



Alternative Splicing

Introduction

One of the ways in which the vertebrate genome is more complex than that of other organisms is by increased use of alternative splicing.

In alternative splicing, more than one protein product is made from one gene.

This explains how vertebrates are able to make 5 times as many proteins as flies or worms, with only 2 times as many genes.

Alternative splicing occurs frequently in vertebrates.

Alternative splicing is estimated to occur for 59% of vertebrate transcripts compared to 22% of nematode transcripts.

Vertebrates have an estimated 3.2 different final transcripts per gene compared to 1.34 for nematodes.

Gene Structure

Eucaryotic genes are composed of interspersed exons and introns.

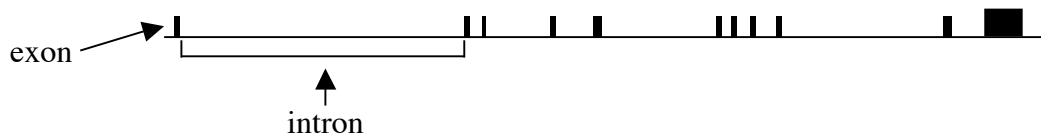
Exons (**expressed sequences**) contain the coding regions of the protein.

Introns (**interspersed sequences**) are removed after transcription.

In vertebrates usually only 5% of the gene is made up of exons.

Below is pictured what the gene structure of a gene looks like on the NCBI website.

Black boxes are exons. Introns are the open areas between the boxes.



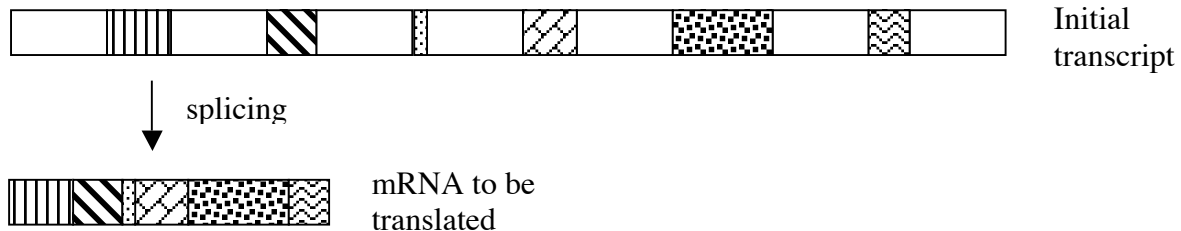
Most genes have 7 or 8 exons, with an average length of 145 bp.

Introns have an average length of 3365 bp.

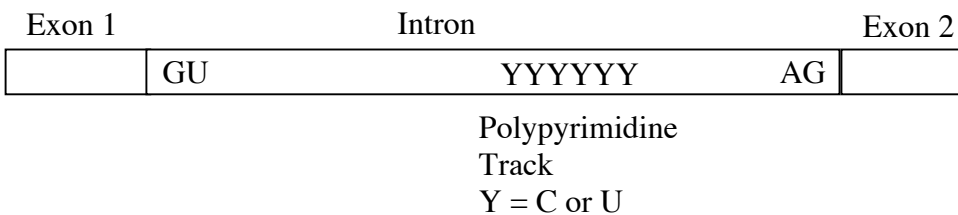
Splicing

Following transcription, introns are removed from the gene by a process called splicing. Splicing results in a joining of all the exons that make up the coding region of a protein.

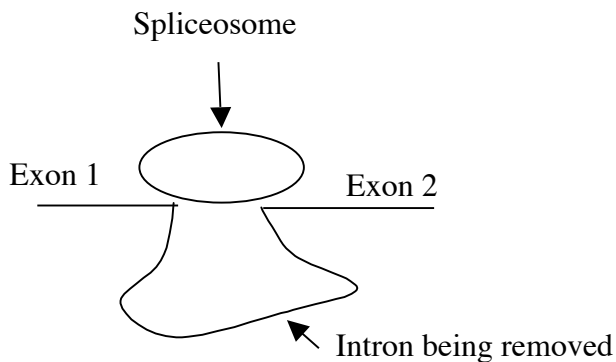
Open boxes represent introns
Filled boxes represent exons



Splicing is mediated by sequences found within the intron; GU immediately following an exon, AG immediately prior to the next exon, and a polypyrimidine track. These sequences are shown below.



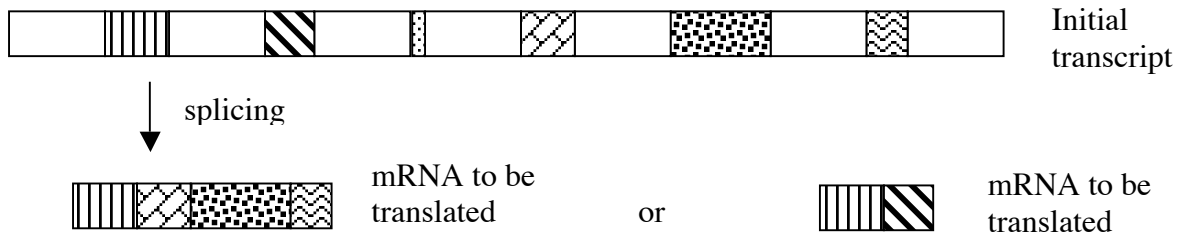
Splicing is carried out by a spliceosome that is made up of a combination of proteins and RNAs. The spliceosome acts as the enzyme that catalyzes splicing. The spliceosome recognizes and binds to sequences found within the introns and causes looping out of the intron.



Alternative Splicing

In alternative splicing, the same gene is processed in two or more ways. When the gene is spliced, different exons may be included or excluded from the final transcript.

For example, the initial transcript shown below could be spliced to produce several different mRNA products, two of which are shown below.



This same initial transcript could be spliced in many additional ways not shown above.

This changes the “one gene, one protein” theory.

Now we see that many proteins can be made from one gene.

Humans have only 30,000 genes. Earlier predictions of gene number based on the number of proteins humans were estimated to produce was 100,000. Vertebrates have an average of 3.2 different final transcripts per gene, compared to 1.34 for nematodes. Thus the human genome with only 30,000 genes can produce 100,000 different proteins with only 30,000 genes. About 59% of our genes are spliced into more than one different mRNA, compared to 22% of nematode transcripts.

Alternative splicing explains how vertebrates have 5 times as many proteins as flies and worms but only two times as many genes.

Examples of Alternative Splicing

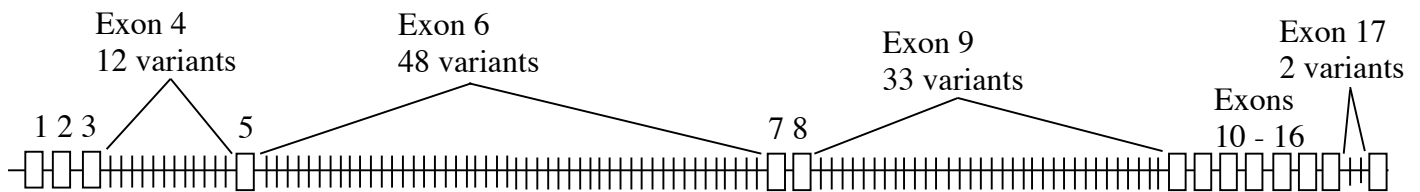
Drosophila melanogaster – axon guidance.

In *Drosophila*, the dscam (Down syndrome cell adhesion molecule) gene encodes an axon guidance receptor which is involved in the migration and connection of neurons during embryogenesis.

The gene contains 24 exons (exons 1 – 18 are depicted below).

Four of the exons have different variants.

Note: this method of numbering is different from the standard numbering. By standard numbering the 12 different variants of exon 4 would be numbered exons 5 – 17. Researchers chose to number these exons differently because of the large number of exons involved and due to the way in which the exons are included in final transcripts.



Only one of each exon is included in the final transcript.

Thus the gene is able to encode $12 \times 48 \times 33 \times 2 = 38,016$ possible proteins. This is 2 – 3 times the total number of genes *Drosophila* has!

It is thought that these many different variants of dscam are responsible for helping neuronal axons find their correct destinations during embryogenesis.

Ways in which alternative splicing can be used

This is just a preview, you will explore some of these different uses in more detail in the activity that follows.

- In the example above, alternative splicing was used to include only one of several versions of an exon into a final protein product. This allows many slightly different versions of the same protein to be made without repeating the whole gene.
- Alternative splicing can also be used to make 2 completely different proteins by inclusion of mutually exclusive coding exons in the final transcript. In this case, alternative splicing allows the cell to use the same cellular localization and transport exons for both proteins.
- Alternative splicing can be used to convert a transcriptional repressor into an activator by including or excluding exons responsible for activating transcription.
- The same protein can be targeted to different places in the cell by including different exons encoding different transport signals in the final transcript.

Activity – Calcitonin-CGRP Splicing

You will be looking at the alternative splicing of one initial transcript to produce mRNAs encoding two very different proteins with very different functions.

Background

The two different proteins produced from this gene are calcitonin and CGRP.

Calcitonin is a hormone produced by thyroid cells when blood calcium levels are high. Calcitonin causes bone to take up calcium, and reduces calcium uptake by the intestines and kidney.

CGRP (calcitonin gene-related peptide) is a neurotransmitter produced by cells of the nervous system, especially sensory neurons.

Gene Structure

The calcitonin/CGRP gene contains 6 exons.

The functions of the various exons in the Calcitonin/CGRP transcript are listed below

- Exon 1 – not translated, and thus not included in the protein. These sequences allow the ribosome to bind and begin translation.
- Exons 2 and 3 together encode a signal peptide that is removed from the protein after it is formed. This signal peptide serves as a tag that ensures the protein is correctly sent to the Golgi Apparatus following translation.
- Exon 4 – calcitonin coding exon
- Exons 5 and 6 together encode CGRP (calcitonin gene-related peptide), a neurotransmitter

In this activity you will work with a chain of beads representing the transcript.

The white beads represent the introns

The colored beads represent the various exons

Exon 1 – Red

Exon 2 – Orange

Exon 3 – Yellow

Exon 4 – Green

Exon 5 – Blue

Exon 6 – Purple

Procedure

Part 1 - Pretend you are a spliceosome

Construct the mRNAs described below by removing the introns and exons that will not be included. You should receive one chain of beads for each of the alternative mRNAs you will be asked to generate.

mRNA 1 - In the thyroid, the transcript is spliced so that exons 1, 2, 3, and 4 are included. Exons 5 and 6 are not included. Below, draw your chain of beads representing this mRNA (You can use either exon colors or numbers, plus the exon function in your drawing).

mRNA 2 – In nerve cells, the transcript is spliced so that exons 1, 2, 3, 5, and 6 are included. Below, draw your chain of beads representing this mRNA.

Part II - Answer the following questions

Look at the functions of the exons included in each of your mRNAs.

What is the function of the protein produced from mRNA 1?

When and/or where is this mRNA generated?

What is the function of the protein produced from mRNA 2?

When and/or where is this mRNA generated?

Part III – Summarize your results for the class. Include the following

A description of your scenario (overview/background).

The different exons and what they encode.

The different alternatively spliced transcripts and what exons they contain.

The different conditions or cell types in which the different mRNAs are produced.

The different proteins that are produced by each of the mRNAs and the functions of these proteins.

Activity – CREM

You will be looking at a situation in which the same transcript can be spliced to produce either an mRNA encoding a transcriptional activator or an mRNA encoding a transcriptional repressor.

Background

CREM is a protein that binds specific promoters within the human genome. After binding to the promoter, CREM either activates or represses transcription. CREM is expressed primarily in the testes of males. When CREM acts as a transcriptional activator it leads to the transcription of many of the proteins involved in spermatogenesis (sperm formation). When CREM acts as a repressor, it binds the promoter and prevents transcription. Proteins involved in spermatogenesis are not transcribed when CREM is acting as a repressor.

Gene Structure

The CREM gene has 8 exons

The functions of the various exons encoded by the CREM transcript are listed below

- Exon 1 – not translated, and thus not included in the protein
- Exon 2 - contains the translational start
- Exon 3 – glutamine rich domain. When one or more glutamine rich domains are present, the phosphate groups can be added to the P-box (see below), thus making it a transcriptional activator.
- Exons 4 and 5 together encode the P-Box. The P-box is the place where phosphates are added to CREM to change CREM from a repressor to an activator.
- Exon 6 – glutamine rich domain. When one or more glutamine rich domains are present, phosphates can be added to the P-box, thus activating CREM.
- Exons 7 and 8 – DNA binding domain – This domain binds the promoter of genes to be regulated by CREM

In this activity you will work with a chain of beads representing the initial transcript.

The white beads represent the introns

The colored beads represent the various exons

Exon 1 – Red

Exon 2 – Orange

Exon 3 – Yellow

Exon 4 – Green

Exon 5 – Blue

Exon 6 – Purple

Exon 7 – Pink

Exon 8 - Black

Procedure

Part 1 - Pretend you are a spliceosome

Construct the mRNAs described below by removing the introns and exons that will not be included. You should receive one chain of beads for each of the alternative transcripts you will be asked to generate.

mRNA 1 – In males prior to puberty the transcript is spliced such that the mRNA includes exons 1, 2, 4, 5, 7, and 8. Exons 3 and 6 are not included. Below, draw your chain of beads representing this mRNA. (You can use either exon colors or numbers, plus the exon function in your drawing).

mRNA 2 – From puberty on, the transcript is spliced so that all 8 exons are included. Below, draw your chain of beads representing this mRNA.

Part II - Answer the following questions

Look at the functions of the exons included in each of your transcripts.

What is the function of the protein produced from mRNA 1?

When and/or where is this mRNA generated?

What is the function of the protein produced from mRNA 2?

When and/or where is this mRNA generated?

Part III – Summarize your results for the class. Include the following

A description of your scenario (background/overview).

The different exons and what they encode.

The different alternatively spliced transcripts and what exons they contain.

The different conditions or cell types in which the different mRNAs are produced.

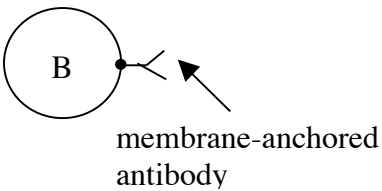
The different proteins that are produced by each of the mRNAs and the functions of these proteins.

Activity – Antibodies

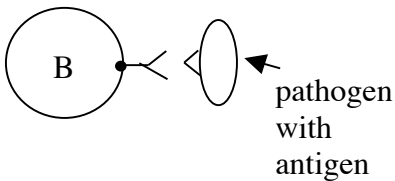
You will be looking at a situation where the same transcript can be alternatively spliced to produce two different forms of an antibody; a form that is anchored in the cell membrane, or a form that is secreted from the cell.

Background

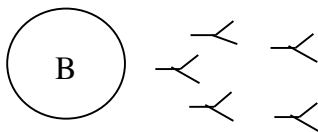
Antibodies are proteins produced by B-cells, a type of white blood cell. Antibodies bind to specific molecules (called antigens) on the surface of invading organisms (pathogens). They help prevent and clear infection by blocking attachment of invading microbes or by directing other components of the immune response against the attacker.



Prior to infection, B-cells produce antibodies that are found on their cell membranes. Each B cell produces a different antibody, which recognizes a different potential pathogen or invader. This antibody is embedded in the cell membrane and protrudes from the



This antibody on the surface of the B-cell acts as the receptor for antigens found on an invading microbe. During infection, antigens on the pathogen surface bind the antibody on the surface of the B-cell. This activates the B cell.



The activated B-cell then starts producing antibodies without the membrane anchor that are secreted outside the B-cell into the blood stream where they can act in fighting infection.

Gene Structure

The antibody gene contains 8 exons.

The functions of the various exons encoded by the antibody transcript are listed below

- Exon 1 – encodes a signal peptide, this peptide is removed after translation. This signal peptide helps target the translated protein to the Golgi Apparatus.
- Exon 2 - encodes the variable region. This is the part of the antibody that recognizes a particular antigen.
- Exons 3 – 6 encode the constant region of the antibody. This is the part of the antibody that is the same in different antibodies recognizing different pathogens.
- Exons 7 – 8 – encode a transmembrane domain that anchors the antibody in the B-cell membrane.

In this activity you will work with a chain of beads representing the transcript.

The white beads represent the introns

The colored beads represent the various exons

Exon 1 – Red

Exon 2 – Orange

Exon 3 – Yellow

Exon 4 – Green

Exon 5 – Blue
Exon 6 – Purple
Exon 7 – Pink
Exon 8 - Black

Procedure

Part 1 - Pretend you are a spliceosome

Construct the mRNAs described below by removing the introns and exons that will not be included. You should receive one chain of beads for each of the alternative transcripts you will be asked to generate.

mRNA 1 – In resting B-cells the antibody transcript contains all 8 exons. Below, draw your chain of beads representing this mRNA. (You can use either exon colors or numbers, plus the exon function in your drawing).

mRNA 2 – Once the B-cell has bound antigen it wants to make antibodies against that antigen. Now the transcript is spliced so that only exons 1-6 are included. Below, draw your chain of beads representing this mRNA.

Part II - Answer the following questions

Look at the functions of the exons included in each of your transcripts.

What is the function of the protein produced from mRNA 1?

When and/or where is this mRNA generated?

What is the function of the protein produced from mRNA 2?

When and/or where is this mRNA generated?

Part III – Summarize your results for the class. Include the following

A description of your scenario.

The different exons and what they encode.

The different alternatively spliced transcripts and what exons they contain.

The different conditions or cell types in which the different mRNAs are produced.

The different proteins that are produced by each of the mRNAs and the functions of these proteins.

Summary

We looked at 3 different examples of alternative splicing.

Calcitonin-CRGP

- Two completely different proteins with different functions are made through mutually exclusive exon inclusion in the different transcripts.
- Calcitonin is encoded on exon 4. It is produced by thyroid cells and is a hormone involved in regulating blood calcium levels.
- CRGP is encoded by exons 5 and 6. It is a neurotransmitter and is produced by sensory neurons.
- Precursors to both proteins included a common signal peptide that is encoded by exons 2 and 3.

CREM

- CREM will act as either a transcriptional activator or transcriptional repressor depending on which exons are included.
- Exons 3 and 6 encode a glutamine rich region. When this glutamine rich region is included in CREM, CREM acts as a transcriptional activator.
- When the glutamine rich regions are not included, CREM acts as a transcriptional repressor.
- In males prior to puberty CREM is expressed as a transcriptional repressor.
- Beginning at puberty CREM is expressed as a transcriptional activator and activates transcription from genes involved in sperm production.
- In reality more than 10 different protein products are produced from this gene. The function of most of them has not been precisely determined but they are likely to be fine-tuning of the on/off switch for spermatogenesis.

CREB (we didn't look at this example)

- CREB is a transcriptional activator that is closely related structurally and functionally to CREM.
- Cells can turn off expression of CREB by including an exon with an in-frame stop codon. When this codon is included, translation will end prematurely and no transcriptional activator is produced.

Antibody

- A membrane bound version and a secreted version of the same antibody are produced from the same gene by alternative splicing leading to inclusion or exclusion of the membrane anchor domain.
- Prior to activation B-cells contain antibodies on their surface. These antibodies are anchored in the membrane by inclusion of a membrane anchor domain. These antibodies on the surface of the B-cell are antigen specific and serve as the receptor for antigen.
- Once antigen has bound the receptor, the B-cell wants to make lots of its antibodies and secrete them into the bloodstream. In this case the transcript is spliced to exclude the membrane anchor. This results in the antibody being secreted into the bloodstream.

What's next?

59% of human transcripts have more than one product. This is over 15,000 genes!

Most of these 15,000 have not been studied.

Even when alternative splicing has been identified and studied, the exact function of all the transcripts is not yet clear.

Thus, there is much work remaining that will undoubtedly lead to many new insights into molecular and cellular processes.