CHROMOSOMES AND INFERTILITY

ESSAY SUBMITTED FOR PARTIAL FULFILLMENT OF
THE MASTER DEGREE (M.Ch.)
IN UROLOGY

BY

SHERIF KHALIL MOUSTAPHA EL KERDANI

SUPERVISED BY

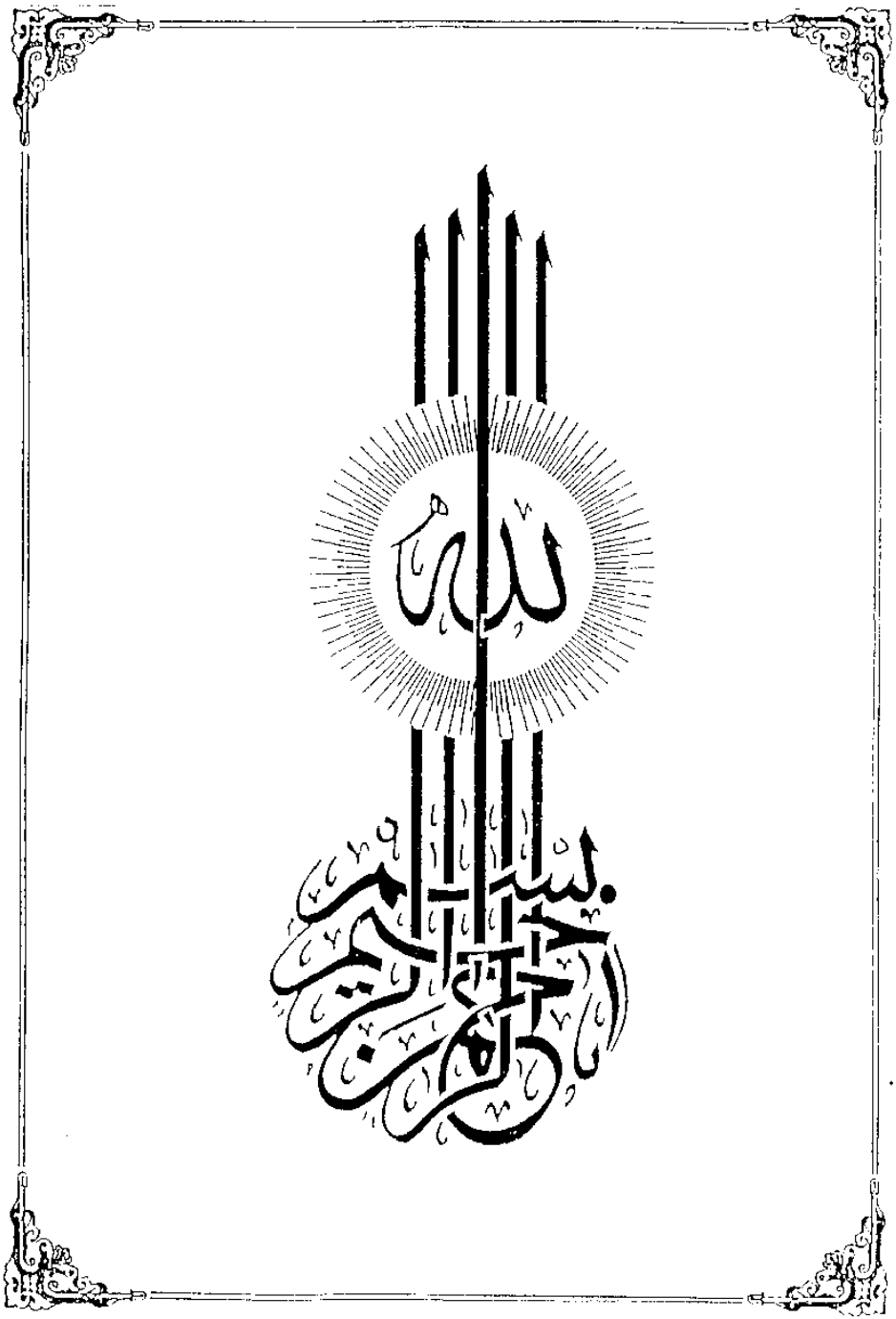
PROFESSOR Dr. MOHAMED AMIN TAHA
PROFESSOR OF UROLOGY
AIN SHAMS MEDICAL SCHOOL

AIN SHAMS UNIVERSITY
1986

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to Professor Dr. **Mohamed Amin Taha** for his kind help and close supervision he gave me during this work. I am indebted to him for his invaluable guidance and also to all the staff of the Department of Urology, Ain Shams Medical School.

I also like to express my deepest thanks to Professor Dr. **Gamal El Baz** for his fatherly guidance and to my colleagues of El Sahel Teaching Hospital for their patience with me.



INTRODUCTION

Infertility in men has long been a very disturbing problem to both man and woman and of course to the consulting physician.

Among the various causes of male infertility, those that are chromosomally related have no definite curative treatment, yet the employment of recent techniques of investigations such as banding techniques and meiotic studies has aided much in the early diagnosis of such errors, and subsequently how to prevent such errors in the offsprings.

This work is a trial to explain the structure and methods of investigating chromosomes, and the application of such methods to diagnose chromosomal related male infertility, hoping that in the future such errors can be completely cured.

One point of importance remains, that counselling rather than treatment alone must be the physician's task in dealing with such patients and their spouses.

CHAPTER I

STURCTURE OF CHROMOSOMES

Crick proposed that DNA existed in the form of a double helix. The basic structure of a chromosome is probably a single long strand of DNA that is tightly coiled and possibly branched.

The DNA double helix may be envisioned as a twisted ladder (Fig. 1) Each vertical column is formed of alternating deoxyribose sugar residues and phosphate residues. These are connected transversely (like ladder rungs) by four nitrogenous bases. The purines adenine (A) or guanine (G) and the pyrimidines thymine (T) or cytosine (C). In RNA uracil (U) is present instead of (T). The transverse connection consists of one purine and one pyrimidine, held together by hydrogen bonds. Adenine is bound to thymine by two hydrogen bonds; cytosine is bound to guanine by three.

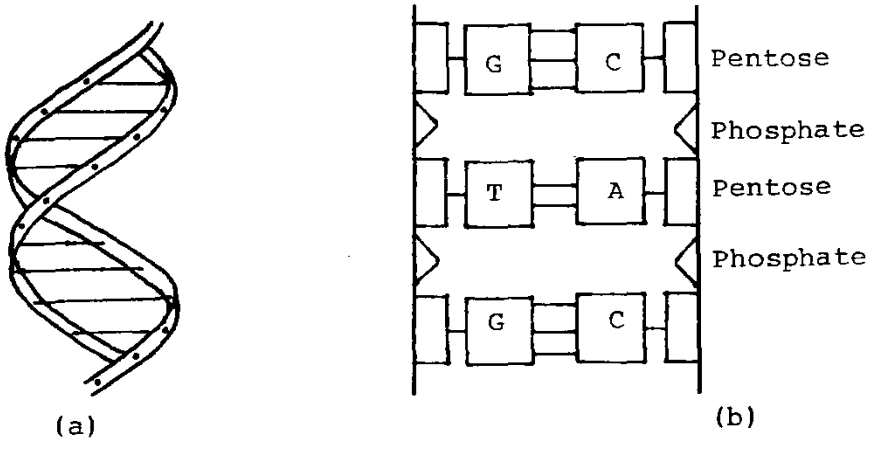


Fig.1: a. Schematic representation of the double helical configuration of DNA. b. Detailed representation showing the alternating nitrogenous bases. From, Simpson, J.L. Disorders of sexual differentiation, 1976a.

The two DNA strands are complementary, thus if one sequence is known, for example ATTGC, the other is deduced, in such a case it is TAACG. A sequence of three bases, called a codon, signifies one of the twenty essential amino acids. However, there are more possible codon permutations namely $4^3 = 64$ than there are essential amino acids, thus different codons can signify the same amino acid.

DNA serves as a template for the formation of messenger RNA (mRNA) which is complementary to the former and is single stranded. mRNA passes to the cytoplasm and attaches itself to ribosomes. Transfer RNA (tRNA) in the cytoplasm which has three free nitrogenous bases attaches itself to mRNA on the ribosome according to the specific codon present, each tRNA carries a specific amino acid which it leaves before leaving the mRNA, thus a specific sequence of amino acids is formed forming a peptide chain which is formed according to the specific sequence of codons in the DNA in the chromosomes inside the nucleus (Fig. 2).

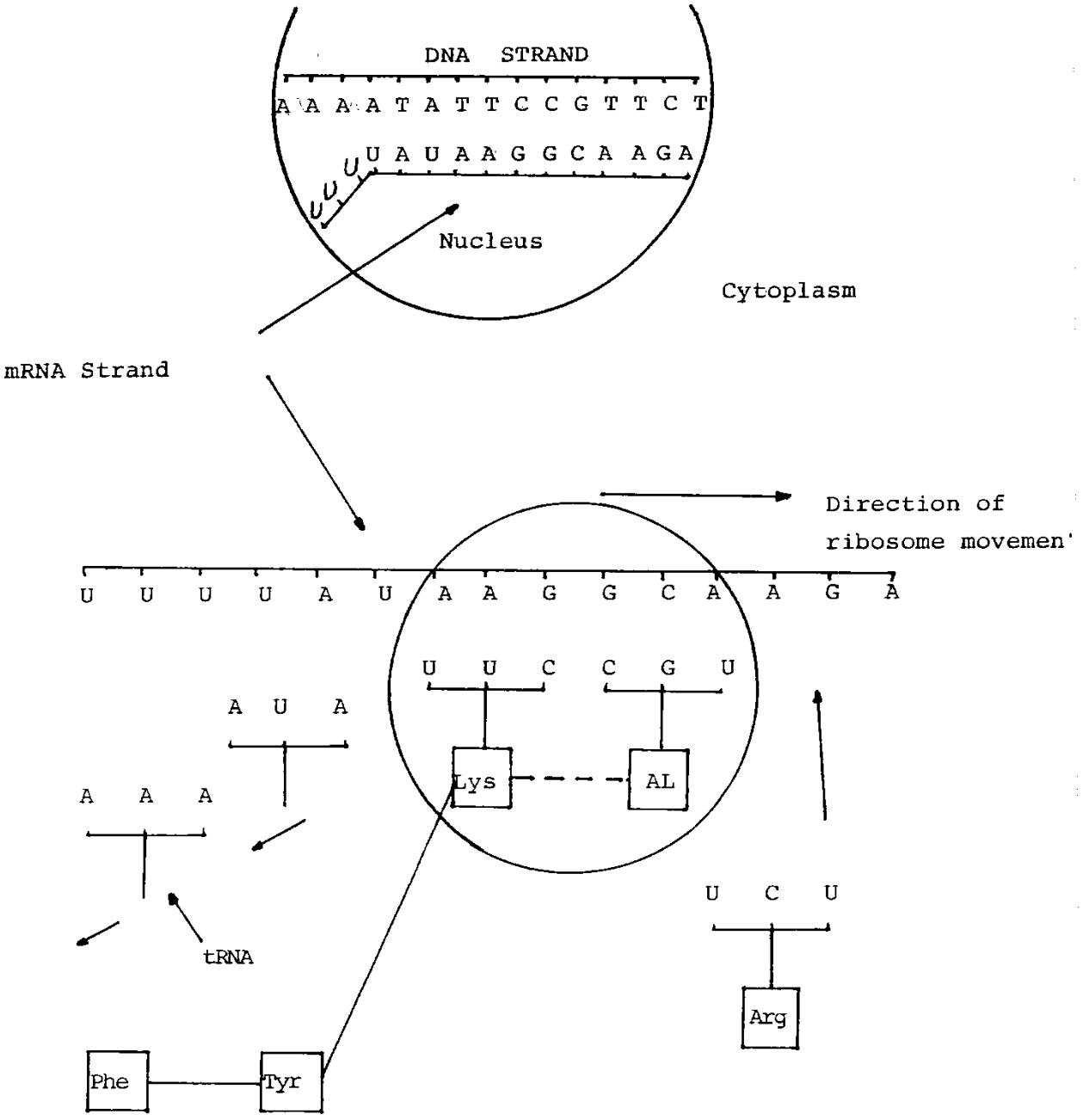


Fig. 2: Schematic representation of the transcription and translation of DNA. From Simpson, J.L. Disorders of sexual differentiation, 1976a.

Some codons signify the initiation of protein synthesis, others signify its termination, and yet others apparently code for nothing (nonsense codons).

Chromosomes display an affinity for certain dyes; thus, they are said to consist of chromatin. **Heterochromatin**, which is highly condensed and stains during interphase and early prophase, has traditionally been considered to represent regions that contain relatively few genes that actively synthesize proteins. Euchromatin, which is less condensed and stains less readily at interphase than heterochromatin, has been said to represent relatively more active regions.

IDENTIFICATION AND MORPHOLOGY OF CHROMOSOMES:

Mammalian chromosomes are best analyzed at metaphase. After accumulation of metaphase figures by addition of the mitotic inhibitor colchicine or diacetyl methyl colchicine (colcemid) and exposure to a hypotonic solution that causes cell swelling, chromosomes can be spread on slides and stained. At metaphase, each chromosome consists of two sister chromatids. The centromere or primary constriction (spindle attachment region) divides a chromosome into a short arm (abbreviated p) and a long arm (q). Based upon

the position of the centromere, one can classify the chromosome as:

1. Metacentric (p and q equal in length).
2. Submetacentric (q slightly greater than p).
3. Acrocentric (q much greater than p i.e., the centromere nearly terminal).
4. Telocentric (the centromere terminal). This type may or may not exist in man.

Chromosomes are designated according to their size and their centromeric position as shown in Fig. 3. The X is submetacentric, intermediate in size between Nos. 7 and 8. The Y is usually slightly larger than Nos. 21 and 22.

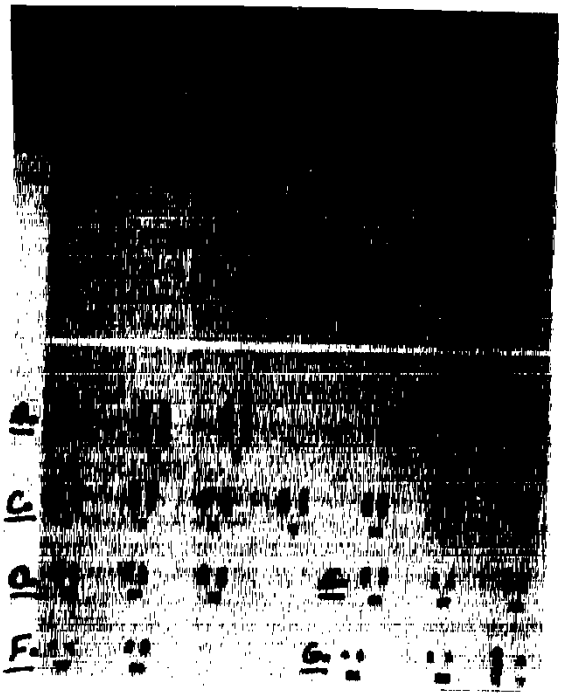


Fig. 3: Metaphase figure and karyotype of a normal male (46, XY). From Simpson, J.L., Disorders of sexual differentiation 1976a.

Human chromosomes are classified into groups: A = Nos 1 - 3; B = Nos. 4 and 5; C = Nos. 6 - X -12; D = Nos. 13 - 15, E = Nos. 16 - 18; F = Nos. 19 and 20; G = Nos. 21 and 22. With conventional staining techniques chromosomes in one group can be distinguished from chromosomes of another group; however, within a given group chromosomes cannot always be distinguished from one another.

ROLE OF CHROMOSOMES IN CELL CYCLE (Simpson, 1976a)

1. MITOSIS:

Mitosis is a process by which daughter cells receive identical copies of the parental genome. Each pair of chromosomes is said to be identical or homologous with respect to their genetic loci and visible structure.

We might first consider the four components of a cell cycle : G_1 (gap 1), S (synthesis), G_2 (gap 2) and D (division or mitosis). In human cells G_1 is usually the longest. During G_1 the cell accumulates nucleotides, amino acids, proteins, and other substances in preparation for replication of its DNA, which occurs during S. At the end of S the DNA is doubled in content. Initially each chromosome consists of a single unit; eventually each chromosome will consist of two units

that are then called sister chromatids, joined at a single centromere. During S, chromosomes replicate asynchronously (German,1964), a phenomenon that can be utilized to identify chromosomes that are morphologically indistinguishable by routine techniques. X chromosomes in excess of one are inactivated and form X-chromatin masses, these inactive X chromosomes are the last of the complement to replicate their DNA (late replicating X). If more than two late replicating X chromosomes are present, they may replicate asynchronously with respect to one another. After DNA synthesis is completed another resting period (G_2) extends until the end of interphase.

The fourth period, division (D) or mitosis, is relatively short, although it is continuous and lacks well demarcated points, yet it is divided into four stages (Fig. 4).

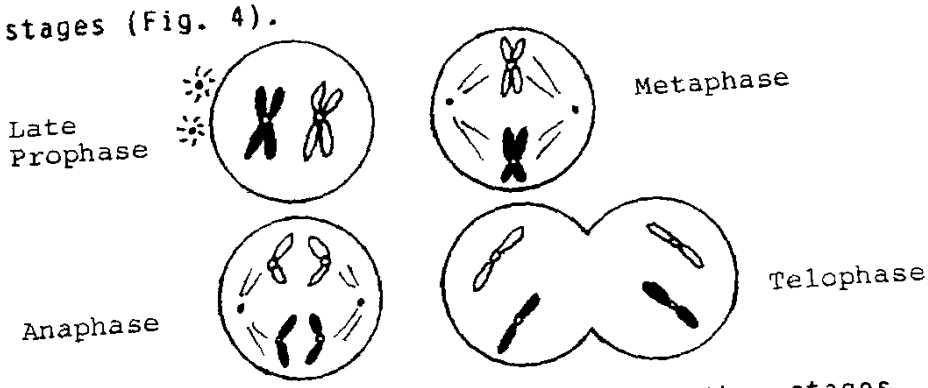


Fig. 4: Schematic representation of the stages of mitosis.

A. **Prophase**, chromosomes are first elongated; then they become shorter, compact, and darkly stained. Although each chromosome is formed of two sister chromatids, by light microscopy they appear as a single strand. Toward the end of prophase, the centriole divides into two, each one migrates to opposite poles in preparation for the formation of the mitotic spindle, on to which chromosomes will become oriented for division.

B. **Metaphase**, the nuclear membrane disappears and the mitotic spindle forms. Division of the centromere along the longitudinal plane occurs, and chromatids pass to opposite poles.

C. The process of chromatid movement to opposite poles constitutes **anaphase**. Occasional failure of sister chromatids to disjoin, thus passing to the same rather than different daughter cells (non disjunction), accounts for a variety of clinical syndromes of chromosomal numerical errors.

D. **Telophase** is characterized by mitotic spindle disappearance, nuclear membrane formation, and two complete cells are thus formed.

2. MEIOSIS:

This stage is divided into meiosis I and meiosis II.

MEIOSIS I:

The germ cells pass through the same period of the cell cycle that somatic cells do: G_1, S, G_2 and D.

There are four meiotic stages, analogous to the four mitotic stages. Meiotic prophase is longer and more complex than mitotic prophase. Several subdivisions of prophase can be identified. During **leptotene** the chromosmesare long, slender, and darkly stained. During **zygotene** homologous chromosomes pair longitudinally with each other, a process known as synapsis, which probably occurs at homologous loci. At this stage, non-disjunction can occur. During **pachytene**, the two sister chromatids of each chromosome become distinguishable, thus each two chromosomes form a group of four chromatids (tetrad). At some sites genes are exchanged between nonsister chromatids, a process known as crossing over. The two sites of contact are called chiasmata, again this is a stage of possible non-disjunction. During **diplotene** one pair of nonsiter chromatids separates from the other; complete separation is prevented by chiasmata. The rest of the stages of

meiosis resemble those of mitosis except that neither centromere divide in meiosis I. In summary, the net result of meiosis I is that the chromosome number is reduced from 2n to n, each daughter cell receives one of the two chromosomes that formerly constituted a tetrad.

MEIOSIS II:

Following meiosis I, and interphase, G_1 may or may not occur prior to meiosis II, no S preiod is required as there is little or no DNA synthesis, and there is usually no prophase stage. The rest of the stages are similar to the corresponding mitotic divisional stages. After completion of meiosis II, an originally diploid cell will have divided into four haploid cells. Theoretically, if recombination occurs, no two cells will be genetically identical.

CHROMOSOMAL ERRORS:

1. NUMERICAL:

A. **Aneuploidy.** This is a condition in which a diploid cell or a haploid gamete lacks its expected number of chromosomes (**2n and n** respectively).

B. **Trisomy** exists if the complement contains one additional whole chromosomes (**2n + 1**).