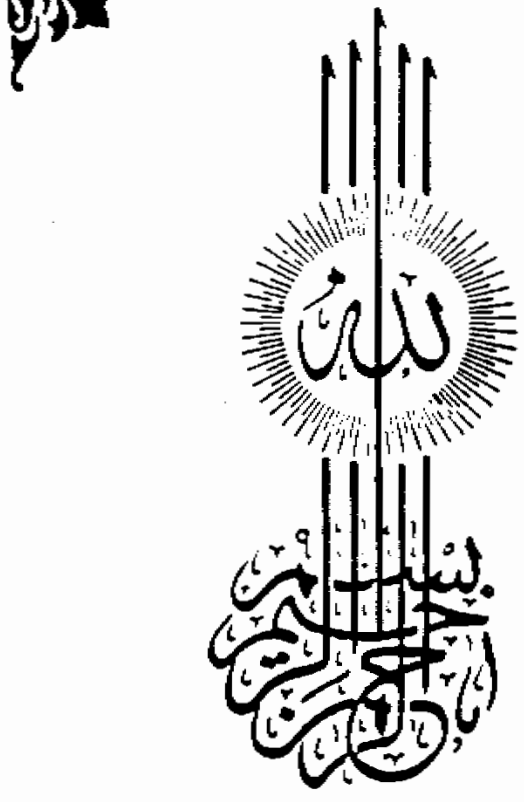


بسم الله الرحمن الرحيم

قالوا سبحانك لا علم لنا إلا ما
علمتنا إنك أنت العليم الحكيم.

صدق الله العظيم
البقرة - ٣٢ -

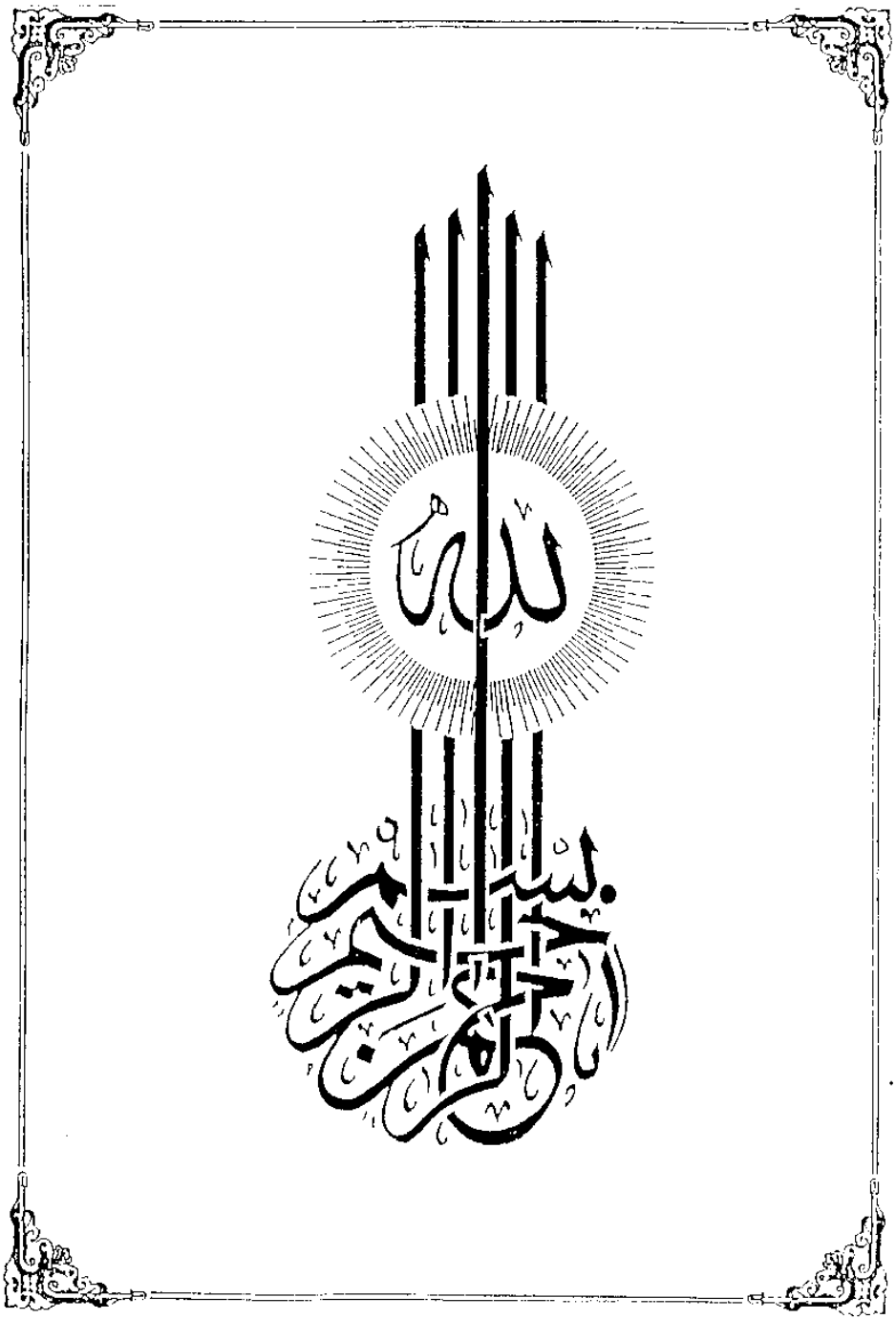
ACKNOWLEDGEMENT

I Would like to express my deepest appreciation and gratitude to Professor Dr. Nehayet Azmy for giving me the privilege of gaining her experience and helpful advice and constructive assistance.

I am also sincerely indebted to Assistant Professor Dr. Saliva Abu El Hana for her honest supervision with a keen interest. She gave me much of her time and fruitful advice throughout this work.

I owe special gratitude to Dr. Azza Sadek for her honest supervision, revision and constructive criticism. It is difficult to imagine how this work could have been delivered to light without her supervision.

Finally, I would like to record my deepest gratitude to my family, especially my mother for her great support.



List of Abbreviations

A	:	Adenine
ALL	:	Acute lymphoblastic leukemia
AML	:	Acute myeloid leukemia
APL	:	Acute promyelocytic leukemia
ASO	:	Allele specific oligonucleotide
α	:	Alpha
β	:	Beta
bcr	:	breakpoint cluster region
bp	:	base pair
C	:	cytosine
cDNA	:	complementary deoxyribonucleic acid
CLL	:	Chronic lymphocytic leukemia
CML	:	Chronic myeloid leukemia
cv	:	coefficient of variation
δ	:	Delta
D	:	Diversity
del	:	deletion
DIC	:	Dessiminated intravascular coagulopathy
DNA	:	Deoxyribonucleic acid
dT	:	Deoxy thmidylate
ε	:	Epsilon
E-coli	:	Escherichia coli
ELISA	:	Enzyme-linked immunosorbant assay
FAB	:	French-American-British collaborative group
FCM	:	Flow cytometer
G	:	guanine
γ	:	gamma
HD.	:	Hodgkins disease
IA	:	Image analysis
Ig	:	Immunoglobulin
IgH	:	Immunoglobulin heavy chain

IgL	:	Immunoglobulin light chain
IL	:	Interleukin
IFN	:	Interferon
inv	:	inversion
ISCN	:	International system for human cytogenetic nomenclature
IVS	:	Intevening sequence
J	:	Joining region
K	:	Kappa
Kb	:	Kilobase
Kd	:	Kilodalton
λ	:	Lambda
μ	:	Mu.
mRNA	:	Messenger ribonucleic acid
NHL	:	Non-Hodgkin's Lymphoma
p	:	Short arm of chromosome
32p	:	Phosphorus-32 (radio-isotope)
PCR	:	Polymerase chain reaction
PDGF	:	Platelet derived growth factor
Ph	:	Philadelphia chromosome
q	:	Long arm of chromosome
RNA	:	Ribonucleic acid
RFLPs	:	Restriction fragment length polymorphism
T	:	Thymine
t	:	translocation
TCR	:	T-cell receptor gene
TLC	:	Total leucocytic count
tRNA	:	transfer ribonucleic acid
U	:	Uracil
U V	:	Ultra-violet
V_k	:	Variable Kappa
V_λ	:	Variable lambda

CONTENTS

INTRODUCTION AND AIM OF WORK

DNA contains all the information required for the formation of cells, the regulation of their proliferation, their assembly into tissues and subsequently the development of these tissues into organs (Beutler, 1991).

DNA is a highly dynamic molecule. Structural rearrangements (insertion, deletion, amplification and translocation) are responsible for the generation of gene diversity as in immunoglobulin and T-cell receptor genes. This instability of DNA carries with it risks and can lead to adverse consequences (Bishop, 1991).

In fact tumoregenesis is considered to be the end result of multiple genetic accidents in somatic cells, ultimately resulting in autonomous cell growth and metastasis (Cairns, 1981; Weinberg, 1989; Bishop, 1991). Evidence for the somatic mutation theory is presented by the occurrence of chromosomal aberrations in cancer cells (Heim and Mitelman, 1987).

The identification and description of protooncogenes and tumor suppressor genes in the human genome and, in particular, their alterations in human tumors provide yet further proof that cancer is a genetic disease (Hunter, 1991).

The diagnosis and classification of malignant lymphomas and leukemias are complex challenges facing diagnostic pathologists. Genetic analysis has emerged as a precise laboratory aid in the identification of both their clonality and lineage. It also serves as a sensitive unique clonal marker to detect early occult recurrence in patients after therapy (Cossman et al., 1983).

DNA aberrations in leukemias and lymphomas can be studied at different levels of genomic organization: First, the total amount of DNA by either flow cytometry and image analysis; second, chromosomal studies if the cells enter the mitotic phases and the DNA is organized into chromosomes, and finally, molecular genetic analysis to investigate the cells at the level of individual genes or even at the level of single base pairs (Cornelisse et al., 1993).

The aim of this study is to review methods of studying DNA at the various levels of genomic organization and emphasize some of the important DNA abnormalities occurring in leukemias and lymphomas with stress on their values in diagnosis and prognosis.

DNA and Gene Expression

DNA Structure

DNA is a linear (i.e unbranched) polymer consisting of a sugar-phosphate backbone and four nitrogenous (purine or pyrimidine) bases which protrude from the backbone of the polymer. The sugar portion of DNA is deoxyribose, and the four bases are adenine (A), guanine (G), thymidine (T) and cytosine (C). DNA exists as a double helix in which A is always paired with T, and G is always paired with C (Fig. 1) (Maniatis et al, 1982).

The fundamental unit of the DNA polymer is the 'nucleotide", which consists of one molecule of deoxyribose linked to a base. The variant part of a DNA chain is the sequence of its basis, which can be in any order along the sugar phosphate backbone. Adjacent nucleotides are linked together by phosphodiester bonds between the 5'carbon of the deoxyribose moiety of one nucleotide and the 3' carbon of the next (Nevins, 1983). A linear strand of DNA, thus, has one end (3' end) having an unlinked sugar position ie. free at 3' carbon, and the other (the 5'end), a free 5' position. There is thus a 'polarity" to the sequence of bases in DNA strand; the same sequence of bases read in a 3' to 5' direction carries a different meaning than if read in 5' to 3' direction. Cellular enzymes that "read" along the DNA sequence tend to do so only in one direction. Nucleic acid synthesizing enzymes add new bases to the strand in a 5' to 3' direction (McKusich, 1987).

DNA molecules are thermodynamically most stable in a double-stranded form, but the double stranded form of DNA exists only if the sequence of bases on one strand is "matched" by a "complementary sequence of bases on the opposite strand. Complementary bases are those

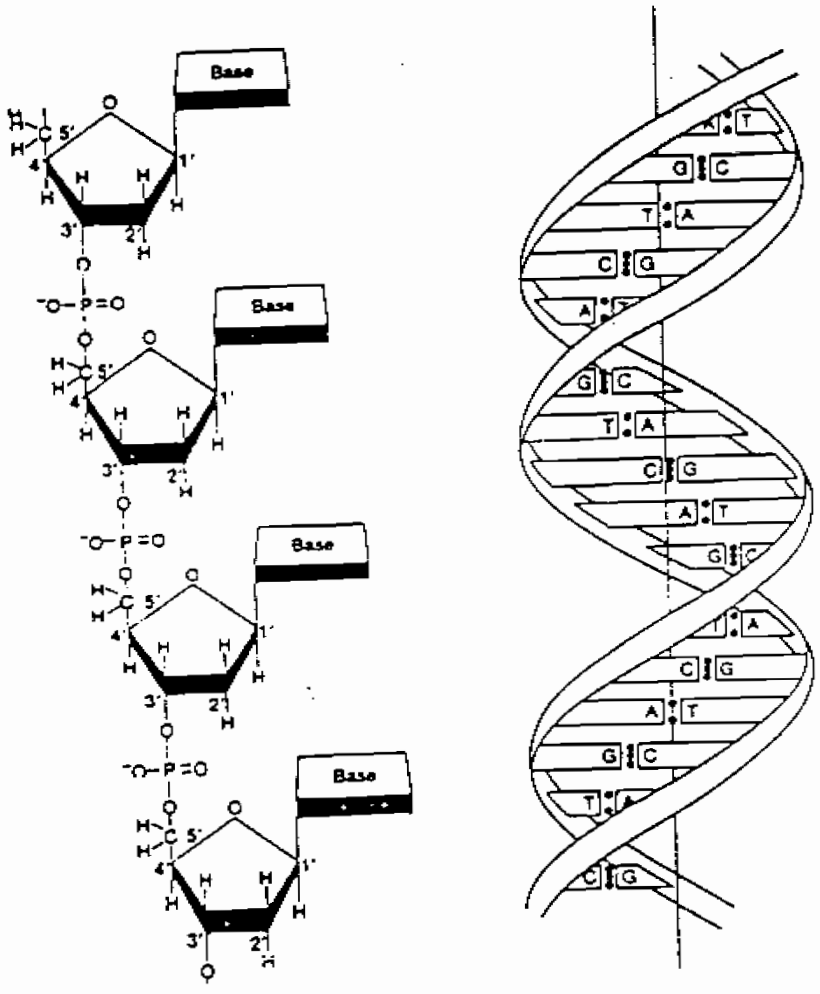


Fig. 1. The Structure of DNA. The DNA double helix. The two bands represent the sugar phosphate backbones of the two strands, which run in opposite directions. The vertical line represents the central axis round which the strands wind. The position of four nucleotide bases, C, A, T, and G, is shown, together with the hydrogen bonds (: ;) which link them together. The diagram on the left represents the structure of part of a DNA chain. It shows the chain-linked deoxyribose and phosphate residues which link them together. (Wetherall, 1985).

which form hydrogen bonds with each other. Thus, adenine will hydrogen bond only to thymidine, while guanine will bond only to cytosine. These bonds are called base pairs, the sequence of bases on one strand immediately dictates the sequence of bases which may occur on the opposite or "complementary" strand (Benz, 1991).

RNA Structure:

Chemically, RNA is similar to DNA except for two differences the sugar of DNA is deoxyribose while in RNA it is ribose, and instead of thymine RNA contains the closely related pyrimidine base, Uracil (U). (High and Benz, 1985).

The synthesis of RNA on DNA templates is very similar to DNA replication, in that it involves the formation of complementary base pairs. As in case of DNA duplication, G pairs with C, but when mRNA is being made on a DNA template A pairs with U instead of T.

Gene Organization

In, eukaryotic species DNA sequences are organized into functional units which are called genes. The total number of base pairs present in human genome is approximately 3 billions existing in the form of 46 long molecules or chromosomes (High and Benz, 1985).

The genetic information contained in the base sequence of a strand of DNA is expressed first by synthesis of a molecule of RNA called messenger RNA (mRNA), and the base sequence in the mRNA molecule is translated into the amino-acid sequence of a protein.

DNA ----------> RNA ----------> Protein
transcription translation

(High and Benz, 1985).

Almost all functional mammalian genes that have been analyzed have their coding sequences (exons) interrupted by sequences of unknown function, called intervening sequences (IVS) or introns, at varying positions along their length. The number and size of these introns, which are considerably longer than the coding sequences or exons, vary from gene to gene (McKussick, 1987).

Genetic Code and Protein Synthesis

Genetic Codon:

The rules by which the base sequence of a DNA strand is converted into amino acid sequence of a protein are summarized by the term "genetic code". The base sequence of the mRNA copy, or transcript, of the DNA coding strand is read as a series of consecutive non-overlapping triplets (High and Benz 1985).

Since there are four code letters (A, C, G and U), so there are (4^3) or 64 possible codons consisting of three bases. Since only 20 amino acids are found in proteins, there are more codons available than there are amino acids to be encoded, so some amino acids are encoded by more than one codon (Table 1) (High and Benz, 1985).

Table (1): The Genetic code: mRNA codons for the amino acids

(High and Benz, 1985)

Alanine 5-GUU- GCC GCA GCG	Arginine CGU CGC CGA AGA AGG	Asparagine AAU AAG	Aspartic Acid GAU GAC	Cysteine UGU UGC
Glutamic Acid GAA GAG	Glutamine GAA GAG	Glycine GGU GGC GGA GGG	Histidine GAU CAC	Isoleucine AUU AUC AUA
Leucine UUA UUG CUU CUA CUG	Lysine AAA AAG	Methionine AUG	Phenylalanine UUU UUC	Proline CCU CCC CCA CCG
Serine UCU UCC UCA UCG AGU AGC	Threonine ACU ACC ACA ACG	Tryptophan UGG	Tryosine UAU UAC	Valine GUU GUC GUA GUG
Chain Termination Codon UAA UAG UGA				