Gene Expression in Eukaryotic cells

Central Dogma

DNA is the the genetic material of the eukaryotic cell.

Watson & Crick worked out the structure of DNA as a double helix.

According to what Francis Crick called the "Central Dogma of Molecular Biology"

- DNA is replicated to make copies of itself.
- The information in DNA is transcribed into RNA
- This information is then translated into protein

The Flow of Genetic Information

The information in DNA is contained in the form of specific sequences of nucleotides.

The DNA inherited by an organism leads to specific traits by dictating the synthesis of proteins.

Gene expression, the process by which DNA directs protein synthesis, includes two stages:

- transcription
- translation

Importance of proteins

Proteins make up much of the physical structure of an organism.

Enzymes exert control over all of the chemical processes inside a cell by turning them on and off at precise times.

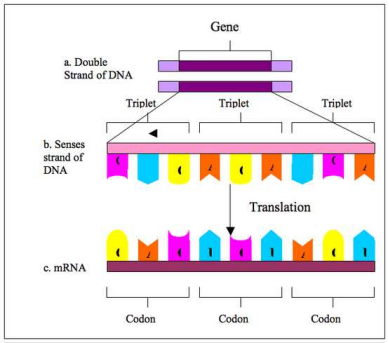
So, all the form and function of a cell is directed by:
- what proteins are made
  or more importantly -
- what genes are expressed by the cell.

Comparison of Eukaryotic and Prokaryotic genes

Prokaryotes have a single small, circular chromosome in their cytoplasm. **Eukaryotes have chromatin fiber contained in a nucleus.**

Prokaryotes regulate their gene expression by using operons that turn genes on and off depending on the chemical environment of the cell. **Eukaryotes have much more complex chromosomes that require multiple levels of regulation.**

Transcription and Translation



a. genes are portions of DNA that code for a particular protein.
b. Triplets, the three-base sequences code for amino acid sequences.
c. Transcribed mRNA codons, which are complementary to the code in DNA triplets.

Transcription and translation occur in Eukaryotes the same as in Prokaryotes, but there are extra steps that help regulate expression

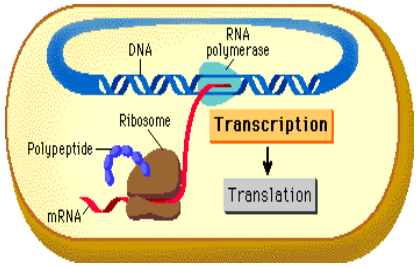
1 A particular triplet of bases in the template strand of DNA is AGT. The corresponding codon for the mRNA transcribed is

- A AGT.
- B UGA.
- C TCA.
- D ACU.
- E UCA

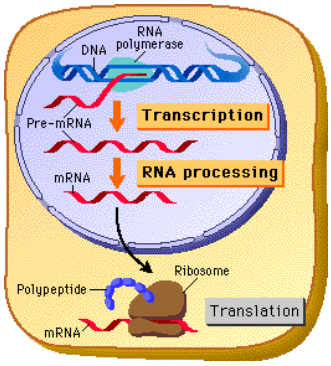
2 A codon

- A consists of two nucleotides.
- B may code for the same amino acid as another codon.
- C consists of discrete amino acid regions.
- D catalyzes RNA synthesis.
- E is found in all eukaryotes, but not in prokaryotes.

Gene expression in Prokaryotes



Gene expression in Eukaryotes overview



Complexity of Eukaryotes

Multicellular Eukaryotes have very high levels of complexity not seen in Prokaryotic organisms. Many cells of different types work together, expressing specific genes in specific situations, to contribute to the survival of the overall organism.

For this reason, eukaryotes must exhibit complex regulation of their genes.

If a mistake is made and genes are expressed in the wrong way, the survival of the organism is put in jeopardy.

All cells in a multicellular eukayote have the same DNA

All cells in a multicellular eukaryote contain the same genome. Every cell has all the genes necessary to make all parts of the organism.

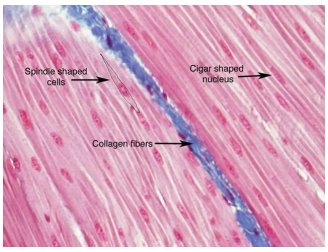
Cells become specialized by only expressing certain genes, a small fraction of all the genes in the genome.

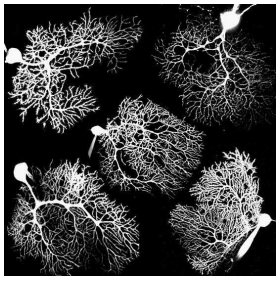
The main factor in this specialization is what genes are "unpacked" so they can be exposed to RNA polymerase.

3 If the triplet CCC codes for the amino acid proline in bacteria, then in plants CCC should code for

- A leucine.
- B valine.
- C cystine.
- D phenylalanine.
- E proline.

All cells in a multicellular eukaryote have the same DNA





These muscle cells and brain cells (neurons) have the same DNA but they are expressing different genes, that is why their structure and function is so different.

Chromatin structure determines a cell's purpose

Most DNA in a nucleus is packed into a structure called **chromatin**. The DNA is tightly wound around proteins called **histones** like thread wrapped on a spool. The combination of eight histones and DNA is called a **nucleosome**.

http://www.youtube.com/watch?v=gbSIBhFwQ4s&feature=related

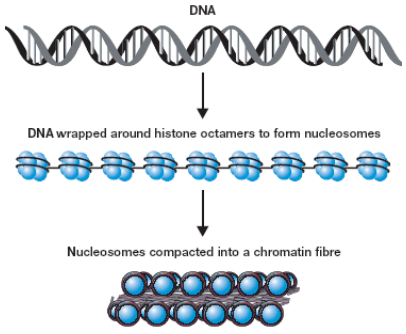
Chromatin structure determines a cell's purpose

When DNA is packed in chromatin it is not accessible to RNA polymerase so transcription can not happen.

The genes that need to be expressed are unwound from histones by **chromatin modifying enzymes** in order to expose their nucleotide sequences.

Genes that are unnecessary to a particular cell will remain packed while the neccessary ones are unpacked.

Chromatin structure determines a cell's purpose



All gene sequences are exposed to RNA polymerase

Some genes exposed

No genes exposed

Once DNA is unpacked it can be transcribed

Eukaryotic RNA polymerase needs the assistance of proteins called **transcription factors** that also help regulate when a gene is expressed.

If all the necessary transcription factors are present for a specific gene, then the gene can be expressed. If any are missing, transcription will not start.

There can be thousands of transcription factors in an organism's cells (3,000 in humans). The kind and number of them present in the nucleus at any given time dictate what genes are expressed.

Transcription factors are essential for the regulation of gene expression

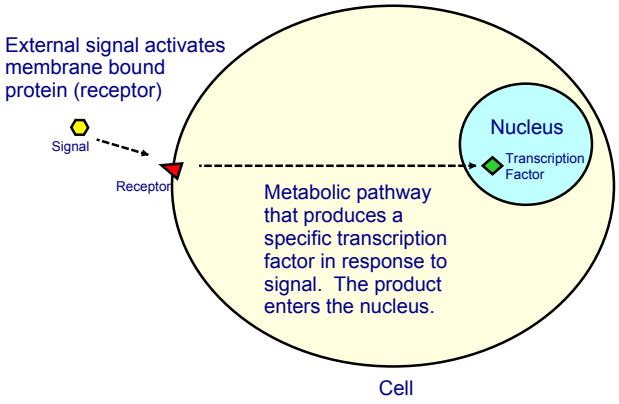
Transcription factors are proteins that are capable of binding with DNA. When they bind to areas near the promoter region of the gene they work with RNA polymerase to begin the transcription of that gene.

They are produced in response to cues from the external environment of the cell.

These proteins make the cell capable of turning on genes in response to external stimulus. This is essential to multicellular eukaryotes because it allows the different cells of the organism to communicate and respond to situations in unison.

http://www.youtube.com/watch?v=vi-zWoobt_Q

Transcription factors are essential for the regulation of gene expression



After transcription, expression can still be regulated

Transcription alone does not account for gene expression. Certain mechanisms can stop or help a transcript of mRNA to be translated.

RNA Processing
Degredation of mRNA
Transport to Cytoplasm
Degredation of Proteins

These mechanisms allows a Eukaryotic cell to rapidly and specifically adjust its gene expression in response to its surroundings.

mRNA Processing

After Transcription, the transcript is known as **pre-mRNA**. Enzymes in the eukaryotic nucleus modify pre-mRNA before the genetic messages are sent to the cytoplasm

During RNA processing, both ends of the primary transcript are altered.

Some interior sequences of the molecule may be cut out, and other parts spliced together.

Alteration of mRNA Ends

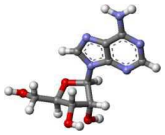
Each end of a pre-mRNA molecule is modified in a particular way The 5`end receives a molecule known as a **nucleotide cap** and the 3` end gets a **poly-A tail.**

These modifications have several functions:

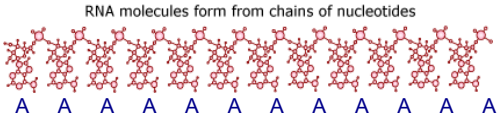
· They facilitate the export of mRNA from the nucleus to the cytoplasm
· They protect mRNA from hydrolytic enzymes once it is in the cytoplasm
· They help ribosomes attach to the mRNA so they can be translated into a protein.

Alteration of mRNA Ends

The 5`cap is a modified guanine molecule (the G in A, T, C, G)



The 3` tail is series of adenosine (A) nucleotides.

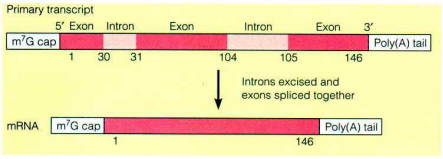


RNA Splicing

Most eukaryotic genes and their RNA transcripts have long noncoding stretches of nucleotides that lie between coding regions. These **noncoding regions** are called intervening sequences, or **introns.**

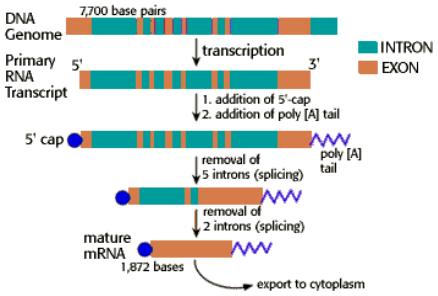
The other regions called **exons** (because they are eventually **ex**pressed), are usually translated into amino acid sequences.

RNA splicing removes introns and joins exons, creating an mRNA molecule with a continuous coding sequence.



Alteration of pre-RNA

This is an example of a pre-mRNA becoming a final transcript.



Alternative RNA splicing

Some genes can code more than one kind of polypeptide, depending on which segments are treated as exons during RNA splicing.

Alternative splicing allows the number of different proteins an organism can produce to be much greater than its number of genes.

Alternative RNA splicing

DNA sequence
AAATTTCCCGGGAAATTTCCCGGG

Pre-mRNA
(Cap)-UUUAAAGGGCCCUUUAAAGGGCCC-(Tail)

Alternate splices
(Cap)-UUU AAA UUU AAA-(Tail) OR (Cap)-GGC CCG GGC-(Tail)

Resulting polypeptide (protein)
Phe - Lys - Phe - Lys OR Gly - Pro - Gly

Alternate splicing can dramatically change the length and/or the sequence of the polypeptide chain that will be made

4 What are the coding segments of a stretch of eukaryotic DNA called?

- A introns
- B exons
- C codons
- D replicons
- E transposons

5 Which of the following helps to stabilize mRNA by inhibiting its degradation?

- A RNA polymerase
- B ribosomes
- C 5' cap
- D poly-A tail
- E both C and D

6 A transcription unit that is 8,000 nucleotides long may use 1,200 nucleotides to make a protein consisting of 400 amino acids. This is best explained by the fact that

- A many noncoding nucleotides are present in mRNA.
- B there is redundancy and ambiguity in the genetic code.
- C many nucleotides are needed to code for each amino acid.
- D nucleotides break off and are lost during the transcription process.

7 Once transcribed, eukaryotic pre-mRNA typically undergoes substantial alteration that includes

- A removal of introns.
- B fusion into circular forms known as plasmids.
- C linkage to histone molecules.
- D union with ribosomes.
- E fusion with other newly transcribed mRNA.

8 A mutation in which of the following parts of a gene is likely to be most damaging to a cell?

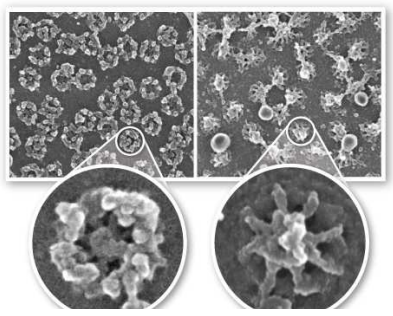
- A intron
- B exon
- C would be equally damaging.

9 Alternative RNA splicing

- A can allow the production of proteins of dramatically different sizes from a single mRNA.
- B can allow the production of proteins of dramatically different amino acid sequences from a single mRNA.
- C Both can happen

Entrance into the Cytoplasm

After the finalized mRNA transcript is complete and correct, the pores in the nuclear envelope allow it to pass to the cytoplasm where it can be transcribed by ribosomes.



The nuclear pore is a protein structure that controls the traffic flow of the nucleus. Each nuclear pore is made up of hundreds of individual proteins that insure only mRNAs with proper caps and tails can make it to the cytoplasm.

Degradation of mRNA

Hydrolytic enzymes in the cytoplasm breakdown mRNA molecules. The length of time an mRNA suvives in the cytoplasm relates to how much protein is made from it. Longer time in the cytoplasm means more translation by ribosomes.

The length of the poly-A tail is one of many factors that determines the time of survival in the cytoplasm. The longer the tail, the longer it's survival.

Protein Degradation

Mechanisms exist in the cytoplasm to breakdown damaged or unused proteins.

The endoplasmic reticulum can label proteins with a carbohydrate to make a glycoprotein. This tag also can be used as a marker to determine when a protein will be broken down.

At some point, the protein will be broken down and it will no longer perform it's function in the cell. The cell must make more proteins of the same type if it wants the function to continue.

Summary of Gene Expression Regulation in Eukaryotes

- The gene must be unpacked from chromatin
- The right transcription factors must be present

Transcription occurs

- Pre-mRNA must be edited (spliced)
- Cap and tail must be added to the mRNA
- Nuclear envelope pores allow passage to the cytoplasm
- mRNA is not broken down by hydrolytic enzymes in the cytoplasm so it can contact a ribosome

Translation occurs

- Protein must survive long enough to perform it's function

Mutations

A **mutation** is a permanent change in the DNA sequence of a gene. Mutations in a gene's DNA sequence can alter the amino acid sequence of the protein encoded by the gene.

Like words in a sentence, the DNA sequence of each gene determines the amino acid sequence for the protein it encodes. The DNA sequence is interpreted in groups of three nucleotide bases, codons. Each codon specifies a single amino acid in a protein.

10 The change a mutation causes to DNA is :

- A temporary
- B always fatal
- C permanent
- D flawed

Substitution Mutations

When a nucleotide in a gene is copied incorrectly during DNA replication, one nucleotide can be substituted with another. This results in the incorrect amino acid sequence, changing the structure of the protein.

Correct DNASequence: AAA TTT CCC GGG AAA TTT CCC GGG

Correct RNA Transcript: UUU AAA GGG CCC UUU AAA GGG CCC

Correct Polypeptide: Phe - Lys - Gly - Pro - Phe - Lys - Gly - Pro

Substitution mutation: AAA TTT CCC GGG ATA TTT CCC GGG

Resulting Transcript: UUU AAA GGG CCC UAU AAA GGG CCC

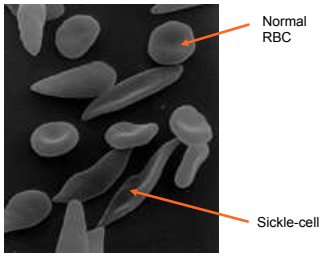
Resulting Polypeptide: Phe - Lys - Gly - Pro - Tyr - Lys - Gly - Pro

11 Changing one nucleotide in a DNA sequence can change _____ in a protein

- A a polypeptide
- B the primary structure
- C the secondary structure
- D the tertiary structure
- E all of the above

Sickle-Cell Disease

Sickle-cell disease is a blood disorder caused by a substitution mutation. It is characterized by red blood cells that assume an abnormal, rigid, sickle shape.



Sickling decreases the cells' flexibility and ability to carry oxygen. It results in a risk of various complications. The sickling occurs because of a mutation in the hemoglobin gene.

12 Sickle-Cell Disease is caused by a mutation in the _____ gene.

- A red blood cell
- B hemoglobin
- C substitution
- D sickle-cell

Reading Frame Shifts

We can think about the DNA sequence of a gene as a sentence made up entirely of three-letter words.

Thesunwashot

If you were to split this sentence into individual three-letter words, you would probably read it like this:

The sun was hot

If this sentence represents a gene then each letter corresponds to a nucleotide base, and each word represents a codon. If you shifted the three-letter **reading frame** it would result in a sentence which is not understandable...

_ _T hes unw ash ot_

Or

Th esu nwa sho t _

Insertion and Deletion Mutations

When a nucleotide in a gene is copied incorrectly during DNA replication, nucleotide can be added or deleted. This results in a reading frame shift and the incorrect amino acid sequence, changing the structure of the protein.

Correct DNASequence:	AAA TTT CCC GGG
RNA Transcript:	UUU AAA GGG CCC
Correct Polypeptide:	Phe - Lys - Gly - Pro

Insertion mutation:	AAA ATT TCC CGG G_ _
Resulting Transcript:	UUU UAA AGG GCC C_ _
Resulting Polypeptide:	Phe - STOP

Deletion mutation:	AAT TTC CCG GG_
Resulting Transcript:	UUA AAG GGC CC_
Resulting Polypeptide:	Leu - Lys - Gly ?

A

13 The result of a deletion mutation in DNA is that it potentially changes the:

- A structure of the protein
- B chromatin
- C DNA backbone
- D number of chromosomes

14 Using AAA TTT GGG AAA as an example, which of the following would be an example of a frame shift mutation?

- A AAA TTT CCC GGG
- B AAA TTT GGG AAA
- C AA ATT TCC CGG G
- D TTT CCC GGG

Tay-Sachs Disease

Tay-Sachs disease is a result of insertion and deletion mutations. It causes a relentless deterioration of mental and physical abilities that commences around six months of age and usually results in death by the age of four.

The disease results from mutations on chromosome 15 in humans. These mutations include base pair insertions and deletions. Each of them alters a protein product, and thus inhibits the function of enzymes in some manner.

Junk DNA and Silent mutations

Junk DNA is the term given to DNA that does not code for proteins. It is the space between genes. A large portion of the DNA in humans is junk DNA, better than 90% in some estimates. That means if a mutation happens in 90% of a humans DNA then the person is unaffected. This is known as a **silent mutation**.

Another way to make a mutation silent is the redundancy in the genetic code. Each amino acid has more than one possible codon. So, if a substitution occurs, the same amino acid may still be coded. This reduces the possibility of a mutation located in a gene causing a change in the protein.

Ala - GCU, GCC, GCA, GCG
Leu - UUA, UUG, CUU, CUC, CUA, CUG
Arg - CGU, CGC, CGA, CGG, AGA, AGG

15 DNA which does not code for proteins is referred to as:

- A Silent DNA
- B Excess DNA
- C Junk DNA
- D Redundant DNA

Are mutations always bad?

Not necessarily...

Very rarely a mutation will cause an individual to become stronger than the rest of its population. Sometimes a mutation can create a polar bear with thicker fur, or a giraffe with a longer neck.

These would be advantages to the individual and they may become fitter to survive in their environment.

Mutagens

A **mutagen** is a physical or chemical agent that can change the DNA of an organism and thus increases the frequency of mutations.

What examples can you think of?

Spontaneous Mutagens

Not all mutations are caused by mutagens.

Spontaneous mutations occur due to errors involving:
· Changes to the Chemistry of the DNA
· DNA replication, repair and recombination.

This shows a DNA strand slipping out of place during replication, causing a mutation in the DNA once it has been repaired.