NUCLEIC ACIDS

Every living organism has intrinsic ability to replicate itself. To do this ,they must possess complete information about themselves. This information is usually stored in nucleic acids. Nucleic acids are non-protein nitrogenous substances made up of monomeric units called nucleotides. Nucleic acids are thus polymers of nucleotides. Two types of nucleic acids abound; ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)

DEOXYRIBONUCLEIC ACID (DNA)

DNA is a polymer of deoxyribonucleotides joined by phosphodiester bonds, that are found in chromosomes, mitochondria and chloroplast. Nuclear DNA is found bound to proteins called histones. DNA is present in every nucleated cell and carries genetic information

ISOLATION OF DNA

DNA can be isolated from viruses, spleen and leucocytes. Homogenize tissue in neutral pH, centrifuge first at low speed to get nuclear pellets. The pellets are then extracted with 2m Nacl which separates DNA from proteins. The DNA in solution is further precipitated with alcohol. Treatment with ribonuclease to destroy RNA leaves DNA in the precipitate.

HISTONES

These constitute a small family of closely related basic proteins found in chromatin.

H_1 Histones; these are easily detectable, least tightly bound and can be removed by salt solution. The organizational unit of this soluble chromatin is nucleosome

TYPES OF HISTONES

Nucleosome contain 4 types of histones. These are; H_2A, H_2B, H_3 and H_4 .The structure of all 4 types of histones are highly conserved between species indicating functional similarities in all eukaryotes.

Structure of Histones

The –COOH terminal 2/3rd of the molecule have a typical random amino acid composition, but the first 1/3rd of the –NH_3 terminal are rich in basic amino acids. These 4 types of histones interact with each other in specific ways as follows;

H_3 and H_4 form tetramers containing two molecules of each $(H_3H_4)_2$.

H_2A and H_2B form dimers (H_2AH_2B)

Under physiological conditions, these histone oligomers associate to form histone octomers in the composition $(H_3H_4)_2(H_2A\text{-}H_2B)_2$

In the nucleosome the DNA is supercoiled in a left handed helix over the surface of the disc-shaped histone octomer. The 146 base pair of DNA consisting of 1.75 super helical turns are in contact with the octomer

FUNCTION

The association of DNA with histone octomer protects DNA from digestion by a nuclease

PRIMARY STRUCTURE OF DNA

Chromosomal DNA consists of a very long DNA molecule (Mw 1.6×10^6-2×10^9)

Each DNA is a polymer of about 10^{10} nucleotides.

Four types of bases are present in DNA (A,G,C,T) and RNA (A,G,C,U)

Nucleotides of each of the two strands of the helical structure are bound to each other by a 3',5' –phosphodiester bond.. Each phosphodiester linkage is formed by an ester bond between a 5' phosphate group of one nucleotide with the 3' OH group of the ribose sugar of the adjacent nucleotide. This gives rise to a linear polydeoxyribonucleotide strand with two free ends, the 3' and the 5' ends.

SECONDARY STRUCTURE OF DNA

This is the double stranded helix formed by two polydeoxyribonucleotide strands around a central axis, known as the Watson and Cricks model (published in nature in 1953 and received a nobel price in 1962)

DNA is a double stranded α-helical structure. Each of the two strands is coiled about a central axis in a right handed helix.

Each of the two strands is coiled around each other about a central axis in a right handed helix. The two sugar phosphate backbones wind around the outside of the bases like the banisters of a spiral staircase and are exposed to the aqueous environment. The phosphodiester bonds in the two interwoven strands run in opposite directions. They are said to be anti-parallel. Hence the polarity of the two strands will be 3'-5' and 5'-3'. The 3' – 5' is called the leading or template strand while the 5'-3' is the non-coding or lagging strand. The aromatic rings of the bases are hydrophobic and they are stacked in the interior nearly perpendicular to the long axis of the helix

Adenine pairs with thymine (two hydrogen bonds) while guanine pairs with cytosine (three hydrogen bonds).

The ratio of purines and pyrimidine bases in any DNA is always equal to one A+G=C+T ie A+G/C+T =1 .This is Chargaff's rule

In DNA, the glycosidic bonds between sugar and bases are not directly opposite each other and two grooves of unequal width form around the double helix. The edge of the helix >180^{o} is the major groove but if <180^{o} is the minor groove.

TYPES OF DNA

DNA often exist in different conformations

B-DNA

Adjacint nucleotides in each chain is rotated by 34.6^{o} to each other

Double helix complete one turn after every 10.4 base pairs

Each base pair increase the length of the double helix by 0.33nm.

One turn of a double helix span a distance of 3.4 nm (the pitch of the double helix).

The diameter of the double helix is 2.73 nm

A-DNA

When DNA crystals are dried or when salt content of crystals is decreased, the long B-DNA molecule become short, stubby molecule called A-DNA

The pitch of A-DNA is 2.46nm and number of base pair is 11. A-DNA is not found under physiological condition

Z-DNA is longer and thinner than B-DNA .It has left handed helix.

One complete turn of Z-DNA has 12 base pairs and the pitch of double helix is 4.5 nm. The diameter of the double helix is 1.84 nm. The major groove in Z-DNA is no more than a convex surface.

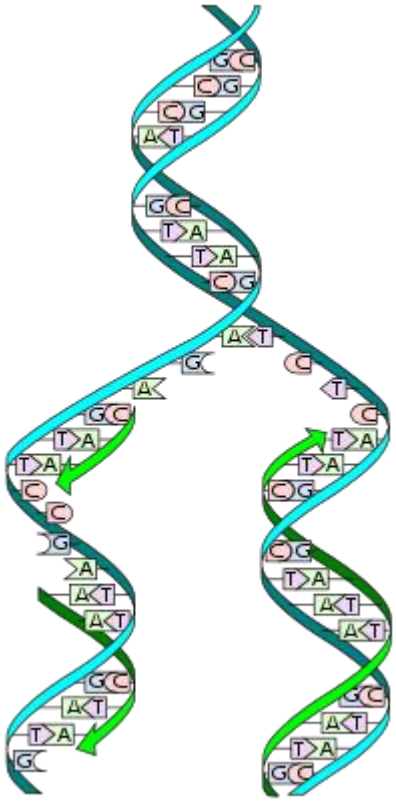
DNA DENATURATION

Two strands 0f DNA unwind or separate during processes such as DNA replication, RNA transcription and genetic recombination. In vitro, complete unwinding of DNA occur, a process called helix to coil transition. Denaturation occurs if H-bonds between base pairs break. Heating also unwinds DNA. Tm is the temperature at which ½ of DNA is denatured. At Tm, absorbance of DNA is increased by 18.5% ie half of 37% at 360nm. This phenomenon is known as hyperchromicity or hyperchromic effect. Increase in G-C base pairs in DNA increases Tm. Hence base content of DNA influence Tm.

ANNEALING

Recombination of denatured DNA occur on cooling. It can occur only at temperatures below Tm ie below 70^oC. Annealing is fastest at temperatures 20^oC below the Tm which is 50^oC.

DNA replication



DNA replication:

The double helix is un'zipped' and unwound,

Each separated strand acts as a template for replicating a new partner strand

Nucleotides (bases) are matched to synthesize the new partner strands into two new double helices.

In molecular biology, DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. DNA replication occurs in all living organisms acting as the basis for biological inheritance. The cell possesses the distinctive property of division, which makes replication of DNA essential.

TYPES OF REPICATION

Conservative Replication; Here the two strands of old DNA are paired and the two new daughter DNA strands are paired together

Semi Conservative replication Each of the old DNA strand is paired with a newly synthesized strand. Meselson and Stahl has demonstrated using isotope labelled DNA that DNA replication in vivo is semi-conservative.

Theta Replication; This is the type of replication that occurs in circular DNA Since DNA functions to transfer genetic information from parents to progeny, it means that DNA replication must be complete and must be carried out with high fidelity to maintain genetic fidelity between species.

DNA POLYMERASE 1 also known as the Konberg Enzyme is primarily a repair enzyme and brings about deoxyribonucleotide polymerization. It has both 5'->3' and 3'->5' exonuclease activities.

When the 5'→3' exonuclease domain is removed, the remaining enzyme molecule retains the polymerization and proofreading activities. Such a fragment is known as the Klenow fragment which is widely used in recombinant DNA technology

DNA POLYMERASES II ; These are major enzymes involved in repair and deoxyribonucleotide polymerization

In Prokaryotes, three types of DNA polymerases abound. They are DNA Polymerase 1, DNA polymerase ll and DNA polymerase lll. In Eukaryotes;There are five typesof DNA polymerases namely α,B,d,€,¥

DNA Helicase Required for unwinding of dsDNA

DNA Premase Required for synthesis of RN A primer

Nick Sealing Enzyme

Topoisomerases

DNA ligases

Single strand binding Proteins (SSB Proteins)

SALIENT POINTS IN DNA REPLICATION

Replication aims at complete information to the progeny

Replication must be complete and with high fidelity

Several proofreading mechanisms needed

Origin of replication must be targeted precisely and unwound to enable formation of replication complex

After replication both parent and daughter DNA must reform dsDNA

In Eukaryotes the replicated DNA must reform domain structures including nucleosomes that existed prior to replication

Replication mechanisms has been worked out in prokaryotes and appears to be the same in Eukaryotes.

ESSENTIAL EVENTS IN DNA REPLICATION

The events of replication can arbitrarily be divided into five processes namely

Identification of Origin of Replication (ori)

Unwinding of dsDNA to provide ssDNA to serve as template

Formation of Replication fork

Initiation and

Chain elongation

Formation of replication bubbles and the ligation of newly synthesized DNA segments

ORIGIN OF REPLICATION

Replication starts at specific sites called origin of Replication (ori)

At ori,

There is association of sequence specific DNA binding proteins with a series of direct repeat DNA sequences.

Adjacint to ori , is A-T rich region.

A specific interaction of the origin binding protein with the origin of replication leads to the denaturation and unwinding of the dsDNA at the A-T rich region near ori.

DIFFERENCE OF PROKARYOTIC AND EUKARYOTIC DNA POLYMERASE

PROKARYOTIC	EUKARYOTIC	FUNCTION
I	α	Gap filling and synthesis of lagging strand
II	€	Proofreading and DNA repair
	B	DNA Repair
	y	Required for mitochondrial synthesis
III	*d*	Helps in leading strand synthesis

UNWINDING OF DNA TO FORM ssDNA WHICH SERVES AS TEMPLATE

Protein interacts with ori and delineates the start point of replication and provides short strands of ssDNA that serves as template

DNA helicase is main unwinding enzyme and allows processive unwinding of DNA

SSB protein binds to stabilize the ssDNA and averts rewinding

Torsional stress caused by DNA Helicase unwinding action creates a nick in one strand to allow further unwinding

The nick is sealed by the nick-sealing enzyme, the Topoisomerase requiring no energy

FORMATION OF REPLICATION FORK

This requires the following 4 component;

DNA Helicase unwinds short segment of dsDNA to form ssDNA

A Primase that synthesizes short RNA primer

DNA polymerase initiates nascent DNA synthesis primed by the RNA primer

SSB Protein binds to ssDNA to prevent annealing to dsDNA

DNA Polimerase synthesizes DNA only in 5'-3' direction Since the two DNA strands run in opposite direction ,synthesis cannot occur in both strands in the same direction simultaneously

No DNA Polymerase can synthesis DNA in 3'-5' direction

Leading strand synthesis goes continuously using one primer

Lagging strand is synthesized discontinuously using many primers

They are synthesized in short spurts 1.5kb fragments called Okazaki fragments

The DNA Helicase associate with the Primase to form a mobile complex called Primosome

This association allows the Primase access to the DNA; which makes the RNA primer needed for DNA synthesis

Note DNA Polymerase cannot initiate DNA synthesis de novo.

When synthesis of an Okazaki fragment is completed, DNA Polymerase is released to allow for the synthesis of another RNA primer.

The same DNA Polymerase remains associated with the replication fork and continues with synthesis of a new Okazaki fragment and so on till several Okazaki fragments are formed on the lagging strand.

INITIATION AND ELONGATION

Initiation of DNA synthesis requires priming by short length of RNA (10-200 nucleotides)

PRIMING requires a nucleophilic attack of the 3'-OH of the RNA Primer on the α-phosphate of the first entering deoxyribonucleotide triphosphate with the release of a ppi.

ELONGATION

Now the 3'-OH group of the newly attached deoxyribonucleotide monophosphate is then free to carry out a nucleophilic attack on the next entering deoxyribonucleotide triphosphate again at its α-phosphate moiety with release of PPi

Note the nascent DNA is always made in the 5'-3' direction because DNA Polymerase can add a nucleotide only to the 3' end of a DNA strand

The selection of the new deoxyribonucleotide triphosphate to be added is strictly governed by base pairing rule.

FORMATION OF REPLICATION BURBLES AND LIGATION OF NEWLY SYNTHESIZED DNA FRAGMENTS

Entire mammalian genome replicates in about 9hours (average time required to form a tetraploid genome from a diploid genome)

IF replication is done from a single ori, it will take approx 150hours

To avoid this; (a) Replication occurs from multiple origins (approx 100 in humans)in each chromosome (b) Replication occurs bidirectionally resulting in the formation of replication bubbles

LIGATION OF SYNTHESIZED DNA

After synthesis of Okazaki fragments in mammals, the RNA primers are removed and filled with properly paired deoxyribonucleotide and the remaining nick is filled by the nick-sealing enzyme the DNA ligase. This process requires energy in form of ATP

REPLICATION IS SEMICONSERVATIVE

DNA is made up of a double helix of two complementary strands.

During replication, these strands are separated.

Each strand of the original DNA molecule then serves as a template for the production of its counterpart, a process referred to as semiconservative replication.

As a result of semi-conservative replication, the new helix will be composed of an original DNA strand as well as a newly synthesized strand.

Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication.

DNA structure

DNA exists as a double-stranded structure, with both strands coiled together to form the characteristic double-helix. Each single strand of DNA is a chain of four types of nucleotides. Nucleotides in DNA contain a deoxyribose sugar, a phosphate, and a nucleobase.The four types of nucleotide correspond to the four nucleobases adenine, cytosine, guanine, and thymine, commonly abbreviated as A, C, G and T. Adenine and guanine are purine bases, while cytosine and thymine are pyrimidines. These nucleotides form phosphodiester bonds, creating the phosphate-deoxyribose backbone of the DNA double helix with the nucleobases pointing inward (i.e., toward the opposing strand). Nucleobases are matched between strands through hydrogen bonds to form base pairs. Adenine pairs with thymine (two hydrogen bonds), and guanine pairs with cytosine (three hydrogen bonds).

DNA STRANDS ARE ANTI-PARALLEL

DNA strands have a directionality, and the different ends of a single strand are called the "3′ (three-prime) end" and the "5′ (five-prime) end".

By convention, if the base sequence of a single strand of DNA is given, the left end of the sequence is the 5′ end, while the right end of the sequence is the 3′ end.

The strands of the double helix are anti-parallel with one being 5′ to 3′, and the opposite strand 3′ to 5′.

These terms refer to the carbon atom in deoxyribose to which the next phosphate in the chain attaches.

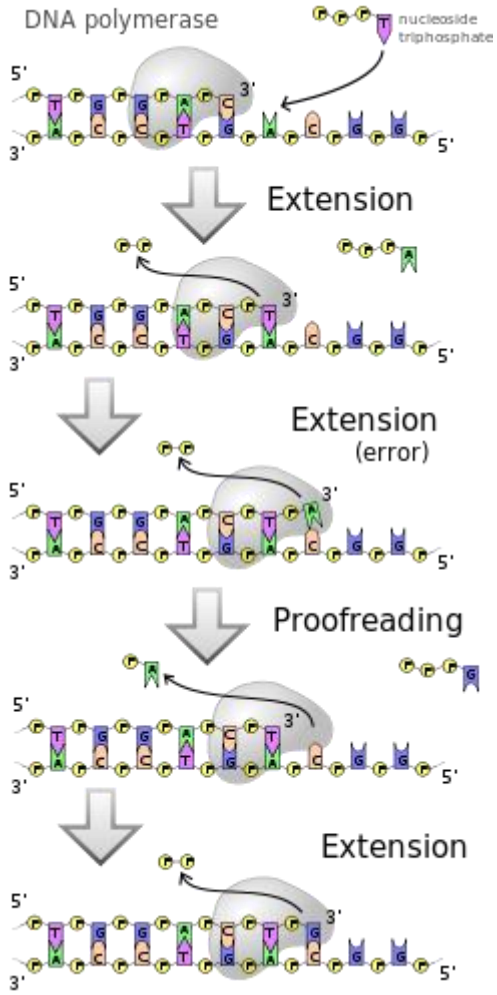
Directionality has consequences in DNA synthesis, because DNA polymerase can synthesize DNA in only one direction by adding nucleotides to the 3′ end of a DNA strand.

The pairing of complementary bases in DNA (through hydrogen bonding) means that the information contained within each strand is redundant.

Phosphodiester (intra-strand) bonds are stronger than hydrogen (inter-strand) bonds. This allows the strands to be separated from one another.

The nucleotides on a single strand can therefore be used to reconstruct nucleotides on a newly synthesized partner strand

DNA polymerase



DNA polymerases add nucleotides to the 3′ end of a strand of DNA. If a mismatch is accidentally incorporated, the polymerase is inhibited from further extension. Proofreading removes the mismatched nucleotide and extension continues.

DNA polymerases

They are a family of enzymes that carry out all forms of DNA replication.

DNA polymerases in general cannot initiate synthesis of new strands, but can only extend an existing DNA or RNA strand paired with a template strand.

To begin synthesis, a short fragment of RNA, called a primer, must be created and paired with the template DNA strand.

DNA polymerase adds a new strand of DNA by extending the 3′ end of an existing nucleotide chain, adding new nucleotides matched to the template strand one at a time via the creation of phosphodiester bonds.

The energy for this process of DNA polymerization comes from hydrolysis of the high-energy phosphate (phosphoanhydride) bonds between the three phosphates attached to each unincorporated base.

Free bases with their attached phosphate groups are called nucleotides; in particular, bases with three attached phosphate groups are called nucleoside triphosphates.

When a nucleotide is being added to a growing DNA strand, the formation of a phosphodiester bond between the proximal phosphate of the nucleotide to the growing chain is accompanied by hydrolysis of a high-energy phosphate bond with release of the two distal phosphates as a pyrophosphate.

Enzymatic hydrolysis of the resulting pyrophosphate into inorganic phosphate consumes a second high-energy phosphate bond and renders the reaction effectively irreversible.

In general, DNA polymerases are highly accurate, with an intrinsic error rate of less than one mistake for every 107 nucleotides added. In addition, some DNA polymerases also have proofreading ability; they can remove nucleotides from the end of a growing strand in order to correct mismatched bases. Finally, post-replication mismatch repair mechanisms monitor the DNA for errors, being capable of distinguishing mismatches in the newly synthesized DNA strand from the original strand sequence. Together, these three discrimination steps enable replication fidelity of less than one mistake for every 109 nucleotides added.

The rate of DNA replication in a living cell was first measured as the rate of phage T4 DNA elongation in phage-infected E. coli. During the period of exponential DNA increase at 37 °C, the rate was 749 nucleotides per second.

The mutation rate per base pair per replication during phage T4 DNA synthesis is 1.7 per 108.

Clamp proteins form a sliding clamp around DNA, helping the DNA polymerase maintain contact with its template, thereby assisting with processivity. The inner face of the clamp enables DNA to be threaded through it. Once the polymerase reaches the end of the template Cor detects double-stranded DNA, the sliding clamp undergoes a conformational change that releases the DNA polymerase. Clamp-loading proteins are used to initially load the clamp, recognizing the junction between template and RNA primers.

DNA replication proteins

At the replication fork, many replication enzymes assemble on the DNA into a complex molecular machine called the replisome. The following is a list of major DNA replication enzymes that participate in the replisome.

Enzyme	Function in DNA replication
DNA Helicase	Also known as helix destabilizing enzyme. Helicase separates the two strands of DNA at the Replication Fork behind the topoisomerase.
DNA Polymerase	The enzyme responsible for catalyzing the addition of nucleotide substrates to DNA in the 5′ to 3′ direction during DNA replication. Also performs proof-reading and error correction. There exist many different types of DNA Polymerase, each of which performs different functions in different types of cells.
DNA clamp	A protein which prevents elongating DNA polymerases from dissociating from the DNA parent strand.
Single-Strand Binding (SSB) Proteins	Bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it, thus

	maintaining the strand separation, and facilitating the synthesis of the nascent strand.
Topoisomerase	**Relaxes the DNA from its super-coiled nature.**
DNA Gyrase	**Relieves strain of unwinding by DNA helicase; this is a specific type of topoisomerase**
DNA Ligase	**Re-anneals the semi-conservative strands and joins Okazaki Fragments of the lagging strand.**
Primase	**Provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand.**
Telomerase	**Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes. This allows germ cells and stem cells to avoid the Hayflick limit on cell division.**

Replication machinery

Replication machineries consist of factors involved in DNA replication and appearing on template ssDNAs. Replication machineries include primosomes are replication enzymes; DNA polymerase, DNA helicases, DNA clamps and DNA topoisomerases, and replication proteins; e.g. single-stranded DNA binding proteins (SSB).

In the replication machineries these components coordinate. In most of the bacteria, all of the factors involved in DNA replication are located on replication forks and the complexes stay on the forks during DNA replication. These replication machineries are called replisomes or DNA replicase systems. These terms are generic terms for proteins located on replication forks. In eukaryotic and some bacterial cells the replisomes are not formed.

Since replication machineries do not move relatively to template DNAs such as factories, they are called a replication factory. In an alternative

figure, DNA factories are similar to projectors and DNAs are like as cinematic films passing constantly into the projectors. In the replication factory model, after both DNA helicases for leading strands and lagging strands are loaded on the template DNAs, the helicases run along the DNAs into each other.

Regulation

cell cycle of eukaryotic cells.

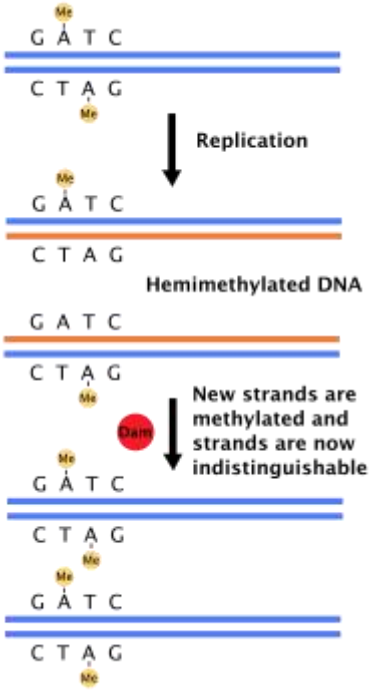
Eukaryotes

Within eukaryotes, DNA replication is controlled within the context of the cell cycle. As the cell grows and divides, it progresses through stages in the cell cycle; DNA replication takes place during the S phase (synthesis phase). The progress of the eukaryotic cell through the cycle is controlled by cell cycle checkpoints. Progression through checkpoints is controlled through complex interactions between various proteins, including cyclins and cyclin-dependent kinases.[29] Unlike bacteria, eukaryotic DNA replicates in the confines of the nucleus.[30]

The G1/S checkpoint (or restriction checkpoint) regulates whether eukaryotic cells enter the process of DNA replication and subsequent division. Cells that do not proceed through this checkpoint remain in the G0 stage and do not replicate their DNA.

Replication of chloroplast and mitochondrial genomes occurs independently of the cell cycle, through the process of D-loop replication.

Bacteria



Dam methylates adenine of GATC sites after replication.

Most bacteria do not go through a well-defined cell cycle but instead continuously copy their DNA; during rapid growth, this can result in the concurrent occurrence of multiple rounds of replication.[31] In *E. coli*, the best-characterized bacteria, DNA replication is regulated through several mechanisms, including: the hemimethylation and sequestering of the origin sequence, the ratio of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), and the levels of protein DnaA. All these control the binding of initiator proteins to the origin sequences.

Because *E. coli* methylates GATC DNA sequences, DNA synthesis results in hemimethylated sequences. This hemimethylated DNA is recognized by the protein SeqA, which binds and sequesters the origin sequence; in addition, DnaA (required for initiation of replication) binds less well to hemimethylated DNA. As a result, newly replicated origins are prevented from immediately initiating another round of DNA replication.[32]

TRANSCRIPTION

The central dogma states that genes are units perpetuating themselves and functioning through their expression as proteins

Genes carry genetic information in DNA sequences. These are perpetuated by replication and expressed by a two stage process

Transcription (Synthesis of RNA from DNA) and

Translation (Protein synthesis from RNA

The flow of information was thought to be irreversible but this has been disproved by reverse transcriptase

TRANSCRIPTION

This is Synthesis of RNA from DNA by RNA Polymerase. RNA sequence is complementary to the DNA strand from which it is transcribed. The DNA strand serves as a template for RNA synthesis.

RNA POLYMERASE

This is the chief enzyme of RNA synthesis

Synthesizes all types of RNA in Bacteria ie mRNA, rRNA and tRNA

Eukaryotes have 3 types of RNA polymerase

RNA Polymerase I

RNA Polymerase II and

RNA Polymerase III

COMPOSITION OF RNA POLYMERASE

Units	No	Mass(Daltons)	location	Function
α	2	40,000 each	Core enzyme	promoter binding

B	**1**	**185000**	**Core Enzyme**	**Nucleotide Binding**
B'	**1**	**160000**	**Core Enzyme**	**Template binding**
d(sigma)	**1**	**85000**	**Sigma Factor**	**Initiator protein**

Holo Enzyme is α_2BB'd.

Sigma factor d polypeptide

1) **It needs a template of double stranded DNA or occasionally single stranded DNA. Four ribonucleotise triphosphates ; CTD, UTP. GTP andATP plus Mg^{2+} ,Mn^{2+}**
2) **Note Only the holoenzyme can initiate transcription, then the sigma factor dissociates and is released after initiation. The core enzyme can continue to polymerize RNA using DNA template but it cannot initiate transcription at the correct site.**

STAGES OF TRANSCRIPTION

Can be divided into 4 stages

a) **Formation of transcription complex (of DNA and RNA Polymerase)**
b) **Initiation**
c) **Elongation and**
d) **Termination**

FORMATION OF INITIATION COMPLEX

a) **RNA Polymerase binds with a specific sequence on the DNA called the Promoter site**
b) **Promoter site is variable in prokaryotes (20-200 bases)**
c) **Core enzyme cannot recognize the promoter site. Sigma factor is needed for recognition and initiation of transcription complex**

Four steps are involved in transcription or initiation complex formation

1) **Sigma factor recognizes the promoter sequence**
2) **RNA polymerase attaches to promoter region**
3) **RNA Polymerase melts the helical structure and separates the two strands of DNA locally**

4) **RNA Polymerase initiates RNA Synthesis**

Point at which the first ribonucleotide is inserted is called the start site or start point

CHARACTERISTICS OF PROMOTER SITE

a) **The Pribnow box is a sequence contained with the promoter region**
b) **It is 5-10 bases to the left ie upstream the first 4 bases that will be copied into RNA**
c) **It orients RNA Polymerase as to the direction and start of RNA synthesis.**
d) **All Pribnow boxes are variants of TATAATG.. sequences often referred to as TATA box**
e) **The T at position 6 (conserved T) is found in every promoter region of the box**
f) **The –'35' sequence is a second recognition site in many promoter regions upstream from Pribnow box. It is thought to be the initial site of *d(sigma) subunit* binding. Typically it contains 9 bases.**
g) **Being a large molecule, RNA Polymerase also comes into contact with the Pribnow box.**
h) **Once bound to the Pribnow box, RNA Polymerase dissociates from the initial binding site.**
i) **The complex is active intermediate in RNA chain initiation. One important event is the melting of DNA duplex which takes place about 10 base pairs upstream of Pribnow box and extending up to the first transcribed base at the start point.**

INITIATION

a) **Core enzyme begin transcription of the unwound section of DNA strand of an initiation complex.**
b) **As the enzyme moves along , the unwound region moves along with it.**
c) **The first base to be copied is always within 6 to 9 bases of the conserved T of the Pribnow box on the unwound portion of the 3'-5' strand.**
d) **The subunit of RNA Polymerase has two specific binding sites for binding of nucleotide triphosphated (NTP)**
e) **H-bonding formation follows the base-pairing rule .The first incoming NTP binds to the RNA polymerase at the start point of initiation site and H-bond to the complementary base on the DNA.**

f) **This site binds only purine NTP ie either A or G. The binding is with 3' end of NTP leaving 5' end to be free.**
g) **The second incoming NTP binds to the elongation site on the RNA Polymerase The NTP is selected in accord with the base pairing rule which can H –bond with the complementary base on the DNA. This dinucleotide tetraphosphate has either PPPA or PPPGs as the 5'terminal nucleotide. After this phosphodiester bond formation , the sigma factor dissosciates and is released.The first base now dissociates from the initiation site marking the completion of initiation**

ELONGATION

a) **The core-RNA polymerase moves in the 3'-5' direction of the coding strand and it adds successive NTPs at the 3' -OH end of the ribonucleotide chain already laid down in 5'-3' direction**
b) **The incoming NTP forms a phosphodiester bond with the 3'-OH group of the preceding ribonucleotide.**
c) **The base is determined by the sense strand by base pairing rule.**
d) **The DNA helix recloses after RNA Polymerase transcribes through it and growing RNA chain dissociates from the DNA.**

TERMINATION

a) **Special sequences on the DNA molecule function as the signal for termination of transcription process.**
b) **The signal could be inverted GC rich regions separated by intervening region followed by AT rich sequences.**

A sequence of Adenine that codes for 6-8 uracil residues. The uracil residues are followed by one Adenine

There is no unique base that codes for termination of transcription, eg for a given promoter, RNA might end with 5U's or 6U's + 1A

Rho (*p*) protein and the sequences mentioned, together bring about termination. At specific termination sites, the new RNA chain may be released . The Rho protein binds very tightly to the RNA (not to the Polymerase and in this bound state it acts like an ATPase

The Rho factor then dissociates RNA and RNA Polymerase from the DNA

POST TRANSLATIONAL MODIFICATION

All RNAs are obtained by transcription The required modifications take place after they are released from polysomes

MODIFICATION OF NUCLEOSIDE

Methyltransferases, deaminases and dehydrogenases may methylate, deaminate or reduce the bases into the 'minor' bases eg 5' methylcytosine,

N^6 –methyladenine, hypoxanthine, dihydrouracil etc. Uridine may be converted to pseudouridine.

LIGATION AND CLEAVAGE OF NUCLEOTIDES

The gene contains exons and introns .The introns need to be separated out and exons must be joined as they are actually amino acids coding sequences. Specific nucleases and ligases bring about this function. These change RNA into functional mRNA.

Additional nucleotides may be added to the end of the mRNA transcript, eg 7 methyl GTP cap is added at 5'end while poly A-tail is added at the 3'end

Different subspecies of r-RNA such as 5.8S, 18S, 28S r-RNA are made after transcription from a precursor RNA

INHIBITION OF RANSCRIPTION

Several substances have been found to inhibit Transcription

RIFAMYCIN; Rifamycin and Streptovaricin binds with *B*-subunit of the polymerase to block the initiation of transcription

ANTIMYCIN D; It forms a complex with double stranded DNA helix .This way it prevents movement of core enzyme and thus inhibits the process of chain elongation

STREPTOGLYDIGIN; It binds with the *B-subunit of prokaryotic DNA* polymerase and thus inhibits elongation

HEPARIN; It is a polyanion that binds to the B'-subunit and inhibits transcription in vitro. The a-subunit has no known role in transcription

GENETIC CODE

Transcription copies the base sequence in DNA and makes it available as mRNA for translation into specific protens. Four kinds of bases exist (A,U,C,G). Also 20 different amino acids are found in proteins So neither one nor two bases can specify all the 20 amino acids.It has been proven that a group of three bases referred to as CODONS specifies or codes for an amino acid.

The 64 combinations of three bases coding for amino acids (initiating and stopping protein synthesis) are arranged in the form of a table commonly referred to as the genetic code

The sequence of coding strand of DNA read in the 5'-3' direction consists of triplets corresponding to the amino acid sequence of the orotein read from the N-terminus to C-terminus

CHARACTERISTICS OF GENETIC CODE

DEGENERACY;

One remarkable feature of genetic code is degeneracy of the 61 codons that codes for the 21 amino acids.

But for Methionine, all other amino acid is coded by more than one codon

Codons that represent one amino acid are called synonyms

Codons representing one amino acid tend to be clustered in groups

Often the base at the third position of the codon tends to be insignificant. This is so because four codons differing only in the third base represent the same amino acid. Sometimes distinction is made only between a purine versus a pyrimidine in this position

This reduced specificity at the third position is is known as third base degeneracy or wobbling phenomenon. This feature together with the endency of similar amino acids to be represented by related codons minimizes the effects of mutations. It increases the probability that a single random base changewill result in no amino acid substitution or in one involving amino acids of similar character

UNAMBIGUITY

Each codon designates only one amino acid and does not lead to the incorporation of any unspecified amino acid into the peptide chain.

UNIVERSALITY

In all living organisms, the genetic code is the same. The onlexception is in mitochondrial genome where AUA codes for methionin andUGA for tryptophan instead of isoleucine and termination or stop respectively

AGA and AGG codes for Arginine in normal condition but in mitochondrialgene, it codes for termination of protein synthesis.

Most of the amino acid substitution in proteins can be accounted for by a change of a single DNA base

COLINEARITY OF GENE AND GENE PRODUCTS

The product of gene is a protein specified by base sequence.

High resolution genetic mapping technique showsthat there is linear correspondence in base sequence in genes and amino acids sequence in protein

NON-OVERLAPPING

All codons re independent sets of 3 bases

There is no overlapping. No base function as a common member of two consecutive codons

COMMALESSNESS

Codons are arranged as a contimuous structure. There is not one or more nucleotides between consecutive codons

The last nucleotide of preceeding codon is immediately followed by the first nucleotide of succeeding nucleotide

TRANSLATION

Protein is a polymer of amino acids joined to each other by peptide bonds. The synthesis of proteins from mRNA is known as translation. During translation, amino acids are incorporated sequentially in a specific number and sequence determined by the sequence of codons in the genetic code of the relevant m-RNA.

MATERIALS REQUIRED FOR PROTEIN SYNTHESIS

Amino acids—at least 20 amino acids

DNA and three RNAs-m-RNA,r-RNA ant t-RNA.

Polyribosomes (Polysomes)

ENZYMES

Amino acyl-t-RNA Synthetase enzyme required for activation of amino acids

Peptide Synthetase (Peptidyltransferase)

FACTORS

Initiation factors-eIF-1, eIF-2, eIF-3, eIF-4A, eIF-4B, eIF-4G, eIF-4E, eIF-5

Elongation factors—EF_1 and EF_2

Release Factors R1 and R2 .

COENZYMES AND COFACTORS

$F.H_4$ –Required in prokaryotes only to formylate o methionine

- Mg^{++}

ENERGY; ATP and GTP.

RIBOSOMES

Protein synthesis occurs on ribosomes which are large particles.

Ribosomes are nucleoproteins with 65% RNA and 35% proteins

Sedimentation coefficient is 70S (prokaryotes) and 80S (eukaryotes)

EDTA chelate Mg^{++} and so split it into two unequal parts. Mg needed to hold the 2 subunits together

The two subunits in prokaryotes are 50S large and 30S small subunit and in eukaryotes 60S and 40S.

50S contains34 proteins(L-proteins) and 2moles of 23S and 5S r-RNA

30S contains 21 proteins S-proteins) and a 16S r-RNA

Most of the ribosomal proteins (S-, and L-) are low molecular weight basic proteins. This enables them to interact well with negatively charged RNA.

The RNAs in ribosomal subunits have a specific well defined secondary structure and they interact with ribosomal proteins in a well defined manner.

Eukaryotic 60S subunit of ribosome contains 45 proteins, and 28S, 5.8S and 5S r-RNA. The 40S contains 30 proteins and 18S RNA

Mitochondrial and prokaryotic ribosomes are similar.

Polysomes or polyribosomes are beaded string-like linear clusters of 5S ribosomes on an m-RNA

Each ribosome has a peptidyl (P) and an aminoacyl (A) site

STEPS OF PROTEIN SYNTHESIS

After transcription, the process of proteinsynthesis can be subdivided into the following headings;

Activation of amino acids

Initiation

Elongation and

Termination

ACTIVATION OF AMINO ACIDS(formation of Aminoacyl-t-RNA