Gene Expression: Transcription and Translation

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Transcription and translation convert a gene into protein. During this process, the genetic information of a gene, the DNA, is converted into RNA so that protein may be realized later on. This article will provide you with exam-related information regarding this process, thus preparing you ideally for the upcoming exams.

Overview of Gene Expression

The first step in transforming the genetic content of DNA into proteins is called transcription. During this first step, a copy (or transcript) of the DNA segment is created via messenger RNA (mRNA). The mRNA, in turn, is transformed into an amino acid sequence, i.e., a protein, via translation. This process uses a sort of adapter called transfer RNA (tRNA), which creates the protein according to a triplet base coding system.

Until the 1970s, a common belief was that transcription of DNA into RNA and the flow of information from gene to protein only took place in this one direction. However, with the discovery of retroviruses, it was found that via an enzyme called reverse transcriptase, a virus can also transfer its RNA genome into DNA. This mechanism is referred to as reverse transcription. It takes place via the formation of telomere sequences by telomere extension at the end of the chromatin, for instance. More information about retroviruses is detailed in another article.
Gene Expression: Transcription, Processing, Translation

DNA carries information for the production of all proteins a cell requires. It is located in sections called structural genes. As not all cells require every protein all the time, control elements manage the regular expression of structural genes.

Gene expression or protein biosynthesis in eukaryotes includes transcription (the creation of an RNA transcript in the form of mRNA), processing (modifying the mRNA) and translation (translating the base sequence of mRNA into an amino acid sequence, which will result in the final protein after further modification). There is no processing with regard to prokaryotes.

Gene Expression I: DNA Transcription

If a cell requires a protein, the appropriate code section of DNA is read in the cell nucleus, and a copy is created. The creation of this transcript is the first step in the protein biosynthesis process and is referred to as transcription. It takes place in 3 steps: initiation, elongation, and termination.

Step 1: Preparing for transcription – Initiation

When preparing a transcript of a specific gene, no primer is needed as it is for replication. Its starting point is a specific DNA sequence – the so-called promoter sequence – which precedes the section that is to be transcribed. It consists of characteristic sequence motifs, i.e., -10 TATAAT, which is referred to as a TATA box. The
preceding ‘-10’ means that this section contains 10 base pairs of the gene to be read.

In order to recognize a promoter, eukaryote RNA polymerases need helper proteins (transcription factors). RNA polymerase II, which synthesizes heteronuclear RNA (hnRNA), requires the transcription factors TFIIA, TFIIB, etc., for this process.

TFIID contains a TATA Box binding protein (TBP) with which it binds to the promoter. Together with other transcription factors and the RNA polymerase, it forms the initiation complex.

The prokaryote RNA polymerase assists a σ-subunit to recognize the promoter sequence. In order to allow this synthesis, the helicase unwinds the preceding section (as in replication), meaning a transcription bubble is created in front of it. Topoisomerases stabilize the DNA unwinding, and single-strand binding proteins (SSB) ensure that the transcription bubble remains open.

**Step 2: RNA synthesis – Transcription elongation**

As the RNA polymerase synthesizes an oligonucleotide with the length of approximately 10 nucleotides, the σ-subunit separates, and elongation is initiated. The DNA-dependent RNA polymerase reads the DNA template in the 3′→5′-direction and synthesizes the RNA transcript in the 5′→3′-direction using ATP, UTP, GTP, and CTP substrates. Just like during replication, the synthesis mechanism consists of a nucleophilic attack with phosphoric acid-forming ester bonds.

Prokaryotes possess one type of RNA polymerase while eukaryotes have four: 3 (I-III) in the cell nucleus and 1 in the mitochondria. In prokaryotes, the polymerase creates the transcript from mRNA. In eukaryotes, however, RNA polymerase II first creates a primary transcript from hnRNA. You can find more information concerning different RNAs in our Lecturio magazine.

While the transcript is being completed, a hybrid helix from DNA and complementary RNA forms for a short period of time.

**Step 3: Transcription termination**

The end of the transcribed section is marked by a certain base sequence called the terminator. This contains a palindromic base sequence that reads the same in both directions, i.e., with regard to matrix DNA as well as the emerging RNA (i.e. 5′ CCATGG 3′). Bases at the beginning and the end of the CG-rich palindrome can pair and spontaneously form hairpin structures. These loosen the bond between RNA and DNA, by causing the RNA to become dissociated from the DNA template strand and from the RNA polymerase later on.

Another termination mechanism that is being used in addition to the terminator sequence is the Rho-(ρ-) protein. During p-dependent termination, the p-protein binds to RNA and moves toward the RNA polymerase. The p-protein can then interact with it and detach it from the DNA template via ATP hydrolysis.

With regard to prokaryotes, transcription takes place in the same cellular compartment as translation, and mRNA represents the ‘mature’, finished transcript. It may directly serve as a template, meaning that the prokaryote translation may begin before the transcription is even finished. With regard to eukaryotes, however, hnRNA must be processed until the final mRNA has been finished, and it can move from the cell nucleus to the cytoplasm where it attaches to ribosomes.
Regulating gene expression

In order to enable cells to react to situational needs and to adjust their protein production according to external conditions, different genetic regulatory mechanisms are in place, which manage the genetic transcription. One mechanism works via **enhancers**, which are DNA sequences located upstream of the promoter regions. They influence transcription via specific ligand binding. The **lac operon** is a type of on/off mechanism for certain genes, through which - only in the presence of lactose - the lactose-reducing enzyme lactase is produced by *Escherichia coli*.

**The Lac Operon**

In the absence of lactose, gene expression is repressed.

**The Lac Repressor**

In the presence of lactose, gene expression is activated.

**CAP-responsive operons**
Activation in the absence of glucose.

In the presence of glucose, cAMP is low, thus, no CAP activation!

Other Operons

The Trp operon

Other operons are repressible. In the absence of tryptophan, the repressor is inactive.

Tryptophan activates the repressor.
Gene Expression II: Post-transcriptional hnRNA Processing

During processing, the eukaryote hnRNA undergoes at least 3 modifications as requirements for these modifications have already been fulfilled via the recruitment of certain factors even before the termination of transcription. The modifications include:

- **The 5′-end is capped**: to prevent hnRNA exonuclease degradation and to function as a recognition sequence for translation, methylated guanylyl residue (GMP) is added to the 5′ end of hnRNA; furthermore, between 1 and 3 ribose molecules of the transcript may be methylated.

- **Removal of introns via splicing**: aside from exons that code for genes, the eukaryote DNA also contains non-coding introns, which make up approximately 45% of the genome in humans. The introns are still present in hnRNA, the primary transcript, but should no longer exist in mRNA. This exclusion process is referred to as **splicing**, where the introns are cut off while the exons are ‘glued’ together. The enzyme **spliceosome**, which is responsible for this process, consists of **small nuclear ribonucleoproteins (snRNPs)** and subunits. These structures can recognize the exon-intron splice sites via certain base sequences and fuse the exons after **two transesterification reactions** - meaning one gene can code for different proteins.

- **3′-end polyadenylation**: At the end of transcription, the 3′-end of each hnRNA adds multiple **adenyl residue (AMP)**, the so-called poly (A) tail, preventing degradation. **Poly(A) polymerase** can recognize such a sequence through various factors and subsequently adds 50-250 adenylyl residues.

Furthermore, the individual sections of hnRNA or mRNA may specifically be changed (RNA editing).

![Overview of the processes of eukaryotic gene expression](Image)
Gene Expression III: Translating mRNA into Amino Acids

During translation, the base sequence of mRNA is translated into amino acids, and these amino acids are, in turn, linked together with peptide bonds. Translation is the last step in the expression process from gene to protein.

The Genetic code

The 4 different bases that makeup mRNA must produce 20 proteinogenic amino acids. In order to accomplish this, they must be combined in code: 3 bases always form a base triplet—the codon. Thus, there are $4^3 = 64$ possibilities to encode those 20 amino acids, and these 64 triplets make up the genetic code.

- The base triplet **AUG** is the **translation start codon** and codes for methionine (Met).
- The triplets **UAG**, **UGA**, and **UAA** are **stop codons** that specify translation termination.
- Under certain circumstances, **UGA** codes for the rare amino acid **selenocysteine** by forming a hairpin structure.
- The remaining codons mostly code for several amino acids (exceptions are: methionine and tryptophan).

The **wobble hypothesis** states that between the 3rd base of the codon and the complimentary 1st base of the anticodon of tRNA, more than 1 base pair is possible. An example would be inosine (I) that is found in tRNA, which pairs with the uracil (U), adenine (A), and cytosine (C) that are found in the mRNA triplet. This means that the first 2 bases are the main determining bases for amino acids. Thus, the number of necessary tRNAs that are suitable as adapters for several base triplets decreases to 31, due to their wobble flexibility.

The genetic code is very tolerant with regard to errors in the 3rd and first codon positions. The most important codon position is the one in the middle, which contains important characteristics for the secondary and tertiary structure of the corresponding amino acids: the U in the 2nd place codes for hydrophobic amino acids, the A in 2nd place codes for hydrophilic amino acids.

Using the codon table

Knowledge of the genetic code, determined by the correct use of the ‘codon table’, is frequently the subject of exam questions. In many cases, you will find an mRNA section, although you should make sure that it is noted in the 5′-3′-direction. If it is a DNA section instead, thymine (T) corresponds to the uracil (U) in mRNA.
In order to determine the amino acid a particular mRNA section codes, the 5′ end of the mRNA should be noted as translation always takes place from 5′ to 3′. The next step is to mark the base triplets, which always contain 3 bases (A, C, G, and/or U) that form a codon. Find the 5′ in the middle of the codon table and choose the 1st letter of your triplet, moving on to the 2nd and 3rd. This is how you translate 1 base triplet after another in your structure.

**Example: AUG codes for the amino acid Met (methionine).**

If the question is about corresponding tRNA, the **anticodon**, please take into consideration that tRNA is complementary to mRNA, meaning in the 3′-5′-direction to the mRNA codon (in the 5′-3′ direction). However, as the base sequence is always shown in the 5′-3′-direction, the tRNA bases must always be read in this order.

**Example: 5′ AUG 3′.**

Here, you should note the complementary bases 3′ UAC 5′ as well as, once again, the correct reading frame 5′ CAU 3′.

**The transcription tool: tRNA**

DNA holds the blueprint for 31 tRNAs that are synthesized by **RNA polymerase III**. After post-transcriptional modification, it has the following characteristics:

- High content of rare bases such as **inosine** and **pseudouridine**
- **Single polynucleotide strand** made up of 70–85 nucleotides
- The single-stranded DNA occasionally forms hydrogen bonds creating the typical **clover-leaf structure**
- Opposite the clover-leaf type is the single-strand base triplet specific to tRNA, the **anticodon**, which is complementary to an mRNA base triplet (codon).
- At the 3′-end is the base sequence **CCA**, whose adenosine monophosphate serves as a **binding site for amino acids**, which it then transports.

The tRNA, therefore, functions as an adapter.
Catalytic RNAs

Catalytic RNAs (ribozymes) catalyze chemical reactions. Ribozymes play roles in ribosomes (Note that it is easy to confuse those two terms). Ribozyme catalyzes a reaction. The ribosome is a structure that makes peptide bonds and proteins. Ribosomes contain ribozymes.

In the figure on the right, there is a ribosome and in this reaction, individual amino acids are being joined, in the formation of peptide bonds. During the formation of those peptide bonds, one of the ribosomal RNAs, specifically the 23S ribosomal RNA in the case of a prokaryotic system, is creating the bond between the adjacent amino acids. That peptide bond formation occurs as a result of the action of a ribozyme within a ribosome.

Other ribozymes play roles in the processing of individual tRNAs, such as a ribozyme known as RNase P. RNase P plays an important function in the final processing of a tRNA. In the left side of the figure on the right, the tRNA can be seen, and at the 5′ position, a black line at the 5′ end (in a rectangular box) is visible and has to be processed and removed because, without the removal of this little piece, the tRNA will not function. That piece is removed by RNase P. So in addition to the many functions that RNAs perform in the process of translation, they also perform functions of catalysis and in some cases, those catalytic functions influence other RNAs. That structure removed by RNase P is simply lost. Despite the small size of the removed piece, it would stop the translation process if it were not removed.

RNA interference

Another recently discovered important process that all RNAs also function in, is RNA interference (RNAi). RNAi is a process that is stimulated by the presence of double-stranded RNA inside the cell. RNAi can occur either as a result of the cell making double-stranded RNA or by viral invasion or by the introduction of double-stranded RNA. The 2 different forms of double-stranded RNA that can exist in the cell, are known as micro RNAs or miRNAs and these have cellular origins.

The siRNAs or silencing RNAs come from an external source like a virus for example. Biotechnologists also use double-stranded RNAs to control genes for biotechnology purposes. That is another way foreign siRNA can get into cells.

The process is quite widespread in eukaryotic cells. It occurs in plants and animals and
plays various roles. The actions performed in this process are called RNAi. These double-stranded RNA molecules result in the interference of the translation of targeted genes.

The ability to use this technology to target and specifically stop the production of certain proteins is important in research. More importantly, though, it allows the cell to both protect cells from invaders and to control its own gene expression. RNAi operates through the silencing of gene expression, and this silencing interferes in the way that proteins are made.

This occurs as a result of the appearance of a double-stranded RNA in the cell. Just like dicer in a kitchen, a dicer enzyme splits the double-stranded RNA into bite-sized chunks and those bite-sized chunks are about 20-base-pairs long. These 20 base-pair chunks at this point are called siRNAs if they come from an external source and miRNAs if they come from a cellular source. These pieces of RNA can then be bound by the RNA-induced silencing complex (RISC).

The figure below illustrates the process:

Two things should be noted here. The process occurring on the right starts with the production of an RNA that makes a double-stranded RNA that is used to create the miRNAs. In the process on the left, the dsRNA in the cell is derived from a foreign source and could be from a viral infection or from deliberate implantation by a researcher.

The cell has encoded within its genome certain sequences that when transcribed produce a structure like can be seen on the right. That double-stranded structure with the tails hanging off of it and the poly-A on it is called a pri-miRNA. That pri-miRNA gets processed and will ultimately become the miRNA. An enzyme (known as drosha) cuts off some of the ins of the pri-miRNA and creates a smaller structure.

The pri-miRNA is then moved out of the nucleus, where it is then attached to the dicer enzyme. At this point, siRNA and miRNA become the same. Dicer takes that double-stranded pri-miRNA or the double-stranded RNA from the foreign source and chops them into the 20 sequenced base pairs.
Dicer, after this chopping into 20 nucleotide blocks, then peels away one of the strands. This leaves a single-stranded siRNA or single-stranded miRNA that is then transported by the RISC. The RISC takes that individual sequence and carries it to an mRNA. The significance of the fact that there is a single strand at this point is due to the fact that this single strand would be complementary to a target mRNA, as we shall see subsequently.

After the RISC has complexed with that single-stranded RNA (miRNA or siRNA; at this point, this does not matter), the RISC-RNA complex then goes and seeks mRNA. mRNAs are coding for individual proteins. If RISC finds a sequence that is complementary to the RNA that it is carrying, it aligns that sequence with the specific region in the mRNA. Then, enzymatic activity in the RISC complex due to the argonaute proteins cleaves the target mRNA.

Now, cleaving of the target mRNA means the destruction of the coding for a protein. In this way, the protein coded for by this mRNA can no longer be made, and this has several implications, including obvious protective effects for the cell against an invading virus. If the invading virus makes a double-stranded RNA in the process of its life cycle, then this siRNA system will stop the production of targeted virus proteins.

The miRNA also plays a role in regulating gene expression because, the miRNA is actually stopping, in this case, the production of a cellular gene that would otherwise make this protein. Moreover, although it might seem very inefficient for this cell to make an mRNA and then degrade the mRNA, that is preferable than continuing to make a protein that the cell would not need.

This miRNA system allows for an additional level of protection or an additional level of control of cellular gene expression. In any event, what happens here is that the translation of the mRNA is stopped. This processing does not have to cut the RNA. It can also involve a simple binding of the miRNA or siRNA sequence to the mRNA and stop translation by the formation of that duplex alone.

The Translation Process
Step 0: Preparation – Loading tRNAs with amino acids

tRNAs have a certain base triplet in their structure called the **anticodon**. As the anticodon represents a certain amino acid, the tRNA has to be loaded with exactly that particular amino acid. This is the responsibility of **aminoacyl-tRNA synthetase**.

For this purpose, the **specific amino acid** is first activated by binding it to an ATP molecule. This process releases pyrophosphate, leaving a product called **aminoacyl adenylate** consisting of the combined amino acid and the remaining AMP. During the next step, the AMP is split off from the amino acid, which then links to the AMP at the 3′-end of tRNA via an ester bond. The tRNA loaded with the amino acid is referred to as **aminoacyl-tRNA** and is ready for translation.

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Step 1: Formation of the 80S Ribosome – Initiating Translation

**Aminoacyl-tRNA** (during initiation, the initiator methionyl-tRNA) forms a ternary (comprised of 3 parts) complex together with the energy carrier **GTP** and a helper protein (during initiation, the **initiation factor eIF-2**; during elongation, the elongation factor eEF1-α). This complex connects to the small ribosomal **40S subunit**, thus forming the 43S *pre-initiation complex*.

The eukaryotic **ribosome** consists of ribosomal RNA (rRNA) and proteins. Its 2 subunits, the **large 60S, and the small 40S subunit**, which together form the **80S ribosome** (S stands for Svedberg units, which cannot be simply added) and are only stored in this way for the purpose of the translation. Otherwise, they exist separately in the cytosol. Prokaryotic 70S ribosomes consist of one large 50S and one small 30S subunit. During translation, several ribosomes often bind directly to one mRNA and hereby form a **polysome**.

The **initiation factor eIF4F** recognizes and binds the mature mRNA at its 5′-cap. A component of this initiation factor, eIF4G, subsequently binds mRNA and the pre-initiation complex to the 48S *initiation complex* via eIF3. This migrates along mRNA to the AUG triplet, the start codon, where eIF2 hydrolyzes GTP and rotates with the other initiation factors.

eIF2 binds GTP while it is active; after hydrolyzing GTP, the **exchange factor eIF2B** is required, in order to turn the inactive eIF2 GDP complex back into an active eIF2 GTP complex, which can again participate in initiation.

Once the starter tRNA has bound to the **start codon AUG** on mRNA, the large ribosomal 60S subunit also binds to the 48S initiation complex via eIF5B. Translation occurs from the 5′-end to the 3′-end of mRNA.

The newly complete **80S ribosome** has 3 binding sites for tRNA: first, there is the **A (aminoacyl) or acceptor site**, to which aminoacyl-tRNA is bound during elongation; further, the **P (peptidyl) site**, where peptidyl-tRNA binds; and lastly, the **E (exit) site**, where deacylated-tRNA is bound prior to its release. The starter methionine-tRNA is bound to the P site for initiation; the A- and E-sites are vacant.

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Step 2: Protein synthesis – Translation elongation

The vacant A-site waits for the next base triplet—in this case, the one after the starter
codon—whose corresponding aminoacyl-tRNA forms a complex with the elongation factor eEF-1α. Just like eIF2 that aids in the initiation, the eEF-1α is a GTP energy carrier. Under hydrolysis, this GTP binds the aminoacyl-tRNA eEF-1α complex to the A-site. The eEF-1α subsequently detaches, and the exchange protein eEF-1β replaces GDP with GTP, thus restoring the elongation factor eEF-1α for a new cycle.

The A- and P-sites of the ribosome are now loaded with tRNA; the E-site is vacant.

Next, the ribosomal peptidyl transferase transfers the amino acid from the P-site of tRNA (to begin with, the starter amino acid methionine) to the amino acid of the A-site by forming peptide bonds. This is why methionine always remains at the starting point of emerging peptides. Peptides grow from the amino terminus to the carboxyl terminus.

After the peptide bonds have been formed, the peptidyl-tRNA migrates from the A-site to the P-site, and the ribosome migrates 3 nucleotides further in the direction of the 3′-end of the mRNA. This, again, occurs via GTP hydrolysis through the elongation factor eEF2, known as a translocase, and a G protein. Therefore, the tRNA is attached to the P-site of the growing polypeptide, the A-site is vacant once again, and the ‘empty’ tRNA on the E-site diffuses and returns again to the cellular tRNA pool.

This cycle is repeated until a stop codon is encountered on an mRNA.

Step 3: Releasing proteins – Translation termination

If one of the 3 stop codons is encountered (UAA, UAG, UGA), translation is terminated. No tRNAs bind to stop codons. Instead, the termination factor eRF1 binds to the ribosomal A-site. The peptidyl transferase now does not add amino acids but, rather, a water molecule to the peptidyl-tRNA. This severs the binding between tRNA and polypeptide, and the polypeptide diffuses into the cytosol where it is processed further. The ribosome releases mRNA and tRNA, breaks down into its 2 subunits and is immediately operational again.

References


