Bio chapter 17 notes gene expression

Science, Genetics

17. 1 Genes specify proteins via transcription and translation * George Beadle and Edward Tatum worked together with mutated (Neurospora crass) bread mold to figure out that they were missing a specific enzyme (gene) that catalyzed and synthesized a pathway required. They concluded that they were missing that enzyme because it was lacking the amino acid that coded for the enzyme, thus was mutated and incapable of growing. Led to the one enzyme-one gene hypothesis. The Products of Gene Expression: A Developing Story * Revisions made: not all proteins are enzymes * Because proteins that are not enzymes are nevertheless gene products, molecular biologists began to think in terms of one gene-one protein. * Beadle and Tatum’s idea was restated as “ one gene-one polypeptide hypothesis. " * BUT: many eukaryotic genes can each code for a set of closely related polypeptides via process call alternative splicing BUT: quite a few genes code for RNA molecules that have important functions in cells even though they are not translated into protein Basic Principles of Transcription and Translation * Genes provide the instruction for making proteins. But a gene does not a build a protein directly. * Bridge between DNA and protein synthesis is the nucleic acid RNA. * Genes are typically hundreds or thousands of nucleotides long, each gene having a specific sequence of nucleotides. * Each polypeptide of a protein also has monomers arranged in a particular linear order (the protein’s primary structure), but its monomers are amino acids. * TRANSCRIPTION: the synthesis of RNA using information in the DNA (General term for the synthesis of any kind of RNA on a DNA template. * For a protein-coding gene, the resulting RNA molecule is a faithful transcript of the gene’s protein-building instructions. * (mRNA)

messenger RNA carries a genetic message from the DNA to the protein-synthesizing machinery of the cell. * Translation: synthesis of polypeptide using the information in the mRNA; during this stage there is a change in language: cell must translate the nucleotide sequence of mRNA molecule into the amino acid sequence of a polypeptide; site is done at ribosomes: complex particle that facilitate the orderly linking of amino acids into polypeptide chains * Difference in Bacteria & Eukaryotes: * Bacteria don’t have nuclear membranes and thus its DNA is not separated by other compartments such as ribosomes and other protein-synthesizing equipment: so for bacteria that allows translation of mRNA to begin while transcription is still in progress * In Eukaryotes, the nuclear envelope separates transcription and translation in space and time: transcription occurs in the nucleus and mRNA is transported to the cytoplasm, but before mRNA is sent out it is modified to produce function mRNA. Transcription of a protein-coding eukaryotic gene results in pre-mRNA, further processing yields the finished mRNA. Those that are not translated into proteins are called primary transcript. * DNA RNA Protein The Genetic Code * Flow of information from gene to protein is based on a triplet code: genetic instructions for a polypeptide chain are written in the DNA as series of non-overlapping, three-nucleotide words. * During transcription, the gene determine the sequence of nucleotide bases along the length of the RNA molecule that is being synthesized * For each gene, only one of the two DNA strands is transcribed, template strand, which provides the pattern for the sequence of nucleotides in an RNA transcript * The Triplet Code: A strand of DNA is blueprint for a particular gene, which is transcribed to mRNA, which is translated by an

outside compartment to particular amino acids that make up polypeptide chain (protein): mRNA is read 5’ 3’ * mRNA is complementary rather than identical to DNA because it follows the base pairing rules, similar the strand of DNA replication but T is replaced with U * The nucleotide triplets are called codons, written in the 5’3’ direction * Non-template strand is sometimes called the “ coding strand", there nucleotide triples are also referred to as codon * Three codons don’t designate amino acids are called “ stop" signals, or termination codons, marking the end of translation. * Genetic messages usually begin with the mRNA codon AUG, “ start" signal, or initiation codon, which signals the protein-synthesizing machinery to begin translating the mRNA at that location * An enzyme may subsequently remove this starter amino acid from the chain * Ability to extract the intended message from a written language depends on reading the symbols in the correct groupings– the correct reading frame 17. 2 Transcription is the DNA-directed synthesis of RNA: a closer look * RNA polymerase: pries the two strands of DNA apart and joins together RNA nucleotides complementary to the DNA template strand, thus elongating the RNA polynucleotide * They do not need a primer (like DNA does) to start a chain. They do it from scratch * Promoter: the DNA Sequence, where RNA polymerase attaches and initiates transcription; in bacteria, the sequence that signals the end of transcription is called the terminator: this is different in eukaryotes * Downstream and upstream are the directions of transcription, or at least that’s what they are referred to as, also used to describe the positions of nucleotide sequences within the DNA or RNA. * Promoter sequence in DNA is said to be upstream from the terminator * Transcription unit: the stretch of DNA that is transcribed into an

RNA molecule * Different Types of RNA: bacteria have a single type of RNA polymerase that synthesizes not only mRNA but also other types of RNA that function in protein synthesis, such as ribosomal RNA; eukaryotes have at least three types of RNA polymerase in their nuclei, the one used for mRNA is called RNA polymerase II: other RNA polymerase transcribe RNA molecules that are not translated into protein Synthesis of an RNA Transcript * There are three stages of transcription: initiation, elongation, and termination of the RNA chain RNA Polymerase Binding and Initiation of Transcription * Promoter of a gene includes within it the transcription start point, the nucleotide where RNA synthesis actually begins. * RNA polymerase binds in a precise location and orientation on the promoter, determining where transcription starts and which of the two strand of the DNA helix is used at the template. * Transcription factors: collection of proteins, mediate the bind of RNA polymerase and the initiation of transcription * Only after the transcription factors are attached to the promoter does RNA polymerase II bind to it * Transcription initiation complex: whole complex of transcription factors and RNA polymerase II bound to the promoter * TATA Box: crucial promoter DNA sequence, crucial in forming the initiation complex at a eukaryotic promoter * Once the appropriate transcription factors are firmly attached to the promoter DNA and the polymerase is bound in the correct orientation, the enzyme unwinds the two DNA strands and starts transcribing the template strand. Elongation of the RNA Strand * The congregation of many polymerase molecules simultaneously transcribing a single gene increases the amount of mRNA transcribed from it, which helps the cell make the encoded protein in large amounts. * A single gene can be transcribed

simultaneously by several molecules of RNA polymerase following each other like trucks in a convoy. * The enzyme adds nucleotides to the 3’ end of the growing RNA molecule as it continues along the double helix. In the wake of this advancing wave of RNA synthesis, the new RNA molecule peels away from its DNA template, and the DNA double helix re-forms. Termination of Transcription * Bacteria: transcription proceeds through a terminator sequence in the DNA, the transcribed terminator functions as the termination signal causing the polymerase to detach from the DNA and release the transcript, which require no further modification before translation * Eukaryotes: RNA polymerase II transcribes a sequence on the DNA called the polyadenylation signal sequence, which codes for a polyadenylation signal (AAUAAA) in the pre-mRNA. Then, at a point about 10-35 nucleotides downstream from the AAUAAA signal, proteins associated with the growing RNA transcript cut it free from the polymerase, releasing the pre-mRNA. 17. 3 Eukaryotic Cells modify RNA after transcription * RNA processing: both end of the primary transcript are altered; certain section of the RNA molecule are cut out and the remaining parts spliced together, producing mRNA molecule ready for translation Alteration of mRNA Ends * The 5’ end is synthesized first; it receives 5’ cap: a modified form of a guanine nucleotide added onto the 5’ end after transcription of the first 20-40 nucleotides * The 3’ end of the pre-mRNA molecule is also modified before the mRNA exits the nucleus * The pre-mRNA is release soon after the polyadenylation signal (AAUAA) is transcribed * At the 3’ end, an enzyme add 50-250 more adenine nucleotides, forming a poly-A tail * 5’ cap and poly-A tail: they seem to facilitate the export of the mature mRNA from the nucleus, they help protect

the mRNA from degradation by hydrolytic enzymes, and they help ribosomes attach to the 5’ end of the mRNA once the mRNA reached the cytoplasm * (UTs) untranslated regions at the 5’ and 3’ ends of the mRNA, will not be translated into protein, but they have other functions, such as ribosome binding Split Genes and RNA * RNA splicing: the removal of large portion of the RNA molecule that is initially synthesized–a cut-and-paste job * The sequence of DNA nucleotides that codes for a eukaryotic polypeptide is usually not continuous; it is split into segments * Introns: the noncoding segments of the nucleic acid that lie between coding regions (intervening sequences) * Exons: segments that are eventually expressed, by being translated into amino acid sequences (sequences of RNA that “ exit" the nucleus) * Intron and exon is used for both RNA sequences and the DNA sequences that encode them. * RNA splicing: RNA polymerase II transcribes both introns and exons from the DNA, but the mRNA molecule that enters the cytoplasm has the introns cut out from the molecule and the exons joined together, forming the mRNA molecule with a continuous coding sequence * Signal for RNA splicing is a short nucleotide sequence at each end of an intron * (snRNPs) small nuclear ribonucleoproteins recognize these splice sites * snRNPs join with additional proteins to form an even larger assembly called a splicesome, interact with certain sites along an intron, releasing it Ribozymes * Ribozymes: RNA molecules that function as enzymes * In some organisms, RNA splicing can occur without proteins or even additional RNA molecules: the intron RNA functions as a ribozyme and catalyzes its own excision! * Three properties of RNA enable some RNA molecules to function as enzymes: * Because RNA is single-stranded, a

region of an RNA molecule may base-pair with a complementary region elsewhere in the same molecule, which gives the molecule a particular three-dimensional structure. A specific structure is essential to the catalytic function of ribozymes, just as it is for enzymatic proteins. * Like certain amino acids in an enzymatic protein, some of the bases in RNA contain functional groups that may participate in catalysis * The ability of RNA to hydrogen-bond with other nucleic acid molecules (either RNA or DNA) adds specificity to its catalytic activity. For example, complementary base pairing between RNA of the spliceosome and the RNA of a primary RNA transcript precisely locates the region where the ribozyme catalyzes splicing. The Function Importance of Introns * Alternative RNA splicing: A single gene can encode more than one kind of polypeptide. Many genes are known to give rise to two or more different polypeptides, depending on which segments are treated as exons during RNA processing * Because of alternative splicing, the number of different protein products an organism produces can be much greater than its number of genes. * Domains: discrete structural and functional regions * One domain might include active site * The presence of introns in a gene may facilitate the evolution of new and potentially beneficial proteins as a result of a process known as exon shuffling. 17. 4 Translation is the RNA-directed synthesis of a polypeptide: a closer look Molecular Components of Translation * Transfer RNA (tRNA): translator, transfers amino acids from the cytoplasmic pool of amino acids to a growing polypeptide in a ribosome * Ribosome, structure made of proteins and RNAs, add each amino acids brought to it by tRNA to the growing end of a polypeptide chain The Structure and Function of Transfer RNA (tRNA) *

Molecules of tRNA are not all identical, and each type of tRNA molecule translate a particular mRNA codon into a particular amino acid * (tRNA) arrives a ribosome with a specific amino acid at one end and a nucleotide triplet called anticodon, which pairs with a complementary codon on mRNA * as an mRNA molecule is move through a ribosome, an amino acid will be added to the polypeptide chain whenever the corresponding codon is presented for translation * ribosome joint the amino acids into a chain * tRNA is a translator in the sense that it can read a nucleic word and interpret it as a protein word, they are also transcribed from DNA templates * aminoacyl-tRNA synthetases: carries out the matching up of tRNA and amino acid; its actives site fits only a specific combination of amino acid and tRNA * there are 20 different synthetases, one for each amino acid * catalyzes the covalent attachment of the amino acid to its tRNA in a process driven by the hydrolysis of ATP, resulting in a charged tRNA * has 45 tRNAs, which means they are able to bind to more than one codon, they’re relaxed about the rules for base pairing between the third nucleotide base of a codon and the corresponding base of a tRNA anticodon * wobble: flexible base pairing at the 3’ end, explains why the synonymous codons for a given amino acid most often differ in their third nucleotide base, but no in other bases Ribosomes * ribosomes consists of a large subunit and a small subunit, each made up of proteins and one or more ribosomal RNAs (rRNAs) * their RNA is transcribed in the nucleolus and processed and assembled with proteins imported from the cytoplasm * 1/3 of mass is proteins and other is either 3 rRNAs for bacteria or 4 for eukaryotes * rRNA is the most abundant type of cellular RNA Building a Polypeptide * Three stages: Initiation, elongation, and

termination Ribosome Association and Initiation of Translation * First, a small ribosomal subunit binds to both mRNA and a specific initiator tRNA, which carries the amino acid methionine. In eukaryotes, the small subunit, with the initiator tRNA already bound, binds to the 5’ cap of the mRNA and then moves, or scans, downstream along with the mRNA until it reaches the start codon, the initiator tRNA then hydrogen-bonds to the AUG start codon. The start codon signals the start of translation. * The union of mRNA, initiator tRNA, and a small ribosomal subunit is followed by the attachment of a large ribosomal subunit, completing the translation initiation complex. * Initiation factors: proteins required to bring all these components together * Cell also expends energy obtained by hydrolysis of a GTP molecule to form the initiation complex. * At the completion of the initiation process, the initiator tRNA sits in the P site of the ribosome, and vacant A site is ready for the next aminoactyl tRNA. * Polypeptide is always synthesized in one direction, N-terminus to C-terminus Elongation of the Polypeptide Chain * Amino acids are added one by one the previous amino acid at the C-terminus of the growing chain 1. Codon recognition: The anticodon of an incoming aminoacyl tRNA base-pairs with the complementary mRNA codon in the A site. Hydrolysis of GTP increases the accuracy and efficiency of this step. (many different aminoacyl tRNAs are present, but only the one with the appropriate anticodon will bind and allow the cycle to progess) 2. Peptide bond formation: An rRNA molecule of the large ribosomal subunit catalyzes the formation of a peptide bond between the amino group of the new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site. This step removes the polypeptide from the tRNA in the P site and attaches it

to the amino acid on the tRNA in the A site. 3. Translocation: The ribosome translocates the tRNA in the A site of the P site. At the same time, the empty tRNA in the P site is moved to the E site, where it is released. The mRNA moves along with its bound tRNAs, bring the next codon to be translated into the A site. Termination of Translation * The release factor causes the addition of a single water molecule instead of an amino acid to the polypeptide chain. * Breakdown of the translation assembly requires the hydrolysis of two or more GTP molecules. 1. When a ribosome reaches a stop codon on mRNA, the A site of the ribosome accepts a “ release factor, " a protein shaped like a tRNA, instead of an aminoacyl tRNA. 2. The release factor promotes hydrolysis of the bond between the tRNA in the P site and the last amino acid of the polypeptide, thus freeing the polypeptide from the ribosome. 3. The two ribosomal subunits and the other components of the assembly dissociate. * Polyribosomes: Strings of ribosomes: when the mRNA attaches to one ribosome and others because to attach as well, but when a ribosome is far enough pas the start codon Completing and Targeting the Functional Protein * Sometimes the process of translation is often not sufficient to make a functional protein. Protein Folding and Post-Translational Modifications * During its synthesis, a polypeptide chain begins to coil and fold spontaneously as a consequence of its amino acid sequence (primary structure), forming a protein with a specific shape * Gene determines primary structure, and it determines shape. * Post-transitional modifications: enzymes may remove one or more amino acids from the leading (amin) end of the polypeptide chain, in some cases cleaved in two or more pieces Targeting Polypeptides to Specific Locations * Free ribosomes are suspended

in the cytosol and mostly synthesize proteins that stay in the cytosol and function there. * Bound ribosomes make proteins of the endomembrane system (nuclear envelope, ER, Golgi apparatus, lysosomes, vacuoles, and plasma membrane) as well as proteins secreted from the cell, such as insulin. * Ribosomes are identical and can switch their status from free to bound. * Polypeptide cues the ribosome to attach to the ER. * Polypeptides of proteins destined for the endomembrane system or for secretion are marked by a signal peptide, which targets the protein to the ER. * It is recognized as it emerges from the ribosome by a protein-RNA complex called signal-recognition particle (SRP) * Functions as an escort that brings the ribosome to a receptor protein built into the ER membrane * The receptor I s part of a multi-protein translocation complex * Polypeptide synthesis continues there * Polypeptide snakes across the membrane into the ER lumen via a protein pore * If the polypeptide is to be secreted by cell, it is released into the lumen, if not, it remains partially embedded in the ER membrane. * It is located near the leading end (N-terminus) of the polypeptide * It is a sequence of about 20 amino acids 1. Polypeptide synthesis begins on a free ribosome in the cytosol. 2. An SRP binds to the signal peptide, halting synthesis momentarily. 3. The SRP binds to a receptor protein in the ER membrane. This receptor is part of a protein complex (a translocation complex) that has a membrane pore and a signal-cleaving enzyme. 4. The SRP leaves, and polypeptide synthesis resumes, with simultaneous translocation across the membrane. (The signal peptide stays attached to the translocation complex) 5. The signal-cleaving enzyme cuts off the signal peptide. 6. The rest of the completed polypeptide leaves the ribosome and

folds into its final conformation. 17. 5 Mutations of one or a few nucleotides can affect protein structure and function * Mutations: changes to genetic information * Point mutations: changes in a single nucleotide pair of a gene Types of Small Scale Mutations: Point Mutations: single nucleotide-pair substitutions & nucleotide-pair insertions or deletions * Nucleotide-pair substitution: replacement of one nucleotide and its partner with another pair of nucleotides * Some have know effect because of the redundancy of the genetic code * A change in nucleotide pair may transform one codon into another that is translated into the same amino acid: silent mutation * Missense mutations: change on amino acid to another one, * may have no effect since that amino acid might not have been essential to protein's function, or it may have similar properties * nonsense mutation: when a point mutation changes a codon for an amino acid into a stop codon, causing translation to be terminated prematurely, shorter polypeptide, leading to nonfunctional proteins * Insertions and deletions: additions or loses of nucleotide pairs in a gene * Frameshift mutation: whenever the number of nucleotides that are downstream of the deletion or insertion will be improperly grouped into codons , resuling in extensive missense, ending sooner or later in a nonsense and premature termination * Unless the frameshift is very near the end of the gene, the protein is almost certain to be nonfunctional * Mutagens: physical and chemical agents that interact with DNA in ways that cause mutations * Nucleotide analogs are chemicals that are similar to normal DNA nucleotides but that pair incorrectly during DNA replication. * Some chemical mutagens interfere with correct DNA replication by inserting themselves into the DNA and distorting the double helix. * Other

mutagens cause chemical changes in bases that change their pairing properties. * Carcinogens (cancer-causing chemicals) are mutagenic 17. 6 While gene expression differs among the domains of life, the concept of a gene is universal Comparing Gene Expression in Bacteria, Archaea, and Eukarya * Archaea and eukaryotes use a complex set of transcription factors, unlike the smaller set of accessory proteins in bacteria. * Transcription is terminated differently in bacteria and eukaryotes. * Archaeal RNA polymerase resembles the three eukaryotic ones * Archaeal ribosomes are the same size as bacterial ribosomes, but their sensitivities to chemical inhibitors more closely match those of eukaryotic ribosomes. * Initiation of translation is slightly different in bacteria and eukaryotes, archaeal is more like that of bacteria. * Most important different between bacteria and eukaryotes with regard to gene expression is that bacterial cell’s lack compartmental organization. * Bacterial cells can simultaneously transcribe and translate. What is a gene? Revisiting the Question * A gene is a region of DNA that can be expressed to produce a final functional product that is either a polypeptide or an RNA molecule. *