**Gene Expression Notes 2019**

**Concept: Genes specify proteins via transcription and translation**

Making Proteins:

Proteins are involved in several organelles including nucleus, ribosomes, endoplasmic reticulum, golgi apparatus, vesicles.

The nucleolus:

-Function to make \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ production

-it builds ribosome subunits from rRNA & proteins

-The subunits exit through nuclear pores to cytoplasm & combine to form functional ribosomes

Ribosomes:

-Function: protein production

-Structure: rRNA & protein. Have 2 subunits combine

Types of Ribosomes:

-Free ribosomes are suspended in cytosol and synthesize proteins that function in cytosol

-Bound ribosomes are attached to endoplasmic reticulum and synthesize proteins for export or
for membranes

The process: DNA is transcribed to RNA. RNA is used to make protein segments at ribosomes. Protein segments are built and modified in the rough endoplasmic protein. Packaged protein segments are sent to the golgi apparatus through vesicles. The golgi finishes making the complex proteins. The complex proteins are sent on its way to other parts of the cell, other cells, or the membranes of the cell.

Metabolism taught us about genes:

 -Inheritance of metabolic diseases suggested that genes coded for enzymes. Each disease (phenotype) is caused by non-functional gene product. Some just lack an enzyme such as Tay sachs, PKU, albinism.

1 Gene-1 Enzyme hypothesis

 -Beadle and Tatum compared mutants of bread mold, Neurospora fungus created mutations by X-ray treatments. X-rays break DNA and damage a gene.

-wild type grows on \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ media. The sugars + required nutrients allows fungus to synthesize essential amino acids

-mutants require added amino acids. each type of mutant lacks a certain enzyme needed to produce a certain amino acid. Creates non-functional enzyme from damaged gene.

- Damage to specific gene, mapped to nutritional mutations

RNA:

-uses ribose sugar. N-bases use \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ instead of thymine

U : A

C : G

-is single stranded

-lots of types of RNAs

mRNA, tRNA, rRNA, siRNA…

**Concept: Transcription is the DNA-directed synthesis of RNA: a closer look**

Transcription:

-Making mRNA; transcribed DNA strand = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ strand

-untranscribed DNA strand = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ strand

-same sequence as RNA

-synthesis of complementary RNA strand

-transcription bubble

-created by enzyme called: RNA polymerase

Transcription in Prokaryotes:

Initiation: RNA polymerase binds to \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ sequence on DNA

Role of promoter is the Starting point. It shows where to start reading. It shows the start of gene. It identifies the Template strand which is the strand to read. It also points the direction on DNA as it always read DNA 3’🡪5’ and build RNA 5’🡪3’

Elongation: RNA polymerase copies DNA as it unwinds

~20 base pairs at a time

-300-500 bases in gene

builds RNA 5’🡪3’

Termination: RNA polymerase stops at \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ sequence

 - RNA GC hairpin turn

Prokaryote vs Eukaryote genes:

Prokaryotes: Eukaryotes

DNA in cytoplasm DNA in nucleus

circular chromosome linear chromosomes

naked DNA DNA wound on histone proteins

no introns introns vs. exons

**Concept: Eukaryotic cells modify RNA after transcription**

Transcription in Eukaryotes:

3 RNA polymerase enzymes

-RNA polymerase 1: only transcribes rRNA genes. They make \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

-RNA polymerase 2: transcribes genes into mRNA

-RNA polymerase 3: only transcribes tRNA genes

-each has a specific promoter sequence it recognizes

Initiation complex

-transcription factors bind to promoter \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ upstream of gene

-suite of proteins which bind to DNA

-turn on or off transcription

-TATA box binding site

recognition site for transcription factors

-transcription factors trigger the binding of RNA polymerase to DNA

Post-transcriptional processing:

Primary transcript (pre-mRNA)

-eukaryotic mRNA needs work after transcription

mRNA processing (making mature mRNA)

-mRNA splicing = edit out introns

-protect mRNA from enzymes in cytoplasm

-add 5’ cap

-add polyA tail

Splicing must be accurate:

-No room for mistakes!

-splicing must be exactly accurate

-a single base added or lost throws off the reading frame

Splicing enzymes:

snRNPs

 -small \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ RNA and proteins (snRNPs)

 -Spliceosome:

 -several snRNPs recognize splice site sequence (cut and paste)

Ribozyme:

 -RNA as ribozyme

 -some mRNA can even splice itself.

 -RNA used as an \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

**Concept: Translation is the RNA-directed synthesis of a polypeptide: a closer look**

Translation in Prokaryotes:

Transcription & translation are simultaneous in bacteria

-DNA is in cytoplasm. There is no mRNA editing

-\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ read mRNA as it is being transcribed

Differences between prokaryotes & eukaryotes

-time & physical separation between processes. It takes eukaryote ~1 hour from DNA to protein. RNA processing

So….. What is a gene?

One gene – one enzyme?

-but not all proteins are enzymes

-but all proteins are coded by genes

One gene – one protein?

-but many proteins are composed of several polypeptides

-but each polypeptide has its own gene

One gene – one polypeptide?

-but many genes only code for RNA (tRNA, rRNA…)

One gene – one product?

-but many genes code for more than one product …

“Defining a gene is problematic because… one gene can code for several protein products, some genes code only for RNA, two genes can overlap, and there are many other complications.”

 – Elizabeth Pennisi, Science 2003

Cracking the Code:

Crick: determined 3-letter (triplet) codon system

Nirenberg (47) & Khorana (17): determined mRNA–amino acid match. They added fabricated mRNA to test tube of ribosomes, tRNA & amino acids

-created artificial UUUUU… mRNA

-found that UUU coded for phenylalanine (phe)

The code:

Code for \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ life!

-strongest support for a common origin for all life

Code is redundant

-several codons for each amino acid

-3rd base “wobble”

Start codon is AUG. It codes for the amino acid methionine

Stop codons: There are 3 UGA, UAA, UAG

Transfer RNA Structure

Has “Clover leaf” structure with \_\_\_\_\_\_\_\_\_\_\_\_\_ on “clover leaf” end and amino acid attached on 3’ end

Loading tRNA:

Aminoacyl tRNA synthetase

-enzyme which bonds amino acid to tRNA would require energy

-ATP 🡪 AMP

-energy stored in tRNA-amino acid bond

-unstable

-so it can release amino acid at ribosome easily

Ribosomes:

Facilitate coupling of tRNA anticodon to mRNA codon

-organelle or enzyme?

Structure ribosomal RNA (rRNA) & proteins. Has 2 subunits (large and small)

\_\_\_\_\_\_\_\_ site (aminoacyl-tRNA site)

holds tRNA carrying next amino acid to be added to chain

­­­­­­­­­\_\_\_\_\_\_\_\_ site (peptidyl-tRNA site)

holds tRNA carrying growing polypeptide chain

\_\_\_\_\_\_\_\_ site (exit site)

empty tRNA to leave ribosome from exit site

Building a polypeptide:

Initiation: brings together mRNA, ribosome subunits, initiator tRNA

Elongation: adding amino acids based on codon sequence

Termination: end codon

Protein targeting: Signal peptide as an address label