The Mystery of the Massively Muscular Myostatin Bull

by
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Part I – The Central Dogma of Biology

Arnold: Jack, you know I've been lifting weights and following an extreme diet for years. Yesterday, I was listening to public radio and heard an incredible story about a child in Germany who had extreme muscle definition and at the young age of five could easily lift 10 pound weights! This makes me wonder how I could enhance my muscles with less time and effort, which would be amazing for my bodybuilding career. What does this kid have that I don't?

Jack: That's interesting. During my biology classes in college, we studied how proteins are made and how they function. It's an interesting process; you should learn more about it because I think it would really help you gain a better understanding of how cells work in relation to muscle growth. I think there's even a picture in my old textbook that shows a bull with extreme musculature; it had something to do with the proteins present in their cells. I wonder if there's any relation to the story that you heard.

Arnold: Do you have your textbook here? I'd love to see what you're talking about, and maybe you can give me an idea about how proteins work since I've only had high school biology classes and I don't have in-depth knowledge about the subject.

Jack: Of course, I have all of my old textbooks; here's the picture (Figure 1). It says that this is a Piedmontese bull with increased muscle mass, called "double muscling" in cattle. This increased muscle growth is called muscle hypertrophy. Can you imagine if all cows had this much skeletal muscle?

Back to your question about how the little boy from Germany may have achieved increased muscle mass. To summarize, the DNA in our cells contains genes, the information that is used to make proteins. DNA is used as a template to generate messenger RNA (mRNA) using a process called transcription. One of the main proteins involved in transcription is RNA polymerase, which incorporates RNA ribonucleotides into the elongating RNA strand. RNA polymerase uses its enzymatic activity to form the phosphodiester bonds needed to hold the individual ribonucleotides together. The mRNA from transcription is then translated with the help of the ribosome. Ribosomes are a complex of proteins and another type

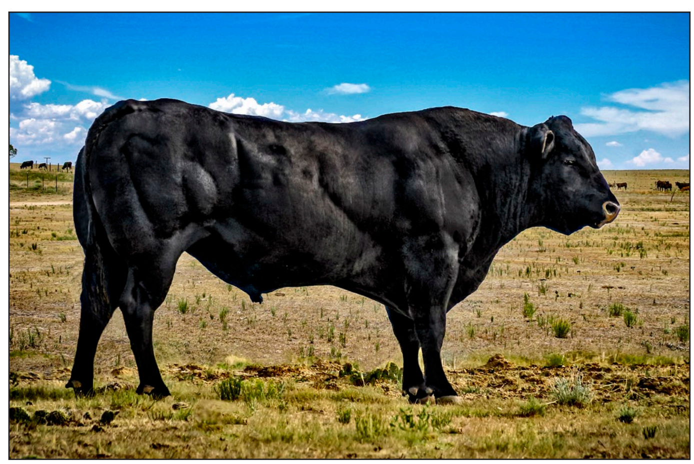

Figure 1. The pictured bull is 75% Piedmontese, exhibiting extreme muscle hypertrophy or "double muscling" due to a variation in the myostatin gene. *Credit:* Betty Wills (Atsme), CC-BY-SA 4.0, <https://commons.wikimedia.org/wiki/File:MLF_Unforgettable_911W.jpg>.

of RNA called ribosomal RNA (rRNA) that plays a role in generating a protein. Another form of RNA called transfer RNA (tRNA) delivers the correct amino acid to the ribosome. They recognize and bind to nucleotides on the mRNA, called codons. The ribosome and its associated rRNAs are then involved in forming the peptide bonds that hold the individual amino acids together, helping to generate the final protein. The process where DNA is transcribed into mRNA and mRNA is then translated into proteins that carry out various functions inside cells is called the central dogma of biology (Figure 2). The proteins generated are responsible for determining the functions of individual cells. Sometimes a mutation in a gene can lead to an altered protein that functions differently from the original protein, or the altered protein may be degraded by the cell. It is possible that a mutation in the DNA could be responsible for the muscle hypertrophy seen both in the boy from Germany and the Piedmontese bull in the picture, but we would need to investigate further.

Arnold: Thank you for giving me a general understanding of the process that cells go through to make individual proteins. I think I'll need to learn more about this subject if this is the key to understanding muscle hypertrophy and would help me understand if there is anything that I could do to mimic this type of change in my body.

Jack: Absolutely, I love learning more about the biology of cells. We can walk through this step by step and figure out how this bull is different from conventional cattle.

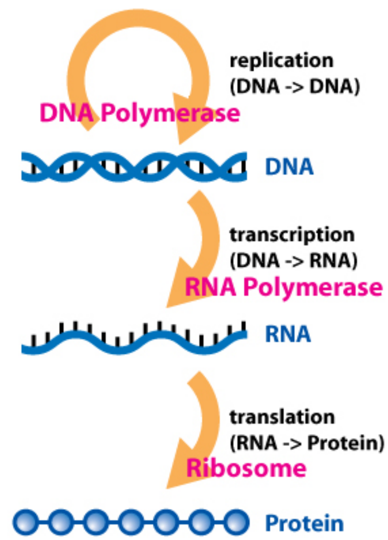

Figure 2. A depiction of the central dogma of biology. DNA is a double stranded molecule made of nucleotides that resides in the nucleus of eukaryotic cells. Portions of DNA are transcribed into a single stranded molecule made of ribonucleotides, termed RNA, by RNA polymerase; mRNA is then translated into a protein using the ribosome and associated rRNAs to form the bonds between amino acids. *Credit:* Daniel Horspool, CC BY-SA 3.0, <https://commons.wikimedia.org/wiki/File:Central_Dogma_of_Molecular_Biochemistry_with_Enzymes.jpg>.

Questions

1. List and describe the roles of the three different types of RNA involved in transcription and translation.

2. List the names and functions of the major proteins involved in transcription.

3. Why do you think the process of DNA being transcribed into mRNA and then translated into protein is referred to as the "central dogma" of biology?

4. Give one scenario that would explain how a mutation in the DNA of the Piedmontese bull could lead to muscle hypertrophy.

5. If more cows had the same musculature as the Piedmontese bull, how would that affect meat production in countries that use cows as a primary meat source?

Part II – Point Mutations in DNA

Jack: Arnold, I already found some information that can help us figure this out. As I mentioned before, the bull in the picture is Piedmontese, and I did a quick search and found that these cattle have a change in the myostatin gene that causes the muscle hypertrophy. To figure out whether this is also true for the child in Germany, I contacted my biology instructor, Professor Ricks, and asked her if there was a way that we could find out more about this phenomenon in humans. Here is her email response.

Jack,
I am delighted that you want to learn more about the basic science behind genetic disorders. I performed a literature search at our university's library and found a case study that I believe refers to the child in Germany that you mentioned. From what I have read, it seems that this child does have changes in the myostatin gene. In this journal article, not only were the child's muscles measured to document the muscle hypertrophy, but his DNA was examined to determine if there were mutations that might have contributed to the increased muscle mass (Schuelke et al., 2004). After reading this article, I believe that the type of mutation that causes extreme musculature in the Piedmontese bull may be similar to the kind of mutation seen in a well-known human disorder, sickle cell anemia. Sickle-cell anemia is a disease that results from a single nucleotide change in the DNA, called a point mutation. The single nucleotide change is in the gene that codes for the hemoglobin protein whose job it is to carry oxygen throughout the body in red blood cells (Ingram, 1957).
Professor Vanessa Ricks

Arnold: OK, so Professor Ricks is saying the increase in muscle mass in the Piedmontese bull could be from one single nucleotide change, similar to a mutation that results in sickle cell anemia? That seems like such a minor change; how does that make such a huge impact?

Jack: Remember how we talked about the general idea of the central dogma of biology, and how DNA is used to make RNA and then that RNA is used to make proteins? If there are differences, or mutations, in the DNA that is used as the template, these mutations would be transcribed into the mRNA and therefore could alter the protein made during translation. Sometimes a point mutation in the DNA does not change the resulting protein at all, and this is termed a silent mutation. In some cases, a point mutation can lead to the termination of translation, and the shortened, or truncated, polypeptide that is made may be degraded and never function inside the cell. Another possibility is a point mutation that leads to the generation of a protein with one amino acid that is different from the normal protein, which is called a missense mutation. A missense mutation can change the function of the protein based on whether or not the resulting amino acid substitution has different properties than the one that was present when no mutation occurred (Figure 3).

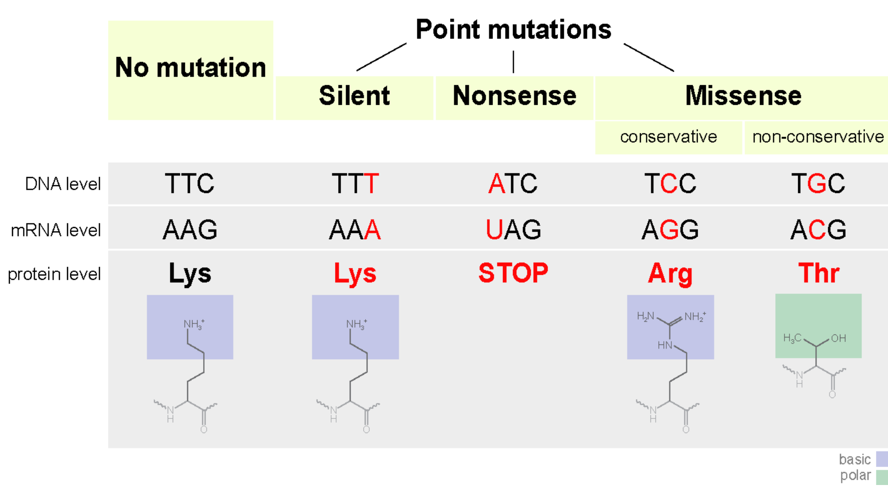

Figure 3. Point mutations in DNA. Single nucleotide changes in DNA can result in no change in the amino acid sequence, termed a silent mutation; an early stop codon in the mRNA, termed a nonsense mutation; or an amino acid substitution that may result in translation using a different amino acid, termed a missense mutation. *Credit:* Jonsta247, CC BY-SA 3.0, <https://commons.wikimedia.org/wiki/File:Point_mutations-en.png>.

Arnold: That's fascinating! So let me try to put this all together now that you have talked me through what is happening at the molecular level. Similar to sickle cell anemia, it is possible that a point mutation in the myostatin gene resulted in change in the myostatin protein and may be linked to muscle hypertrophy?

Jack: Absolutely, you've got a really good grasp of these concepts. Let's look more closely into why this Piedmontese bull has the double muscling.

Questions

1. Describe how it would be possible for a single point mutation in DNA to result in early termination during translation.

2. What is a possible consequence of a single nucleotide addition to the DNA used to generate a protein?

3. Use the codon table provided (Figure 4) to determine the impact of the following point mutation:

   Original sequence: AUG UUU UAU UGU

   Mutated sequence: AUG UUU UAC UGC

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

Figure 4. This table is used to determine the appropriate amino acid sequence of a polypeptide generated during translation based on a known mRNA sequence. *Credit:* CNX OpenStax, CC BY 4.0, <https://commons.wikimedia.org/wiki/File:OSC_Microbio_11_04_GenCode.jpg>.

Part III – The Role of Myostatin in Muscle Growth

Jack: Arnold, I contacted Professor Ricks again to help us figure out whether the Piedmontese bull with double muscling was the result of a change in the myostatin gene. She told me to do some research to try and figure out what portion of the gene might be important for the protein to function correctly. I performed a literature search and found this portion of the myostatin gene that is present in Holstein cattle (Figure 5). Holstein cattle do not exhibit muscle hypertrophy like the Piedmontese, so maybe this particular DNA sequence from the myostatin gene is different to the one found in the Piedmontese bull.

5′ … TGCTCTGGAGAATGTGAATTTGTATTT … 3′

3′ … ACGAGACCTCTTACACTTAAACATAAA … 5′

Figure 5. Portion of DNA (Exon 3) from the myostatin gene in conventional Holstein cattle (McPherron & Lee, 1997). The "…" represents the nucleotides not shown that exist on either end of the sequence.

Arnold: You make this sound so interesting. I wish I had taken classes like this in college. Maybe I'll consider enrolling in one so that I can learn some more. For now, we should walk through this process again. At the very least if I understand the process of transcription and translation, maybe we could determine whether that Piedmontese bull has changes in the myostatin protein that cause such defined musculature.

Jack: Sure, let's transcribe and translate this sequence and then maybe we can compare it to the one in the Piedmontese bull.

Questions

Transcribe and translate the portion of the myostatin gene from Figure 5. Assume the bottom strand is the template strand with the understanding that the promoter region in the DNA and the start and stop codons in the mRNA are not depicted (present in the sequence on either end of what is shown).

1. Holstein mRNA sequence: ____

2. Holstein polypeptide sequence: ___________________________________

Arnold: Now that we've gone through the process, I think I understand how DNA is transcribed and the resulting mRNA is translated into protein. I would like to understand a little bit more about how the myostatin protein works in cells so that I can understand how a mutation might result in muscle hypertrophy."

Jack: I'll email Professor Ricks about this, I think we may need her help in understanding the role of myostatin in cells.

Email from Professor Ricks:

Jack,

I am proud that you are delving deeper into understanding the role of myostatin. I will just give you a brief overview of its function in cells. Myostatin is involved in inhibiting muscle growth by binding to other proteins called receptors on the surface of skeletal muscle cells. When the myostatin protein is not functional, the result is increased muscle mass. I hope this is enough information for you to work with.
Sincerely,
Professor Vanessa Ricks

Jack: Arnold, I think we have enough basic knowledge to understand that the normal myostatin protein helps to inhibit muscle growth, and when the myostatin gene has a mutation that causes it to be unable to work with the receptor, this regulation may be lost, resulting in increased muscle growth (Figure 6.)

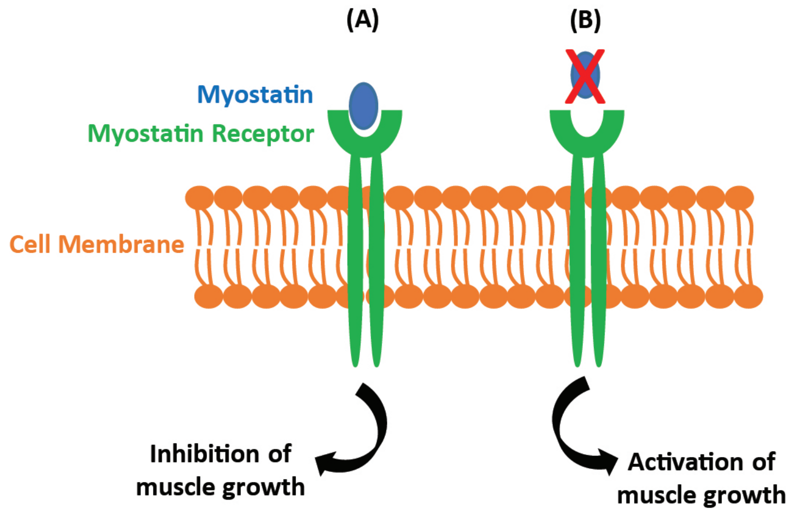

Figure 6. The role of myostatin in muscle growth. (A) The binding of myostatin (blue) to its corresponding receptor (green) on the plasma membrane of cells (orange) can lead to the inhibition of muscle growth. (B) In the absence of a functional myostatin protein, muscle growth is activated.

Arnold: OK, so if myostatin can't bind to its receptor, then it will not inhibit muscle growth and that might result in muscle hypertrophy! Can we try and figure out whether there actually is a mutation in the myostatin gene in the Piedmontese bull?

Jack: In the paper that I found the portion of the myostatin gene from the Holstein cattle (McPherron & Lee, 1993), it was directly compared to the one found in Piedmontese cattle. Let's take a look.

5′…TGCTCTGGAGAATGTGAATTTGTATTT …3′

3′…ACGAGACCTCTTACACTTAAACATAAA… 5′

Portion of Exon 3 from the myostatin gene in conventional Holstein cattle. At each end of the sequence "…" represents the nucleotides not shown that exist on either end of the sequence.

5′…TGCTCTGGAGAATATGAATTTGTATTT …3′

3′…ACGAGACCTCTTATACTTAAACATAAA… 5′

Portion of Exon 3 from the myostatin gene in Piedmontese cattle. At each end of the sequence, "…" represents the nucleotides not shown that exist on either end of the sequence.

Questions

3. Identify the mutation in the Piedmontese myostatin gene and put a box around it.

4. Transcribe and translate the portion of the gene from Piedmontese cattle with the understanding that the promoter region in the DNA and the start and stop codons in the mRNA are not depicted (present in the sequence on either end of what is shown).

   Piedmontese mRNA sequence: ____

   Piedmontese polypeptide sequence: ___________________________________

5. Compare the polypeptide sequence for the Holstein cattle (Question 2 above) to that for the Piedmontese. What kind of mutation is in the DNA from the Piedmontese that may have led to muscle hypertrophy?

6. CRISPR is a new biotechnology that targets DNA sequences, allowing us to alter specific DNA sequences. For example, it can be used to delete portions of the genome. How could you use the CRISPR technology to treat a muscle wasting disease?

References

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