

Transcription of RNA and Protein Synthesis

The exercises in this laboratory demonstrate how our genetic molecule, DNA, is used in the process of transcription to direct the synthesis of messenger RNA, and how messenger RNA is translated to direct the synthesis of protein.

As you have studied, the genetic information for living organisms is stored in the structure of deoxyribose nucleic acid (DNA). Our DNA molecules carry the genetic code for inherited characteristics in the triplet-coded sequences of its nitrogen bases.

DNA molecules contain a coded set of information to direct the metabolic activities of living organisms. A sequence of DNA that stores a specific set of information is known as a gene. In order for our cells to use this information, the DNA in the nucleus serves as a template to manufacture molecules of RNA by a process called **transcription**. Molecules of RNA then direct the synthesis of specific proteins at the ribosomes in the cytoplasm according to the specifications of the RNA molecules' codes.

The enzyme, RNA polymerase, catalyzes transcription by directing the construction of an RNA molecule that is complementary to a specific gene sequence of the DNA molecule. There are three types of RNA: ribosomal RNA (rRNA), transfer RNA (tRNA), and messenger RNA (mRNA). All three types are transcribed in the same manner, although each type of RNA has its own function, as you have discussed and read in your text.

Pop-beads are used to represent the RNA nucleotides (nitrogen base-sugar-phosphate molecules) in this set of laboratory exercises, as well as the amino acids and transfer RNA molecules.

Pink beads represent Ribose sugar
Red beads represent the Phosphate group
Orange beads represent Adenine (A)
Green beads represent Guanine (G)
Blue beads represent Cytosine (C)
Purple beads represent Uracil (U)

Exercise I: Transcription of RNA

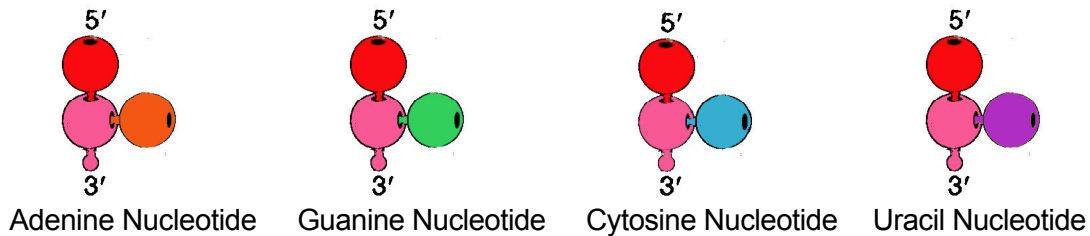
In this exercise you will construct a mRNA molecule that carries the genetic code for the assembly of proteins in the cell from the nucleus to the ribosomes.

Materials needed per lab group

- 6 Adenine (A) ribose nucleotides (each comprised of one orange adenine bead, one pink ribose bead and one red phosphate bead)
- 6 Guanine (G) ribose nucleotides (each comprised of one green guanine bead, one pink ribose bead and one red phosphate bead)
- 6 Cytosine (C) ribose nucleotides (each comprised of one blue cytosine bead, one pink ribose bead and one red phosphate bead)
- 6 Uracil (U) ribose nucleotides (each comprised of one purple uracil bead, one pink ribose bead and one red phosphate bead)
- 1 paper RNA polymerase enzyme
- 1 paper DNA template

Procedure

1. Be sure that each nucleotide is assembled correctly, with the phosphate group (red bead) attached to the 5' position of the ribose sugar (pink bead) and the nitrogen base (adenine, guanine, cytosine, or uracil) to the 1' position of the same sugar.



2. If not done previously, cut out the elliptical RNA polymerase with scissors provided and partially separate the two strands of the DNA template by horizontally cutting between the nitrogen bases. Do not totally separate the two strands of the DNA template; end the cut at the pre-punched holes. The cut should stop about 6 cm from either end, leaving the last two nucleotide base pairs at each end attached to each other.
3. The genetic code carried in the nucleotide sequence of the DNA molecule is transcribed to an RNA molecule by the enzyme, RNA polymerase. Separate the two DNA strands by sliding an RNA polymerase paper enzyme between them. Leave the DNA sense strand exposed and align the cut line of the template with the dotted line of the RNA polymerase (Figure 1).

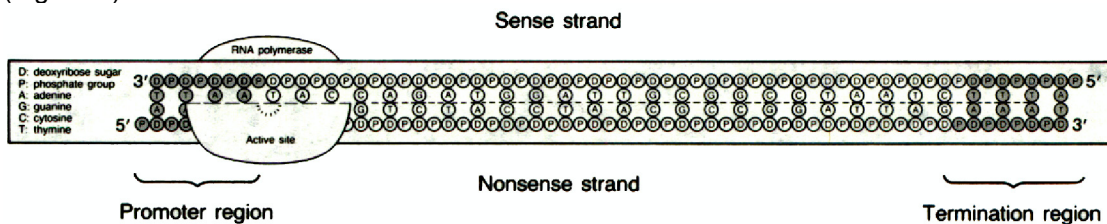


Figure 1. Attaching RNA polymerase to the DNA molecule

4. RNA transcription begins at a region known as the promoter. At the first DNA nucleotide **after** the promoter region, RNA polymerase temporarily attaches a complementary RNA nucleotide to the DNA template. During the copying process RNA polymerase moves along the length of the DNA template from the 3' end toward the 5' end, bringing in successive complementary RNA nucleotides to form the growing RNA molecule. The synthesis of the new RNA molecule is complete when RNA polymerase arrives at a region of the DNA strand recognized as the terminator.
5. Construct the RNA molecule coded by the DNA sense strand by bringing complementary RNA nucleotides to the active site of the RNA polymerase. Beginning immediately after the promoter region, RNA nucleotides should pair with the DNA nucleotides of the template (Figure 2). Recall that uracil replaces thymine in molecules of RNA, so RNA uracil nucleotides will bond to the adenine nucleotides on the DNA template. RNA adenine nucleotides will bond to thymine nucleotides on the DNA template; cytosine bonds to guanine, and vice versa. Move the RNA polymerase down the DNA template one nucleotide at a time bringing the proper complementary RNA nucleotide to the active site. Align the RNA nucleotide antiparallel (in the opposite direction) to the DNA sense strand. Growth of the new RNA molecule occurs in a 5' to 3' direction by connecting the phosphate group (red bead) of new RNA nucleotides to the 3' peg of the last ribose (pink) bead already on the RNA strand. RNA polymerase moves down the template one nucleotide at a time, while the growing RNA strand remains stationary beneath the DNA sense strand.

Materials needed per lab group

- The mRNA molecule assembled in Exercise I.
- 1 paper ribosome
- White oval beads with tRNA labels which represent the appropriate transfer RNA molecules
- White twisted beads with amino acid labels which represent the corresponding amino acids

Procedure

1. Take the mRNA molecule constructed during the RNA Synthesis (Exercise I) and place it on your desk with the 5' end to the left and the 3' end to the right. Align all the nitrogen bases along the upper edge of the mRNA strand.
2. Write each of the eight codons below in the appropriate order. Using Table 1, determine the specific amino acid each of the codons codes for. Fill in the blank directly under each codon with the correct amino acid abbreviation. You'll find that the last codon (number 8) is a termination codon for the synthesis, and not a codon for an amino acid.

Table 1. Codon Table

First Base	Second Base				Third Base
	U	C	A	G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC	UCC	UAC	UGC	C
	UUA Leu	UCA	UAA term.	UGA term.	A
	UUG	UCG	UAG term.	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC	CCC	CAC	CGC	C
	CUA	CCA	CAA Gln	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC	ACC	AAC	AGC	C
	AUA	ACA	AAA Lys	AGA Arg	A
	AUG* Met	ACG	AAG	AGG	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC	GCC	GAC	GGC	C
	GUA	GCA	GAA Glu	GGA	A
	GUG*	GCG	GAG	GGG	G

*If present as the initiation codon in mRNA, AUG and GUG code for N-formylmethionine, not Met and Val respectively.

Ala	Alanine	Gln	Glutamine	Leu	Leucine	Ser	Serine
Arg	Arginine	Glu	Glutamic Acid	Lys	Lysine	Thr	Threonine
Asn	Asparagine	Gly	Glycine	Met	Methionine	Trp	Tryptophan
Asp	Aspartic Acid	His	Histidine	Phe	Phenylalanine	Tyr	Tyrosine
Cys	Cysteine	Ile	Isoleucine	Pro	Proline	Val	Valine

Codon 5' _____ 3'

Amino acid

1 2 3 4 5 6 7

- Each amino acid is carried to the site by a specific tRNA molecule. Find the 7 labeled tRNA molecules (oval pop beads) and their corresponding labeled amino acids (twist pop beads) that match your 7 codons. The amino acid-tRNA pop beads should be in plastic bags in the designated location. Attach the amino acid to its tRNA as shown in Figure 3.

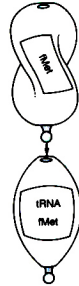


Figure 3. Amino Acid - tRNA attachment

- Bring your ribosome to your mRNA strand on the desk top, sliding the ribosome under the mRNA molecule as shown in Figure 4.
- Each tRNA molecule has a special anticodon loop at its base which recognizes its complement mRNA codon. The tRNA molecules will attach to the mRNA codons in an ordered progression, directed by the P (donor) and A (attachment) sites on the ribosome. Bring your tRNA-amino acid complexes to the ribosome and attach the first matching tRNA to the middle bead of the first mRNA codon at the P site (Figure 5).

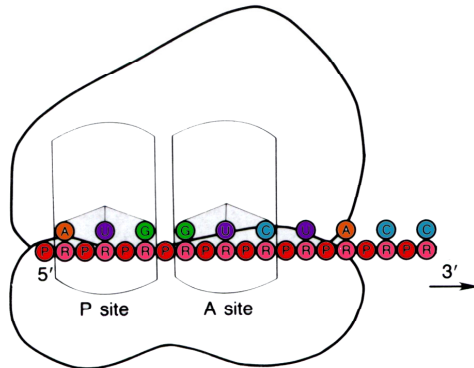


Figure 4. Ribosome and mRNA

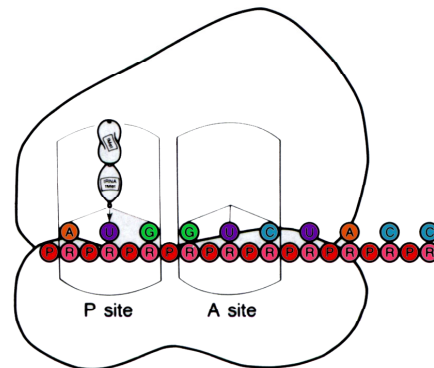


Figure 5. Bonding tRNA to mRNA

- Now bring your second tRNA-amino acid complex to the A site on the ribosome and attach the tRNA to the middle bead of the codon at the A site. In reality this places the two amino acids in a position to form a peptide bond. Detach your P-site amino acid from its tRNA and attach it to the amino acid at the A site on the ribosome, forming a "peptide bond" (Figure 6).

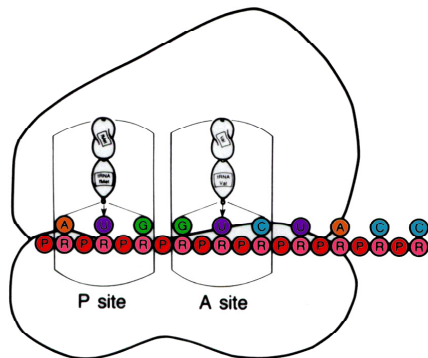


Figure 6. Attaching the second tRNA.

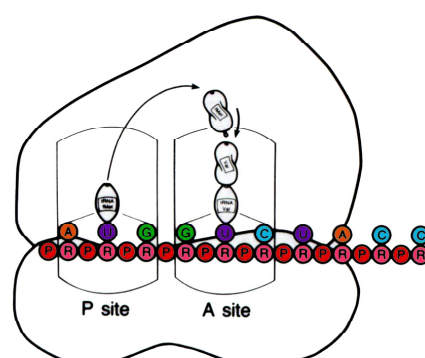


Figure 7. Bonding amino acids at A site

7. Move your ribosome down the mRNA molecule so that the P site is now at the second codon, and the A site is at the third codon. Bring the next tRNA-amino acid complex to the codon at the A site and attach the tRNA to the middle bead of the codon (Figure 7).
8. Move the two bonded amino acids from the P site and attach them to the third amino acid at the A site on the ribosome forming another peptide bond (Figure 8). Your amino acid chain is growing! Continue to shift the ribosome along the mRNA molecule and repeat the process until all of the amino acids have been used, and you reach the end of the mRNA molecule. By the way, as the ribosome moves along the mRNA molecule, and tRNA molecules are freed from the ribosome, the tRNA molecules are detached from the mRNA and are now able to complex with additional amino acid molecules in the cytoplasm.

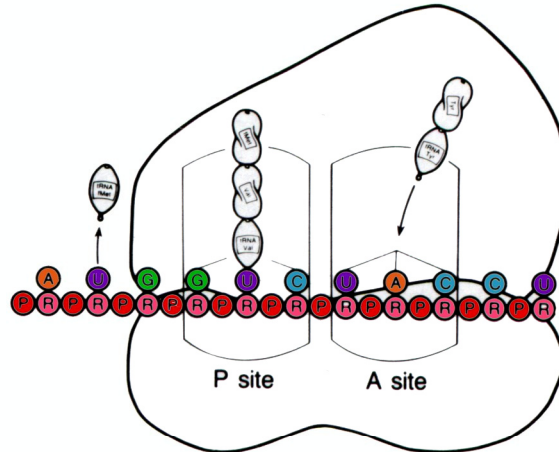


Figure 8. Shifting along the mRNA molecule/ribosome complex

9. When you finish, you may want to rearrange the nucleotides in your original mRNA and repeat this exercise.

At the completion of the exercise, please detach all assembled pop-beads, place the nucleotides and tRNA-amino acids in the appropriate containers, and return other materials to the designated locations.

This laboratory exercise is adapted from Carolina Biological Supply's RNA Simulation Biokit # 17-1035.