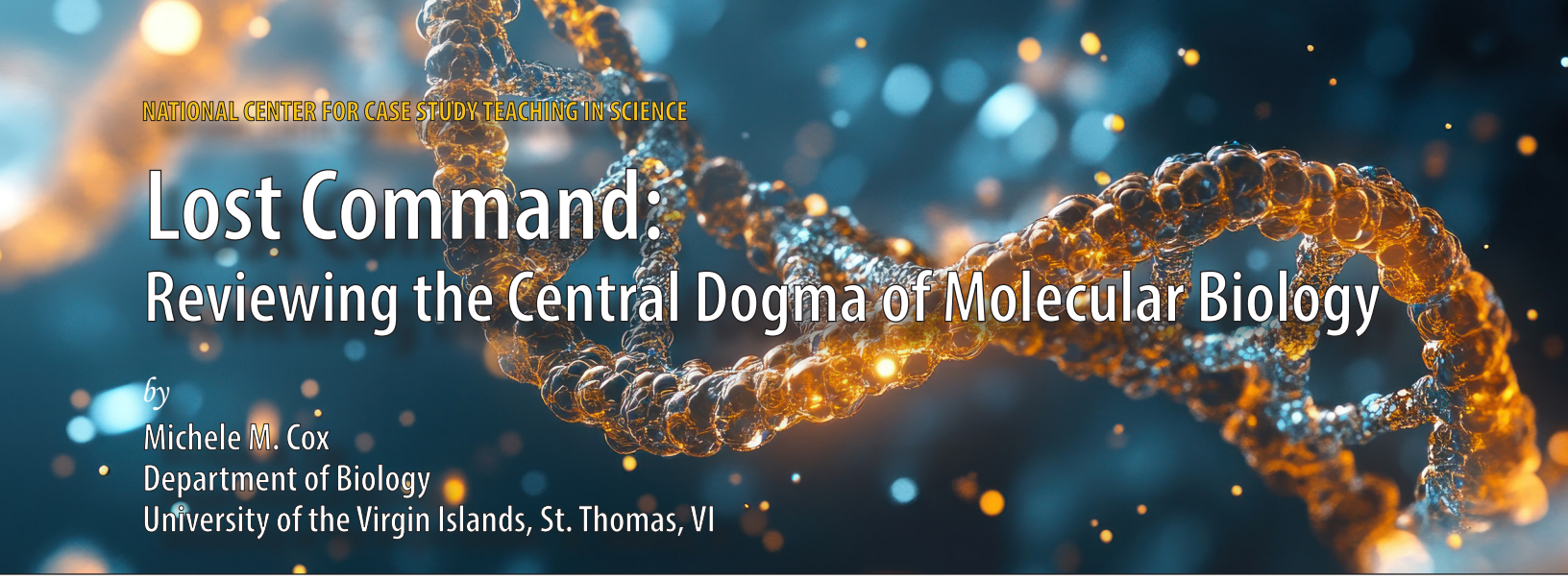

NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Lost Command:
Reviewing the Central Dogma of Molecular Biology

by
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Introduction

It had been a long week, and Chrissy was waiting at the library for her group members to arrive to start their final project. Soon Lisa, Anna, and Philip arrived and sat down.

"I wonder what horrors await us," said Lisa, pointing to a folder with bolded words on it: **Group 3 – Final Project**.

"I don't think it will be that bad," said Anna.

They all turned to Anna with sour looks.

"One thing's for sure, it won't be easy," replied Philip.

Chrissy sighed and opened the envelope. She pulled out the instruction sheet from inside, which read:

> *For your final project, review the medical case information of a six-month-old patient, "Amanda," who was recently brought to the hospital, and answer the following:*
>
> *A) Identify the disease the patient has and describe what it is.*
>
> *B) Explain the cause of the disease.*
>
> *C) Compare and contrast the normal bodily process and that of this disease.*
>
> *D) Connect your understanding of the disease with the results from the WBCs, MRI, radiography, and histology (3 out of 4).*
>
> *E) Describe the prognosis and treatment for this disease.*
>
> *It is expected that each member of the group be able to explain all aspects of this project during your presentation.*

"Well so much for divide and conquer," Philip said, pulling out his laptop. Anna and Lisa pulled out theirs as well.

"Okay, let's hear it," said Lisa while turning on her laptop.

Chrissy continued reading the instruction sheet out loud. "The patient is six-months old, and upon visual inspection Dr. Connor notices that she has abnormal skeletal development, coarse facial features, and restricted joint movement. Dr. Connor reviews the child's medical history with her parents and learns that the child has developmental delays, growth failure (decrease in weight and length), and skin changes. Afterwards, the doctor proceeds to do a physical examination of the child's hepatomegaly and musculoskeletal abnormalities (joint stiffness and claw hand deformities). Dr. Connor orders several tests to be performed, including WBCs, MRI, radiography, and histology (see Table 1)."

Chrissy placed Amanda's test results on the table for the others to look over (see Table 1, next page).

Table 1. Amanda's test results.

Clinical Tests Performed	*Results*
White blood cell count (WBC)	• Deficiency of the enzyme-acetylglucoaminyl-1-phosphotransferase activity.
Magnetic resonance imaging (MRI)	• Normal myelination. • Cerebral atrophy. • Nonspecific white matter changes.
Radiography	• Tubular bones of the upper extremities are short and widened. • Phalanges are bullet-shaped.
Histology	• Numerous intracytoplasmic inclusions in cells.
Note: Parents and older brother show no signs of the disease.	

Question

1. Fill in the chart below by matching the term to its definition. Select from the following: *cerebral atrophy, histology, intracytoplasmic inclusions, magnetic resonance imaging, myelination, phalanges, radiography, white blood cell count*.

Term	*Definition*
	Tiny particles freely suspended and floating in the cytoplasmic matrix.
	Creates images of internal body structures using x-rays.
	Creates detail images of internal body structures using magnets and radio waves.
	Loss of neurons and connections between neurons.
	Bones of fingers and toes.
	Measures the number of white cells in the blood.
	Branch of anatomy dealing with the microscopic structure of tissues.
	Formation of the myelin sheath around a nerve to allow for improved conduction.

Part I – The Genetics of Mucolipidosis Type II

"I'll look up diseases that cause developmental delays, growth failure, and skin changes," chimed Anna as she typed these keywords into Google.

"No, too many diseases will come up in the search. Narrow it down, add 'child hepatomegaly, musculoskeletal abnormalities, joint stiffness, and claw hand deformities,' " said Lisa.

"That takes it down to three possibilities. Let me add 'deficiency of the enzyme-acetylglucoaminyl-1-phosphotransferase activity and intracytoplasmic inclusions,' " said Anna as she typed in the additional keywords.

"Eureka! That was easy; it's mucolipidosis type II, which is also called I-cell disease," exclaimed Anna as she twirled her laptop around for the others to see. "It came down to mucolipidosis type II or mucolipidosis type III, but I eliminated type III because the symptoms usually don't present themselves until the child is about three years old. Our patient is six months old, which is consistent with the presentation of symptoms for type II."

"Muco-what?" asked Philip.

Lisa looked at her computer screen and read, "Mucolipidosis type II is a rare autosomal-recessive disorder caused by a mutation in a gene called *GNPTAB*. This mutation causes a deficiency of the enzyme UDP-N-acetylglucosamine, N-acetylglucosaminyl-1-phosphotransferase, which catalyzes the initial step in the synthesis of the mannose 6-phosphate. This enzyme helps target proteins to the lysosomes so they can function properly. It's classified as a lysosomal storage disease."

"Wait! What? I don't understand, what does autosomal recessive mean? How did she get it?" asked Anna.

"Here let me show you," said Chrissy turning her laptop around to show a human female (XX) karyotype (Figure 1).

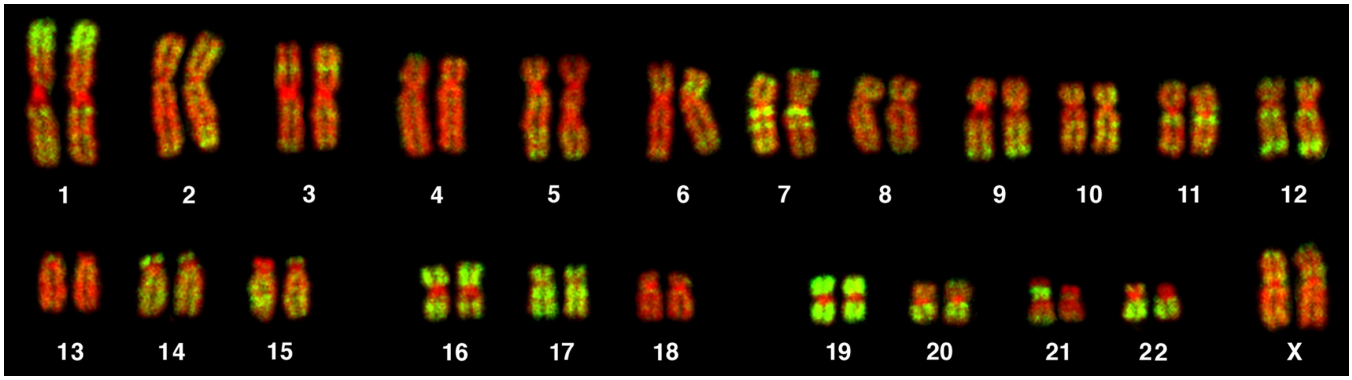

Figure 1. Human female (XX) karyotype. *Credit:* Andreas Blozer et al. (2005). Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. *PLOS Biology* 3(5), e157. CC BY, <https://doi.org/10.1371/journal.pbio.0030157.g007>

"We all have 46 chromosomes or 23 pairs of chromosomes. One pair consists of our sex chromosomes, which determine the sex of the individual. The other 22 pairs are called autosomal and cover a wide spectrum in terms of their information content, from information on various inherited traits to information on protein synthesis. Pairs of autosomes are called homologous chromosomes because they contain the same genes arranged in the same order. For each of these pairs, one member of the pair is donated from our mother and the other is donated by our father."

"At specific locations on our chromosomes, we have genes. Genes are composed of sequences of DNA and contain the genetic information for various traits we have, like eye color. We have different versions of our genes, known as alleles. Here, look at this schematic of the *OCA2* gene located on chromosome 15 (see Figure 2, next page). The *OCA2* gene produces a protein that plays a key role in the amount and quality of melanin that is present in the iris. A lot of melanin present in the iris tends to lead to brown eyes and less melanin present tends to lead to lighter eyes. In this figure the father (parental chromosome) carries the instruction to produce a lot of melanin while the mother (maternal chromosome) carries instructions to produce less melanin. The versions of the gene, called alleles, are different."

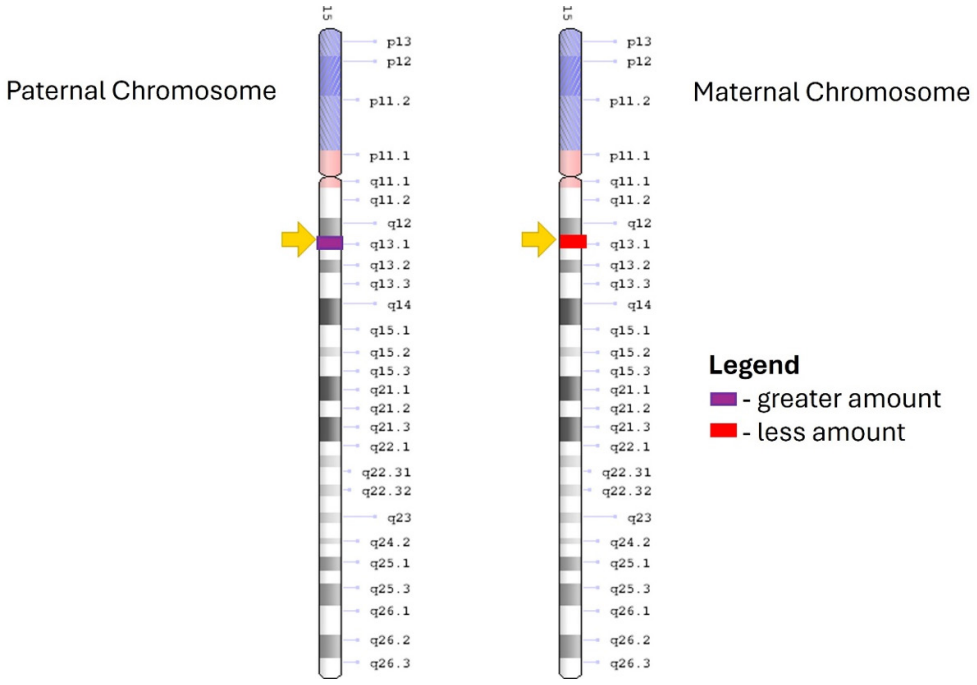

Figure 2. Schematic of OCA2 location on chromosome 15.
Credit: National Institutes of Health, adapted, PD.

"Chrissy, you have brown eyes; do both of your parents also have brown eyes?" asked Philip.

Chrissy shook her head. "My mom has blue eyes, and my dad has brown eyes."

"In your case, your mother gave you the *OCA2* light eye allele and your dad gave you the *OCA2* brown eye allele. This is your genetic makeup for this trait or genotype. You have one of each, making you heterozygous. The *OCA2* blue eye allele is there but not being expressed and could be passed on to your children," said Philip.

"Yes. There are other genes that participate in eye color, but I do have brown eyes," said Chrissy, batting her eyelids. "Expression of a trait is known as your phenotype and the blue eye phenotype is usually recessive to the brown eye phenotype."

"How does any of this have to do with this patient and her disease?!" exclaimed Anna.

"Mucolipidosis type II is an autosomal recessive disease. Autosomal recessive is one of several ways that a trait or disease can be inherited or passed down through families. Mucolipidosis type II is a disease found on one of the other 22 pairs of chromosomes and not on the sex chromosomes. The disease is recessive, meaning you need to inherit two mutated copies of the allele to get the disease. She got it from her parents," answered Lisa. "Watch this video I found; I think it may help."

- *Understanding Autosomal Dominant and Autosomal Recessive Inheritance.* Produced by Zero to Finals, 2017. Running time: 7:05 min. <https://youtu.be/lm0RXMFIenU>

"Just like the video, in Figure 3 both parents have an unaffected and an affected allele for this gene to produce an enzyme. Remember we get half our genes from our moms and half from our dads. What version of the gene that we get is random. So, in this case the dad gives

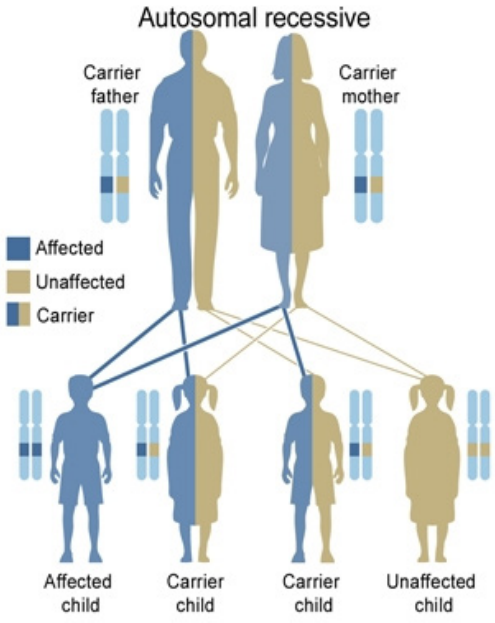

Figure 3. Autosomal recessive inheritance for an affected enzyme. *Credit:* GenesInLife, CC BY 3.0.

two of his children the affected allele and the other two the unaffected. Same goes for the mother in this case, two of her kids received the affected and the other two received the unaffected allele. The inheritance pattern for this enzyme is recessive, meaning to have an affected enzyme you need both copies of the affected allele.

"Look again at Figure 3," continued Lisa. "The first child received both copies of the affected allele and therefore has the affected enzyme; one from each parent. The second and third child each received one copy of each allele. The girl got the affected copy from her dad, but the unaffected copy from her mom. The boy had the reverse; he got the unaffected copy from his dad and the affected copy from his mom. Since both of these two children have each allele, they are heterozygotes and carriers; meaning they could pass on the affected enzyme to their children like their parents, but do not have the affected enzyme themselves. The fourth child received unaffected copies and therefore does not have the affected enzyme and cannot pass the affected enzyme to her children," concluded Lisa.

Questions

1. What is mucolipidosis type II?

2. What are some of the physical or musculoskeletal symptoms/abnormalities of mucolipidosis type II?

3. Fill in the chart below comparing the various terms.

	Gene	*Allele*	*Dominant*	*Recessive*	*Genotype*	*Phenotype*	*Homozygous*	*Heterozygous*
Definition								
Pairing								
Example								

4. Draw a Punnett square using standard symbols. Label the genotype of both parents and all the possible genotypes for their children. Label the one that is Amanda's.

5. Draw a pedigree using standard symbols. Label the genotype of both parents, Amanda, and all possible genotypes for her older brother.

6. Based on the Punnett and pedigree you drew, explain the following:

   a. Why does Mr. and Mrs. Nichols' daughter, Amanda, have mucolipidosis type II?

   b. Why don't the parents have mucolipidosis type II?

   c. Why does Amanda's older brother not have the disease?

7. What test could be utilized to verify this prediction about Amanda's brother?

Part II – DNA to RNA

Pointing to the Punnett square she just created (Figure 4), Lisa continued, "Both of Amanda's parents are heterozygotes for this gene, meaning they each contain one mutated copy of the allele (m) and one unmutated copy of the allele (M). Since they each have one of each, one unmutated and one mutated, they don't have the disease. Remember that you must have two mutated copies of the allele to have mucolipidosis. However, each of them contributed a copy of the allele to Amanda, the mutated allele to be exact. Amanda received a mutated copy from her mom and a mutated copy from her dad. Having the two mutated copies of the allele is why Amanda has mucolipidosis type II."

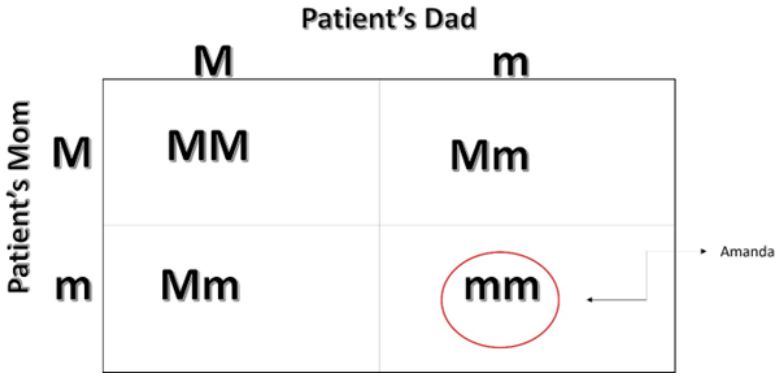

Figure 4. Punnett square illustrating the genotype of the patient and her brother.

"Then why doesn't her brother have the disease?" asked Anna.

"I think I can explain that," said Philip showing her a pedigree he just drew of the Nichols family (Figure 5). "Her brother must have either MM or Mm, based on the pedigree. He either received two copies of the unmutated alleles, MM, making him homozygous for the gene, or he received one of each, Mm, making him heterozygous for that gene. Either way, because he has at least one unmutated allele, he doesn't have the disease."

"So, the cause is genetics, specifically autosomal recessive inheritance pattern," said Chrissy as she typed on her laptop. "We can use this Punnett and pedigree to illustrate the cause."

"Well, we can check off A and B from the list for our final project. Let's have a look at C," said Philip grabbing the sheet for a closer look. "'Compare and contrast the normal bodily process and that of this disease.' Well, to answer that, I guess we need to find out more about what this *GNPTAB* gene does."

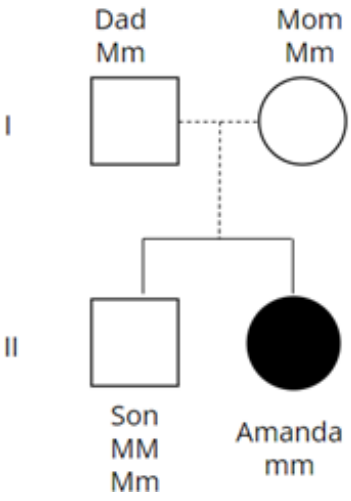

Figure 5. Pedigree illustrating the genotype of the patient and her brother.

Lisa quickly typed on her laptop and replied, "The gene encodes for the synthesis of an enzyme that helps target proteins to the lysosomes allowing them to function properly."

"Target? How does an enzyme help target proteins?" asked Lisa.

"To answer that, I think we need to go back to the very beginning and understand how a gene encodes an enzyme," said Chrissy.

Philip and Chrissy looked through their notebooks for information, while Anna and Lisa searched the internet.

After some time had passed, Chrissy exclaimed, "Hey guys, I think I found something!" The others stopped and turned to listen to her. "It all goes back to the blueprint in our cells: DNA and its structure."

"Does anyone remember what the structure of DNA is?" asked Anna. "I sure don't."

"I remember something about a ladder," said Philip frantically looking through his notes.

"Yes, a twisted ladder! The structure of our DNA is a double helix, and it resembles a twisted ladder," said Lisa. "The rails (outside) of the DNA ladder are made from alternating phosphates and sugars, called deoxyribose (sugar-phosphate-sugar-phosphate…). The rungs (inside) of the ladder are made up of four different kinds of nitrogen containing bases (nucleotides), with one base hanging off the sugar portion of each rail," Lisa continued.

"That's right! Our genetic information or instructions are ingrained in our DNA, using these four nucleotides. The nucleotides are adenine, thymine, cytosine and guanine," said Chrissy.

"Annabelle Thompson's Corner Grocery!" declared Anna.

The others looked at Anna in confusion.

"What?" asked Philip.

"Annabelle Thompson's Corner Grocery. It's how I memorized the four nucleotides and their pairing," smiled Anna.

"How'd you figure that?" asked Lisa.

"Oh, now I get it; Annabelle for adenine, Thompson for thymine, corner for cytosine, and grocery for guanine. That's clever, but why a grocery?" asked Chrissy.

"DNA is present in almost all living cells of all living things. This includes the food that we eat. So where do we get our food? From a grocery store. I was hungry when I made it up," explained Anna.

The others laughed.

"Anna is correct, DNA is in almost all living cells in all living things; that includes not just us, but in everything from my pet goldfish to plants; it's the same molecule. Nucleotides are like the alphabet of DNA. This alphabet tells our cells what to make, what not to make, when to make it, where it needs to go, and when to dispose of it when it's no longer needed," said Chrissy

"Here, I found a picture in my old textbook," said Philip as he passed his book to the group (Figure 6).

"The nucleotides are broken two into groups: purines (adenine and guanine) and pyrimidines (thymine and cytosine). Adenine only binds to thymine (A-T) and cytosine only binds to guanine (C-G) in DNA. This is known as complementary base pairing," said Philip.

"Look, here's an example of complementary base pairing," said Anna pointing at Philip's textbook. "So, if we know the nucleotides on one strand, we can figure out the nucleotides on the other. If we have a sequence of AGCGGTTA on one strand, the other strand would read TCGCCAAT," continued Anna.

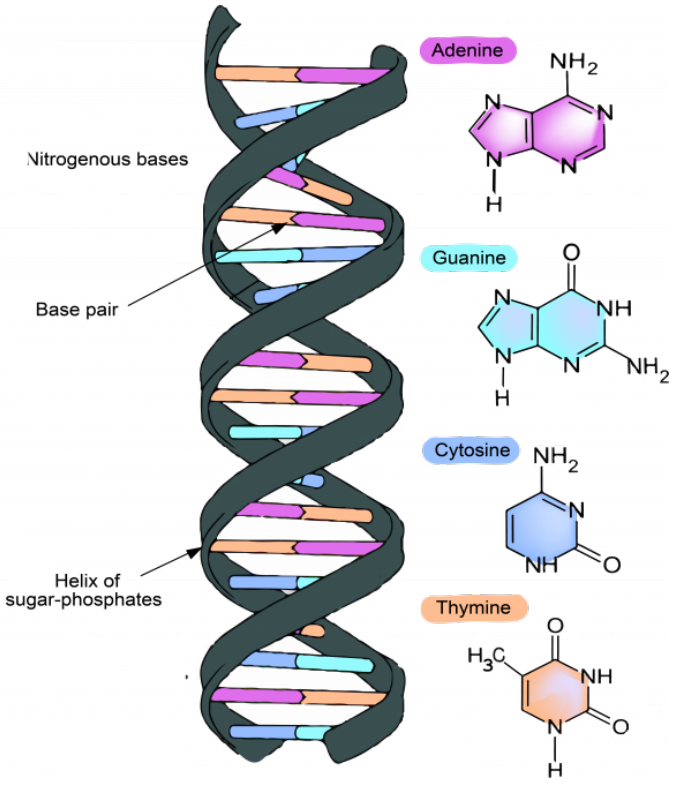

Figure 6. Structure of DNA. *Credit:* Windy Zheng, CC BY-NC-ND 2.0.

"So far this all makes sense. What else does it say in the book?" asked Lisa.

Philip read from the textbook, "Although this may seem like random letters to you this sequence is the key to providing all the information that keeps us alive. However, the genetic information or code (DNA) is trapped in the nucleus of our cells."

"Trapped! How is the DNA trapped?" asked Anna.

"Remember, the DNA is a double helix. It's too big to pass out of the nucleus," answered Lisa.

"For these instructions to be received by other organelles in our cells, they must be passed on to a messenger. In fact, the genetic information is passed on to messenger RNA (mRNA) through a process known as transcription," said Chrissy (Figure 7).

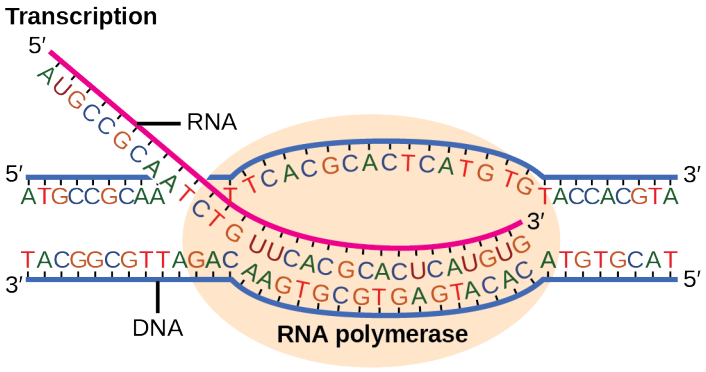

Figure 7. Transcription. *Credit:* Clark, M.A., M. Douglas, & J. Choi. (2018). *Biology 2e.* OpenStax. Access for free at <https://openstax.org/books/biology-2e/pages/1-introduction>. CC BY 4.0.

"There are several steps that take place for the genetic information to pass from DNA to RNA. The first step is initiation," said Philip.

"What happens during initiation?" asked Anna.

"The DNA is tightly coiled to fit in the nucleus of our cells. For conversion of information to begin, the DNA must uncoil to expose its sequence. This is accomplished with the help of transcription factors (gene-activating chemicals) to loosen up the DNA. Once the DNA is uncoiled, additional transcription factors bind to a special sequence that contains the start sequence so the gene can be transcribed; this is known as the promoter," said Chrissy.

"But doesn't the promoter also identify which strand of the double-stranded DNA will be used as the template in the creation of the mRNA (template strand)?" asked Lisa.

"Yes, it has two roles," said Philip.

"What happens next?" asked Anna.

"RNA polymerase (protein) binds to the DNA at the promoter site. The binding of RNA polymerase allows the DNA to unwind before it. Next, RNA polymerase moves across the DNA from the 5′ end to the 3′ end of the strand, adding nucleotides via complementary base pairing to the growing mRNA. This step is known as elongation," concluded Chrissy.

"It's very important to remember that guanine binds to cytosine and adenine binds to thymine during DNA replication. However, during the process of elongation in transcription, adenine (A) does *not* bind to thymine (T), but to uracil (U) instead to create mRNA. This is one of the differences between DNA and RNA," added Philip.

"How does the process end? I mean, it can't keep adding nucleotides forever," stated Anna.

"Once RNA polymerase reaches a stop sequence, it is released from the DNA strand. This final step is called termination. The mRNA, which is only single stranded and is composed of ribose sugar instead of deoxyribose sugar, undergoes modifications and eventually leaves the nucleus to deliver the genetic information. Since mRNA is only single stranded, it can pass out of the nucleus with the genetic information," finished Chrissy.

Questions

1. Determine the complementary DNA sequence for each of the following:
   a. ATGATCTCGTAA

   b. GTTCATTTGTATCCTGAAGAA

   c. TACGACAAGTTCGAGGATGTCTCTGTCTGGATATGGATGGATAGGGTGTCCATATGA

2. Identify three structural differences between DNA and RNA molecules in the table below.

DNA	*RNA*

3. Using your answer from Question 1c, write the complementary mRNA sequence.

Part III – RNA to Protein

"Okay, so the genetic information is transcribed from DNA to messenger RNA (mRNA), which then leaves the nucleus. I'm still not seeing a connection with Amanda and her disease," said Anna.

"Amanda has mucolipidosis type II, which causes a deficiency in the production of an enzyme, a protein. Where are proteins synthesized?" asked Lisa.

A look of understanding flashed across Anna's face as she exclaimed, "Now I get it! Proteins are synthesized in the cytoplasm. The genetic information is in the DNA, which is trapped in the nucleus; by transcribing the information to mRNA, a smaller molecule, the information can get out of the nucleus and reach the cytoplasm where the information is used to synthesize the protein."

"I think she's got it," answered Lisa.

"So, in Amanda's case, the mRNA isn't reaching the cytoplasm with the instructions to make the enzyme?" said Anna.

"No, that isn't the reason she has mucolipidosis," said Philip. "Because it doesn't fit with the WBC results. There was a deficiency of the enzyme activity, meaning there is some, but not a lot of it. If the mRNA wasn't reaching the cytoplasm, then there would be *no* enzyme activity."

"I agree. I think we need to look at what happens once the mRNA reaches the cytoplasm," said Chrissy.

Lisa nodded her head. Anna sighed.

"Once in the cytoplasm of the cell, the information must be translated or read for the proper events to occur, specifically the synthesis of proteins. When mRNA reaches the cytoplasm, it meets up with two other types of RNA, rRNA (ribosomal) and tRNA (transfer)," finished Chrissy.

"What do rRNA and tRNA do? I'm blanking on their functions," said Lisa.

"I know that rRNAs join together small and large proteins in the cytoplasm, forming a ribosome. mRNAs then bind to the ribosome. This process is known as translation," said Philip looking at Chrissy for confirmation.

Chrissy nodded. "The ribosome moves along the mRNA reading each base (…A, U, G, C,…) in groups of threes, also known as a triplet or codon. Each codon represents an amino acid, the building blocks of proteins. When the ribosome reads the codon AUG, also known as the start codon, another type of RNA, known as transfer RNA (tRNA for short) binds to the ribosome, bringing with it the amino acid for that codon."

"I remember the tRNA; it has a funny shape," said Anna (Figure 8).

"That's right. tRNA is clover shaped. At one end, known as the head, is a triplet code (anticodon) that is complementary to the mRNA and allows it bind based on comple-

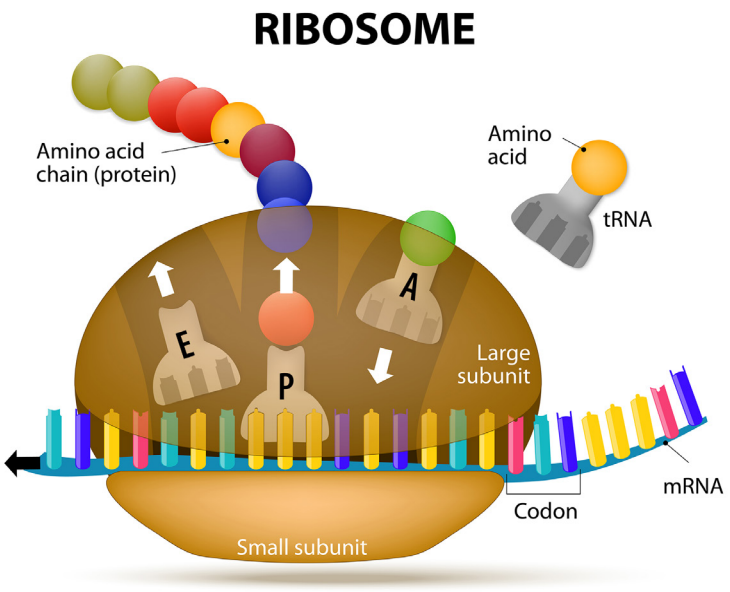

Figure 8. Ribosome. The letters E,P, and A indicate binding sites. The A site is where the t-RNA carrying the amino acid will bind to the ribosome, the P site is where the amino acid detaches from the t-RNA and binds to the growing polypeptide chain, and the E site is where the t-RNA exits.
Credit: Licensed image © Designua | Dreamstime, ID 55156376.

mentary base pairing. For example, if the mRNA triplet is GAG, then the anticodon would be CUC. This anticodon corresponds with the amino acid attached to the other end of the tRNA, in this case, cysteine," said Chrissy.

"Here's a picture of a tRNA," said Philip pointing to an image in his textbook (Figure 9). "You see the tRNAs continue to bring amino acids based on the mRNA codon attaching its amino acid to the previous one, forming a chain until it reaches the stop codon. The ribosome detaches from the mRNA and the synthesized protein is released into the cytoplasm."

"I'm still a bit confused," explained Anna.

"Hey, I found a video that shows translation." Chrissy turned her laptop around so the others could watch.

- *How are Proteins Made? Transcription and Translation Explained.* Produced by Cognito, 2020. Running time: 11:20 min. <https://youtu.be/ubdoUqmNF98>

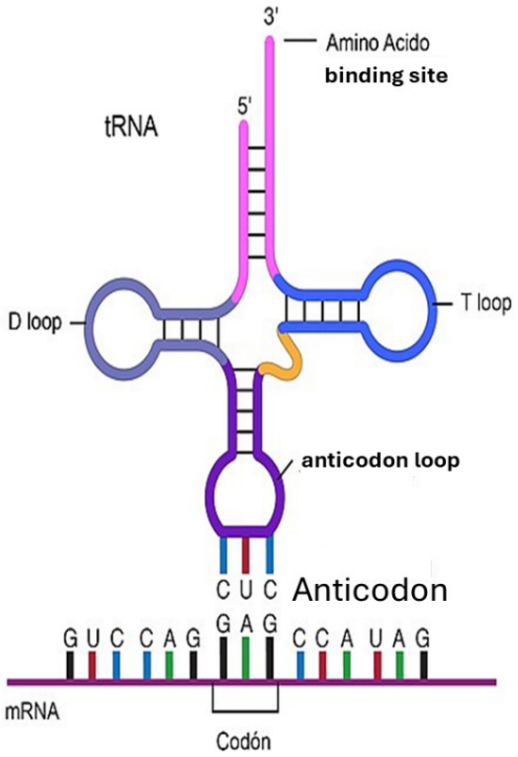

Figure 9. Transcription of a sequence of three amino acids thanks to tRNA (adapted).
Credit: xtec.cat | Wikimedia Commons, CC BY-SA 4.0.

First Base	Second Base: U	Second Base: C	Second Base: A	Second Base: G	Third Base
U	UUU, UUC — Phe; UUA, UUG — Leu	UCU, UCC, UCA, UCG — Ser	UAU, UAC — Tyr; UAA, 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 or Start	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 10. Codon chart.
Credit: Sarah Greenwood | Wikimedia Commons, CC BY-SA 4.0.

Questions

1. Use a codon chart (Figure 10) to determine the amino acid sequences below. Remember to read through the strand and *only* start on AUG and *stop* when it tells you to stop. Circle all the amino acids only. Follow the example below.

   Example:

   DNA ⇨ AGA CGG TAC CTC CGG TGG GTG CTA GTC TGT ATC CTT CTC ATG ACT

   mRNA ⇨ UCU GCC AUG GAG GCC ACC CAC GAU CAG ACA UAG GAA GAG UAC UGA

   protein ⇨ met – glu – ala – thr – his – asp – glu – thr – stop

a. DNA ⇨ TAA ACT CGG TAC CTA GCT TAG ATC TAA TTA CCC ATC

   mRNA ⇨

   protein ⇨

b. DNA ⇨ CCT CTT TAC ACA CGG AGG GTA CGC TAT TCT ATG ATT ACA CGG TTG CGA TCC ATA

   mRNA ⇨

   protein ⇨

c. DNA ⇨ TAC CTT GGG GAA TAT ACA CGC TGG CTT CGA TGA ATC CGT ACG GTA CTC GCC ATC

   mRNA ⇨

   protein ⇨

2. Based on the DNA sequence in Figure 11 below, fill in the mRNA sequence, anticodon, and the amino acid attached to the anticodon.

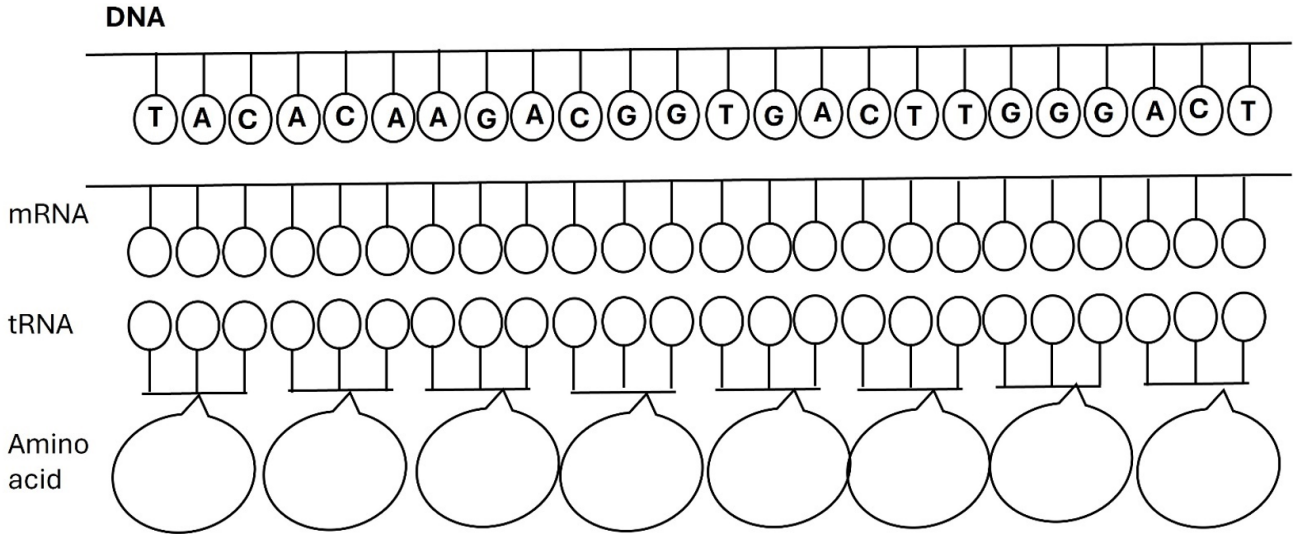

Figure 11. DNA sequence and corresponding mRNA sequence, anticodon, and amino acid.

3. Suppose a person has a mutation in their DNA codon that reads GCA instead of the normal codon GCC. What amino acid does the mutant DNA and the normal DNA code for? Will this mutation have an influence on the individual? Explain your answer.

Part IV – Conclusion

"Okay, let's review what we have already. We know that genes are encoded in our DNA. Our genes code for many things, including proteins. However, this information is "trapped" in the nucleus because DNA is too big to leave the nucleus. Am I right so far?" asked Anna.

The others nodded their heads.

"The genetic information is transcribed from DNA to RNA, specifically mRNA, which is small enough to leave the nucleus. In the cytoplasm the mRNA, along with tRNA, rRNA and the ribosomal subunits, translate the genetic code from the mRNA to synthesize proteins," continued Anna.

"Exactly" said Chrissy.

"We also know based on the WBC results of the patient that the enzyme, which is a protein, is present but low. That means that the gene is being expressed and transcribed. The mRNA can leave the nucleus with the coded information and gets translated in the cytoplasm. So, the problem isn't with transcription or translation. But then where *is* the problem?" asked Anna.

"Protein sorting!" exclaimed Chrissy.

All eyes turned to Chrissy.

"It's right here where the professor posted all her lectures. Right after translation she has 'Intracellular Compartments and Protein Sorting'," said Chrissy.

"Well then read what's in the lecture," said Philip.

Chrissy cleared her throat and began to read:

> *Normally these proteins have a signal on them that delivers them to the rough endoplasmic reticulum and then the Golgi apparatus for modification. While in the Golgi these proteins that are bound for the lysosomes have an additional signal that helps these proteins bind to a specific enzyme, phosphotransferase, which in turn catalyzes the formation of a recognition marker, mannose-6-phosphate. This recognition marker is what allows these proteins to reach the lysosomes, where they can break down substances and keep the cells healthy.*

"I still don't get it," sighed Anna.

"Think of it this way. When you send a package you need to put a mailing label on the package. The mailing label contains information that tells the postal personnel where to send the package so that it can reach its destination. Now, what would happen if that mailing label was missing? Your package wouldn't get delivered. In fact, it would end up at the 'lost and found' and eventually be destroyed," said Philip.

"What does happen to those proteins that don't have the recognition marker? Where do they end up?" asked Lisa.

Chrissy shrugged, "Hmm... that's a good question. I don't know."

Anna gasped. "Chrissy doesn't know the answer!? Never thought I'd ever hear those words."

The group laughed.

"Let's look on the internet to find out," said Lisa

"It says here that the lysosomal enzymes missing the recognition marker are missorted. Instead of going to the lysosomes they are transported to the cell membrane where they are secreted into the blood," finished Philip.

"If I remember correctly, lysosomal enzymes are acidic hydrolases, which help to breakdown undigested macromolecules in our cells. So…," said Lisa.

"So, if these enzymes are not reaching the lysosomes, then those substances are building up in Amanda's cells, maybe even to toxic levels," said Anna looking surprised.

"We must be on the right track because it says here in this paper that inclusions are vacuoles which, though not fully studied, contain polysaccharides, disaccharides, lipids, and B cells. Obviously, these inclusions contain the products that are from within the cell or imported into the cell via endocytosis to be degraded. Since they aren't degraded, they just accumulate in the cells and is why we can see them under a microscope. It's also a unique trademark of this disease," concluded Philip.

"That matches with the histology results, numerous intracytoplasmic inclusions in the cells," said Lisa.

"This also connects with the MRI results," said Chrissy. "The cerebral atrophy is because neurons are cells just like any other cells; they have lysosomes. The neurons are dying off due to the accumulation of substances in the neuronal bodies," stated Chrissy.

"But why does it seem that the musculoskeletal system is most affected?" asked Philip.

"Great question, but we only need to connect three out of the four results for the assignment. We only have a few more days before the presentation," stated Lisa.

"Yeah, I don't have the mental capacity to go for extra credit," laughed Anna.

Philip looked up at the ceiling, "Just curious."

"Thank goodness, the final part. What is the prognosis and treatment for this disease?" asked Lisa.

"I'm afraid the prognosis is not good. There is no cure, doctors can manage some of Amanda's specific symptoms with various medications. Children with mucolipidosis type II typically succumb within the first decade of their lives," said Chrissy sadly.

"What! You mean she's going to die? All this because of a few missing proteins!" said Anna bewildered.

"All this because of a lost command," said Philip.

Questions

1. Draw and label the route of lysosomal proteins once they have been synthesized in the cytoplasm.

2. Describe how mucolipidosis type II interferes with lysosomal protein sorting.

3. Lysosomes contain up to 50 different enzymes, each for a specific function. If there is a deficiency in one specific enzyme would the symptoms experienced by that individual be the same if that person had a deficiency in a different enzyme?

Part IV – Conclusion (Extended Version)

"Okay, let's review what we have already. We know that genes are encoded in our DNA. Our genes code for many things, including proteins. However, this information is "trapped" in the nucleus because DNA is too big to leave the nucleus. Am I right so far?" asked Anna.

The others nodded their heads.

"The genetic information is transcribed from DNA to RNA, specifically mRNA, which is small enough to leave the nucleus. In the cytoplasm the mRNA, along with tRNA, rRNA and the ribosomal subunits, translate the genetic code from the mRNA to synthesize proteins," continued Anna.

"Exactly" said Chrissy.

"We also know based on the WBC results of the patient that the enzyme, which is a protein, is present but low. That means that the gene is being expressed and transcribed. The mRNA can leave the nucleus with the coded information and gets translated in the cytoplasm. So, the problem isn't with transcription or translation. But then where *is* the problem?" asked Anna.

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"Well then read what's in the lecture," said Philip.

Chrissy cleared her throat and began to read:

> *Most proteins bound for incorporation into the ER, Golgi apparatus, lysosomes, or plasma membrane, or for secretion are initially targeted for the ER. In our cells, these proteins are usually imported into the ER while they are being translated.*

"Wait, I thought ribosome subunits were floating in cytosol. How are they able to translate the mRNA sequence and import the newly synthesized protein into the ER at the same time?" asked Philip.

"Our professor posted a video with the lecture. Maybe it can shed some light on this," said Lisa as she turned her computer around for all to see.

- *Cotranslational Targeting of Secretory Proteins to ER*. Produced by Sinauer Associates. Running time: 2:29 min. <https://youtu.be/FRph3TGkIAE>

"I still don't get it," sighed Anna.

"The ribosome begins translation just like we discussed earlier, but somewhere in the first 16 to 30 amino acids the ribosome hits a signal sequence, which tells it where this new protein is supposed to go. This signal sequence is recognized by the signal recognition particle or SRP. The SRP binds to the ER signal sequence on one end and the other end blocks the binding site between the small and large ribosome subunits, preventing translation," said Chrissy.

"But why does it need to block translation?" asked Anna.

"Think about it," said Philip. "The ribosome is in the cytoplasm. By blocking translation, it gives the ribosome time to get to the ER."

"It also prevents the newly synthesized protein from being released in the cytoplasm instead of in the ER," added Lisa.

"Yeah, that makes sense. What happens next?" asked Anna.

"Once the signal sequence binds to the SRP, the SRP exposes its binding site for the SRP receptor. The SRP is a transmembrane protein complex in the rough ER membrane," continued Chrissy.

"Transmembrane. Doesn't that mean that the protein spans the entire width of the membrane?" asked Lisa.

Chrissy nodded and continued, "The SRP will bind to its receptor, bringing the ribosome with it. Then the whole SRP-ribosome complex is transported to an unoccupied translocator on the membrane. The signal sequence opens a pore channel. Translation begins again, pushing the signal sequence and eventually the whole newly synthesized protein into the lumen of the ER. Once the ribosome reaches the stop sequence on the mRNA, translation ends. The SRP, SRP receptor, and ribosome are released from each other to be recycled."

"Well, that gets the protein to the ER. Is the same signal used again to get it to the lysosome?" asked Philip.

"No, the signal sequence is cleaved or cut off, allowing the newly synthesized protein to undergo modifications," said Chrissy.

"Modifications! Like what?" asked Anna.

"The protein would need to be folded into its tertiary structure. Right now, it's only in its primary structure, which is just a string of amino acids," answered Lisa.

"The protein has to fold and twist into its final 3-D shape," said Philip.

"This shape is held together by the interactions between R groups of amino acids. It is this tertiary structure that determines the function of the protein," added Lisa.

"Also, proteins bound for the lysosomes undergo initial glycosylation in the ER. It's during this modification that mannose monomers are added to these proteins," stated Chrissy.

"Wait ... mannose ... Amanda's disease is caused by a deficiency of an enzyme that catalyzes the initial step in the synthesis of mannose. This is it!" exclaimed Anna. "There aren't enough enzymes to add the mannose monomers. We did it! We figured it out," said Anna triumphantly.

"Um, no we didn't," said Lisa.

Anna gave her a questioning look.

"According to this paper, mannose monomers are initially added in the ER, but further modifications occur in the Gogli. The enzyme UDP-N-acetylglucosamine, N-acetylglucosaminyl-1-phosphotransferase is found in the Golgi, not the ER," said Lisa turning her laptop around and pointing to the screen.

Anna let out a loud sigh.

"Okay, I understand how the protein gets into the ER and while in the ER the protein undergoes various modifications, which includes getting folded into its tertiary structure. In addition, proteins bound for the lysosomes undergo glycosylation, where mannose monomers are initially added. How does it get to the lysosome? Is there another signal somewhere?" Anna asked jokingly.

"Exactly!" shouted Chrissy.

"I was only joking," said Anna suprised.

"Well, I guess the joke's on you!" laughed Philip.

"Chrissy how does the protein now with the mannose monomers attached get to the lysosomes?" asked Lisa.

"First, the protein must be transported to the Golgi. It gets there via vesicular transport," stated Chrissy.

"I just read about that in this article," chimed Lisa.

"Well, what does it say?" asked Philip.

Lisa read the passage out loud:

> *Entry into vesicles that leave the ER can be either a selective process or happen by default. Most membrane proteins are actively recruited into these vesicles, where they are concentrated. These membrane proteins display a transport signal on their cytosolic tails. This transport signal is recognized by adaptor proteins located on the inner coat of the vesicle, Coat Protein Complex II (COP II). COP II vesicles will bud off from exit sites on the ER carrying with them their protein cargo. Eventually these vesicles shed their outer coat and begin to fuse with one another forming vesicular tubular clusters. Vesicular tubular clusters move along microtubules from the ER toward the Golgi. This is known as anterograde transport. The vesicular tubular clusters will fuse with the cis-Golgi membrane, unloading their cargo.*

"Anterograde transport? cis-Golgi? What are you talking about?" asked Anna.

"Anterograde transport just means transport from the ER to cis-Golgi. Transport from the cis-Golgi to the ER is known as retrograde," answered Philip.

"The Golgi has two sides. The side facing the ER is called the cis-Golgi and the side facing the plasma membrane is known as the trans-Golgi," said Lisa.

"How am I supposed to remember all this?" said Anna placing her head on the table.

"Easy trick, just think of the alphabet," said Philip.

"What?" said Anna.

"C comes before E, so cis-Golgi and ER. P comes before T, so plasma membrane and trans-Golgi. Simple, but it works for me," stated Philip.

"Hmm, you know that makes a lot of sense and if you forget all you have to do is sing the alphabet," giggled Lisa.

"What happens once the proteins reach the Golgi?" asked Philip.

All eyes turned to Chrissy, who simply laughed. After a few minutes typing on her laptop Chrissy looked up at the group.

"Remember those mannose molecules that were added to the protein in the ER?" asked Chrissy.

The others nodded.

"Well, normally in the Golgi the enzyme UDP-N-acetylglucosamine, N-acetylglucosaminyl-1-phosphotransferase recognizes the mannose molecules and adds a phosphate group to them, which is known as phosphorylation. Now, this is key because the phosphorylated mannose, also known as mannose-6-phosphate is the signal or recognition marker to target the protein to the lysosome. Vesicles transporting proteins from the trans-Golgi to the lysosomes bind to mannose-6-phosphate and package the proteins in the vesicles. The vesicles then bud off from the Golgi and bind to late endosomes. The low pH environment of the late endosome causes the protein to dissociate from the receptor. The late endosome eventually fuses with a lysosome forming an endolysosome; the lysosomes then re-form by a maturation process. The even lower pH environment of the lysosome causes the protein to become active," finished Chrissy.

"What happens to those proteins that don't have the recognition marker? Where do they end up?" asked Lisa.

Chrissy shrugged, "Hmm... that's a good question. I don't know."

Anna gasped. "Chrissy doesn't know the answer!? Never thought I'd ever hear those words."

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"Yeah, I don't have the mental capacity to go for extra credit," laughed Anna.

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Part V – Extra Credit

A couple of day later, Chrissy, Lisa, and Anna were sitting in the library going over their presentation.

"Where is Philip? He was supposed to be here an hour ago," said Chrissy.

"Yeah, I left the cafeteria early and I missed getting dessert. They close in 20 minutes," said Anna with her arms crossed in frustration.

A few minutes later Philip came rushing over, completely out of breath.

"Where have you been?" asked Lisa.

"Sorry guys, but great news," said Philip as he took a seat with the group and placed down his laptop. "I figured it out!"

"Figured what out?" asked Chrissy.

"The skeletal deformities seen in Amanda's results," stated Philip

"Whoa, I told you I don't have the capacity to do extra credit," said Anna.

Lisa added, "Yeah, we will present tomorrow. We don't have time to recreate a whole new presentation."

"We're not creating a new presentation, but just adding a few extra slides and tying everything together," said Philip.

"Nope, that's going to take too long, and I want to get to the cafeteria before they run out of that triple layer chocolate cake with chocolate frosting, chocolate sprinkles, and chocolate chips," stated Anna.

"Oh, they ran out of that about 15 minutes ago," replied Philip nonchalantly.

Anna looked like she was about to cry.

"But," continued Philip "I was able to snag this extra-large piece before they ran out and three forks. You guys ready to go for extra credit?"

Anna nodded her head like a bobble head doll. Lisa giggled and Chrissy smiled saying, "We're in."

Philip placed the cake in the middle of the table and handed out the forks.

"Okay, what have you got?" said Anna with a mouthful of cake.

Philip opened his laptop and began. "Amanda's radiography results showed that her tubular bones of the upper extremities were short and widened and her phalanges were bullet shaped. Tubular bones are a classification of either the long bones or short bones. Examples of a long bone are the humerus (funny bone) or femur (thigh bone). The short bones refer to the bones in the hand and feet. Since the results say, "tubular bones of the upper extremities" we can conclude that we are looking at the short bones of Amanda's hand."

Philip looked around to see if the others were following his logic. The girls nodded as they continued to eat the chocolate cake.

Philip continued, "The phalanges refer to the bones of the fingers and toes. Since the results didn't mention upper or lower, we may be talking about the fingers, toes, or both. The reason for these changes in Amanda's bones based on my research is due to bone turnover."

"What is bone turnover?" asked Lisa.

"Bone turnover is a natural process that happens throughout our lives. It involves the resorption of old bone and the replacement by new bone with very little shape change," concluded Philip.

"I don't get it," said Anna.

"No worries, I got a video here to help. Eat your cake and watch," said Philip.

- *Bone Remodeling and Modeling*. Produced by Amgen, 2011. Running time: 4:12 min. <https://youtu.be/0dV1Bwe2v6c>

"Let me see if I understand. During our growth and development, but particularly during childhood, our bones are growing both in length and width. The process of this growth is due to bone resorption and bone deposition, which is known as bone remodeling." Chrissy looked at Philip, who nodded.

Chrissy continued, "During bone resorption, bone cells known as osteoclasts attach to the surface of bone creating the sealed zone. The sealed zone is an acidic microenvironment that dissolves the mineral content of the bone. This is followed by the release of enzymes, collagenases that remove collagen from the bone matrix completing the resorption process."

"Then," continued Lisa, "the osteoblasts come in. They synthesize and deposit osteoid an organic matrix filling in the depressions and grooves created by the osteoclasts, thereby remodeling the bone."

"Wait, what are osteo whatever and collagen? This sounds like an anatomy and physiology course, which I didn't take," said Anna.

"Calm down, Anna. Osteoclasts are a special type of multinucleated bone cells that initiate the resorption process of bones. Osteoblasts are another type of bone cells that form new bones and grow and heal existing bone. They are both important for the maintenance, repair, and remodeling of our bones," explained Philip

"Some osteoblasts get trapped within the matrix they created and become osteocytes, while others undergo programmed cell death, known as apoptosis. Still others will revert to cells that line the surface of the bone. Osteoclasts undergo apoptosis once they complete resorption," continued Lisa.

"Collagen is a protein that provides structural support because of its rigidity and resistance to stretching. It is found in the matrix, not just bones, but in tendons, ligaments and skin," added Chrissy.

"And why does our bones need to be remodeled?" asked Anna.

"Bone remodeling serves several purposes. First, it adjusts the construction of bone to meet our changing mechanical needs, like when we grow and develop. Second, it helps repair damage to bone matrix and prevents the accumulation of old bone. Lastly, it helps to maintain the plasma calcium homeostasis. Remember the video, it stated that during resorption calcium is released into the blood," finished Philip.

"Philip, this is great information, but I'm having a hard time seeing how this connects with Amanda's disease," said Lisa.

"Yeah, you yell 'Bone Turnover, not Bone Remodeling!'" exclaimed Anna.

Philip laughed. "Bone turnover is the rate of resorption and remodeling. You see, with bone resorption and remodeling under normal conditions old bone is replaced with new bone with very little shape change. However, what would happen if the rate were sped up or increased?"

"Well, then wouldn't there be constant removal and depositing of bone causing not small shape changes, maybe causing large changes to the bone?" asked Chrissy.

"Okay, what would happen if the rate were to slow down or decrease?" replied Philip

"Then there would be an accumulation of old bone and possible reduction of repairs to the bone," answered Lisa.

"Finally, what if one process, let's say resorption was elevated and remodeling was reduced?" asked Philip

"Then we would have weaker bones, because we are removing bones faster than we can replace them, right?" questioned Anna.

"Exactly; changes to bone turnover can lead to deterioration of the microarchitecture of the bone. I found several papers that demonstrated elevated bone resorption with reduced bone formation in patients with mucolipidosis II," said Philip triumphantly.

"That's great. Let's type that into the presentation and go home!" shouted Anna.

"Wait, I can't see the link to Amanda and her disease," said Chrissy.

"Me either. I mean this is great information, but I don't see the connection," said Lisa.

Anna glared at both.

"Okay, I will take a step back. Mucolipidosis II is a lysosomal storage disease, in which lysosomal enzymes are missing their recognition marker. Instead of going to the lysosomes, these enzymes are missorted, where many are secreted into the blood. This causes an accumulation of substances in the cell causing the cell to die. Well, what are osteoblasts?" finished Philip.

"Oh my goodness, that makes perfect sense, why didn't I see that," said Chrissy.

"Make sure we say that in the presentation, as it will make it so much clearer to everyone," added Lisa.

"Um…not so clear to me, little help here," said Anna.

"Osteoblasts are bone cells. The reduction of the osteoblasts as seen in patients with mucolipidosis II could be due to the buildup of substances causing the osteoblasts to die because of the lost recognition marker. Reduced numbers of osteoblasts will lead to a reduction of bone formation. This is how it ties back to Amanda," stated Chrissy.

"Did you read anywhere about changes to the osteoclasts?" asked Lisa.

"One paper stated that the osteoclasts themselves showed no signs of morphological or functional abnormalities. However, osteoclastogenesis, the formation of osteoclasts, was increased," said Philip.

"So, let me get this straight. Amanda's disease is causing an accumulation of substances in the osteoblasts. This is causing them to die and leading to a reduction in bone formation, so less new bone is being formed. At the same time osteoclasts are being formed at an elevated rate, which means bone resorption is increased. So, Amanda is losing more bones than she can rebuild, which leads to deformities in her bone structure and shape, and why we see that her upper extremities are short and widened and her phalanges are bullet-shaped, correct?" asked Lisa

Philip gave her a thumbs up.

"But shouldn't something be controlling this?" asked Anna.

"Great question! Bone remodeling is regulated both systemically and locally. The main systemic regulator is the parathyroid hormone (PTH), which maintains calcium levels in the blood by increasing calcium reabsorption at the kidneys. Other hormones such as growth hormone, glucocorticoids, thyroid hormones, and sex hormones are also involved in the regulation. At the local level, bone remodeling is controlled by several cytokines and growth factors that affect bone cell functions. Most papers I read focused on PTH, though," said Philip.

"So, what is it, too much or too little PTH?" asked Anna.

"That's the thing, they don't really know. Some papers showed normal levels of PTH in the serum of mucolipidosis II patients and others showed increased levels," explained Philip.

"So how do we explain that in our presentation?" asked Lisa.

"The exact causes of the elevated bone turnover has yet to be elucidated," said Anna with a smile.

The others looked at her in surprise.

"I learned that from seminar class. Whenever a guest scholar came to talk and couldn't answer a question because it hadn't been determined yet, that is what they said. If it can work for them, it can work for us," stated Anna.

"Sounds good to me," said Chrissy.

Lisa and Philip both nodded.

"Let's add this last bit to the presentation and call it a night," said Lisa.

The next day, Chrissy, Lisa, Philip and Anna walked out of class. Anna jumped for joy.

"I can't believe we got an A+!" exclaimed Anna. "Philip, my GPA and I thank you for pushing us to go for extra credit."

Philip blushed, "You're welcome."

"Let's celebrate! I hear they're serving jerk chicken and coconut cake at the cafeteria today," said Chrissy.

"Coconut cake! Last one there is a rotten egg," yelled Anna as she ran for the stairs.

Philip, Lisa, and Chrissy laughed and took off behind her.

Assignment

Submit a two-page paper in which you a) identify a disease that is caused by either an increase or decrease in bone turnover, b) describe the clinical features or symptoms of the disease, and c) connect clinical skeletal features or symptoms to bone remodeling and turnover.