of DNA
28 Å
A form
B form
Z form
B-DNA
A-DNA
Z-DNA
A
B
Z

Conditions Favoring A-form, B-form, and Z-form of DNA

Whether a DNA sequence will be in the A-, B-or Z-DNA conformation depends on at least three conditions. The first is the ionic or hydration environment, which can facilitate conversion between different helical forms.

A-DNA is favored by low hydration, whereas Z-DNA can be favored by high salt.

The second condition is the DNA sequence: A-DNA is favored by certain stretches of purines (or pyrimidines), whereas Z-DNA can be most readily formed by alternating purine-pyrimidine steps.

The third condition is the presence of proteins that can bind to DNA in one helical conformation and force the DNA to adopt a different conformation, such as proteins which bind to B-DNA and can drive it to either A-or Z forms.

In living cells, most of the DNA is in a mixture of Aand B-DNA conformations, with a few small regions capable of forming Z-DNA.

Other rare forms of DNA

C-DNA

Formed at 66% relative humidity and in presence of Li+ and Mg2+ ions.

Right-handed with the axial rise of 3.32A° per base pair

33 base pairs per turn

Helical pitch 3.32A°×9.33°A=30.97A°.

Base pair rotation=38.58°.

Has a diameter of 19 A°, smaller than that of A-&B- DNA.

The tilt of base is 7.8°

DIFFERENT TYPES OF RNA

Coding RNA (messenger RNA; mRNA)

Messenger RNA (mRNA) carries the genetic code from DNA in a form that can be recognized to make proteins. The coding sequence of the mRNA determines the amino acid sequence in the protein produced. Once transcribed from DNA, eukaryotic mRNA briefly exists in a form called “precursor mRNA (pre-mRNA)” before it is fully processed into mature mRNA. This processing step, which is called “RNA splicing”, removes the introns—non-coding sections of the pre-mRNA. There are approximately 23,000 mRNAs encoded in human genome.

Non-coding RNA (ncRNA)

Ribosomal RNA (rRNA):

Ribosomal RNA is the catalytic component of the ribosomes. In the cytoplasm, rRNAs and protein components combine to form a nucleoprotein complex called the ribosome which binds mRNA and synthesizes proteins (also called translation).

Transfer RNA (tRNA):

Transfer RNA is a small RNA chain of about 80 nucleotides. During translation, tRNA transfers specific amino acids that correspond to the mRNA sequence into the growing polypeptide chain at the ribosome.

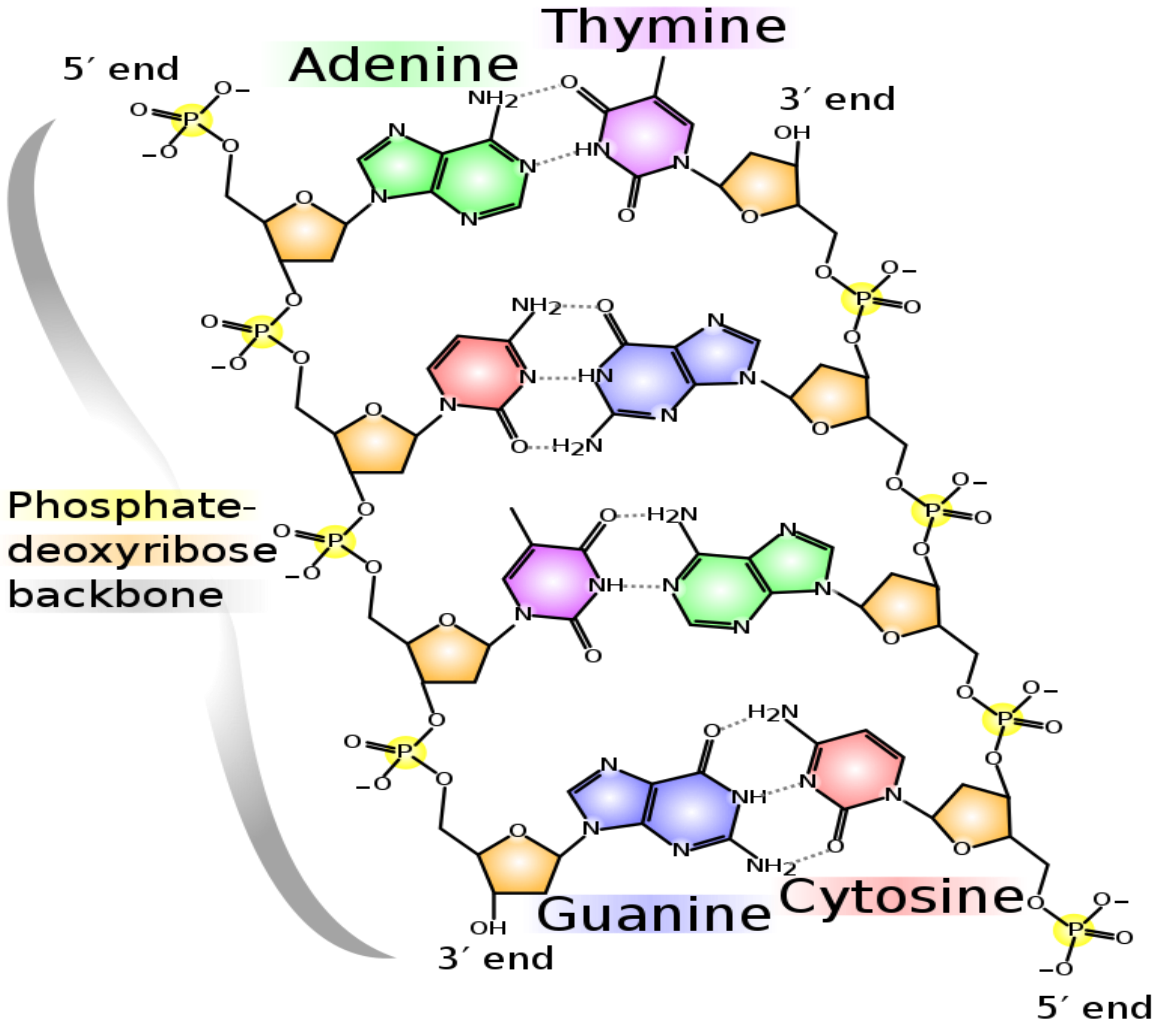
COMPLEMENTARITY OF DNA

In nature complementarity is the base principle of DNA replication and transcription as it is a property shared between two DNA or RNA sequences, such that when they are aligned antiparallel to each other, the nucleotide bases at each position in the sequences will be complementary, much like looking in the mirror and seeing the reverse of things. This complementary base pairing allows cells to copy information from one generation to another and even find and repair damage to the information stored in the sequences.

The degree of complementarity between two nucleic acid strands may vary, from complete complementarity (each nucleotide is across from its opposite) to no complementarity (each nucleotide is not across from its opposite) and determines the stability of the sequences to be together. Furthermore, various DNA repair functions as well as regulatory functions are based on base pair complementarity. In biotechnology, the principle of base pair complementarity allows the generation of DNA hybrids between RNA and DNA, and opens the door to modern tools such as cDNA libraries. While most complementarity is seen between two separate strings of DNA or RNA, it is also possible for a sequence to have internal complementarity resulting in the sequence binding to itself in a folded configuration.

DNA and RNA base pair complementarity

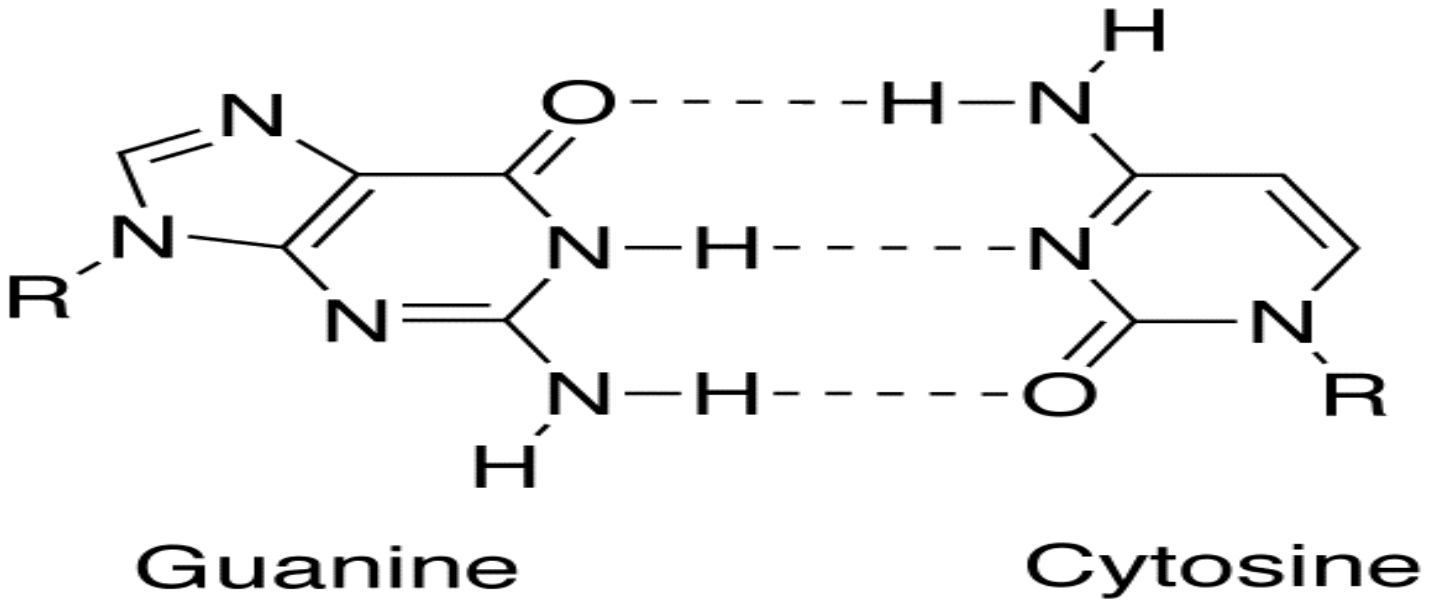
Complementarity is achieved by distinct interactions between nucleobases: adenine, thymine (uracil in RNA), guanine and cytosine. Adenine and guanine are purines, while thymine, cytosine and uracil are pyrimidines. Purines are larger than pyrimidines. Both types of molecules complement each other and can only base pair with the opposing type of nucleobase. In nucleic acid, nucleobases are held together by hydrogen bonding, which only works efficiently between adenine and thymine and between guanine and cytosine. The base complement A=T shares two hydrogen bonds, while the base pair G≡C has three hydrogen bonds. All other configurations between nucleobases would hinder double helix formation. DNA strands are oriented in opposite directions, they are said to be antiparallel



Complementarity between two antiparallel strands of DNA. The top strand goes from the left to the right and the lower strand goes from the right to the left lining them up.

A=T T=A G≡C C≡G	5'-ATGCGCTAGCTCATTT-3' 3'-TACGCGATCGAGTAAA-5'

Left: the nucleotide base pairs that can form in double-stranded DNA. Between A and T there are two hydrogen bonds, while there are three between C and G. Right: two complementary strands of DNA.



Match up between two DNA bases (guanine and cytosine) showing hydrogen bonds (dashed lines) holding them together

A complementary strand of DNA or RNA may be constructed based on nucleobase complementarity. Each base pair, A=T vs. G≡C, takes up roughly the same space, thereby enabling a twisted DNA double helix formation without any spatial distortions. Hydrogen bonding between the nucleobases also stabilizes the DNA double helix.

Complementarity of DNA strands in a double helix make it possible to use one strand as a template to construct the other. This principle plays an important role in DNA replication, setting the foundation of heredity by explaining how genetic information can be passed down to the next generation

Complementarity is also utilized in DNA transcription, which generates an RNA strand from a DNA template. In addition, human immunodeficiency virus, a single-stranded RNA virus, encodes an RNA-dependent DNA polymerase (reverse transcriptase) that uses complementarity to catalyze genome replication. The reverse transcriptase can switch between two parental RNA genomes by copy-choice recombination during replication.

DNA REPAIR MECHANISIM such as proof reading are complementarity based and allow for error correction during DNA replication by removing mismatched nucleobases. In general, damages in one strand of DNA can be repaired by removal of the damaged section and its replacement by using complementarity to copy information from the other strand, as occurs in the processes of mismatch repair, nucleotide excision repair and base excision repair.

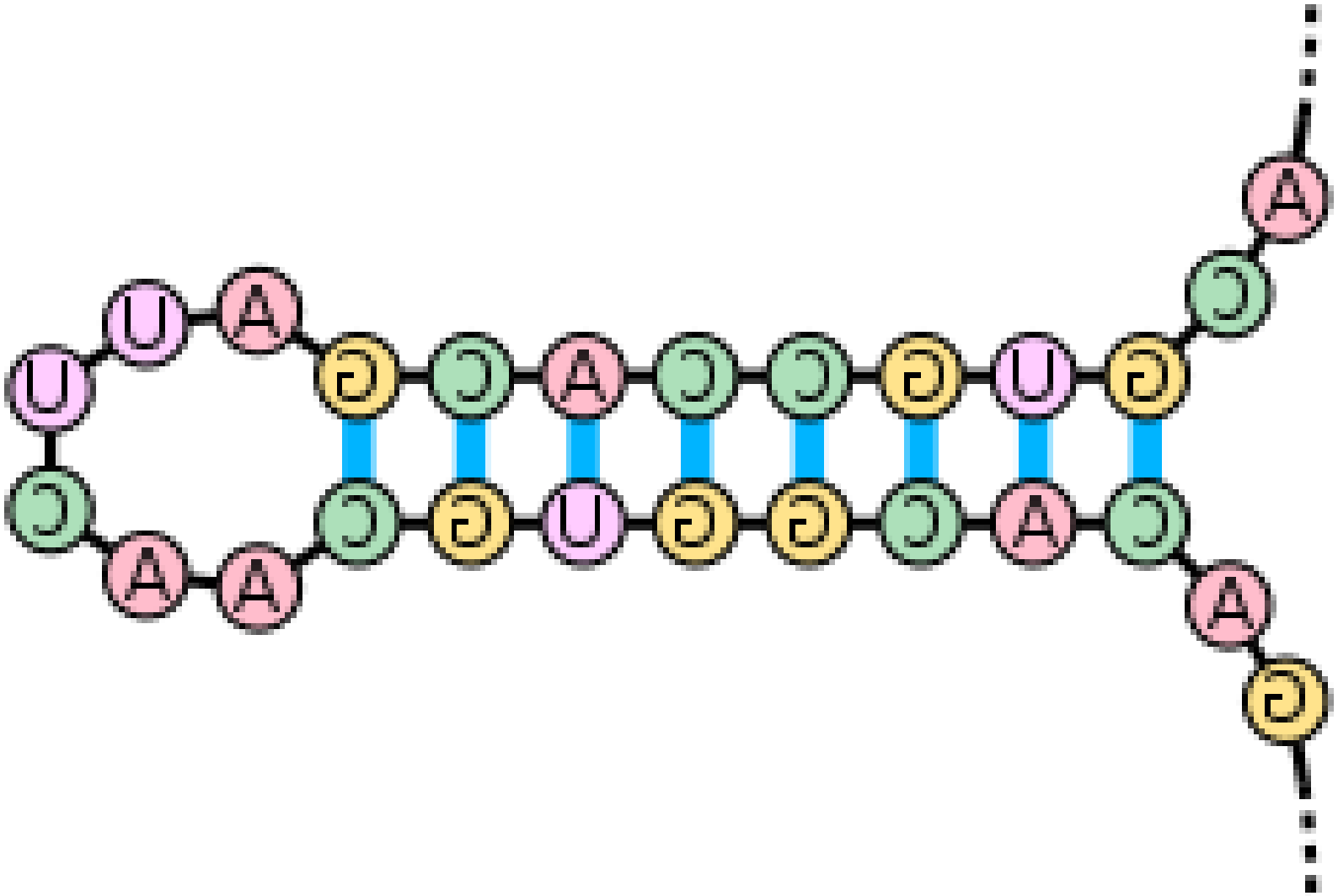
Nucleic acids strands may also form hybrids in which single stranded DNA may readily anneal with complementary DNA or RNA. This principle is the basis of commonly performed laboratory techniques such as the polymerase chain reaction, PCR

Two strands of complementary sequence are referred to as sense and anti-sense.

The sense strand is, generally, the transcribed sequence of DNA or the RNA that was generated in transcription. While the anti-sense strand is the strand that is complementary to the sense sequence.

Self-complementarity and hairpin loops

Self-complementarity refers to the fact that a sequence of DNA or RNA may fold back on itself, creating a double-strand like structure. Depending on how close together the parts of the sequence are that are self-complementary, the strand may form hairpin loops, junctions, bulges or internal loops. RNA is more likely to form these kinds of structures due to base pair binding not seen in DNA, such as guanine binding with uracil.



A sequence of RNA that has internal complementarity which results in it folding into a hairpin

Regulatory functions

Complementarity can be found between short nucleic acid stretches and a coding region or an transcribed gene, and results in base pairing. These short nucleic acid sequences are commonly found in nature and have regulatory functions such as gene silencing

Bioinformatics

Complementarity allows information found in DNA or RNA to be stored in a single strand. The complementing strand can be determined from the template and vice versa as in cDNA libraries. This also allows for analysis, like comparing the sequences of two different species. Shorthands have been developed for writing down sequences when there are mismatches (ambiguity codes) or to speed up how to read the opposite sequence in the complement (ambigrams).

cDNA Library

A cDNA library is a collection of expressed DNA genes that are seen as a useful reference tool in gene identification and cloning processes. cDNA libraries are constructed from mRNA using RNA-dependent DNA polymerase reverse transcriptase (RT), which transcribes an mRNA template into DNA. Therefore, a cDNA library can only contain inserts that are meant to be transcribed into mRNA. This process relies on the principle of DNA/RNA complementarity. The end product of the libraries is double stranded DNA, which may be inserted into plasmids. Hence, cDNA libraries are a powerful tool in modern research

HYPO-HYPERCHROMACITY OF DNA

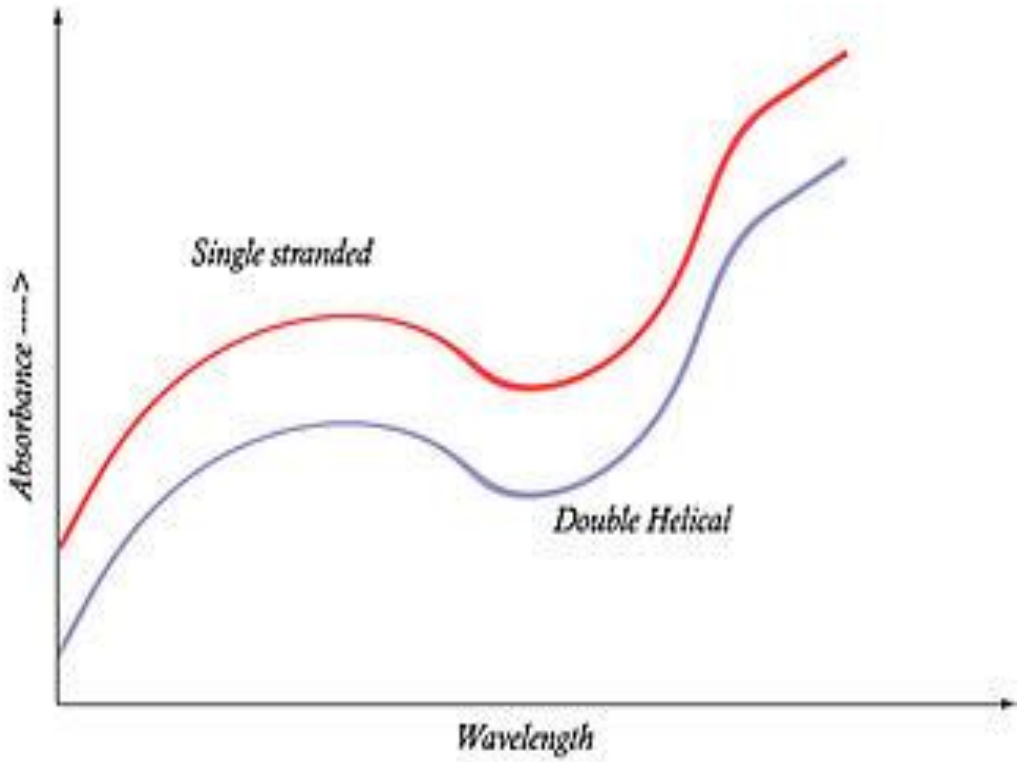
Hypochromicity describes a material's decreasing ability to absorb light. Hyperchromicity is the material's increasing ability to absorb light. The Hypochromic Effect describes the decrease in the absorbance of ultraviolet light in a double stranded DNA compared to its single stranded counterpart.

Hypochromicity

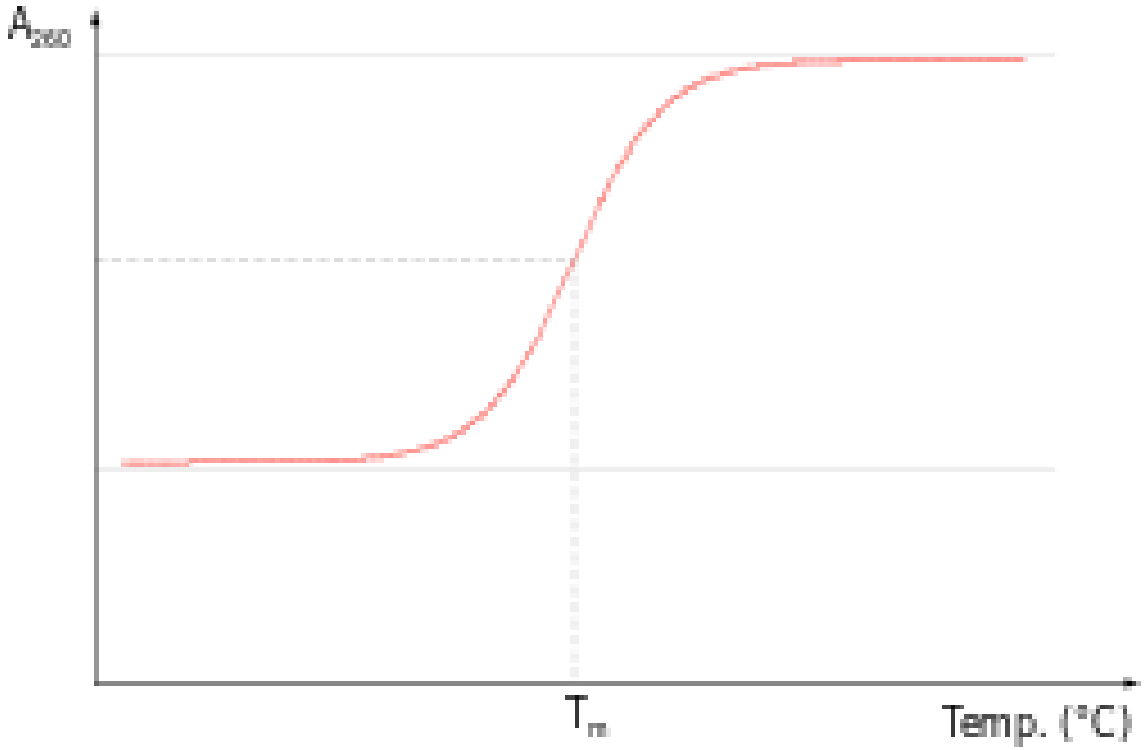
Hypochromicity describes a material's decreasing ability to absorb light. Hyperchromicity is the material's increasing ability to absorb light.

The Hypochromic Effect describes the decrease in the absorbance of ultraviolet light in a double stranded DNA compared to its single stranded counterpart. Compared to a single stranded DNA, a double stranded DNA consists of stacked bases that contribute to the stability and the hypochromicity of the DNA.

When a double stranded DNA is denatured, the stacked bases break apart and thus becomes less stable. It also absorbs more ultraviolet light since the bases no longer forms hydrogens bonds and therefore are free to absorb light. Ways to denature DNA include high temperature, addition of denaturant, and increasing the pH level.



Difference in absorbance of ultraviolet light between single stranded and double stranded DNA



Nucleic acid melting curve showing hyperchromicity as a function of temperature

Importance of Hypochromic Effect

The measurement of absorption of light is important in monitoring the melting and annealing of DNA. At the melting temperature (Tm), the DNA is half denatured and half double stranded. By lowering the temperature below the Tm, the denatured DNA strands would anneal back into a double stranded DNA. When temperature is above the Tm, the DNA is denatured.

Because melting occurs almost instantly at a certain temperature, monitoring the absorbance of the DNA at various temperature would indicate the melting temperature. By being able to find the temperature at which DNA melted and annealed, scientists are able to separate DNA strands and anneal them with other DNA strands. This is important in creating hybrid DNAs, which consists of two DNA strands from different sources. Since DNA strands can only anneal if they are similar, the creation of hybrid DNAs can indicate similarities between genomes of different organisms.

Hyperchromicity

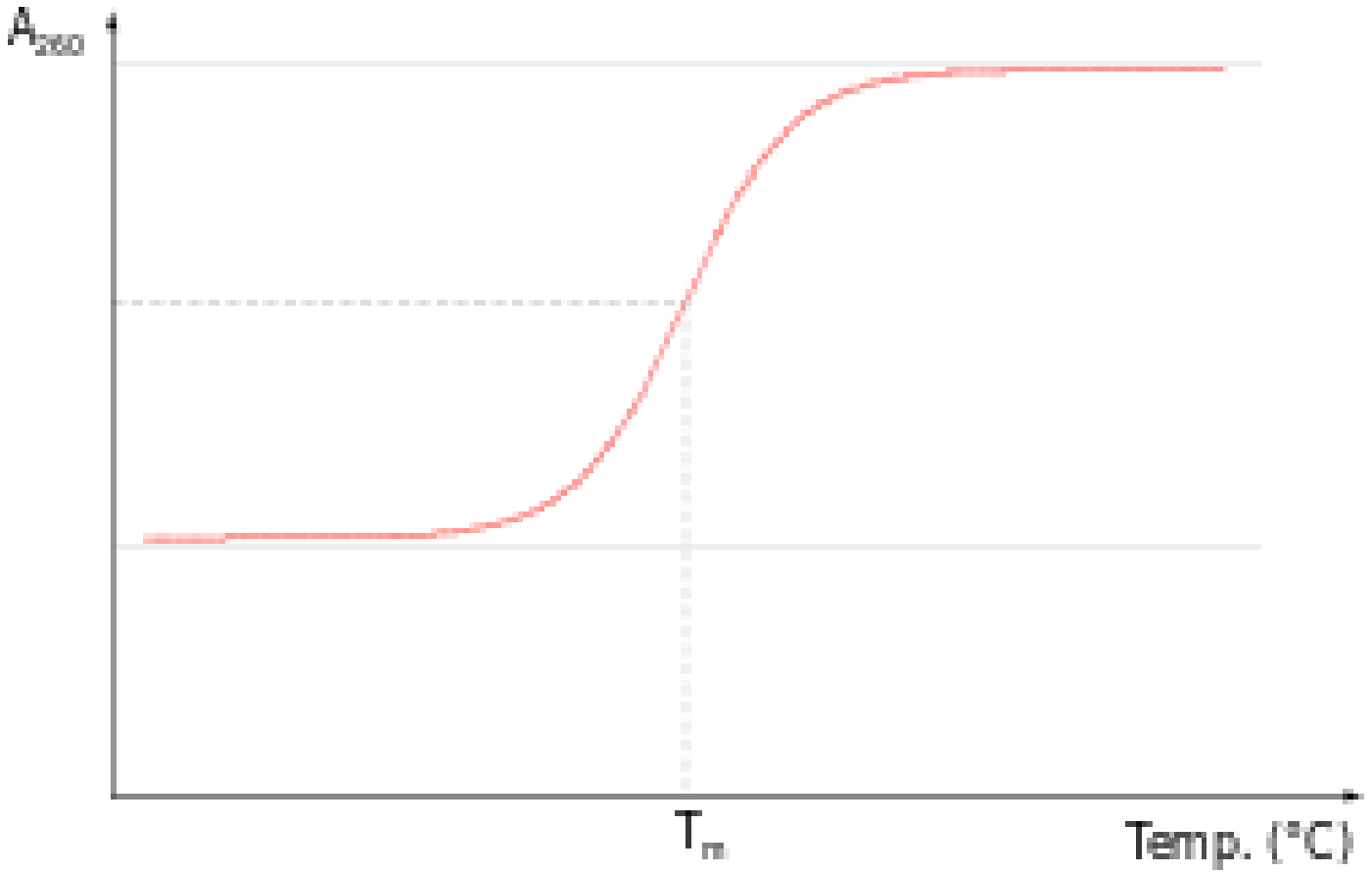
Hyperchromicity is the increase of absorbance (optical density) of a material. The most famous example is the hyperchromicity of DNA that occurs when the DNA duplex is denatured. The UV absorption is increased when the two single DNA strands are being separated, either by heat or by addition of denaturant or by increasing the pH level. The opposite, a decrease of absorbance is called hypochromicity.

Hyperchromicity in DNA denaturation

Heat denaturation of DNA, also called melting, causes the double helix structure to unwind to form single stranded DNA. When DNA in solution is heated above its melting temperature (usually more than 80 °C), the double-stranded DNA unwinds to form single-stranded DNA. The bases become unstacked and can thus absorb more light. In their native state, the bases of DNA absorb light in the 260-nm wavelength region. When the bases become unstacked, the wavelength of maximum absorbance does not change, but the amount absorbed increases by 37%. A double stranded DNA strand dissociating to two single strands produces a sharp cooperative transition.

Hyperchromicity can be used to track the condition of DNA as temperature changes. The transition/melting temperature (Tm) is the temperature where the absorbance of UV light is 50% between the maximum and minimum, i.e. where 50% of the DNA is denatured. A ten fold increase of monovalent cation concentration increases the temperature by 16.6 °C. The hyperchromic effect is the striking increase in absorbance of DNA upon denaturation. The two strands of DNA are bound together mainly by the stacking interactions, hydrogen bonds and hydrophobic effect between the complementary bases.

Nucleic acid melting curve showing hyperchromicity as a function of temperature



The hydrogen bond limits the resonance of the aromatic ring so the absorbance of the sample is limited as well. When the DNA double helix is treated with denatured agents, the interaction force holding the double helical structure is disrupted. The double helix then separates into two single strands which are in the random coiled conformation.

At this time, the base-base interaction will be reduced, increasing the UV absorbance of DNA solution because many bases are in free form and do not form hydrogen bonds with complementary bases. As a result, the absorbance for single-stranded DNA will be 37% higher than that for double stranded DNA at the same concentration

THANK YOU