Molecular Biology II: DNA Transcription

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Learning Goals:

To work with a physical model of DNA and RNA in order to help you to understand:

- rules for both DNA & RNA structure
- transcription including promoters & terminators
- translation including start & stop codons

To work with a computer program in order to help you to understand:

- how to begin to analyze nucleic acid sequences

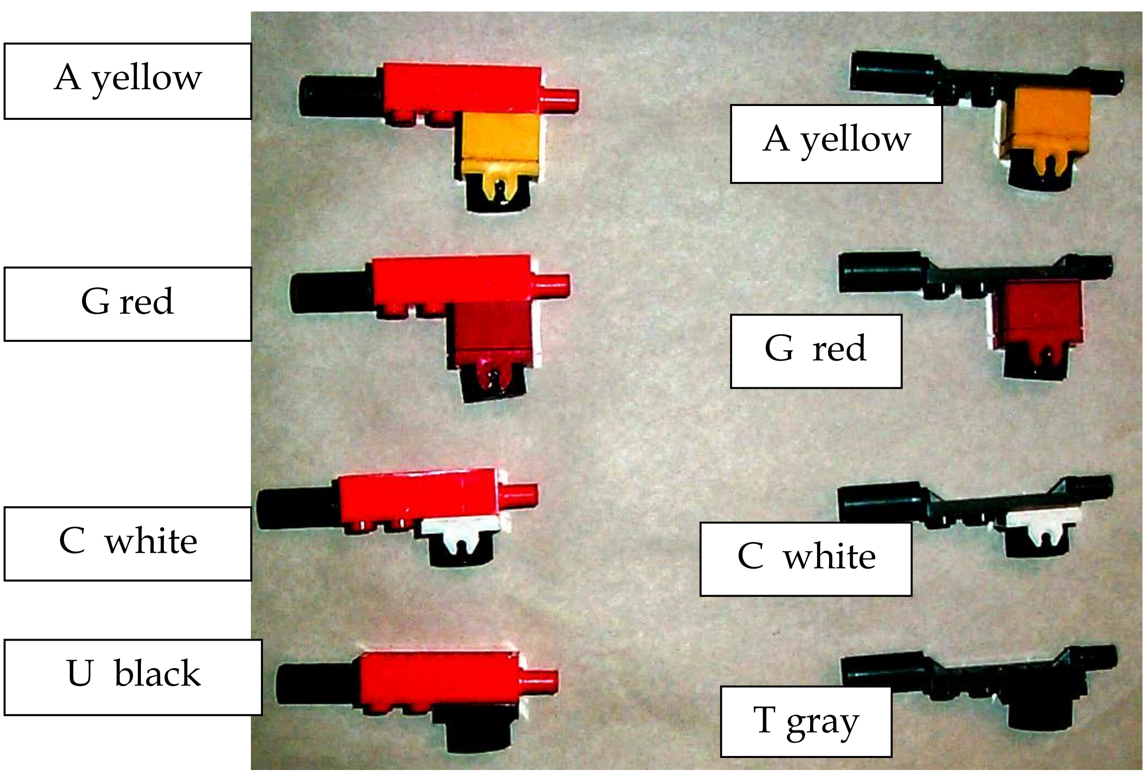
Introduction:

Last week in lab, you modeled DNA Replication using Lego model kits. In order to thoroughly understand Nucleic Acid translation and transcription we will work through some simple problems using LEGO DNA models again this week. This week you will build a small gene and simulate how it produces a protein.

For a more detailed review each part of a nucleic acid modeled by the LEGO models refer to the first two pages of last week's lab, Molecular Biology I, DNA Replication. You should check your kit to be sure that you have the following assembled pieces:

RNA nucleotides — DNA nucleotides

5′ end sugar 3′ end — 5′ end sugar 3′ end



Recognize that:

The correct bases pair via hydrogen bonds simulated by the black magnets on each base.
The backbone is connected by covalent bonds simulated by the plug on the 3′ end and the socket on the phosphate on the 5′ end.

Procedure: 1) Check your kit. You should have:

- 12 DNA A's (yellow base)
- 12 DNA G's (red base)
- 12DNA C's (white base)
- 12 DNA T's (black base)
- 6 RNA A's (yellow base)
- 6 RNA G's (red base)
- 6 RNA C's (white base)
- 6 RNA U's (gray base)

They should be set up as in the pictures above. That is, the phosphate should be on the 5′ end - the end farthest from the base.

A Small Gene

In this part, you will build a small gene and simulate how it produces a protein.

1) Build the gene. Build a single-strand of DNA with this sequence (the spaces are to make it easier to keep your place in the sequence - they are not gaps in the backbone):

```
5'-CTATA AGCAT GCCCC TATGA GGGT-3'
```

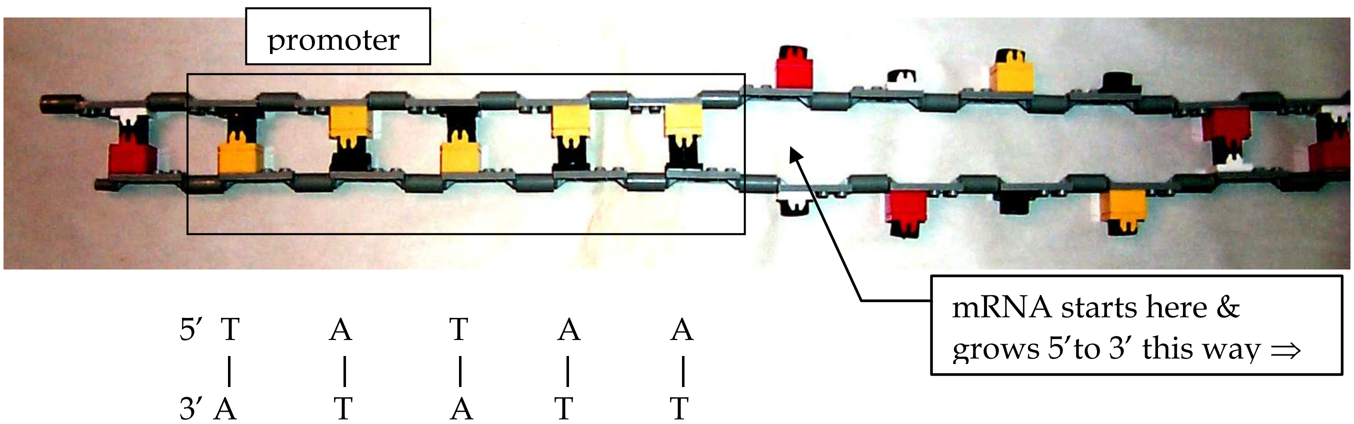
2) Build the corresponding other strand of DNA. If you have got the sequence exactly right, you will use up all of your DNA nucleotides.

Transcription

Transcription in this simulated organism starts at the first nucleotide after a promoter. In this organism, promoters have this sequence:

DNA bases	LEGO Colors (B = black (T), Y = yellow (A))
5′-TATAAx.....-3′	5′-BYBYYx....-3′
||||||	||||||
3′-ATATTy.....-5′	3′-YBYBBy....-5′

The 5′ end of the mRNA starts at base pair x-y. This is shown below:



Transcription in this organism ends at the base-pair just before the terminator. In this simulated organism, terminators have the following sequence:

DNA bases	LEGO Colors (B = black (T), Y = yellow (A), R = red (G), W = white (C))
5′-xGGGT.....-3′	5′-xRRRB...-3′
|||||	|||||
3′-yCCCA.....-5′	3′-yWWWY...-5′

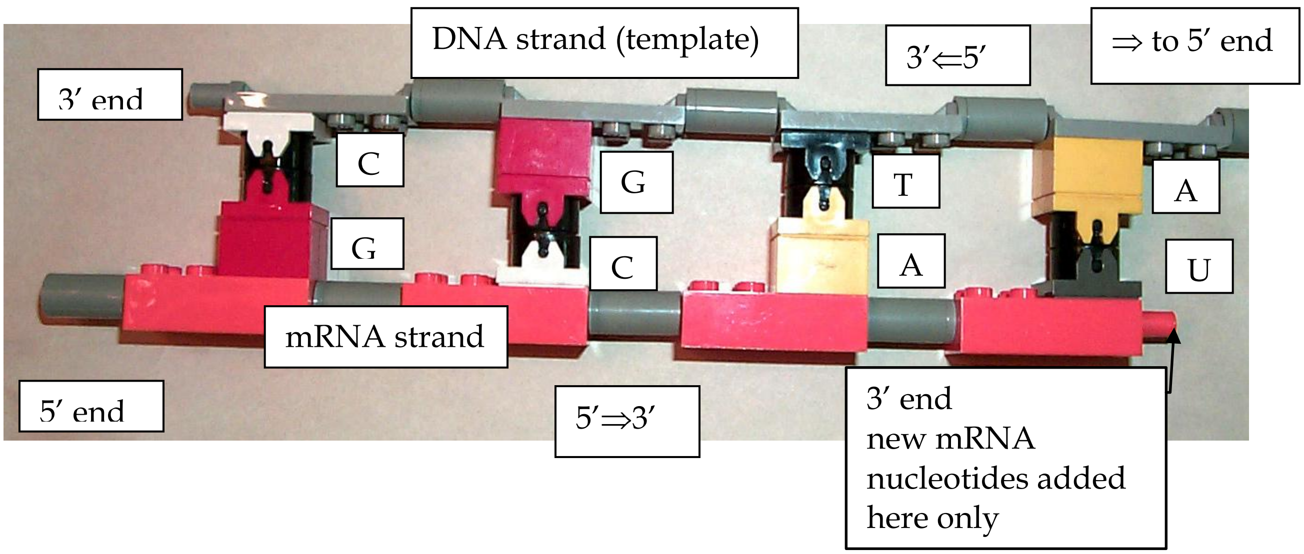
The 3′ end of the mRNA ends with base pair x-y.

13) Unzip the base-pairs from the end of the promoter to the start of the terminator - don’t forget to flip the bases out or they will re-pair. This is shown in the picture above.

14) Make the mRNA using the following rules:

- the strands must be anti-parallel (run 5′ ⇒ 3′ in opposite directions)
- A pairs with U (yellow with gray) the magnets won't let you pair it any other way
- G pairs with C (red with white) the magnets won't let you pair it any other way
- RNA polymerase can <u>only</u> add nucleotides to a 3′ end.

Correct RNA-DNA base-pairs are shown below:



Notice that only one mRNA strand can be made that follows these rules.

What is the sequence of this mRNA?

5′-____-3′

<u>Translation</u>

15) Your mRNA should look something like this:

Ribosomes in all organisms start at the 5′ end of the mRNA and look for the first start codon. This is 5′-AUG-3′ or 5′-yellow-gray-red-3′ and encodes the N-terminal methionine. Translation ends with a stop codon. A table of the genetic code can be found on page 101 of the lab manual.

Acting as a ribosome, translate this mRNA.

What is the resulting protein sequence?

N-____-C

A mutant gene

Mutations are alterations in DNA sequence. You can simulate their effects by changing the LEGO bases at a particular place in your simulated gene.

16) The 12th base-pair in your gene is a G-C base pair. Change it to a C-G base-pair. That is, the original DNA in that region **was**:

```
5'-...TGCCC...-3'
       |||||
3'-...ACGGG...-5'
```

Change it to (the altered base-air is **bold-underlined**)

```
5'-...TGGCC...-3'
       |||||
3'-...ACCGG...-5'
```

What is the resulting mRNA sequence? 5′-_________________________________-3′

What is the resulting protein sequence? N-___________________________-C

17) Disassemble your DNA and mRNA molecules and sort them into the kits. Remember to keep the phosphates on the 5′ ends.

Lego Lab Exercise II : "Designer Genes"

Make up a protein that is 5 amino acids long. You will then design a gene to produce that protein.

1) Give the sequence of your protein. Be sure to indicate the N and C termini.

2) Give the sequence of the mRNA that would encode your protein. Note that more than one answer is possible here; give only one. Be sure to indicate the 5′ and 3′ ends.

3) Give the structure of the double-stranded DNA molecule that would produce this mRNA. Use the format:

```
5′-GT-3′
   ||
3′-CA-5′
```

Be sure to indicate the 5′ and 3′ ends of both strands.