

LE 55
no. 244
cap. 2

WHY STUDY SPERM DNA?

Arthur S. H. Wu

Department of Animal Science

Oregon State University, Corvallis, Oregon



Living organisms are unique in that they are able to synthesize living matter characteristic of their own and are also able to transmit various traits to their offspring. They owe these capacities largely to deoxyribonucleic acid (DNA) which occurs in most living cells and acts as hereditary blueprints to determine the ultimate structure of life. The study of DNA has therefore been pursued by scientists of many disciplines and the research on sperm DNA is indeed of vital importance in animal reproduction.

Chromosomes, Genes and DNA

Through the efforts of scientists, many biological phenomena have become more clearly understood. It is known that growth, development and reproduction depend upon the function of the numerous cells that make up the organism. In the nucleus of each cell there are structures known as chromosomes which consist of protein and DNA molecules. The proteins in the chromosome are genetically inert while the DNA in the chromosome controls heredity by acting as an "information carrying" device of the cell. According to its stored information, DNA dictates the protein syntheses of cells. DNA can also replicate itself so that genetic information is transmitted from one cell to its progeny and from one generation to the next. Each gene, or the unit of inheritance, is now considered to be a segment of a DNA molecule and each chromosome contains a large

number of genes that determine the many inherited traits of an organism.

Chromosomes occur in pairs in the somatic or body cells. The number varies among species but for a given species the number of chromosomes normally remains constant from generation to generation. Since chromosomes carry the units of inheritance, chromosome constancy is of paramount importance. This constancy is maintained by two types of cell division—mitosis and meiosis. In the formation of body cells, the cells divide by mitosis whereby each of the progeny cells receives the same number of chromosomes as found in the parental cell. In the formation of germ cells, the cells divide by both mitosis and meiosis. As a result of meiosis, each sperm or egg receives only half the chromosome number (one member from each pair) found in each of the body cells. Following fertilization the nuclear material of spermatozoon and egg are combined. The fertilized egg resumes the number of chromosomes characteristic of the species and contains, therefore, a complete set of genetic information. When the fertilized egg divides by mitosis, in the formation of new individual, its DNA molecules also replicate so that each of the new progeny cells will also carry DNA like that found in the parental cells. The DNA of the new cells will then either direct protein synthesis or replicate itself. As a result of protein synthesis, the cells will eventually differentiate to form tissues and organs which will carry on their specific tasks. By DNA replication the parental cells will pass their "genetic codes" to their new cells.

The animal will also be able, after reaching puberty, to produce germ cells through which the genetic information carried in the DNA molecules can be transmitted to the offspring.

Chemistry of DNA and Genetic Code

The unique functions of DNA can be explained by its chemical structure. A DNA molecule consists of two long strands of polynucleotides wound around each other into a double helix resembling a spiral staircase. The polynucleotide strand is composed of a large number of nucleotides. Each nucleotide consists of a phosphate, a sugar (deoxyribose) and one of the four main bases (adenine, guanine, cytosine or thymine). In the DNA molecule, the nucleotides are joined together by phosphate bonds connecting the deoxyribose in one nucleotide and the deoxyribose of the next nucleotide in sequence. The bases are attached as side groups of the sugar molecules. The two strands of polynucleotides are held together by hydrogen bonds extending between either adenine in one polynucleotide and thymine in the other, or guanine in one and cytosine in the other. Thus, each strand of polynucleotide consists of a backbone of repeating units of phosphate-deoxyribose with one of the four different bases attached on each of the phosphate-deoxyribose units. Because the phosphate-deoxyribose units are alike in all DNA molecules, the structure in DNA to account for the numerous genetic codes must depend on the sequence of the four bases in the polynucleotide.

With only four different bases one may wonder how DNA

Special Report No. 244, Oregon Agricultural Experiment Station. Study on sperm DNA at O.S.U. is partly supported by the National Association of Animal Breeders and Curtiss Breeding Service.

molecules could represent the large number of genes that determine the numerous traits of an animal. Each DNA molecule, however, is very large and consists of a large number of these four different bases. The sequences of the different bases in the DNA molecules will vary significantly enough to spell out the extensive genetic information. The chromosome of the bacteria *Escherichia coli*, for instance, is probably a single DNA molecule with a molecular weight of about 2 to 4×10^9 . Even a DNA with a molecular weight of 10^6 will have approximately 1,500 nucleotide pairs. The number of sequence permutations (the different arrangements) in such DNA molecules is 4^{1500} , a value far greater than the number of genes found in any one organism.

The pairing of bases between the two polynucleotide strands of the DNA molecule is highly specific, i.e. adenine always with thymine, and guanine with cytosine. This specificity of base pairing is also important in DNA function. By such base pairing mechanism the sequences are faithfully replicated to produce either new DNA molecules or ribonucleic acid (RNA) tem-

plates for protein synthesis.

DNA Replication and Protein Synthesis

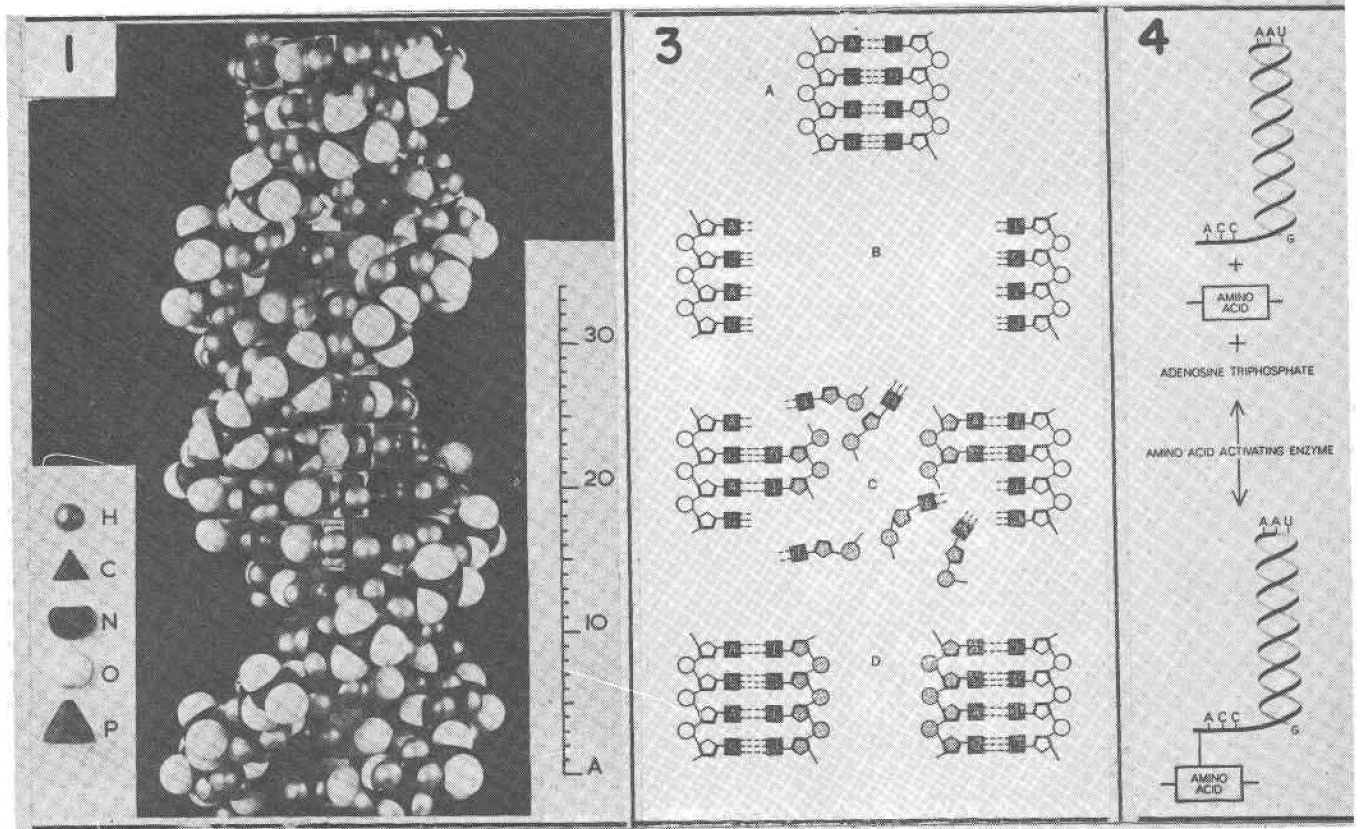
When the two strands of polynucleotide are wound together the DNA molecule is in a resting stage. For replication, the two strands gradually unwind and each separate strand then serves as a template. By base pairing, a complementary copy of polynucleotide strand is formed from each strand. Thus, a new DNA molecule will be formed from a parental molecule. This process of replication is essential for maintaining the DNA or chromosome constancy in new cells.

In protein synthesis, DNA in the nucleus carries the "master plan," and dictates the kind of protein to be synthesized. DNA first synthesizes the substance known as messenger ribonucleic acid (mRNA). The mRNA carries the plan of protein synthesis from DNA to the cytoplasm of the cell. Upon receiving the plan of protein synthesis, a substance known as transfer ribonucleic acid (tRNA) of the ribosomes in cytoplasm will put the type and number of amino acids together in correct sequence and synthesizes the protein according to the specification from the DNA molecule.

Proteins are essential to life. They are not only important cellular constituents but also serve as enzymes to maintain and regulate the activity and function of all cells, including eggs and spermatozoa.

Research on Sperm DNA

Leuchtenberger and her associates (1953) demonstrated a correlation of DNA deficiency in sperm nuclei and one type of sterility in man. They found that in contrast to the constant and uniform DNA content found in spermatozoa of fertile males, the DNA of individual sperm from infertile males was significantly lower and variable. Over 90% of all sperm cells from fertile men had a DNA content within 10% of the mean value, 2.5×10^{-9} mg. per spermatozoon. In 18 infertile men, only 15% of the sample had a normal DNA content, whereas the remaining 85% had an abnormally low DNA content. Similar findings have been observed in some cattle (Leuchtenberger, 1959). Cytophotometric DNA analysis has also been carried out on sperm from Santa Gertrudis bulls by Welch, et al. (1960, 1961). They found that bulls with the poorest sperm motility exhibited also the lowest content of DNA in



their sperm nuclei. Salisbury and his associates (1961) reported a marked progressive decrease in the Feulgen-DNA content of stored bovine spermatozoa. This decrease approximately parallels the known decrease in fertility and increase in apparent embryonic mortality resulting from the use of aged spermatozoa for artificial insemination. At Oregon State University, the effect of radiation on sperm DNA has been observed (Wu and Prince, 1964). We are interested in detecting the factors that may affect sperm DNA and thereby affect animal fertility and inheritance.

One may recall that the biological roles of DNA depend largely on the sequences of its constituent bases and the specificity of the base pairing mechanism. Although DNA molecules replicate with remarkable accuracy, occasional errors do creep in and may lead to biological defects due to an alteration of the genetic code. The disease "sickle cell anemia," for instance, involves a defect in DNA instruction. Similar to other proteins, the hemoglobin of red blood cells is also synthesized under the direction of the molecule of DNA. It has been found that the hemoglobin in the sickle cell anemia patient differs from that of a normal individual in only one minute detail. In such a patient, the glutamic acid of hemo-

globin is replaced by valine. Thus, a change of a single amino acid among the 140 odd amino acids that made up one of the two chains of the hemoglobin molecule leads to a disease with significant defect in the red blood cells.

In studies with DNA, one may then ask what are the factors that may induce errors in the DNA genetic code? How can these happenings be prevented? With the advancement of science, perhaps someday DNA molecules can be controlled at will. Through such controls of the DNA molecule, physical and mental defects may eventually be eliminated from all forms of life, including man!

EXPLANATION OF FIGURES

Figure 1. A space-filling model of double-helical DNA.

Figure 2. A section of the two polynucleotide strands in DNA molecule. Pairs of bases, adenine (A) and thymine (T), or guanine (G) and cytosine (C), join the linear chains consisting of alternate units of deoxyribose (d) and phosphate (P). The sequence of bases acts as the genetic code and each DNA molecule contains thousands of base pairs.

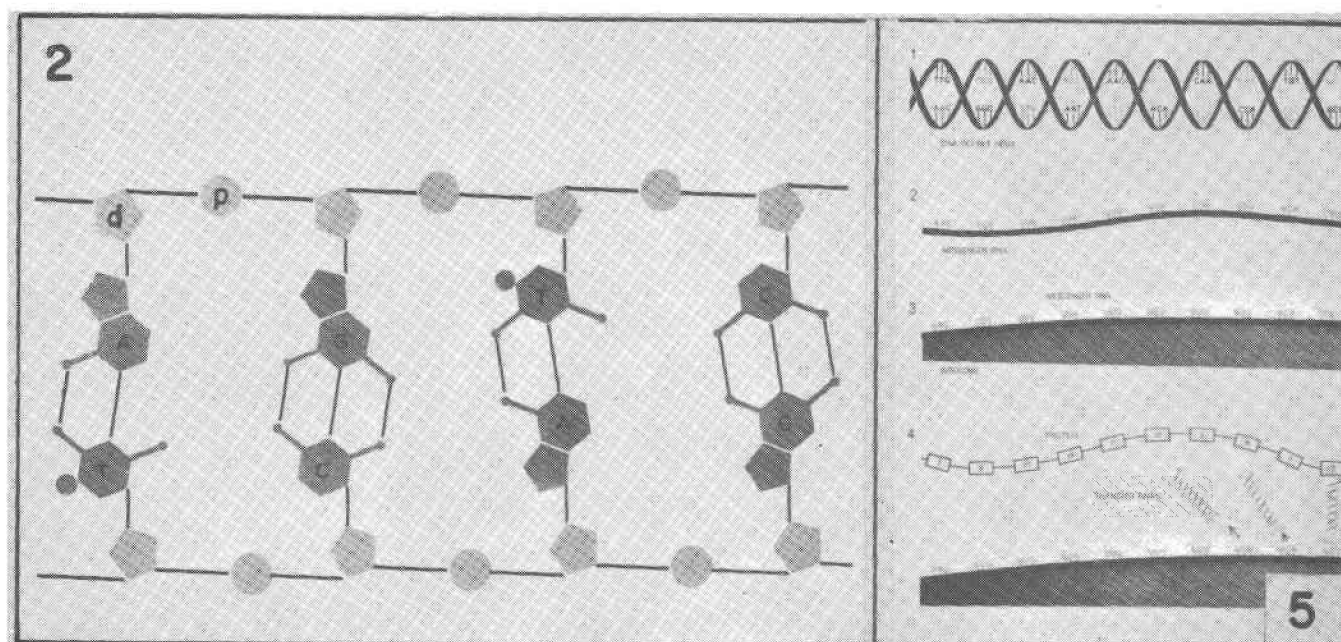
Figure 3. DNA replication. The polynucleotide strands of DNA molecule (A) separate in the region undergoing replication (B). Each strand acts as a template to

link the DNA precursor units at proper sites (C) and directs the synthesis of a polynucleotide strand complementary to itself (D).

Figure 4. Transfer RNA has a sequence of bases complementary to certain sequence of bases in the mRNA molecule. Each tRNA also has a structure capable to "recognize" a particular amino acid. There is at least one tRNA for each of the 20 amino acids. All tRNA seem to carry the bases ACC where the amino acids attached and the base G at the opposite end. The attachment requires a specific enzyme and also energy from ATP.

Figure 5. Syntheses of protein.

1. A section of polynucleotide strands in DNA.
2. The genetic code in DNA is transcribed into mRNA. RNA is similar to DNA except RNA contains ribose instead of deoxyribose and uracil (U) instead of thymine. The single-stranded mRNA is formed by pairing of bases complementary to those on one of the two polynucleotide strands in DNA molecule. The message of mRNA shown in the diagram is derived from the DNA strand bearing the base sequence TTGGG-CAACAAT etc.



Figures—With permission and courtesy of: 1. After N. H. F. Wilkins, from Watson, 1965; W. A. Benjamin, Inc. 2. Crick, 1957; Srb, Owen and Edgar, 1965. W. H. Freeman and Company. 3, 4 and 5. Nirenberg, 1963. W. H. Freeman and Company.

3. The mRNA finds its way into the cytoplasm and attaches itself to ribosomes.
4. The molecules of tRNA carry amino acids to their proper sites on mRNA according to the coded message. i.e., the base sequence on mRNA molecule. The amino acids linked together to form peptides, polypeptides and eventually protein molecules.

LITERATURE CITED

¹ Crick, F. H. C. 1954. The Structure of the Hereditary Material. *Scientific American*, October, 1954. W. H. Freeman and Co., San Francisco.

² Crick, F. H. C. 1962. The Genetic Code. *Scientific American*, October, 1962. W. H. Freeman and Co., San Francisco.

³ Kendrew, John C. 1966. *The Thread of Life*. Harvard University Press, Cambridge, Massachusetts.

⁴ Leuchtenberger, C., F. Schrader, D. R. Weir and D. P. Gentile. 1953. The Deoxyribonucleic Acid (DNA) content in Spermatozoa of Fertile and Infertile Human Males. *Chromosoma*, 6:61.

⁵ Leuchtenberger, Cecilie. 1959. The Relation of the Deoxyribonucleic Acid (DNA) of Sperm Cells to Fertility. Supplement, *J. Dairy Sci.*, 43:31.

⁶ Mann, Thaddeus. 1964. *The Biochemistry of Semen and of the Male Reproductive Tract*. Methuen and Co. Ltd., London.

⁷ Nirenberg, M. W. 1963. The Genetic Code: II. *Scientific American*, March, 1963.

⁸ Salisbury, G. W., W. J. Birge, L. De La Torre and J. R. Lodge. 1961. Decrease in Nuclear Feulgen-Positive Material (DNA) Upon Aging in In Vitro Storage of Bovine Spermatozoa. *J. Biophys. Biochem. Cytol.*, 10:353.

⁹ Srb, A. M., R. D. Owen and R. S. Edgar. 1965. *General Genetics*. W. H. Freeman and Co., San Francisco.

¹⁰ Stahl, Franklin W. 1964. *The Mechanics of Inheritance*. Prentice-Hall, Inc., Englewood Cliff, New Jersey.

¹¹ Summerhill, W. R., Jr. and D. Olds. 1961. Levels of Deoxyribonucleic Acid in Bovine Spermatozoa and Their

Relationship to Fertility. *J. Dairy Sci.*, 44:548.

¹² Thompson, J. S. and M. W. Thompson. 1966. *Genetics in Medicine*. W. B. Saunders Co., Philadelphia and London.

¹³ Watson, James D. 1965. *Molecular Biology of the Gene*. W. A. Benjamin, Inc. New York.

¹⁴ Welch, R. M., E. W. Hanly, W. Guest and K. Resch. 1960. A Cytophotometric Analysis of the Deoxyribonucleic Acid (DNA) Content in Germ Cells from Santa Gertrudis Bulls. M. R. Wheeler (ed.), *The University of Texas Publication*, No. 6014.

¹⁵ Welch, R. M., E. W. Hanly and W. Guest, 1961. The Deoxyribonucleic Acid (DNA) Deviation in the Semen Spermatozoa of Bulls of Unknown Fertility Under Two Years of Age and Its Relationship to Motility, Count and Morphology. *J. Morphology*, 108:145.

¹⁶ Wu, S. H. and J. R. Prince. 1963. Effect of Ionizing Radiation on Spermatozoa Metabolism. *Proceedings, International Symposium on the Effect of Ionizing Radiation on the Reproductive System*. Ed. by W. D. Carlson and F. X. Gassner. Pergamon Press, N.Y.

Reprinted from A.I. DIGEST, March, 1968.