At the 1996 Olympics in Atlanta some 15 international athletes tested positive for anabolic steroids. At the 2000 Sydney games so far 15 athletes have tested positive. Athletes have been taking substances to enhance their performances for centuries. The use of anabolic steroids dates from the second world war when they were given to German soldiers to increase their aggressiveness and ferocity in battle. The first recorded use in athletics was by the Russians in 1954. During the 1960's and 70's their use increased significantly. The height of steroid notoriety peaked at the 1988 Seoul Olympics, when Canadian sprinter Ben Johnson was stripped of his 100 meter world record & gold medal.

The five major classes of steroid hormones: progesterones, glucocorticoids, mineralocorticoids, androgens, and estrogens, are all derived from cholesterol. Progesterones maintain pregnancy; Glucocorticoids, as cortisol, promote gluconeogenesis and glycogen formation and enhance the degradation of proteins & fats. Mineralocorticoids, as aldosterone, help the kidney maintain osmotic balance. The androgens, testosterone, develop male sex characteristics, while the estrogens development female secondary sex characteristics.

The efficacy of anabolic steroids in enhancing performance was finally clearly established this summer, just before the Atlanta Olympic Games, when a research group from the National Institute of Health showed that taking the androgenic steroid, testosterone, in combination with strength training lead to increases in muscle size, muscle mass, and strength in normal men. 43 normal men were assigned to 4 different groups.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Testosterone (600 mg per week) No exercise</td>
<td>Placebo with exercise (bench press &amp; squats)</td>
<td>Testosterone (600 mg per week) with exercise (bench press &amp; squats)</td>
</tr>
<tr>
<td>No exercise</td>
<td>81 mm²</td>
<td>424 mm²</td>
<td>501 mm²</td>
</tr>
<tr>
<td>triceps size</td>
<td>131 mm²</td>
<td>607 mm²</td>
<td>1174 mm²</td>
</tr>
<tr>
<td>quadriceps</td>
<td>1 kg</td>
<td>9 kg</td>
<td>22 kg</td>
</tr>
<tr>
<td>bench press</td>
<td>3 kg</td>
<td>16 kg</td>
<td>38 kg</td>
</tr>
<tr>
<td>squats</td>
<td></td>
<td>p&lt;.05</td>
<td>p&lt;.05</td>
</tr>
<tr>
<td>bench press</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>squats</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


For the first time scientifically, the efficacy of androgenic - anabolic steroids, like testosterone, has been firmly established, even though runners like Ben Johnson, swimmers like Jengi Li, and weight lifters like David Jenkins have known for years of the power of these drugs.

What do athletics and anabolic steroids have to do with protein synthesis. Well, let's take a look at how anabolic steroids work. We'll need to know a few things about muscle growth. Muscle growth is a cyclic process of simultaneously synthesizing and breaking down proteins. Muscle are made primarily of 4 different kinds of proteins: actin, myosin, troponin, and tropomyosin. Muscle growth is the balance between the amount being made and the amounts being broken down. If one wants to have bigger and stronger muscles, their going to have to make a significant alteration in the body's current protein cycles. You're going to have to speed up the rate of synthesis of new muscle protein, or slow the rate of protein breakdown.

Anabolic steroids work by altering the body's cycle of muscle protein balance. They function on both ends of the
cyclic spectrum. Much of the muscle building effects of steroids is due to their ability to inhibit protein degradation. Steroids interfere with the action of the hormone cortisol, thereby slowing protein break down.

The other half of the anabolic effect of steroids is due to their enhancing new protein synthesis. Steroids, like testosterone, traverse the plasma membrane and bind to cytoplasmic receptor proteins. These hormone-receptor complexes migrate to the nucleus, where they bind to specific DNA gene sequences called hormone response elements (HRE’s). The HRE’s regulate the transcription of nearby genes. HRE’s for steroid receptors are palindromes. A palindrome is a word, phrase, verse, or sentence that reads the same backward or forward, and in nucleic acids a palindrome is a segment of double-stranded DNA in which the nucleotide sequence of one strand reads in reverse order to that of the complementary strand. These palindrome sequences induce the transcription of particular sets of 50 to 100 genes. These new mRNA’s synthesize new muscle proteins, thus their anabolic effects.

TRANSLATION or PROTEIN SYNTHESIS

The synthesis of a new protein by a cell may be the most complex and detailed biochemical reaction that cells undertake. In the process of translation a cell must accurately interpret a DNA genetic message and build an intricate 3-dimensional functioning protein. Conceptually, translation is not unlike trying to understand another language. In reading a sentence in French we must convert the French words into English and then interpret their meaning. In protein synthesis the transfer of information is from an RNA molecule into a polypeptide, which involves a change in language from nucleic acid to amino acids.

Exercise 1

In all over 100 different molecules are involved in protein synthesis. Each member of your Learning Community should mentally review the events concerning the Central Dogma of Molecular Biology [DNA --> RNA --> PROTEIN]. After a quick review, each member of the group, on a separate piece of paper, should list all the MAJOR molecules or components which you can think of that are required to synthesize one new protein in the process of translation. Do not list the molecules needed for replication or transcription, only those needed for translation. Now consult together and create a master list of all the molecules required. You may want to refer to pages 304 to 311 in the 5th edition of Campbell’s Biology book to find ones you may have not considered.

Now fill in the Table below with the major molecules, listing a function for each. When your list is complete, compare it to the list the facilitator is using. Review the specific functional role of all the molecules before you begin the rest of today workshop.

<table>
<thead>
<tr>
<th>Required Molecule or Component</th>
<th>Major Function of Component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Required Component of Protein Synthesis and their Function.

Exercise 2. The **Adapter Hypothesis**: Proposed in 1954-55 by F. Crick, the hypothesis suggested there
must be a class of molecules that linked mRNA and the amino acid. The adapter molecule would read the code of the mRNA and deliver the appropriate amino acid to the site of protein synthesis. In 1957 Paul Zamecnik of Harvard discovered molecules (later called tRNA) which bound specific amino acids. Presumably the code was being read by this amino acid carrying RNA adapter molecule. Have your group speculate about what the result of the following experiment might be and what its significance is?

We have a synthetic mRNA (see Figure 1.) with its listed appropriate mRNA codons, tRNA anticodons, and amino acids. The tRNA-Cys anticodon is ACA. Suppose that we chemically modify the cysteine amino acid attached to its tRNA-Cys by reacting it with the metal catalyst, Raney nickel, which removes the -SH sulfur (thiol) group from the cysteine replacing it with a hydrogen atom [look up the structure of cysteine on page 75 - figure 5.15 of 4/e of Campbell].

**a.** Which amino acid is cysteine converted into by removing the -SH _____________? If we replace the normal cystyl-tRNA-cys with this modified one, would you expect the modified tRNA to place this modified amino acid into the growing protein according to the synthetic mRNA’s codon from Fig.2 (Y or N) _________? If so, WHY? and If not, WHY NOT?

This exact experiment was performed in 1962 by F. Chapeville, who proved that it was the codon-anticodon pairing that specified the proper amino acid sequence and not the amino acid itself.

Since the key concept in genetic information transfer and correct protein synthesis is proper **codon-anticodon pairing** let’s practice using the genetic code. Use the Genetic Code on page 299 (figure 17.4) of the 5th edition of Campbell’s Biology book. Work as a group in answering the following questions and problems.

**b.** What does antiparallel mean? Select a member of your group to draw on a piece of paper a double stranded representation of DNA in which the proper ends of the molecule are depicted using the number 3’ and 5’. What do the number 5’ & 3’ refer to? Have another member of the group describe what antiparallel means in a codon-anticodon pairing between mRNA and tRNA.

**c.** In the codon-anticodon pairing depicted in figure 2 what is the anticodon? Fill-in the shaded boxes in Table 3 below with the correct anticodon. Have each member of your group make up random codons and then write the proper anticodons in the shaded boxes numbered 2 in Table 3. Now make up anticodons and then write the proper codons in the shaded boxes numbered 3 in Table 3. Remember proper polarity.
Table 3. Codons and anticodons.

<table>
<thead>
<tr>
<th>tRNA anticodon</th>
<th>tRNA anticodon</th>
<th>tRNA anticodon</th>
</tr>
</thead>
<tbody>
<tr>
<td>3' ----&gt; 5'</td>
<td>3' ----&gt; 5'</td>
<td>3' ----&gt; 5'</td>
</tr>
<tr>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>U G C</td>
<td>U G C</td>
<td>U G C</td>
</tr>
</tbody>
</table>

mRNA codon      mRNA codon      mRNA codon
5' -----> 3'    5' -----> 3'    5' -----> 3'

Table 3. Codons and anticodons.

d. Analogy exercise. Humans often learn best by an analogy, by representing new things as though it resembles something they already know. Let’s give it a try in relationship to the concept of antiparallel base pairing. Select 6 members of your group. Assign 3 members of the group any RNA nucleotide codon letter (either an A, U, G, or a C); then assign the other 3 members the complimentary anticodon nucleotide, respectively (either U, A, C or G). Now think of a way to line up these 6 members of your group to demonstrate the antiparallel pairing of a codon and an anticodon.

e. Divide your group into 3 teams. (use a black board if you have one – if desired)
   1. Team 1 is to make up a DNA molecule; both strands which have about 30 nucleotides per strand. Write out your DNA molecules neatly on a piece of blank paper. You may want to write the letters along the long edge (landscape format) so that we can fill in the transcription and translation products below it. [Keep this DNA sequence handy, we’ll use it later]

2. Team 2 is to take this DNA molecule sequence and transcribe it into an mRNA molecule. Team 2 has to decide which strand of the 2 DNA strands to use as the template for making a mRNA. If Team 1 did its work properly, they will have remembered to include in one of their DNA strands, the so-called sense strand, a codon sequence that will produce the proper ***initiator codon***. If they have not, then team 2 should change the first codon at the 3' end (reading left to right) to the DNA sequence that will give the initiator codon. **What is the initiator codon _________________? What would be its anticodon (i.e., DNA sequence) _________________? Discuss among each team why there needs to be an initiator codon.**

Team 2 should also make sure that the last 3 nucleotides in the sense strand code for a STOP or terminator codon. **What are the STOP codons. What is the purpose of a STOP codon.** To transcribe the DNA sense strand you only need to follow Chargaff’s rules. [Keep this mRNA sequence, we’ll use it alter]

3. Team 3 is to translate the mRNA molecule made by Team 2 into a polypeptide. You will need to use the Genetic Code for this portion of the exercise. It can be found on page 303 (figure 16.5) of the 4th edition of Campbell’s Biology book. In order to insure that you have not made any coding or coping errors have your Facilitator check over your written results.

**Exercise 3.** A Protein Synthesis Analogy. As we suggested above analogies allow learners to think about complex and abstract subjects in simpler or more familiar terminology. Use of an analogy can sometimes provide a bridge to access the abstract concept.

Biology is full of anthropomorphic terms, which describe everything from messenger RNA to daughter cells, each with its related imagery. The Genetic Code is given as a four letter alphabet, the structure of DNA is given as a helical staircase, the plasma membrane is given as a gatekeeper, the mitochondria as a power plant. Neurobiologists compare the human brain to a computer, while computer scientists are trying to design computers that are patterned after the neural net (i.e., the human brain... sort of a double reverse anthropomorphic analogy).
The complex and abstract nature of protein synthesis may be a good place to try some analogy. The following analogy is adapted from T.K. Meyer and C.H. Powers (1994).

<table>
<thead>
<tr>
<th><strong>BIOPOLY Candy Factory</strong></th>
<th><strong>Protein Synthesis</strong></th>
<th><strong>Your Group’s Analogy</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Boss give candy recipe to mailroom messenger</td>
<td>mRNA is transcribed from DNA</td>
<td></td>
</tr>
<tr>
<td>mail messenger leaves through bosses door &amp; goes to factory floor to workstations</td>
<td>mRNA exits through nuclear pore to cytoplasm &amp; ribosomes</td>
<td></td>
</tr>
<tr>
<td>line workers pick up candy ingredients</td>
<td>tRNA bind amino acids</td>
<td></td>
</tr>
<tr>
<td>line workers assemble candy ingredients at work stations</td>
<td>loaded tRNA bind to mRNA at the ribosome</td>
<td></td>
</tr>
<tr>
<td>candy ingredients are combined according to recipe</td>
<td>polypeptide chain grows in response to mRNA codons</td>
<td></td>
</tr>
<tr>
<td>candy assembly is finished; most is wrapped for shipment, spilled pieces are eaten by workers</td>
<td>completed proteins are either used by the cell or packaged for export</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Protein Synthesis Analogy.

Divide your group into 2 teams. From the table above have the members of Team 1 discuss the imaginary process of making candy at the “BIOPOLY Candy Factory”. Talk about the factory boss who sits in her office all day long handing out candy recipe cards to mailroom messengers, who take them to the different assembly lines on the factory floor, and directs the assembly of the candy ingredients that are dictated by that recipe.

Now have Team 2 discuss how proteins are made in cells. Go through each of the protein synthesis steps depicted in the table above.

Both teams together should now decide what cellular organelle or molecule each of the following candy factory components represents: 1) the boss’ office ____________, 2) the work stations ___________, 3) the line workers___________, 4) the mailroom messengers__________, and 5) the candy ingredients ____________.

In order that you may better visualize the protein synthetic process it would be best if you developed your own analogy. Both teams 1 and 2 working together should design an anthropomorphic analogy filling in the boxes in the table above. It would be easiest if you just picked another factory model, such as a Harley Davison factory in Milwaukee; but, the real challenge is to come up with another analogy that is not based on a factory model. Think of other circumstances where direction, assembly, queuing, and specificity are all involved. Good luck.

**Exercise 4. A Simulation of Protein Synthesis.** (adapted from J.D. Ruhl, 1995)

Since most of us like to role play in life, a simulation of a process may help you to visualize the events in the sequence more clearly. This simulation activity is designed to have students walk through the coding of a protein in order to facilitate their understanding of how codons and anticodons work.

Materials Preparation:

Divide your group into 2 teams again. *Isn’t it about time that you switched your groups around a little bit.* Make new combinations of teams that have not worked together yet. Team 1 is to take a piece of note paper &
cut it into small pieces; **Write the anticodon** for each of the amino acid codons of the Genetic Code on one side. On the other side of these tRNA papers **write the appropriate amino acid**.

Team 2, meanwhile, should take a couple of pieces of note paper and tear it into 2 long paper strips. Using a marker pen, label one strip as double-stranded DNA and the other paper strip as single-stranded mRNA.

Your facilitator will now orchestrate the simulation process for you.

1. One member of the group is selected to be the double-stranded DNA. You may fill out this paper strip with the DNA sequence from *Exercise 2* above.
2. One member of the group is selected to be the single-stranded mRNA. You may fill out this paper strip with the mRNA sequence from *Exercise 2* above.
3. One member of the group may use a single 8.5’ x 11’ note paper to draw 2 circles to representing the subunits of the ribosome.
4. The single-stranded mRNA person places the mRNA to the page with the ribosome drawn on it.
5. Team 1 now randomly distributes the tRNA pieces to all the members of the group. Group members now individually match their anticodon to the correct mRNA codon. Stick the index to the paper strip mRNA with tape (amino acid side out) until a chain of amino acids is constructed.
6. While it may seem like a trivial exercise it is important that every member of the group have a complete understanding of the concepts in the protein synthesis process. Discuss among the group any questions or points of the process that you do not follow or understand.

**The sequence of events during Translation:**

*Exercise 5.* Formation of the **Initiation Complex & Elongation** - Each and every different Biology text book uses its own symbols and stick figures to represent the events of translation and Campbell 4/e is not unique. Figure 3, given below, uses the same figure patterns as the Campbell textbook.

**a.** Each member of the group is to attempt to label the various parts of this representation of the formation of an initiation complex in figure 3.

1. _____________________ 2. _____________________ 3. _____________________
4. _____________________ 5. _____________________ 6. _____________________

Then, as a group discuss exactly what happens during the formation of a protein synthesis initiation complex. These figures do not include all the needed components. Two major pieces are missing: What are they.

**b.** ___________________________  **c.** ___________________________.

**d. Elongation.** Identify and fill-in each of the alphabetic labels in Figure 4 below.

**Group discussion:**

Each member of the Learning Community should individually and independently:

1. name the Stages 1 to 4.
2. identify the components a to k.

Now, in turn, each member is to take one of the stages and one or more of the components at that stage, and tell the rest of the community, what that component does or what is happening at that stage. Do not go onto the next stage until everyone in the community understands the events as they occur at each stage.
Exercise 6. Peptide Bond Formation

Activation links an amino acid to its tRNA and consumes one molecule of ATP for each amino acid activated. The formation of an initiation complex is facilitated by initiation factors, protein that promote binding between the components of the initiation complex and requires one molecule of GTP. The elongation stage has an aminoacyl-tRNA binding to the A-site, adjacent to the peptidyl-tRNA and the P-site. Peptide bond formation is followed by the translocation of the new peptidyl-tRNA from the A-site to the P-site. This process requires 3 elongation factors and the consumption of two molecules of GTP. The termination of protein synthesis requires the participation of a release factor and the consumption of one molecule of GTP. Various steps of protein synthesis are inhibited by antibiotics.

a. Calculate the number of high energy phosphoanhydride bonds (ATP equivalents) that are hydrolyzed during
the synthesis of a 600 amino acid protein. Do not include the energy required to synthesize the amino acids, the mRNA, the tRNA, or the ribosomes themselves, only the energy required for peptide bond formation.

Exercise 7. GROUP DISCUSSION QUESTIONS

As a group, discuss among yourselves the concepts and solutions to each of the following problems; then look at the suggested answers and see if your ideas were similar. Use your facilitator as a resource to clarify the question if it is not clear to the group.

a. The process of translation and protein synthesis in cells is a very complex, multi-stepped process involving many different molecular components. Why do you think evolution has favored having individual molecules as mRNA’s, tRNA’s, and various enzymes associate with a large, complicated structure (the ribosome) to bring about protein synthesis? Wouldn’t it be more efficient to allow the components to freely associate by diffusion in a cytoplasmic environment?

b. Why do you think that non-standard base pairing (wobble), which lets other tRNA’s than codon-anticodon pairing would dictate, is allowed during protein synthesis?

c. The fact that the process of translation (protein synthesis) is universal, i.e., the genetic code is nearly identical in all cells on Earth, leads one to an important conclusion about life. What is that inescapable conclusion?