

## 1 From Gene to Protein

Chapter 17

## 2 Proteins are the link between genotype and phenotype

- Beadle and Tatum studied *Neurospora*, a bread mold
- Minimal medium: support medium that is mixed only w molecules required for the growth of the wild type organism
- Nutritional mutants cannot survive on minimal medium
- Complete growth medium supplemented with all 20 amino acids and some other nutrients

## 3 Auxotrophic Mutants

- Cannot survive on minimal medium
- Beadle and Tatum identified specific metabolic deficits (from mutations) by transferring auxotrophic mutants from complete growth medium to minimal mediums containing only one additional amino acid
- Experimented to describe the defect and explain the multistep pathway that synthesizes arginine, an amino acid

## 4 Arginine Synthesis

- Precursor—(Gene → enzyme A) →
- Ornithine—(Gene B → enzyme B) →
- Citrulline—(Gene C → enzyme C) →
- Arginine

## 5 Detecting the metabolic pathway

## 6 Beadle and Tatum Conclusions

- Each mutant each lacked a different enzyme and were blocked at different steps in the arginine synthesis pathway
- Class I lacked enzyme A
- Class II lacked enzyme B
- Class III lacked enzyme C
- Concluded one gene: 1 enzyme

## 7 One gene : one polypeptide

- New evidence since Beadle and Tatum's ground breaking experiment
- Many proteins are not enzymes, but they are gene products
- Many proteins are comprised of two or more polypeptide chains, each chain specified by a different gene
- Now, hypothesis is restated as one gene: one polypeptide

## 8 Transcription and translation

- Transcription is synthesis of mRNA, a copy of DNA
- Translation is synthesis of a polypeptide using the mRNA directions
- RNA is different from DNA in 2 ways:
- Ribose is the sugar in RNA, not deoxyribose
- Uracil is the nitrogenous base that replaces thymine

9  Role of Transcription

10  Transcription in Eukaryote

11  Where is heredity?

- The sequence of nucleotides in DNA determine the linear sequence of amino acids in a protein
- Each gene has a specific linear sequence of the 4 possible nitrogenous bases
- Proteins are made of 20 types of amino acids linked in a particular sequence to form a specific protein

12  Site of translation

- Ribosomes are the site where translation occurs
- Ribosomes are composed of rRNA and a protein (both of which are coded by DNA)
- Both prokaryotes and eukaryotes have ribosomes

13  Differences in translation between prokaryotes and eukaryotes

- Prokaryotes don't have a nucleus or nuclear membrane, there is no separation of DNA from ribosomes allowing translation to begin as soon as transcription is done
- Prokaryotes don't have introns and the RNA needs no processing prior to the onset of translation

14  Triplet Codons

- There are 4 nitrogenous bases and 20 amino acids, so there is not a 1:1 correspondence
- If there was a 2:1 correspondence, only 16 of the amino acids could be specified
- By using 3:1 correspondence, 64 amino acids could be specified; codons have 3 base combinations
- Codons are redundant but not ambiguous-each codon only codes for 1 amino acid
- 1 amino acid may be coded for by 2 or 3 codons – redundant, but ambiguous

15  DNA and transcription

- Genes are transcribed as codons for amino acids in a polypeptide
- For each gene, only one of the two DNA strands is used as the template and will be transcribed
- The complementary nontemplate strand is the parental strand for making new templates when DNA replicates
- The same DNA strand can be the template strand for some genes and the nontemplate strand for others

- mRNA is complementary to the DNA strand: if A is on DNA, then U will be on mRNA

16  **Cracking the code**

- Marshall Nirenberg of NIH deciphered the first codon in 1961
- He synthesized an mRNA by linking three uracil bearing RNA nucleotides, UUU
- He added this poly U to a test tube mixture containing the components necessary for protein synthesis
- The artificial mRNA (poly U) was translated into a polypeptide
- The only polypeptide in the tube was phenylalanine

17  **Codons with special functions**

- AUG has a dual function: it is the code for methionine and it is the START signal for translation
- STOP codons are: UAA, UAG, UGA

18  **Triplet Codon**

19  **Triplet Codon Dictionary**

20  **Reading frame**

- The correct grouping of adjacent nucleotide triplets into codons that are in the correct sequence on mRNA
- If the sequence of amino acids is:
  - Trp-Phe-Gly-Arg-Phe then the correct reading frame is:
  - UGGUUUGGCCGUUUU
- What happens in a deletion? Or an insertion? Or a duplication or translocation?

21  **Common Genetic Language**

- Genetic code is shared nearly universally among organisms; indicates code was established very early in life's history
- CCG is proline in all organisms whose genetic codes have been examined
- Technologically, possible to transfer genes from one organism to another
- Insulin in bacteria; bacteria make lots of insulin rapidly and ~ inexpensively
- Several ciliates have slight differences
- Mitochondrial genetic codes vary even among organisms

22  **Transcription: Initiation**

- Catalyzed by RNA polymerase which untwists the DNA and links RNA nucleotides as they base-pair along the DNA template
- Nucleotides are added only to the 3' end, mRNA molecules grow in the 5' to 3' direction
- Prokaryotes have only one type of RNA polymerase that synthesizes all types of RNA (mRNA, tRNA, rRNA)
- Eukaryotes have 3 RNA polymerases that transcribe genes RNA polymerase II catalyzes mRNA synthesis

- 23  **Transcription unit**
- Transcription unit is the nucleotide sequence on the template strand of DNA that is transcribed into a single RNA molecule
  - It includes the initiation and termination sequences
  - In eukaryotes, a transcription unit contains a single gene (translated into a single polypeptide)
  - In prokaryotes, a transcription unit may contain several genes, consequently a single mRNA may code for different, although functionally related, proteins
- 24  **3 steps of transcription**
- Binding and initiation by RNA polymerase
  - Elongation
  - termination
- 25  **Initiation of Transcription**
- RNA polymerase binds to DNA at region called promoter region
  - Promoter: region of DNA that include the site where RNA polymerase binds and initiation site where transcription begins
  - In eukaryotes promoter is about 100 nucleotides long and includes transcription factors
  - Transcription factors help RNA polymerase to recognize the promoter and bind to specific DNA nucleotide sequences
- 26  **Transcription: Initiation I**
- 27  **Transcription Factors**
- Transcription factor binds to a region on the promoter called a TATA box
  - TATA box is a short nucleotide sequence that is rich in thymine and adenine and is located about 25 nucleotides upstream from the initiation site
  - RNA polymerase II recognized the complex between the bound TATA transcription factor and the DNA binding site
- 28  **Promoter Region w TATA Box**
- 29  **Transcription: Elongation**
- RNA polymerase untwists and opens short segment of DNA about 10 nucleotide bases in length; one of the DNA strands is the template for base-pairing with RNA nucleotides
  - It links incoming RNA nucleotides to the 3' end
  - mRNA grows about 30-60 nucleotides per second
  - mRNA peels away from its DNA template
  - The nontemplate strand of DNA reforms the double helix by pairing w template
- 30  **Elongation**
- Working in series, several molecules of RNA polymerase II can simultaneously transcribe the gene

- Specified protein can be produced in large amounts
- The growing RNA strands hang free from each polymerase

31  Transcription: Elongation

32  Close up Elongation

33  Transcription: Termination

- Terminator sequence is a DNA sequence that signals RNA polymerase to stop transcription and release the RNA molecule and the DNA template
- Additional proteins may cooperate with RNA polymerase in termination
- In eukaryotes, the most common terminator sequence is AATAAA

34  Transcription: Termination

35  Modification of RNA after Transcription

- Both ends are covalently altered and introns are removed & the exons are spliced
- 5' cap is modified guanine added shortly after transcription
- The 5' cap protects the mRNA from degradation by hydrolytic enzymes and helps the small ribosomal subunits recognize the attachment site on mRNA's 5' end
- A leader sequence is noncoding of mRNA from the 5' end to the start codon

36  Poly-A tail

- Sequence of about 200 adenine nucleotides added to the 3' end of mRNA before it leaves nucleus
- May inhibit degradation of mRNA in the cytoplasm
- May regulate protein synthesis by facilitating mRNA's export from the nucleus to the cytoplasm
- Is not attached to the stop codon, but to an untranslated trailer segment of mRNA found between the stop codon and the poly-A tail

37  Adding 5' Cap and Poly-A Tail

38  RNA Splicing

- Original transcript RNA is heterogeneous nuclear RNA (hnRNA)
- RNA splicing removes introns, noncoding sequences in DNA that have been transcribed,
- RNA splicing occurs posttranscription of mRNA, tRNA, and rRNA
- Each end of an intron has a short boundary sequence that accurately signals the RNA splicing sites
- Small nuclear ribonucleoproteins (snRNPS) participate in splicing

39  RNA splicing

40  Spliceosome

- Small nuclear ribonucleoproteins (snRNPs) are complexes of at least 7 proteins

and small nuclear RNAs (less than 300 nucleotides) that are found only the nucleus

- Spliceosome is a large molecular complex that catalyzes RNA splicing reactions
- The spliceosome precisely cuts the RNA transcript at specific splice sites at either end of the intron
- The intron is excised as a LOOP
- The exons are spliced together by the spliceosome

#### 41 Ribozymes

- Ribozymes are RNA molecules that can catalyze reaction by breaking and forming covalent bonds
- RNA is acting as an enzyme, not all enzymes are proteins
- rRNA also functions as an enzyme during translation

#### 42 Role of Spliceosomes

#### 43 Translation

- During translation, proteins are synthesized
- Transfer RNA, tRNA, is the interpreter between the mRNA and the amino acid sequence in polypeptides
- tRNA aligns the amino acids to form a new polypeptide
- tRNA transfers amino acids from the cytoplasm's amino acid pool to a ribosome and it has to recognize the correct codons in mRNA

#### 44 Exons and Proteins

#### 45 Function of tRNA

- Molecules of tRNA are specific for only one amino acid & may be used repeatedly
- One end of tRNA attaches to amino acid
- The other end attaches to an mRNA codon by base pairing with its anticodon
- anticodon is the nucleotide triplet in tRNA that base pairs with a complementary nucleotide triplet codon in mRNA
- tRNAs decode the genetic message

#### 46 tRNA Structure

- tRNA is a single-stranded RNA about 80 nucleotides long
- If folded, forming several double-stranded regions where short base sequences hydrogen bond with other complementary bases
- In a single plane, it looks like a clover leaf
- It is truly 3D and has an overall L shape
- The loop at one end of the L has a specialized sequence of 3 bases called the anticodon
- The amino acid attaches at the 3' sites on the other end of the L

#### 47 tRNA Structure

48  **Anticodon**

49  **wobble**

- There are 45 distinct types of tRNA
- Some tRNAs recognize two or three mRNA codons specifying the same amino acid
- Base-pairing rules are relaxed between the third base of the mRNA codon and the base of a tRNA anticodon- wobble
- Some tRNAs contain a modified base called inosine (I) which is in the anticodon's wobble position and can base pair with U, C or A in the third position of an mRNA codon

50  **Aminoacyl-tRNA synthetase**

- Enzyme that catalyzes the attachment of an amino acid to its tRNA
- Each of 20 amino acids has specific aminoacyl-tRNA synthetase
- Attaches an amino acid to its tRNA in 2 steps:
- Activation of the amino acid with AMP (adenosine monophosphate), the amino acid binds to ATP which loses 2 phosphate groups
- Attachment of the amino acid to tRNA by covalent bonds which displaces AMP from enzyme's active site
- The tRNA is then released from the enzyme

51  **Aminoacyl-tRNA synthetase**

52  **Ribosomes**

- Ribosomes coordinate the pairing of tRNA anticodons to mRNA codons
- 60% rRNA and 40% protein
- The large and small subunits are constructed in the nucleolus
- Prokaryote and eukaryote ribosome structure is different
- Tetracycline and streptomycin can be used to combat bacterial infections because they inhibit bacterial protein synthesis without affecting the ribosomes of the eukaryote host

53  **Ribosome Structure**

- The small subunit has the mRNA binding site
- The large unit has the A & P sites (&E)
- The A site holds the tRNA carrying the next amino acid to be attached
- The P site holds the tRNA carrying the growing polypeptide chain

54  **Binding Sites**

55  **Translation: initiation**

- mRNA and an initiator tRNA bind to a small ribosomal subunit
- In eukaryotes the first tRNA has anticodon UAC (carries methionine)
- The small ribosomal subunit next binds to the 5' end of mRNA base pairing with the complementary sequence on rRNA
- Initiator tRNA base pairs with the start codon on mRNA, AUG
- Initiation complex: small ribosomal subunit, initiator tRNA and mRNA requires protein initiation factors and 1 GTP molecule

56  **Initiation: 2<sup>nd</sup> part**

- Initiation factors attached to the small ribosomal subunit are released, allowing the large subunit to bind with the small subunit
- The initiator tRNA fits into the P site on the ribosome
- The A site is ready for the next aminoacyl-tRNA

57  **Translation 1**

58  **Translation: Elongation 1**

- Codon recognition: mRNA codon in the A site of the ribosome forms hydrogen bonds with the anticodon of a tRNA carrying the next amino acid to be added to the chain
- An elongation factor directs tRNA into the A site; GTP provides energy

59  **Elongation 2**

- Peptide bond formation: an enzyme peptidyl transferase, catalyzes the formation of a peptide bond between the polypeptide in the P site and the new amino acid in the A site
- Peptidyl transferase is part of the large ribosomal subunit and consists of ribosomal proteins and rRNA
- The polypeptide separates from its tRNA and is transferred to the new amino acid carried by the tRNA in the A site

60  **Elongation 3**

- Translocation: the tRNA in the P site releases from the ribosome, and the tRNA in the A site is translocated to the P site
- The codon and anticodon remain bonded so the mRNA and the tRNA move as a unit, bringing the next codon to be translated into the A site
- mRNA is moved through the ribosome only in the 5' to 3' direction
- GTP hydrolysis provides energy for each translocation step
- Each addition takes about 60 milliseconds

61  **Translation 2**

62  **Translation: termination**

- Termination codon (stop codon) is on mRNA and signals the end of translation
- Stop codons are UAA, UAG, and UGA
- Stop a protein release factor binds to the codon
- Peptidyl transferase hydrolyzes the bond between the completed polypeptide and the tRNA in the P site
- Both the polypeptide and the tRNA can release from the ribosome
- The ribosomal subunits dissociate from mRNA and separate back into a small and a large subunit

63  **Ending it all**

- DNA has terminator sequence signaling end of transcription
- RNA has termination or stop codons signaling the end of translation

64  Translation 3

65  Polyribosomes

- Single ribosomes can make average sized polypeptides in less than a minute; usually clusters of ribosomes simultaneously translate an mRNA
- Polyribosome is a cluster of ribosomes simultaneously translating an mRNA molecule
- Once a ribosome passes the initiation codon, a second ribosome can attach to the mRNA
- Several ribosomes may translate an mRNA at once, making many copies of a polypeptide

66  Prokaryotic translation: polyribosome

67  Prokaryotic translation

68  From Polypeptide to Functional Protein

- Genes determine the primary structure
- Primary structure determines how a polypeptide chain will spontaneously coil and fold to form a 3 D molecule with secondary and tertiary structure
- Some proteins undergo post-translational modification
- Sugars, lipids, phosphate groups, or other additives may be attached to some amino acids
- One or more amino acids may be enzymatically cleaved from the leading end of the polypeptide chain
- Single polypeptide chains may be divided into two or more pieces
- Two or more polypeptides may join as subunits of a protein that has quaternary structure

69  Ribosomes: Free or Attached to ER

- Free and attached ribosomes are structurally identical
- proteins made by free will remain in cytoplasm and proteins made by attached are destined for membrane inclusion or for export
- mRNA for secretory proteins codes for an initial signal sequence of 16-20 hydrophobic amino acids at the amino end of the forming polypeptide
- When protein begins to be synthesized, the ribosome moves to the ER membrane by a mechanism that involves SRP, signal recognition particle which attaches both to the signal sequence and to the receptor protein in the ER, and SRP receptor, which is built into the ER membrane and docks with the receptor binding the ribosome to the ER membrane

70  Signal Sequence

- The signal sequence for ribosomes that will be threaded into the cisternal space will be removed by an enzyme
- Different signal sequences may send proteins to specific sites other than the ER, for example, to the mitochondria or to chloroplasts

71  Signaling Mechanism

72  Translation

73  Functional Importance of Introns

- Introns may play a regulatory role in the cell control gene activity
- The splicing process itself may regulate the export of mRNA to the cytoplasm
- Introns may allow a single gene to direct the synthesis of different proteins if the same RNA transcript is processed differently among various cell types in the same organism
- All introns may be removed from a particular transcript in one case, but in another, one or

more of the introns may be left in place to be translated

#### 74 Evolutionary Importance of Introns

- Play an important role in the evolution of protein diversity by increasing the probability that recombination of exons will occur between alleles
- In split genes, coding sequences can be separated by long distances, so they have high recombination frequencies than continuously coded genes without introns
- Exons of a split gene may code for different domains of a protein that have specific functions, such as, an enzyme's active site or a protein's binding site
- Protein domains are continuous polypeptide sequences that are structural and functional units
- Genetic recombination can occur in just one exon resulting in the synthesis of a novel protein with only one altered domain

#### 75 Point Mutations

- Point mutations are limited to about one or two nucleotides in a single protein
- 2 categories: base-pair substitution and base-pair insertions or deletions

#### 76 Base-pair substitutions

- Replacement of one base pair with another; occurs when a nucleotide and its partner from the complementary DNA strand are replaced with another pair of nucleotides according to base-pairing rules
- Could result in little or no change in the protein
- Redundancy in the genetic code is why some substitution mutations have no effect
- The new amino acid may have similar properties to the one it replaces, or it may be in a part of the protein where the exact amino acid sequence is not essential to its activity

#### 77 Problem substitutions

- Alteration of a single amino acid in a crucial area of a protein will significantly alter protein activity
- Rarely a mutation will produce a protein that is improved or has capabilities that enhance success of the mutant organism and descendants
- Mutations usually produce a less active or inactive protein that impairs cell function
- Missense mutation: alters an amino acid codon to a new codon that codes for a different amino acid
- Nonsense mutation: changes an amino acid codon to a chain termination codon or vice versa; nearly all are nonfunctional proteins

#### 78 Point Mutation

#### 79 Sickle Cell Mutant

#### 80 Insertions or deletions

- Base-pair insertion is the insertion of one or more nucleotide pairs into a gene
- Base-pair deletion is the deletion of one or more nucleotide pairs from a gene
- These result in frameshift mutations
- Results in extensive missense, which will end in nonsense
- Frameshift will produce a nonfunctional protein unless the insertion or deletion is very near the end of the gene

#### 81 Point Mutation:

deletion

82  **Mutagens**

- Mutagenesis may be a naturally occurring event causing spontaneous mutations or mutations caused by exposure to mutagens
- Mutagens are physical or chemical agents that interact with DNA to cause mutations
- Radiation most common in nature
- Chemical mutagens are sometimes base analogues, mimic DNA bases but base pair incorrectly
- Ames test is used for measuring the mutagenic strength of various chemicals
- Test is also used to screen for chemical carcinogens

83  **Summary of Translation**