Molecular structure of dna and rna

Science, Genetics

MOLECULAR STRUCTURE OF DNA AND RNA -Adapted from Chapter 9 in Genetics: Analysis and Principles (Robert J. Brooker) Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display IDENTIFICATION OF DNA AS THE GENETIC MATERIAL - To fulfill its role, the genetic material must meet several criteria — 1. Information: It must contain the information necessary to make an entire organism — 2. Transmission: It must be passed from parent to offspring — 3. Replication: It must be copied - In order to be passed from parent to offspring — 4. Variation: It must be capable of changes - To account for the known phenotypic variation in each species Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display A little history on the identification of genetic material In early 1900’s, it was generally agreed that the genetic material must be ‘ something’ from the chromosome A chromosome consists of DNA & proteins! Is the genetic material DNA or Protein? -DNA consists of only four different subunits (A, T, C, G) -Chromosomes contain less DNA than protein by weight -Chromosomes contain more protein than DNA by -weight -Protein has 20 different subunits (amino acids) so it has a greater potential for variety of combinations -Diverse proteins were identified Experiments demonstrating that DNA is the genetic material -Frederick Griffith experiments with Streptococcus pneumoniae -The experiments of Avery, MacLeod and McCarty -Hershey and Chase experiments with Bacteriophage T2 Frederick Griffith Experiments with Streptococcus pneumoniae - Griffith studied a bacterium (Diplococcus pneumoniae) now known as Streptococcus pneumoniae - S. pneumoniae comes in two strains —S Smooth - Secrete a polysaccharide capsule — Protects bacterium from the immune system of animals - Produce smooth colonies on solid media —R Rough - Unable to secrete a capsule - Produce colonies with a rough appearance 9-5 - In addition, the capsules of two smooth strains can differ significantly in their chemical composition Figure 9. 1 In 1928, Griffith conducted experiments using two strains of S. pneumoniae: type IIIS and type IIR 1. Inject mouse with live type IIIS bacteria Mouse died 2. Inject mouse with live type IIR bacteria Mouse survived 3. Inject mouse with heat-killed type IIIS bacteria Mouse survived 4. Inject mouse with live type IIR + heat-killed type IIIS cells Mouse died Figure 9. 2 9-8 - Griffith concluded that something from the dead type IIIS was transforming type IIR into type IIIS — He called this process transformation - The substance that allowed this to happen was termed the transformation principle — Griffith did not know what it was - Research began for the identity of the “ transformation principle" 9-9 The Experiments of Avery, MacLeod and McCarty - To determine the nature of the transformation principle - Avery, MacLeod and McCarty realized that Griffith’s observations could be used to identify the genetic material - They carried out their experiments in the 1940s — At that time, it was known that DNA, RNA, proteins and carbohydrates are major constituents of living cells - They prepared cell extracts from type IIIS cells containing each of these macromolecules Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-10 Understanding Avery et al's experiments: -S. pneumoniae bacteria can take up extracellular DNA and to be ‘ transformed’ by this DNA -Type IIR bacteria can take up DNA from Type IIIS bacteria -An antibody for Type IIR bacteria bound to and precipitated these bacteria - Figure 9. 3 Avery et al conducted the following experiments — To further verify that DNA, and not a contaminant (RNA or protein), is the genetic material Type IIIS Type IIIS Type IIIS Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-11 Hershey and Chase Experiment with Bacteriophage T2 - In 1952, Alfred Hershey and Marsha Chase provided further evidence that DNA is the genetic material Figure 9. 4 - They studied the bacteriophage T2 — It is relatively simple since its composed of only two macromolecules - DNA and protein Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display Inside the capsid Made up of protein 9-12 Figure 9. 5 Life cycle of the T2 bacteriophage 9-13 - The Hershey and Chase experiment can be summarized as such: — Used radioisotopes to distinguish DNA from proteins - 32P labels DNA specifically - 35S labels protein specifically — Radioactively-labeled phages were used to infect nonradioactive Escherichia coli cells — After allowing sufficient time for infection to proceed, the residual phage particles were sheared off the cells - => Phage ghosts and E. coli cells were separated — Radioactivity was monitored using a scintillation counter Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-14 The Hypothesis — Only the genetic material of the phage is injected into the bacterium - Isotope labeling will reveal if it is

DNA or protein Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display Figure 9. 6 9-16 Figure 9. 6 9-17 Interpreting the Data - These results suggest that DNA is injected into the bacterial cytoplasm during infection — This is the expected result if DNA is the genetic material 9-19 RNA Functions as the Genetic Material in Some Viruses - In 1956, A. Gierer and G. Schramm isolated RNA from the tobacco mosaic virus (TMV), a plant virus — Purified RNA caused the same lesions as intact TMV viruses - Therefore, the viral genome is composed of RNA (does NOT have DNA) - Since that time, many RNA viruses have been found - Notable human pathogenic RNA viruses: SARS and Influenza viruses Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-21 NUCLEIC ACID STRUCTURE - DNA and RNA are large macromolecules with several levels of complexity — — — — 1. Nucleotides form the repeating units 2. Nucleotides are linked to form a strand 3. Two strands can interact to form a double helix 4. The double helix folds, bends and interacts with proteins resulting in 3-D structures in the form of chromosomes Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-22 Three-dimensional structure Figure 9. 7 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-23 Nucleotides -A Nucleotide has three components: a phosphate group, a pentose sugar and a nitrogenous base 1 2’ 6 3 7 3 1 Figure 9. 8 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-25 -The structure of nucleotides found in (a) DNA and (b) RNA A, G, C or T A, G, C or U Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-26 - Base + sugar nucleoside — Example - Adenine + ribose = Adenosine - Adenine + deoxyribose = Deoxyadenosine - Base + sugar + phosphate(s) nucleotide — Example - Adenosine monophosphate (AMP) - Adenosine diphosphate (ADP) - Adenosine triphosphate (ATP) Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-27 Base always attached here Phosphates are attached there Figure 9. 10 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-28 Phospho-ester bond formation - Nucleotides are covalently linked together by phosphodiester bonds — A phosphate connects the 5’ carbon of one nucleotide to the 3’ carbon of another - Therefore the strand has directionality — 5’ to 3’ - The phosphates and sugar molecules form the backbone of the nucleic acid strand Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-29 -The bases project from the backbone Figure 9. 11 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-30 A Few Key Events Led to the Discovery of the Structure of DNA - In 1953, James Watson and Francis Crick discovered the double helical structure of DNA - The scientific framework for their breakthrough was provided by other scientists including — Linus Pauling — Rosalind Franklin and Maurice Wilkins — Erwin Chargaff Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-31 Linus Pauling - In the early 1950s, he proposed that regions of protein can fold into a secondary structure Î±-helix — Pauling also suggested a helical structure for DNA Figure 9. 12 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-32

Rosalind Franklin - The X-ray diffraction pattern of DNA fibers she obtained suggested several structural features of DNA — Helical — More than one strand — 10 base pairs per complete turn Erwin Chargaff's Experiment - It was already known then that DNA contained the four bases: A, G, C and T - Tetranucleotide hypothesis: all four bases in DNA were in equal amounts - He analyzed the base composition of DNA in different species Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display The Data Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-39 Interpreting the Data - The compelling observation was that — Percent of A (adenine) = percent of T (thymine) — Percent of C (cytosine) = percent of G (guanine) - This observation became known as Chargaff's rule — It was a crucial evidence that Watson and Crick used to elucidate the structure of DNA - There are rare exceptions Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display Watson and Crick - Familiar with all of these key observations, Watson and Crick set out to solve the structure of DNA — They tried to build ball-and-stick models that incorporated all known experimental observations - A critical question was how the two (or more strands) would interact — An early hypothesis proposed that the strands interact through phosphate-Mg+ + cross links — Refer to Figure 9. 15 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-41 Figure 9. 15 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-42 DNA Double Helix Key Features: - Two strands of DNA form a right-handed double helix -The bases in opposite strands hydrogen bond according to the A/T and G/C rule -The two strands are antiparallel with regard to their 5’ to 3’ directionality -There are ~10. 0 nucleotides in each strand per complete 360º turn of the helix -Hydrogen bonding -van der Waals force Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-48 The DNA Double Helix - General structural features (Figures 9. 17 & 9. 18) — The double-bonded structure is stabilized by - 1. Hydrogen bonding between complementary bases — A bonded to T by two hydrogen bonds — C bonded to G by three hydrogen bonds - 2. Base stacking (van der Waals force) — Within the DNA, the bases are oriented so that the flattened regions are facing each other Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-46 DNA Can Form Alternative Types of Double Helices - The DNA double helix can form different types of secondary structure — The predominant form found in living cells is B-DNA — However, under certain in vitro conditions, A-DNA and Z-DNA double helices can form Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-50 - A-DNA — — — — Right-handed helix 11 bp per turn Occurs under conditions of low humidity Little evidence to suggest that it is biologically important - Z-DNA — Left-handed helix — 12 bp per turn — Its formation is favored by - GG-rich sequences, at high salt concentrations - Cytosine methylation, at low salt concentrations — Evidence from yeast suggests that it may play a role in transcription and recombination Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-51 Bases substantially tilted relative to the central axis Bases substantially tilted relative to the central axis Bases relatively perpendicular to the central axis Sugar-phosphate backbone follows a zigzag pattern Figure

9. 19 9-52 Sugar-phosphate backbone follows a zigzag pattern B DNA is right-handed Z DNA is left-handed Figure 9. 19 DNA Can Form a Triple Helix - In the late 1950s, Alexander Rich et al discovered triplex DNA — It was formed in vitro using DNA pieces that were made synthetically - In the 1980s, it was discovered that natural doublestranded DNA can join with a synthetic strand of DNA to form triplex DNA — The synthetic strand binds to the major groove of the naturally-occurring double-stranded DNA - Refer to Figure 9. 20 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-53 - - Triplex DNA formation is sequence specific The pairing rules are: - Triplex DNA has been implicated in several cellular processes — Replication, transcription, recombination Figure 9. 20 9-54 RNA Structure - The primary structure of an RNA strand is much like that of a DNA strand - RNA strands are typically several hundred to several thousand nucleotides in length - In RNA synthesis, only one of the two strands of DNA is used as a template Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display 9-57 Figure 9. 22 9-58 - Although usually single-stranded, RNA molecules can form short double-stranded regions — This secondary structure is due to complementary basepairing - A to U and C to G — This allows short regions to form a double helix - RNA double helices typically — Are right-handed - Different types of RNA secondary structures are possible — Refer to Figure 9. 23 Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display Complementary regions Held together by hydrogen bonds Figure 9. 23 Noncomplementary regions Have bases projecting away from double stranded regions Also called hair-pin Copyright ©The McGraw-

Hill Companies, Inc. Permission required for reproduction or display 9-60 - Many factors contribute to the tertiary structure of RNA — For example - Base-pairing and base stacking within the RNA itself - Interactions with ions, small molecules and large proteins Molecule contains single- and doublestranded regions These spontaneously interact to produce this 3-D structure Figure 9. 24 - Figure 9. 24 depicts the tertiary structure of tRNAphe — The transfer RNA that carries phenylalanine Copyright ©The McGraw-Hill Companies, Inc. Permission required for reproduction or display