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VCE Biology ¾
Gene Expression & The trp Operon [1.3]
Workbook

Outline:

Study Design: Gene Expression and Regulation

The steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes.

The basic elements of gene regulation: the prokaryotic trp operon as a simplified example of a regulatory process.

Learning Objectives:

- **BI34 [1.3.1]** - Identify and recall the process of gene expression in eukaryotes, comparing how it differs in prokaryotes.
- **BI34 [1.3.2]** - Describe the processes of transcription, mRNA processing and translation, recognising the significance of each step to the final product.
- **BI34 [1.3.3]** - Explain how a single gene can give rise to multiple proteins.
- **BI34 [1.3.4]** - Identify and recall the general principles and reasons for gene regulation in both prokaryotes and eukaryotes.
- **BI34 [1.3.5]** - The regulation of the trp operon through the action of the repressor protein.
- **BI34 [1.3.6]** - Describe the regulation of the trp operon through attenuation in high trp environments.

Section A: Overview of Gene Expression

Sub-Section: Introducing Gene Expression and Proteins

Recall!

Active Recall: What is the purpose of DNA?

genetic instructions

↳ chromosome

Active Recall: What is a gene?

↳ A section of DNA that codes for a protein

Discussion: What are proteins and what do they do?

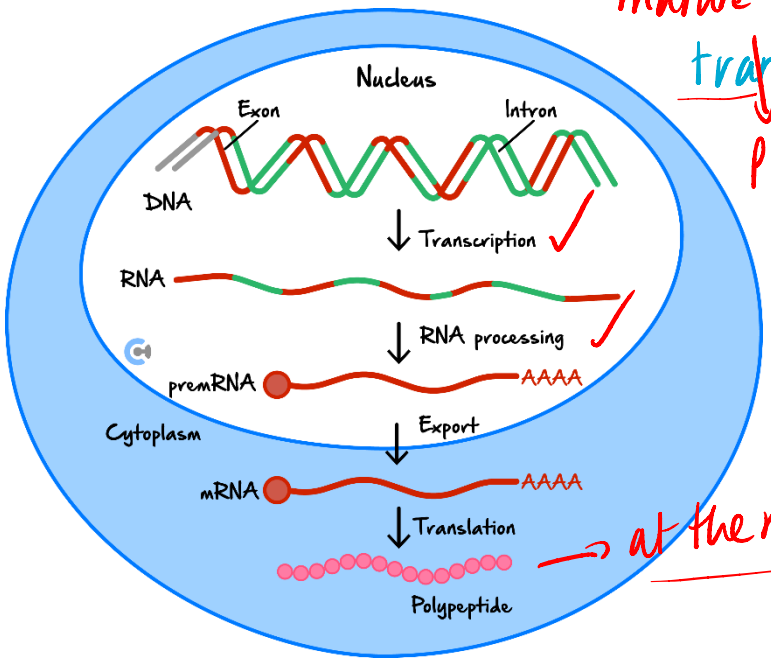
↳ functional

DNA → Protein

How are Proteins Made?

- DNA is the set of instructions that a cell requires to function.
  - It allows for the production of proteins, which are responsible for essentially all the functional capabilities of the cell.
- There are a number of key steps that are required to follow these instructions - known as gene expression.
  - transcription
  - mRNA processing
  - translation

DNA
transcription
preMRNA
mRNA processing
mature mRNA
translation
protein



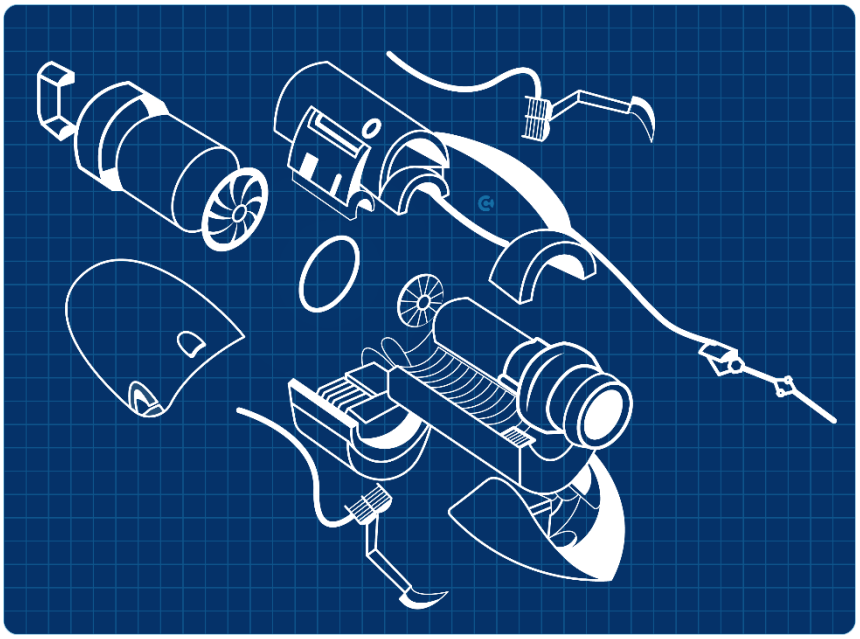
→ at the ribosome

Exploration: Why would RNA be a useful intermediate to have between DNA and proteins?

↳ carries a copy of genetic information outside of the nucleus

↳ Temporary / Shorter

Analogy: The Masterplan



- Let's go back to the 1900s and you are in charge of building Australia's tallest skyscraper – you've created an excellent blueprint after hours of painstaking drawing.
- When the plumber needs to fix a clogged pipe in the 21^{st} floor, are you going to give him the entire blueprint?

NOTE: This idea talked about above is also known as the central dogma of cell biology!

NOTE: This process above is for EUKARYOTES!

How could this process be different in prokaryotes?

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Sub-Section: Comparing Gene Expression in Eukaryotes and Prokaryotes

Active Recall: What is the difference between a eukaryote and a prokaryote?

Prokaryotes do not have a NUCLEUS!

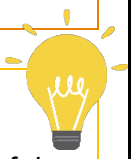
↳ membrane bound organelles

Exploration: What are the differences in gene expression in prokaryotes and eukaryotes?

- NO NUCLEUS
- NO INTRONS

NO RNA PROCESSING

TRANSCRIPTION → TRANSLATION



TIP: The concepts and processes we will be looking at might be complicated to wrap your head around, but whenever you are stuck it always helps to come back to this overview. It is always helpful to ground yourself with the basics - what is the process actually trying to achieve???

REMINDER: Some of the details of the processes we will be talking about maybe beyond the course but have been helpful for understanding purposes - look out for the key takeaways box on how VCAA wants you to answer these questions!

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Section B: Transcription

How does DNA get turned into RNA?

Overview

- Transcription is the process by which an mRNA copy of DNA is made in the nucleus of the cell.
- A complex multi-step process, it is initiated by a key enzyme – RNA Polymerase.
- Three steps: Initiation, Elongation, Termination

START

STOP/END

Context

- What do we know about enzymes?

→ speeding up the rate of chemical reaction

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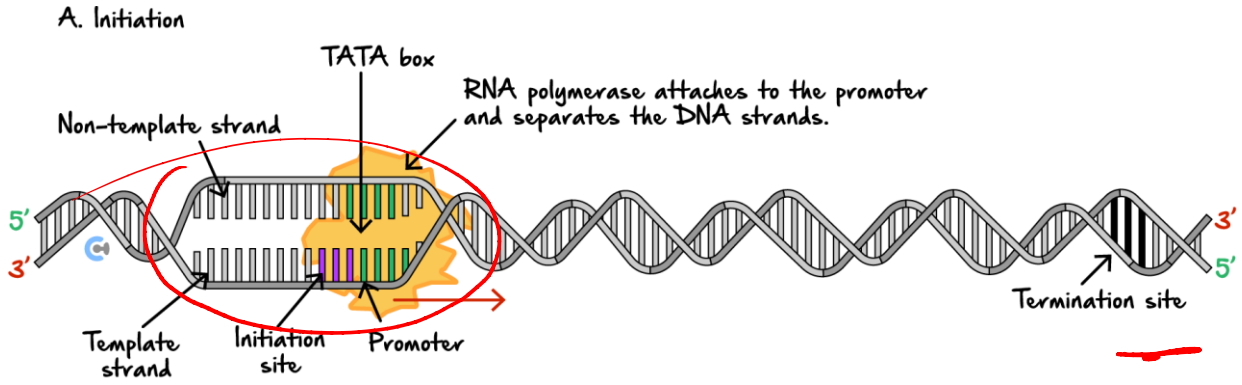
Sub-Section: Initiation

Initiation

- To begin the process, transcription factors assist in the binding of RNA polymerase to the promoter region.
- This initiates the unwinding of DNA, achieved by breaking the hydrogen bond between the 2 strands, allowing the formation of the mRNA strand to occur.



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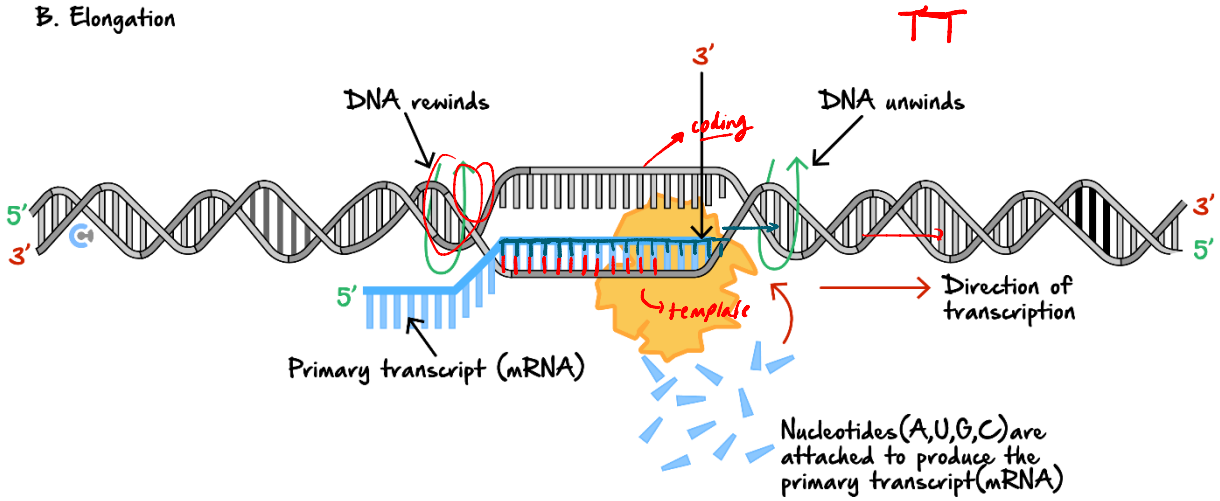
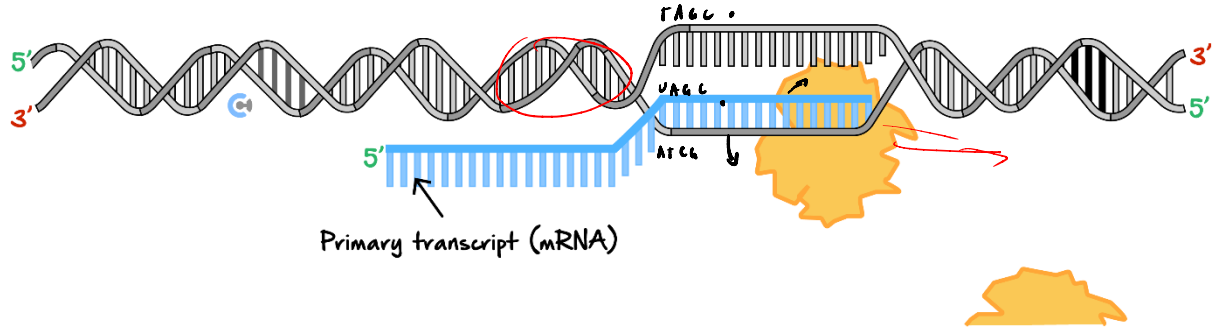
Sub-Section: Elongation

Elongation

- RNA polymerase then moves along the ___template___ strand of DNA - free-floating nucleotides will come and base pair with the exposed ___template___ strand, and as they are "held in place" by this bonding, RNA polymerase will catalyse the ___condensation polymerisation___ reaction to join the ___sugar-phosphate___ backbone, thus forming the pre-mRNA strand.



- As the RNA polymerase moves along, it rezips the section of DNA that has already been transcribed.



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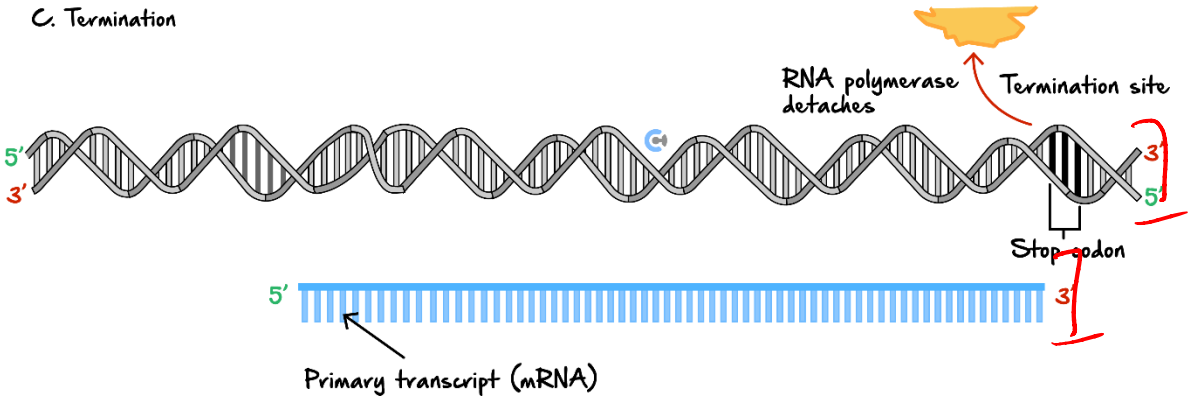
Sub-Section: Termination

Termination

- The elongation process continues until a termination sequence is reached at the end of a gene.



- The DNA will rezip and rewind, returning it back to normal.

Exploration: What is the relationship between the new mRNA strand and the DNA?

coding → same as mRNA except U replaces T

template → complementary as to the mRNA

- What direction is transcription occurring in and why is this the case?

mRNA → produced in a 5' to 3' direction

template - read 3' to 5' (antiparallel to mRNA)

coding read 5' to 3' (antiparallel to template)

free nucleotides can only be added to 3' end

TIP: VCAA does not assess these concepts in this manner, but it is useful to use this IET framework for understanding purposes!

Sample Response: Answering Questions – Transcription

- RNA polymerase binds to the promoter region and the DNA unwinds and unzips.
- RNA polymerase then catalyses the production of the mRNA strand by joining together complementary RNA nucleotides. mRNA is complementary to the DNA template strand – adenine pairs with uracil in RNA instead.
- Continues until a termination or stop sequence is reached.

TIP: Past exam reports can be an amazing resource to determine what VCAA really wants in their answers! They are the "perfect answers" in their eyes.

Question 1 (2 marks)

Explain what RNA Polymerase is and its function.

- RNA polymerase is an enzyme that produces mRNA from DNA
- It does so by joining complementary RNA nucleotides together via condensation polymerisation

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Key Takeaways

- ☑ RNA polymerase binds to the promoter region and the DNA unwinds and unzips.
- ☑ RNA polymerase then catalyses the production of the mRNA strand by joining together complementary RNA nucleotides. mRNA is complementary to the DNA template strand – adenine pairs with uracil in RNA instead.
- ☑ Continues until a termination or stop sequence is reached.

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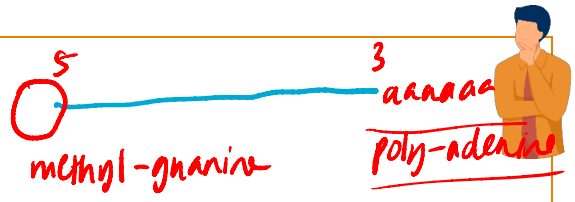
Section C: Pre-mRNA Processing

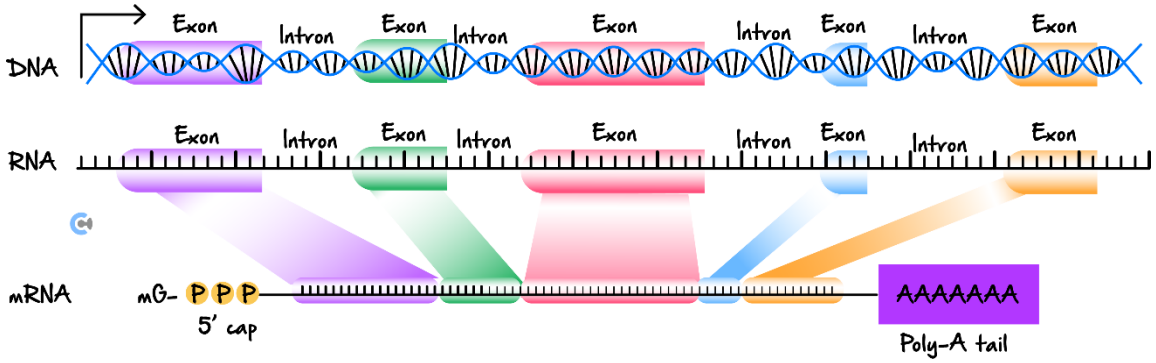
Overview

- This is the second step of the process of gene expression, occurring in the nucleus.
  - The goal is to make the pre-mRNA molecule into _____ mRNA - which is capable of then leaving the nucleus via a pore.

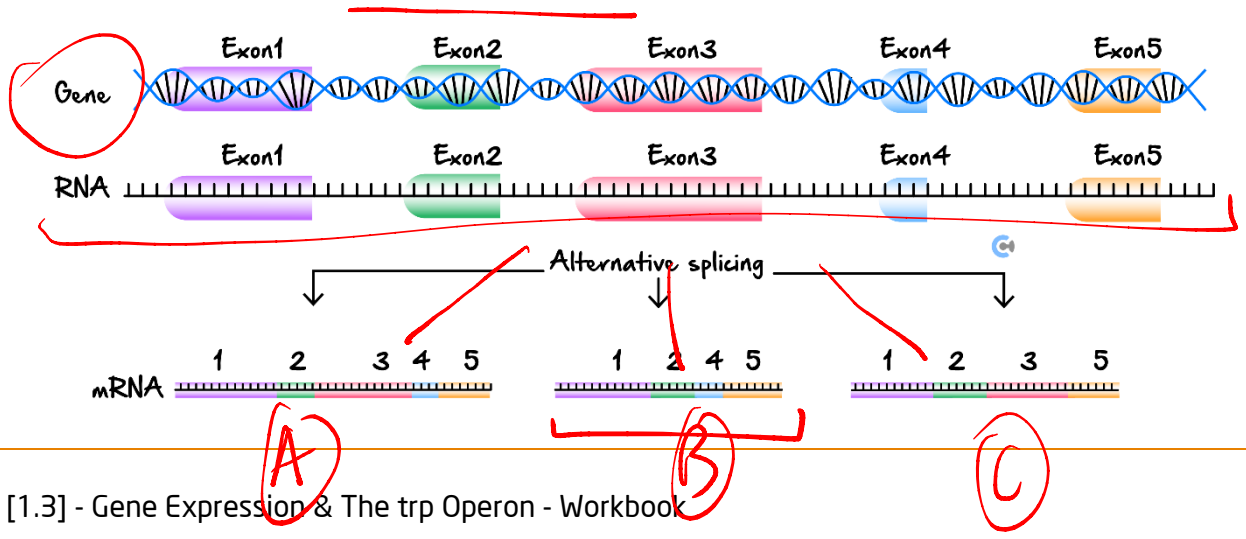
Exploration: The Process



- There are 3 key events that occur in mRNA processing.
  - Addition of a methyl cap to the _____ end.
  - Addition of a poly-A tail to the _____ end.
  - Splicing - _____ are retained and _____ are cut out of the mRNA strand, by a complex molecule known as a spliceosome.

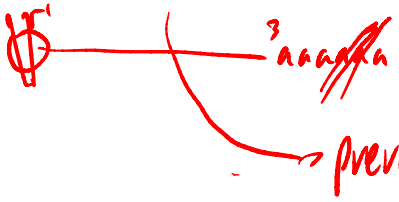


- **Alternative Splicing:**
  - Exons can be removed, introns can be retained or the order of the exons can be shuffled around.



Discussion: What could be the point of the methyl cap and the poly-a tail?

1. Protection



2. Allows detection of the mRNA by the ribosome

Discussion: What is the significance of alternative splicing?

↳ A single gene can be spliced into many unique mRNAs

→ These will code for unique proteins ∴ -

A single gene can code for multiple proteins

NOTE: Sometimes, mRNA processing is grouped together with transcription, as they both occur in the nucleus.

Key Takeaways

- ☑ **Addition of Methyl 5′ Cap:**
  - Added to the 5′ end of the mRNA strand.
  - Prevents degradation.
  - Enables the ribosome to detect the RNA.
- ☑ **Addition of Poly-A Tail:**
  - Added to the 3′ end of the mRNA strand.
  - Composed of adenine bases.
  - Prevents degradation and increases mRNA stability.

- **Splicing: Exons and Introns**
  - Splicing removes introns (non-coding regions) and retains exons (coding regions) in the mRNA strand.
  - This process is performed by a complex molecule called a spliceosome.
- **Alternative Splicing:**
  - Allows flexibility in mRNA processing.
    - Exons can be removed, introns can be retained, or the order of exons can be shuffled.
  - Significance:
    - Creates multiple mRNA variants from a single gene.
    - Leads to the production of diverse proteins from the same gene.

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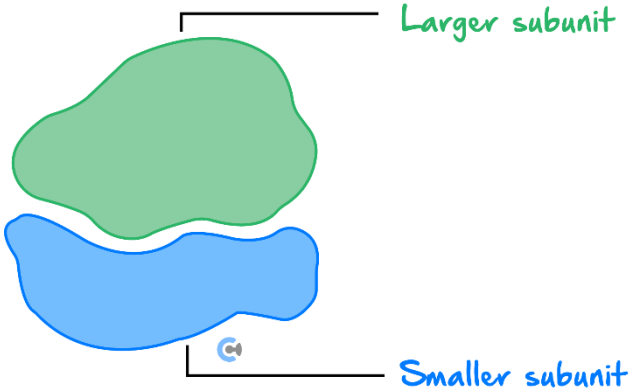
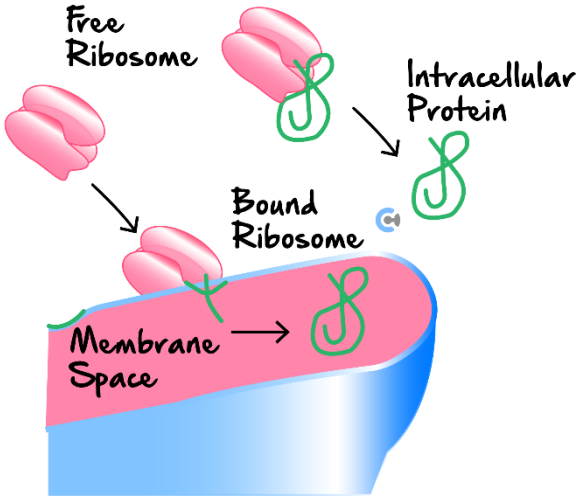
Section D: Translation

How can we change nucleic acids to become proteins?

Overview

- This is the final step of the process - converting the mature mRNA strand to a polypeptide.
  - Polypeptide - amino acids joined together to form a polymer, this then folds later on elsewhere to become a functional protein.
  - Instructions have been followed to the point where they are no longer nucleic acid.
- This occurs outside the nucleus, at the ribosome - they can be free-floating or embedded in the rough endoplasmic reticulum in eukaryotes.
- Structurally, the ribosome can be described as having 1 big unit and a small unit.





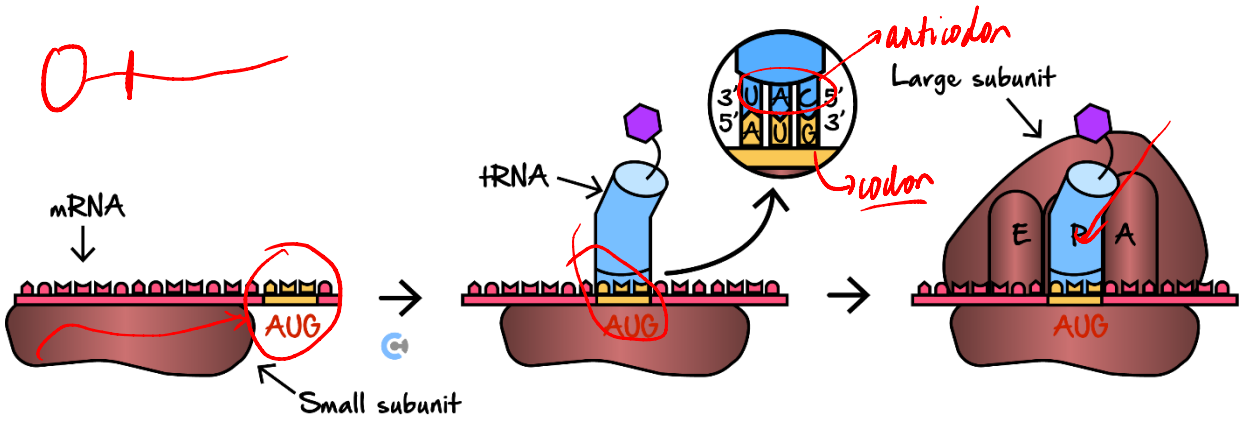
Analogy: Translating a language!

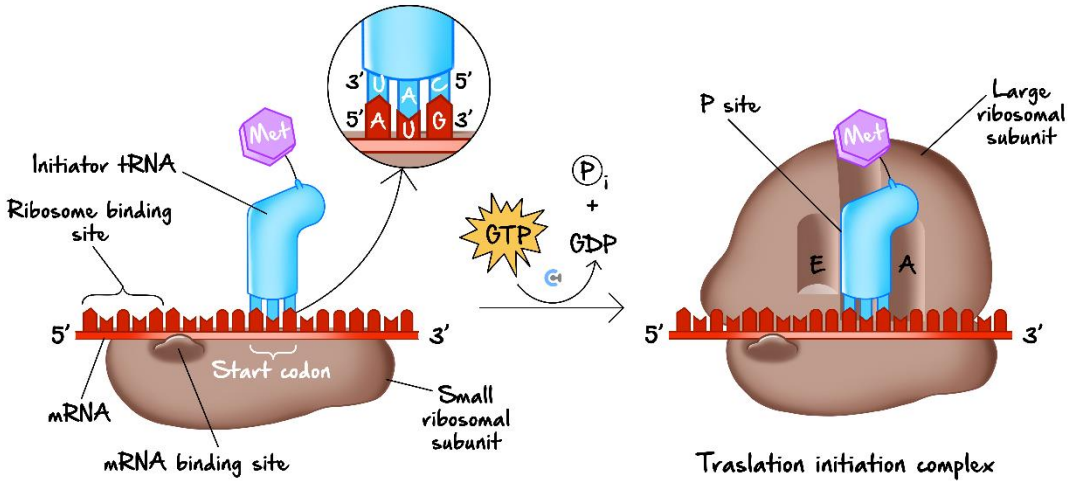
Contour Translate

Sub-Section: Initiation

Initiation

- The mRNA molecule will leave the nucleus via a nuclear pore, and then travel to the ribosome.
- The 5' end of the mRNA will then bind to the ribosome's small subunit, which will move along until it reaches a start codon.
- A tRNA molecule, which will have complementary anticodon, will come and bind to the mRNA, carrying its specific amino acid.
- A large subunit of the ribosome will also then bind, to form the translation complex.





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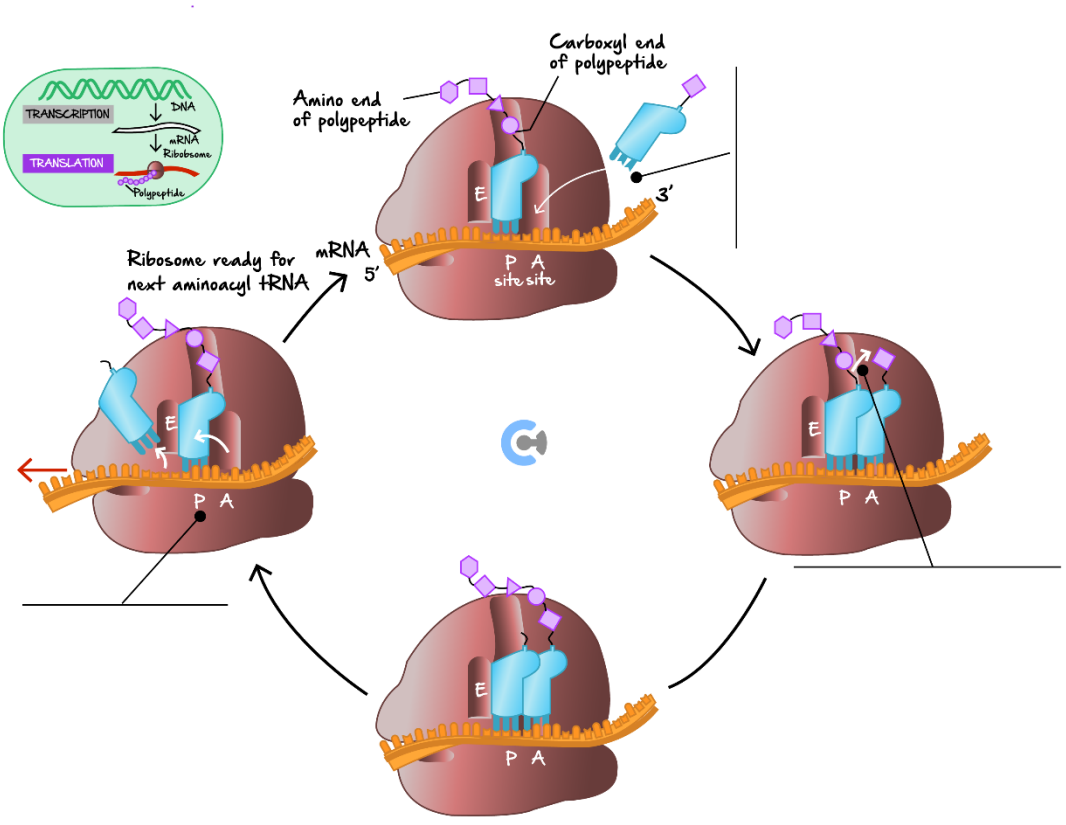
Sub-Section: Elongation

Elongation

- The binding of both the subunits of the ribosome means 3 distinct sites from - the A site, the P site and the exit site.
- As the ribosome moves along, tRNA molecules move along the sites, entering at the A site, shifting to the P site as their amino acid is added to the chain via condensation polymerisation, and then exiting via the exit site as the ribosome moves along.
- The uncharged tRNA will then go back and bind to its specific amino acid.



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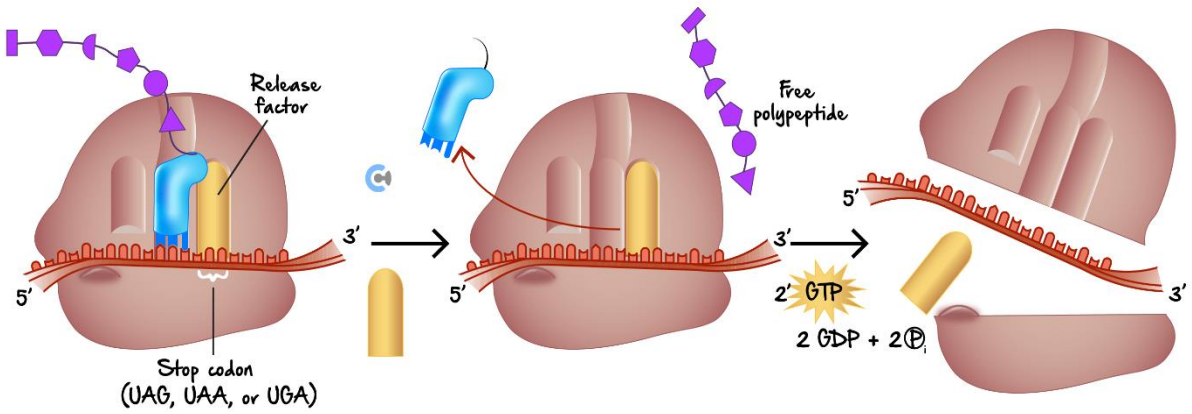
Sub-Section: Termination

Termination

- This continues until the ribosome reaches a stop codon, which will bind to a release factor instead of a tRNA, at which point the polypeptide is released from the ribosome.



Key Takeaways

- ☑ mRNA molecule binds to the ribosome at the 5' end.
- ☑ tRNA anticodons complementary to the mRNA codons, delivering specific amino acids in their correct order to the ribosome.
- ☑ Adjacent amino acids are joined together by condensation polymerisation by the ribosome.
- ☑ Translation ends when the stop codon is reached.

TIP: In biology, a mark usually indicates 1 key point to make in your answers – for transcription and translation it will most likely be 3 marks.

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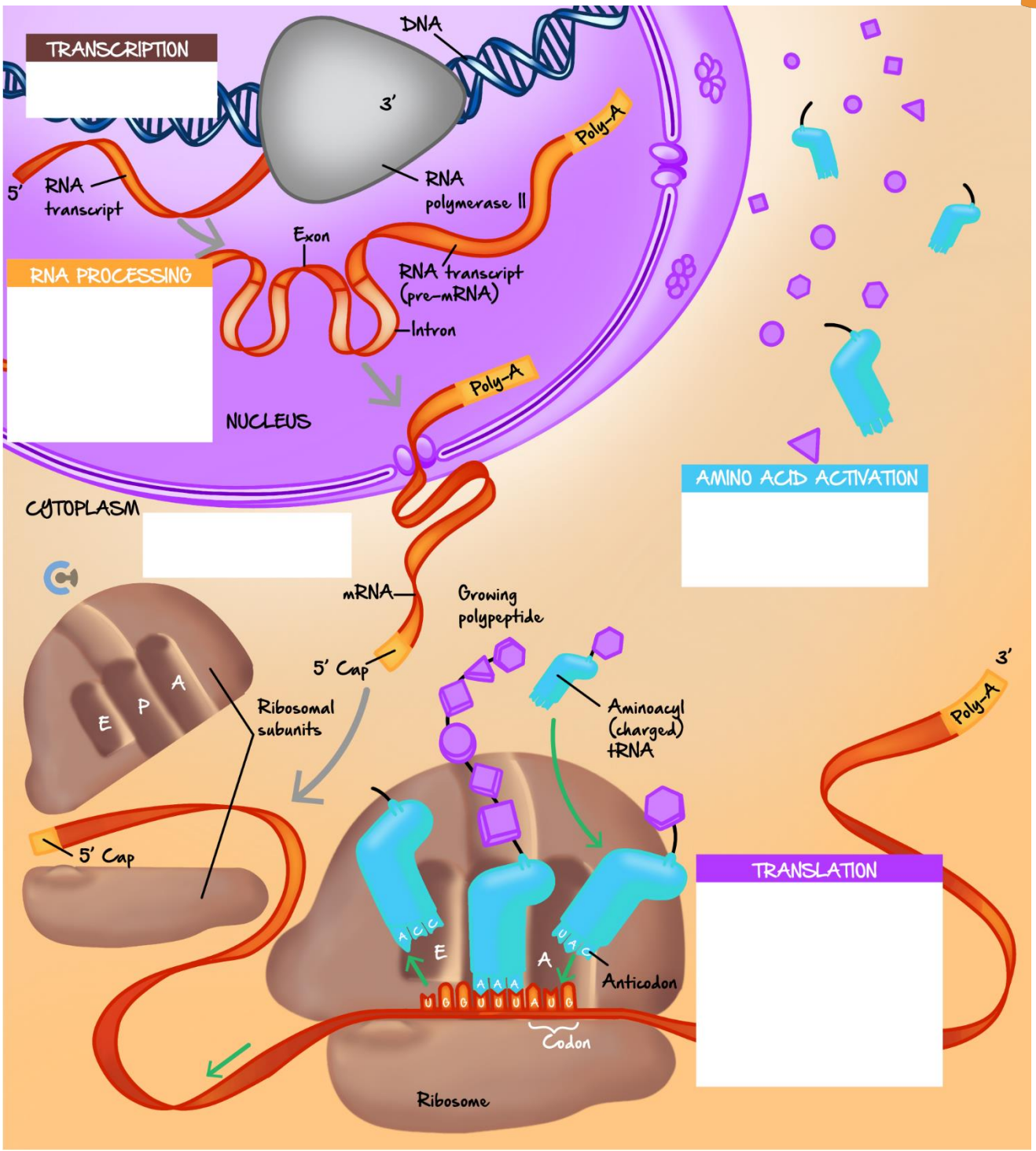
Sub-Section: Summary of Gene Expression

Exploration: Let's bring this all together!



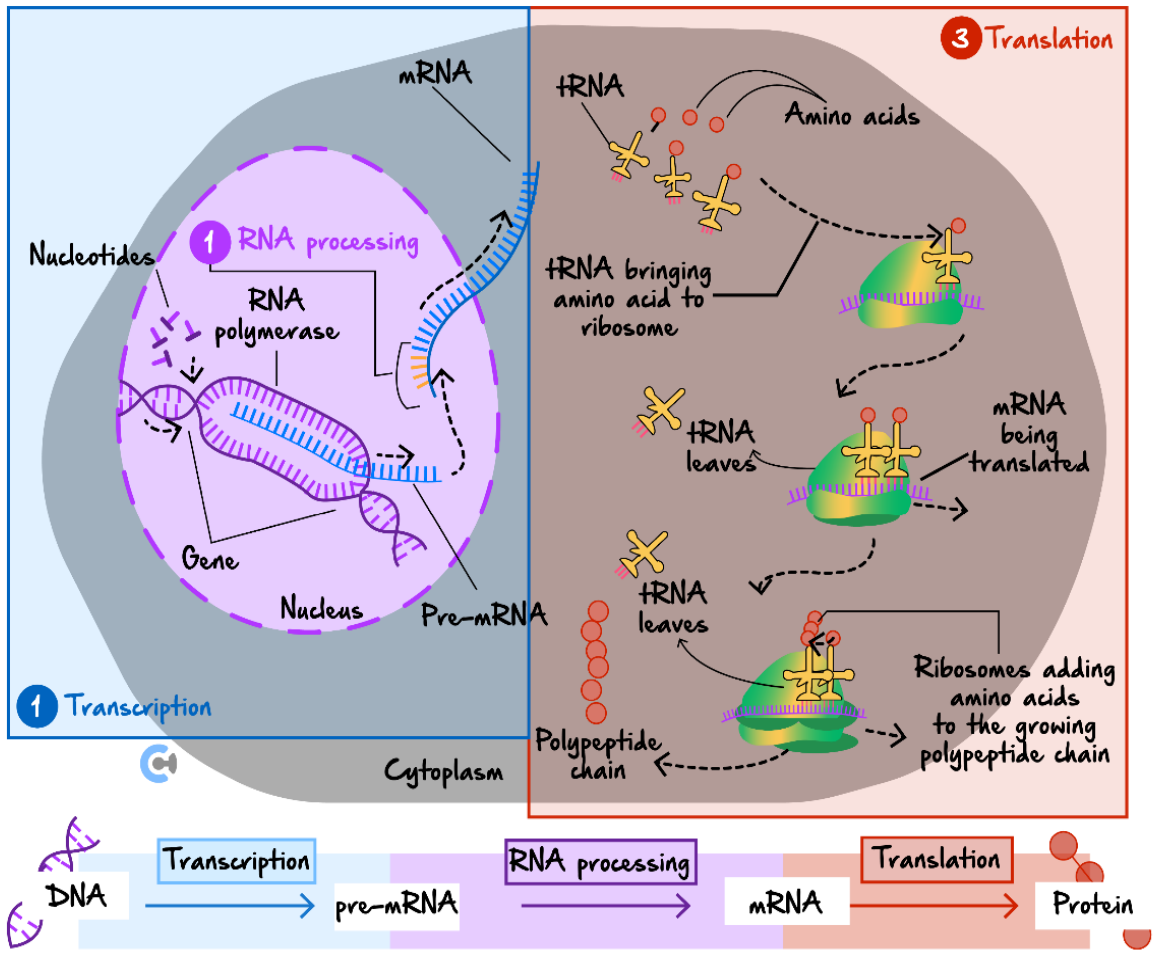


Figure: Summary of transcription, RNA processing and translation

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Section E: Regulation and The trp Operon

Sub-Section: Introducing Gene Regulation

Do we need to make all proteins, all the time?

Gene Regulation

- Gene expression is tightly controlled, and this concept is known as gene regulation.
- Structural genes are genes which code for proteins which contribute to a function.
- Regulatory genes are those which code for transcription factors which control the expression of those structural genes.

Discussion: Why do you think it's important to control gene expression?

1. Conserve energy / resources ✓
2. Too much of protein can be bad
3. Specialisation

How can we control gene expression?

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specific example of gene regulation

Sub-Section: The trp Operon

The trp Operon

- What is an operon? When multiple genes are under the control of a single promotor.
- In E. Coli, this codes for the production of enzymes that will synthesise tryptophan.
  - Necessary for the production of proteins as an amino acid.
  - Regulated through 2 main ways - repression and attenuation.

NOTE: This is a PROKARYOTIC model of gene regulation! Operons are not present in eukaryotic cells.

Discussion: Why do you think operons are beneficial?

↳ Allows control of multiple genes at once

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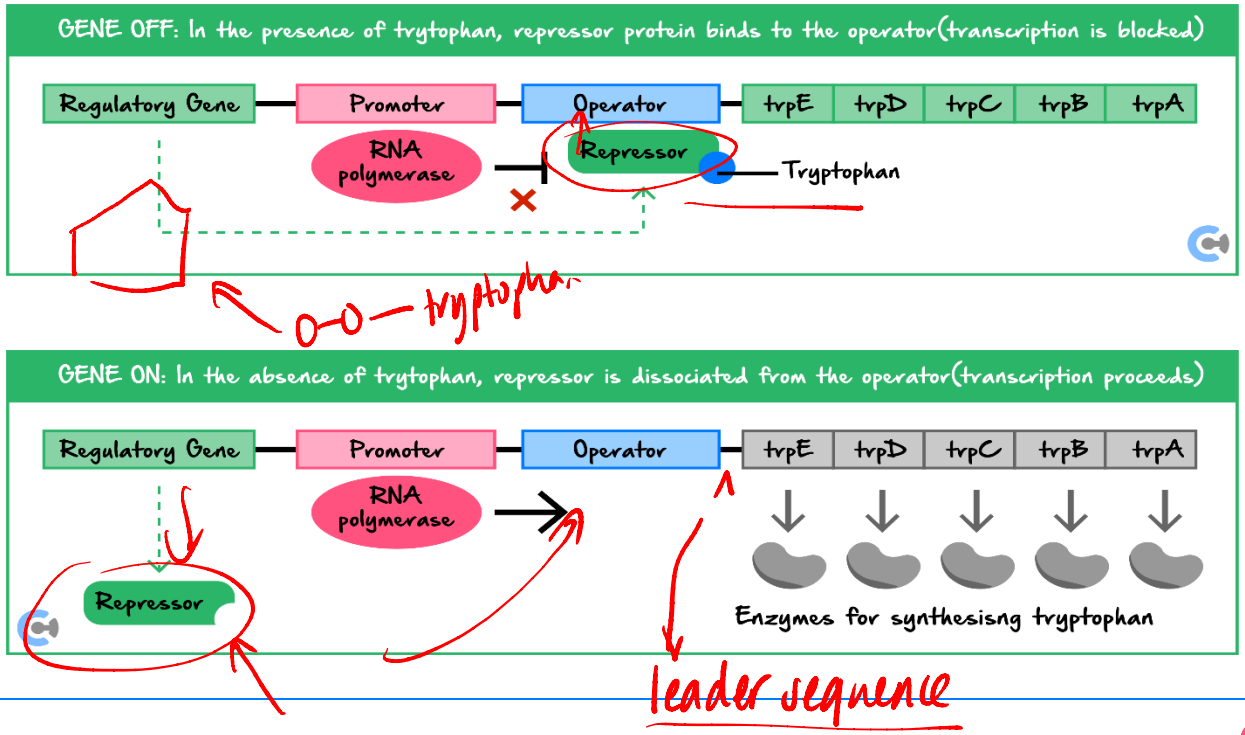
HIGH TRP – no production – Gene/Operon OFF

LOW TRP – production → ON

Sub-Section: Repression

Regulation through the transcription factor

- The trp operon is controlled by a regulatory gene upstream of the actual operator for the operon itself.
  - Produces the trp repressor protein, which will bind to operator.
- This repressor can only bind to the operator when 2 tryptophan is bound to it – this causes a conformational shape change.
- This repressor-operator association will fade after a while.



Discussion: Why is it important that the tryptophan repressor disassociates after a while?

This allows the E. coli to RESUME expression of the trp operon after if levels of trp fall

Sample Response: Repression

- In situations of high tryptophan;

1. In the abundant presence of tryptophan, two trp molecules will bind to the repressor protein expressed by the trpR gene, causing a conformational shape change, hence making the repressor active and complementary to the operator.

2. The repressor will bind to the operator, thus preventing RNA polymerase from binding and transcribing the structural genes required for trp synthesis.

- In situations of low tryptophan;

1. When there is less abundant tryptophan in the cell, it will NOT bind to the repressor and hence it will remain inactive.

2. The repressor will **NOT** bind to the operator, thus allowing RNA polymerase to bind and transcribe the structural genes producing enzymes required for trp synthesis!

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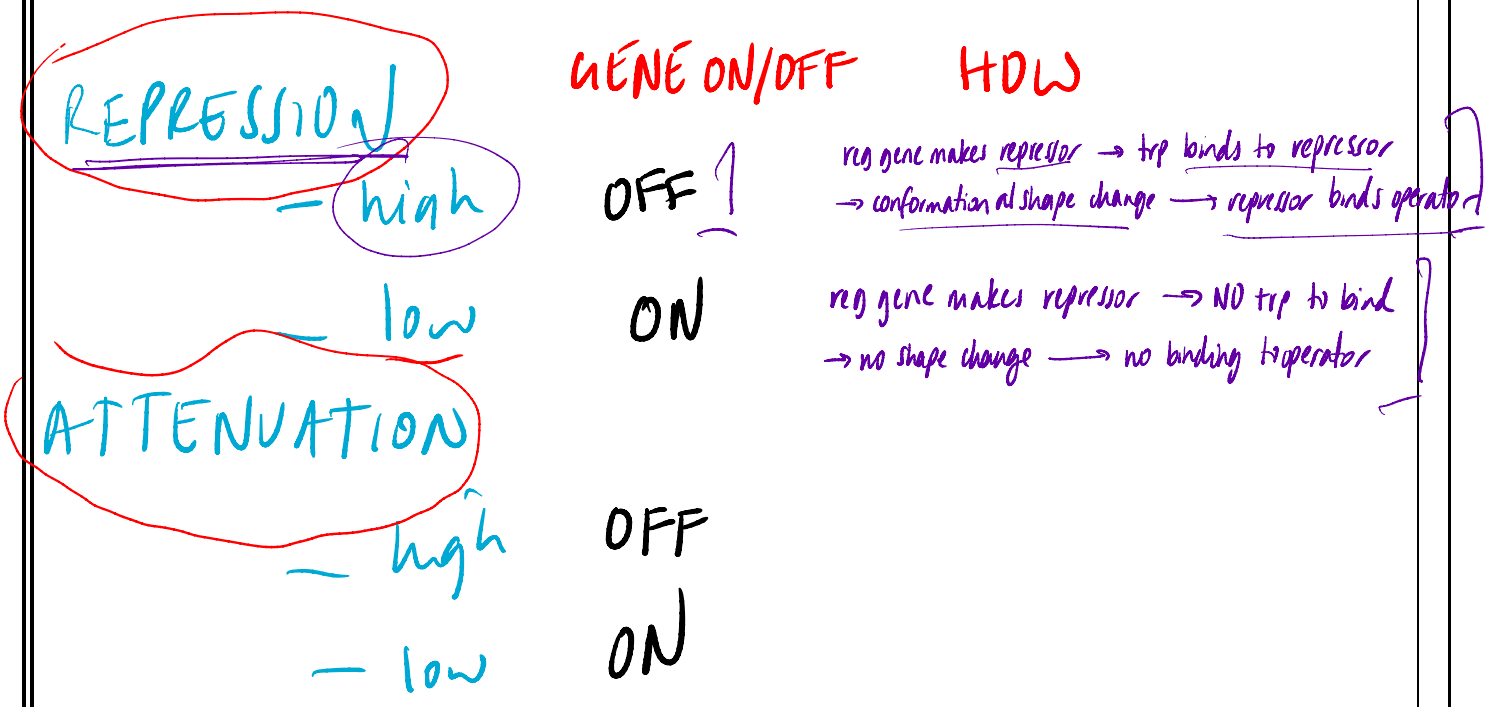
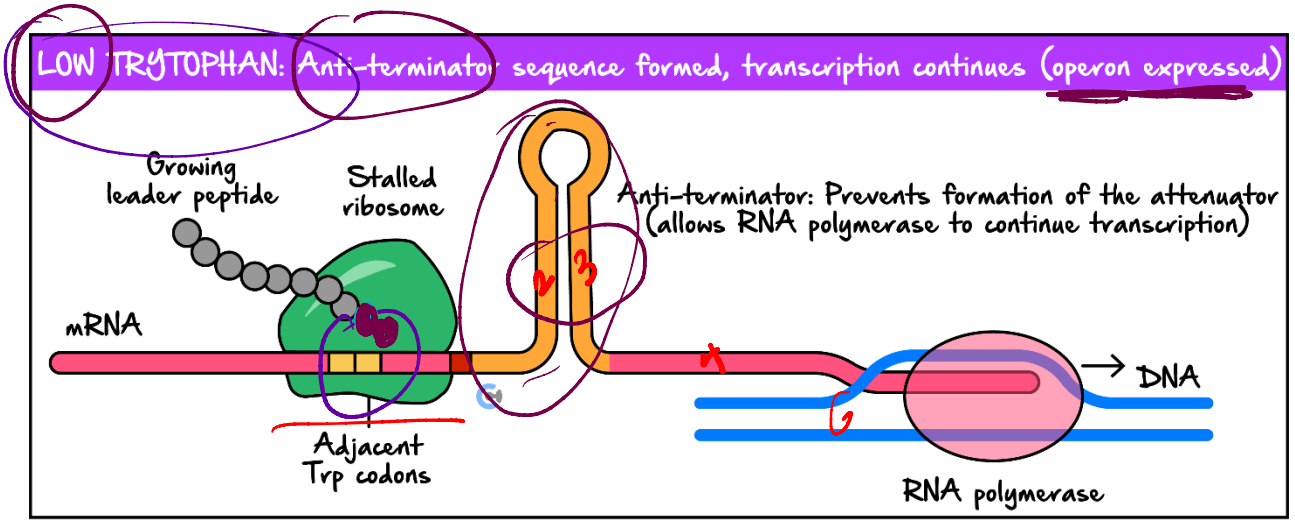
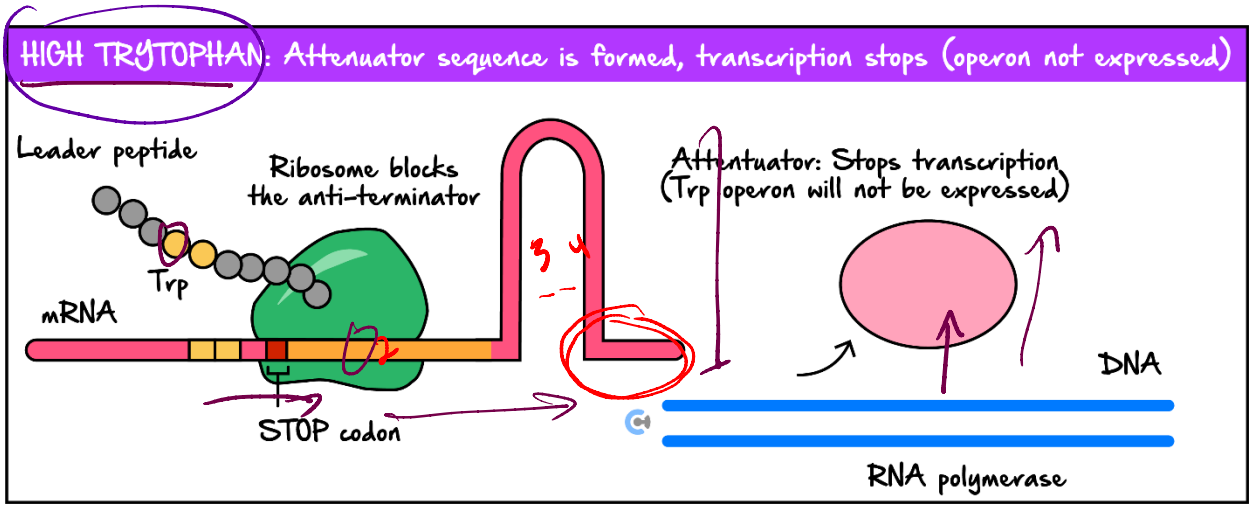


Sub-Section: Attenuation

Attenuation

- This is another method by which the trp operon can be regulated.
  - Occurs "during" transcription and translation - in prokaryotes this can occur at the same time.
- This will block the completion of transcription and translation.
- Between the operator and the trp genes, is a leader sequence that has 2 trp residues.
  - When this is transcribed, it can form 2 hairpin loops.
  - Attenuator loop - formed when there is high trp - stops transcription + translation
  - Anti-terminator loop - prevents the termination of to transcription + translation
- Which loop forms, depends on the progress of the ribosome through the leader sequence.
  - When there is low trp, the ribosome will have to pause, allowing time for the anti-terminator to form. → before structural gene
  - When there is high trp, the ribosome will not pause, therefore causing the formation of the attenuator.





NOTE: You do not need to know the regions specific for tryptophan, and you do not need to know attenuation in low trp environments according to VCAA.

Sample Response: Attenuation

In prokaryotes, transcription and translation can occur simultaneously due to a lack of processing and a nucleus.

In between the operator and the structural genes, there is a leader sequence.

This leader sequence contains the code for two trp residues. If there are low levels of tryptophan, then the ribosome will be stalled while translating this sequence.

Following the leader sequence, there is an attenuator – if the ribosome is stalled then this attenuator will form an anti-terminator loop and allow transcription to occur.

If there are high levels of trp then the ribosome will not be stalled – thus resulting in a terminator loop to be formed, preventing full transcription of the structural genes.

↳ attenuator

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HIGH → ribosome moves FAST (doesn't wait) → forms attenuator → transcription stops

LOW → ribosomes moves SLOW → form antiterminator
↳ stalls at 2 trp codons ↳ transcription continues

What does VCAA say about how much you need to know?

- Can generally just use as described above – however, the FAQs also give a decent template.
- Are students required to know about both repression and attenuation in relation to the regulation of the trp operon in Unit 3?
  - Yes, students should understand that in some prokaryote cells, such as $E. coli$, there are two mechanisms that regulate the expression of the structural genes of the trp operon when the level of tryptophan in the cell is high: first, repression, which involves the trp repressor protein, expressed by the regulatory gene, in its active form (bound to two tryptophans) binding to the operator sequence and blocking the initiation of transcription; and second, attenuation, which prevents the completion of transcription. Students should understand that there is a low basal rate of transcription of the trp operon because the trp repressor periodically stops binding to the operator region. The second mechanism, attenuation, allows the cell to terminate transcription of the trp operon when the level of tryptophan in the cell is high without the repressor protein binding to the operator region.
- Do students need to know how the hairpin loops form as part of the regulation of the trp operon?
  - Students should understand that within the trp operon, and before the five structural genes, there is a segment called the leader segment. This segment features two adjacent trp codons, and when transcribed has regions that undergo base pairing to form hairpin loops. Students should understand that when the level of tryptophan is low (where the cell requires more tryptophan to be synthesised), the ribosome pauses at the two adjacent trp codons and waits for the arrival of tRNA carrying tryptophan. The stalled ribosome causes a hairpin loop to form that does not stop transcription, and the five structural genes are expressed. When the levels of tryptophan are high and the repressor protein is not bound to the operator region, the ribosome does not pause at the two adjacent trp codons and a different hairpin loop forms, causing transcription to be terminated. Students do not need to know which specific segments of the leader region are paired to allow each hairpin loop to form.
- To what depth do students need to understand attenuation?
  - In relation to attenuation, students should understand that when the level of tryptophan is high (and the cell does not need to synthesise more tryptophan), the ribosome does not pause at the two adjacent trp codons, allowing a hairpin loop to form that acts as the transcription termination signal. The consequence is that the ribosome detaches from the short (attenuated) mRNA transcript strand, transcription stops prematurely and the five structural genes are not expressed. Students do not need to know how attenuation occurs when the level of tryptophan in the cell is low. Students should also understand that attenuation of the trp operon is made possible in prokaryotes because transcription and translation in prokaryotes take place very close to each other in the cytoplasm, as the two processes are not separated by a nuclear membrane.

Key Takeaways

- **Gene Regulation**:
  - Ensures specific genes are expressed only when required, conserving energy and resources.
  - **Structural genes**: Code for proteins that perform specific functions (e.g., enzymes).
  - **Regulatory genes**: Code for transcription factors that control the expression of structural genes by interacting with DNA sequences like promoters or operators.
- **Purpose of Gene Regulation:**
  - Avoids unnecessary production of proteins that may not be needed.
  - Responds to environmental changes (e.g., nutrient availability, stress).
  - Maintains cellular efficiency and resource management.
- **What is an Operon?**
  - A cluster of genes with related functions, controlled by a single promoter.
  - Includes a promoter, an operator (binding site for regulatory proteins) and structural genes.
  - Example: The *trp operon* in *E. coli* codes for enzymes that synthesise the amino acid tryptophan.
- **Benefits of Operons**:
  - Simplifies the regulation of multiple related genes.
  - Allows a coordinated response to environmental changes (e.g. nutrient levels).
  - Common in prokaryotes but absent in eukaryotes.
- **Key Components of the *Trp* Operon**:
  - **Promoter:** The site where RNA polymerase binds to start transcription.
  - **Operator:** The DNA segment where repressor proteins bind to regulate transcription.
  - **Structural Genes:** Code for enzymes required in the tryptophan synthesis pathway.
  - **Regulatory Gene:** Produces the *trp* repressor protein.

- **Repression**:
  - A regulatory gene upstream of the operon produces the *trp* repressor protein.
  - When tryptophan levels are high:
    - Tryptophan binds to the repressor protein, causing a conformational change that activates it.
    - The active repressor binds to the operator, blocking RNA polymerase from transcribing the structural genes.
    - This halts the production of enzymes needed for tryptophan synthesis, conserving resources.
  - When tryptophan levels are low:
    - Tryptophan dissociates from the repressor, rendering it inactive.
    - RNA polymerase can bind to the promoter and transcribe the structural genes.
    - Enzymes for tryptophan synthesis are produced, allowing the cell to make more tryptophan.
- **Importance of Repressor Dissociation**:
  - Prevents unnecessary repression when tryptophan levels drop.
  - Ensures enzymes are available to synthesise tryptophan when needed.
- **Attenuation:**
  - Unique to prokaryotes because transcription and translation occur simultaneously.
- **Mechanism**:
  - The leader sequence between the operator and structural genes contains codons for two tryptophan residues and a region that can form hairpin loops in the mRNA.
  - Depending on tryptophan availability, the mRNA forms one of two types of loops:
    - **Attenuator Loop** (transcription stops):
      - Forms when tryptophan is abundant.
      - Ribosome does not stall at tryptophan codons, quickly moving past the leader sequence.
      - The loop prevents RNA polymerase from continuing transcription.

- **Anti-Terminator Loop** (transcription continues):
  - Forms when tryptophan is scarce.
  - Ribosome stalls at the tryptophan codons in the leader sequence due to insufficient tryptophan.
  - This stalling allows the formation of the anti-terminator loop, enabling transcription to continue.

- **Low tryptophan levels**:
  - Ribosome stalls at leader sequence → Anti-terminator loop forms → RNA polymerase continues transcription → Structural genes are expressed.

- **High tryptophan levels**:
  - Ribosome does not stall → Attenuator loop forms → RNA polymerase is stopped → Structural genes are not expressed.

Space for Personal Notes

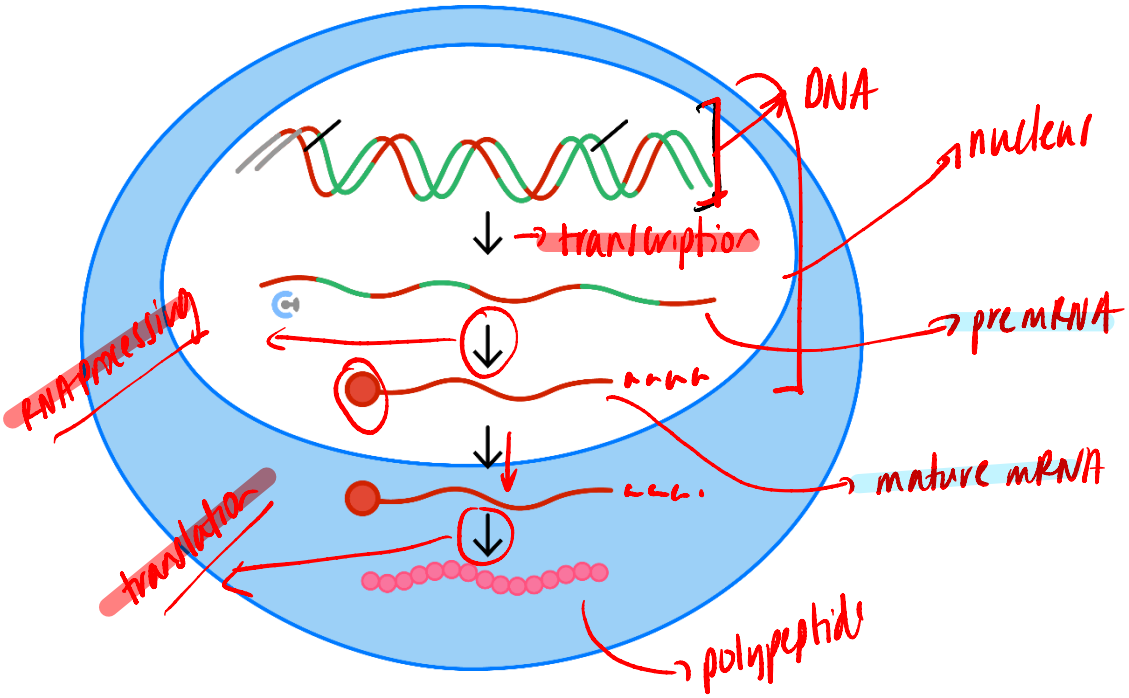
Contour Check

Learning Objective: [1.3.1] – Identify and recall the process of gene expression in eukaryotes, comparing how it differs in prokaryotes

Study Design

The steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes.

Key Takeaways



- How does this differ in prokaryotes?

Learning Objective: [1.3.2] – Describe the processes of transcription, mRNA processing, and translation, recognising the significance of each step to the final product, and [1.3.3] Explain how a single gene can give rise to multiple proteins

Study Design

The steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes.

Key Takeaways

- RNA polymerase binds to the _____ region and the DNA _____.
- _____ then catalyses the production of the mRNA strand by joining together complementary RNA nucleotides. mRNA is _____ to the DNA template strand – adenine pairs with _____ in RNA instead.
- Continues until a termination or stop sequence is reached.
- **Addition of Methyl 5′ Cap:**
  - Added to the **5′ end** of the mRNA strand.
  - Prevents _____.
  - Enables the _____ to detect the RNA.
- **Addition of Poly-A Tail:**
  - Added to the **3′ end** of the mRNA strand.
  - Composed of _____ bases.
  - Prevents _____ and increases mRNA stability.
- **Splicing: Exons and Introns:**
  - Splicing removes _____ (non-coding regions) and retains _____ (coding regions) in the mRNA strand.
  - This process is performed by a complex molecule called a spliceosome.
- **Alternative Splicing:**
  - Allows flexibility in mRNA processing:

- Exons can be removed, introns can be retained, or the order of exons can be shuffled.

- Significance:
  - _____.
  - Leads to the production of diverse proteins from the same gene.

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Learning Objective: [1.3.4] - Identify and recall the general principles and reasons for gene regulation in both prokaryotes and eukaryotes

Study Design

The basic elements of gene regulation: prokaryotic trp operon as a simplified example of a regulatory process.

Key Takeaways

- **Gene Regulation**:
  - Ensures specific genes are expressed only when required, conserving energy and resources.
  - **Structural genes**: _____.
  - **Regulatory genes**: _____.
- **Purpose of Gene Regulation:**
  - _____.
  - _____.
  - _____.
- **What is an Operon?**
  - A cluster of genes with related functions, controlled by a single promoter.
  - Includes a promoter, an operator (binding site for regulatory proteins), and structural genes.
  - Example: The *trp operon* in *E. coli* codes for enzymes that synthesise the amino acid tryptophan.
- **Benefits of Operons**:
  - _____.
  - _____.
  - _____.

Learning Objective: [1.3.5] – Describe the regulation of the trp operon through the action of the repressor protein

Study Design

The basic elements of gene regulation: prokaryotic trp operon as a simplified example of a regulatory process.

Key Takeaways

- **Repression**:
  - A _____ gene upstream of the operon produces the *trp* repressor protein.
  - When tryptophan levels are _____:
    - Tryptophan binds to the repressor protein, causing a _____ that activates it.
    - The active repressor binds to the operator, blocking RNA polymerase from transcribing the structural genes.
    - _____.
  - When tryptophan levels are _____:
    - Tryptophan dissociates from the repressor, rendering it inactive.
    - RNA polymerase can bind to the _____ and transcribe the structural genes.
    - Enzymes for tryptophan synthesis are produced, allowing the cell to make more tryptophan.
- **Importance of Repressor Dissociation**:
  - _____.
  - _____.

Learning Objective: [1.3.6] – Describe the regulation of the trp operon through attenuation in high trp environments

Study Design

The basic elements of gene regulation: prokaryotic trp operon as a simplified example of a regulatory process.

Key Takeaways

- **Attenuation:**
  - Unique to prokaryotes because _____.
- **Mechanism**:
  - The leader sequence between the operator and structural genes _____ _____ and a region that can form hairpin loops in the mRNA.
  - Depending on tryptophan availability, the mRNA forms one of two types of loops:
    - **Attenuator Loop** (transcription _____):
      - _____.
      - _____.
      - _____.
    - **Anti-Terminator Loop** (transcription _____):
      - _____.
      - _____.
      - _____.
  - **Low tryptophan levels**:
    - Ribosome stalls at leader sequence → _____ → RNA polymerase continues transcription → Structural genes are expressed.
  - **High tryptophan levels**:
    - Ribosome does not stall → _____ → RNA polymerase is stopped → Structural genes are not expressed.