

# Tazswana's Story: How Alternative mRNA Splicing Leads to Genetic Disease

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## Handout 1—"Junk DNA"

Ms. Williams picked up her copy of the *Science Newsette* from the coffee table, flipped to a page with a catchy headline, and began to read:

News Report: (*Science Newsette*)

### What You Thought Was "Junk" May Be as Precious as Your Own Life

by S. Catherine Silver Key

Did you know that 50% of all genetic diseases are caused by mutations in portions of genes considered "junk DNA"? That's right, these pieces of DNA that were in the past considered unimportant are now known to be responsible for roughly half of inherited diseases including cancer, cystic fibrosis, and a blood disease called thalassemia. Why? Well, as you know, genes are made of DNA that is then transcribed into pre-messenger RNA (pre-mRNA) in the nucleus, but before the pre-mRNA leaves the nucleus to be translated into protein it has to undergo a few alterations. One of these alterations removes portions of the pre-mRNA. Much like one would remove faulty frames of a movie film and then splice the two good ends back together to make one continuous properly edited movie, pre-mRNA is also "spliced" to make the correct mRNA and the subsequent protein. What "junk" is left behind **IN** the nucleus is referred to as "**IN**trons". The "good" portions of mRNA are "spliced" together to form mRNA and are allowed to **EXIT** the nucleus; they are referred to as "**EX**ons". The exon-containing mRNA encodes the protein-to-be. So, if the "junk" DNA that becomes "junk" mRNA (introns) does not code for the proteins that perform essential functions for the cell, how do they cause genetic disease?

To go back to the film analogy, if you do not remove the "bad" film or portion of the movie that does not add to the plot, the film length will be longer: it will be the wrong length. Leaving in a scene that

really needs to be edited out may actually change or detract from the overall plot of the movie.

Alternatively, if you do cut the appropriate region of film but paste together the wrong ends, you could have a Western with an upside-down cowboy riding on his horse.

Similarly, mRNA must be spliced correctly in order to avoid mistakes in the final product. In the case of the cell, that's a functional protein. Without the correctly sized and pasted together RNA, the resulting protein product will be defective. This is what happens roughly in half of all genetic diseases. It could be a lot worse than an upside-down cowboy: it could be fatal.

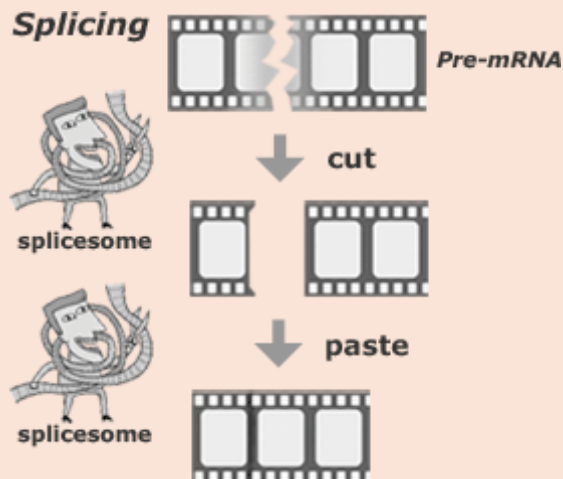
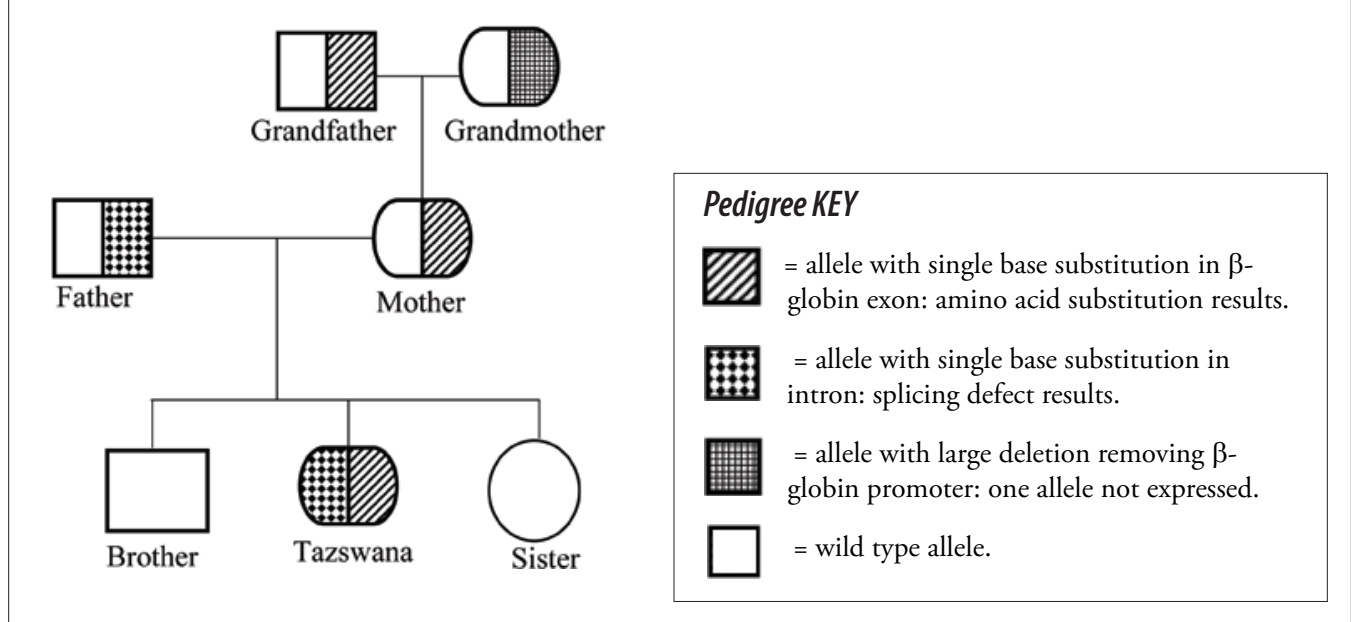


Figure 1. Pre-mRNA is cut and spliced to form mRNA. Within human cells, machinery called the spliceosome (splīs-a-sōm) performs the job of scissors and tape; removing introns and splicing together exons to form mRNA.

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## Handout 2— $\beta$ -Globin Inheritance Pattern

Figure 2-1. Tazswana Williams's Family Pedigree



### Questions

1. The predominant inheritance pattern for  $\beta$ -thalassemia major and intermedia are inherited in an autosomal recessive pattern. List the individuals in Tazswana's pedigree chart who are *carriers* for  $\beta$ -thalassemia. (Note: assume Tazswana is the only family member with symptoms.)
2. List the individuals in the chart who are indicated as heterozygous for the  $\beta$ -globin gene. Explain your answers. (Note: heterozygous = more than one type of allele; "wild-type" is a normal allele; each type of mutation is an allele. Terms to learn: *heterozygote*, *compound heterozygote* (two mutant alleles).)
3. Explain why only Tazswana has the disease.
4. Speculate on why a mutation in the *non-coding, intron* region of the  $\beta$ -globin gene could produce a defective  $\beta$ -globin protein.

## Handout 3—Beautiful Baby

“Hmmm...,” Mrs. Williams mused. “That *Science Newsette* article was interesting. Now I understand better what Dr. Kernodle was talking about....”

Mrs. Williams returned to her weekend chores, which included caring for her daughter, Tazswana.

Tazswana Williams was born a healthy baby girl on August 1, 2004. Labor and delivery proceeded without complication and she was perfect—all 10 fingers and toes. To her parents she was the most beautiful creature they had set their eyes on—their first baby girl. On her first birthday, Tazswana was a vivacious yet petite toddler who loved to sneak up on her Mom and say “Boo!” to which her mom would reply, “Peek-a-boo!” This would send Tazswana into a gleeful, waddling run to the nearest hiding place.

However, at 13 months of age, Tazswana had stopped running around. Coincidentally, she began to look pale and lost her appetite. Concerned, her mother took Tazswana to the family pediatrician.

After a routine exam, Dr. Vivian Kernodle reassured Mrs. Williams. “According to the growth chart, her weight and length are both in the 25<sup>th</sup> percentile, which is still just within the normal range. Additionally, you and Mr. Williams are petite, so Tazswana comes by it naturally. She *is* exhibiting symptoms that are consistent with a recent flu bug that’s been going around. No need to be concerned at this point. If her appetite and color don’t return by late next week, give us a call.”

“O.K., thank you doctor,” responded Mrs. Williams.

After a week, Tazswana’s symptoms had not subsided. Mrs. Williams called the doctor’s office Friday and made another appointment for the following Monday. During Tazswana’s second examination, Dr. Kernodle expressed concern. “Mrs. Williams, Tazswana’s spleen seems slightly enlarged. This typically indicates that it is working extra hard to produce immune cells to fight off infections. However, it could be an indication of other conditions involving the blood stream. I would like to go ahead and draw some of Tazswana’s blood and yours to run a few tests. Would you agree to that?”

Mrs. Williams replied, “I’d be glad to give it, if it will help my baby, but why do you need my blood?”

“Since you and your family have sub-Saharan African ancestry, I just want to check your blood to see if what is causing Tazswana’s listlessness could be an inherited blood disorder.”

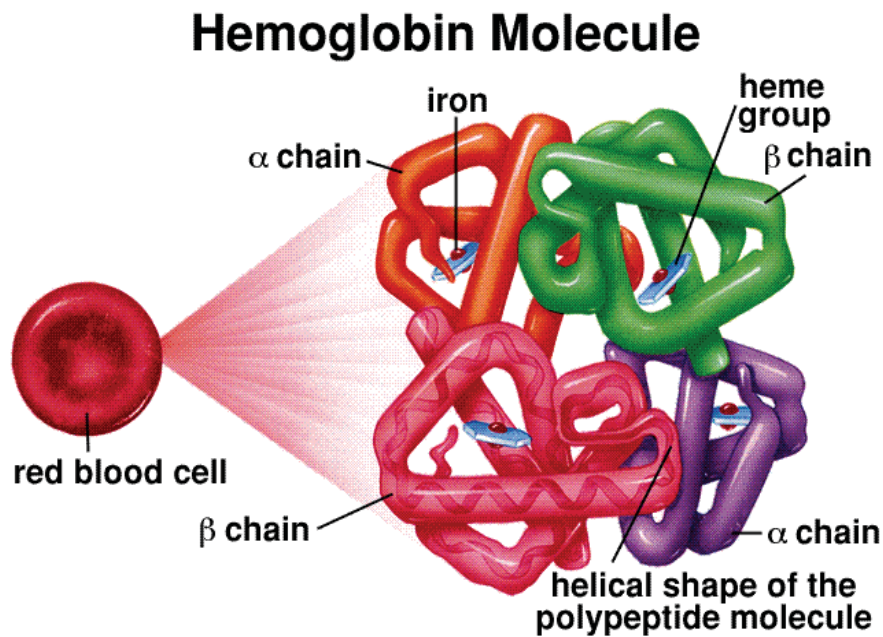
“Well, certainly doctor... do you want my blood now?”

“Yes, I’ll just walk you down to the phlebotomist’s office now.”

*Examine Handout 4 showing the blood test results and discuss and answer the questions on the handout.*

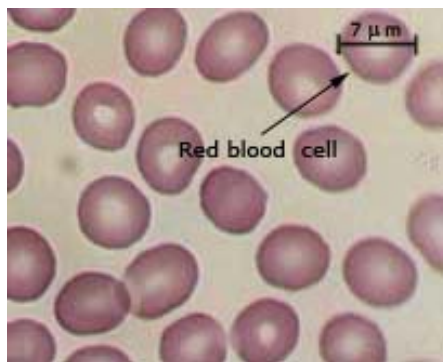
## Handout 4—Tazswana’s Blood Test

Figure 4-1. Hemoglobin consists of 2  $\beta$ -globin proteins and 2  $\alpha$ -globin proteins. Illustration used with permission from McGraw-Hill. Sylvia S. Mader, *Inquiry into Life*, 8<sup>th</sup> ed. © 1997 The McGraw-Hill Companies, Inc. All rights reserved.



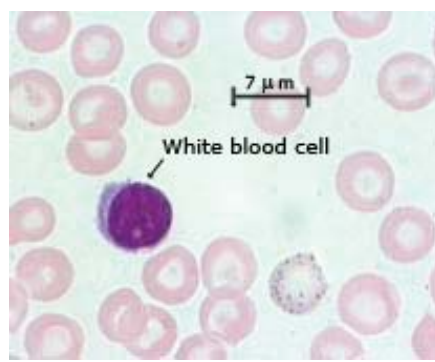
The hemoglobin forms a lattice structure on the cytoplasmic side of the RBC plasma membrane; thus, affecting the shape of the RBC, as shown in Panels A, B, and C of Figure 4-2 below.

Figure 4-2. Three different RBC samples. The iron-containing heme group is pigmented. Cells with less heme have less pigmentation and are said to be hypochromic. *Note:* mature RBC lack a nucleus. *Also note:* The following image location is the reference for the 7 micron size of a normal red blood cell: <http://static.howstuffworks.com/gif/artificial-blood-7.jpg>



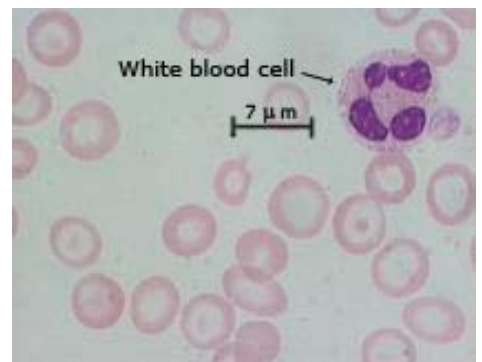
**Panel A: Normal sample**

Credit: Copyright © The American Society for Clinical Laboratory Science. Used with permission.



**Panel B: Mrs. Williams' sample**

Credit: Donald Innes, M.D. Used with permission.



**Panel C: Tazswana's sample**

Credit: Donald Innes, M.D. Used with permission.

## Questions

1. Compare the size, shape and color intensity of the red blood cells. Record your observations in the chart below:

		SAMPLE		
		Panel A: Wild-type	Panel B: Mrs. Williams	Panel C: Tazswana
PHENOTYPE	Size			
	Shape			
	Color Intensity			

2. Thinking about the function of  $\beta$ -globin and the shape and color of Tazswana's red blood cells, explain why Tazswana has severe symptoms compared to her mother or a normal person.

## Handout 5—Bad News

At the next office visit, Dr. Kernodle sat Tazswana's mother down and gently gave her the bad news:

"Tazswana's blood tests indicate that she has thalassemia. Roughly, this means that the  $\beta$ -globin protein in her red blood cells is defective, which causes the red blood cells to have an abnormal shape. Her blood cells cannot properly carry oxygen to the body tissues because the abnormal protein cannot bind oxygen. As a consequence, even with treatment, Tazswana will be physically limited. She won't be able to run and play as other children."

Devastated that her sweet little daughter might never have enough energy to play "Peek-a-boo" again or run with other children, Mrs. Williams managed to ask:

"How is it treated ... is there a cure?"

"Often  $\beta$ -thalassemia patients are treated with frequent transfusions which require frequent hospital visits, but this is not a cure. Additionally, your daughter has a severe form of  $\beta$ -thalassemia and her quality of life would be minimal with such treatment and she would most likely die in her mid-teens. There are two possible cures, one is a bone marrow transplant ..."

Mrs. Williams interjected: "She can have my bone marrow!"

"I know you want to help your daughter, Mrs. Williams, but we may be able to fix her own bone marrow stem cells ... There is one possible experimental gene therapy I'd like you to consider, but we will have to test RNA from Tazswana's blood cells. If Tazswana's test indicates that she has a certain type of abnormal  $\beta$ -globin, then she may qualify for the experimental study."

Mrs. William asked: "I don't know, Doctor, *experimental* gene therapy ... sounds risky. I'll think about it. How would Tazswana qualify?"

"We can determine whether Tazswana qualifies by looking for this specific change in her RNA."

"R.N. what?"

"RNA: it's related to DNA and it can tell us more details about Tazswana's condition. Altered mRNA is easily detectable using a specific scientific test called a Northern blot—the altered mRNA would be a larger size than normal mRNA."

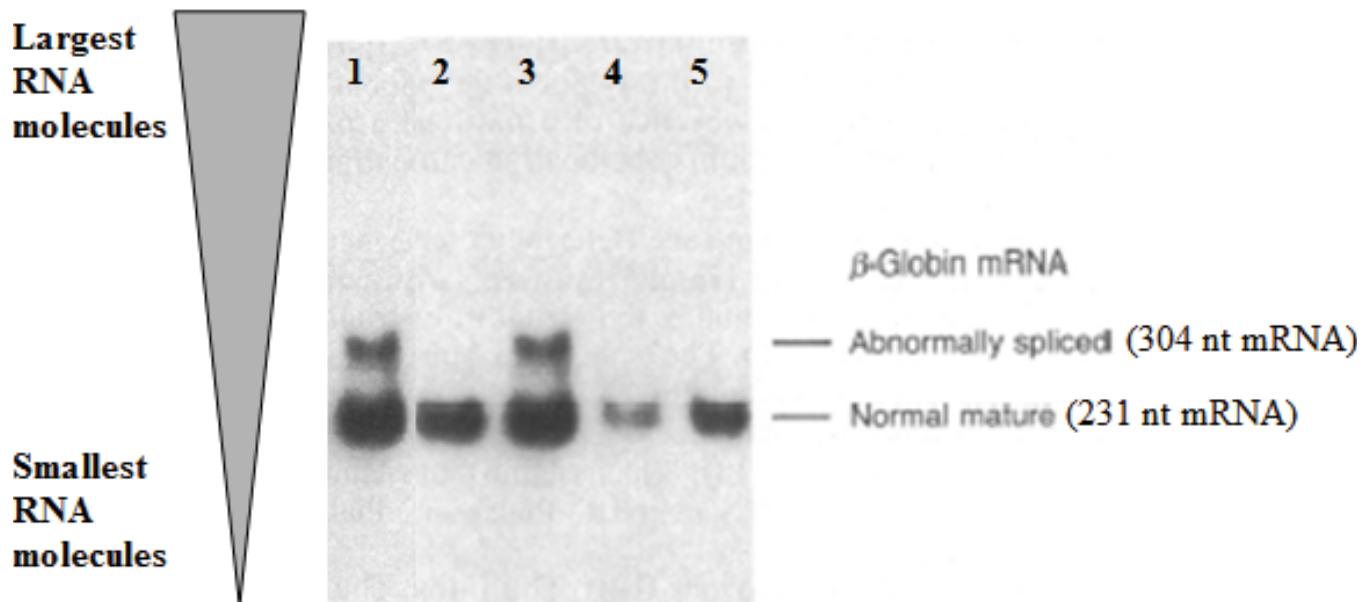
"O.K., more information is always good. Can you tell me what the larger RNA size means?"

"Sure I can, Mrs. Williams. This larger size would be due to incorrect splicing of the  $\beta$ -globin pre-mRNA. Rather than having a  $\beta$ -globin mRNA with exons spliced directly together, part of an intron would be retained between the two coding sequences. In other words, part of the  $\beta$ -globin *non*-coding information would have become part of the code for her  $\beta$ -globin protein. Consequently, the  $\beta$ -globin protein would *not* be translated correctly and would cause her symptoms. If this is the case, we will need to run a second test (sequencing) to verify that she has the specific intron mutations that the gene therapy is designed to correct. We would look at the sequence of her  $\beta$ -globin pre-mRNA. If this checks out, she would be an excellent candidate for the pre-mRNA gene therapy!"

"O.K., Dr. Kernodle ... I don't completely follow you, but anything that might help my baby girl."

*Examine Handouts 6–8 and discuss and answer the questions listed on each of the handouts.*

## Handout 6—Tazswana’s RNA Test



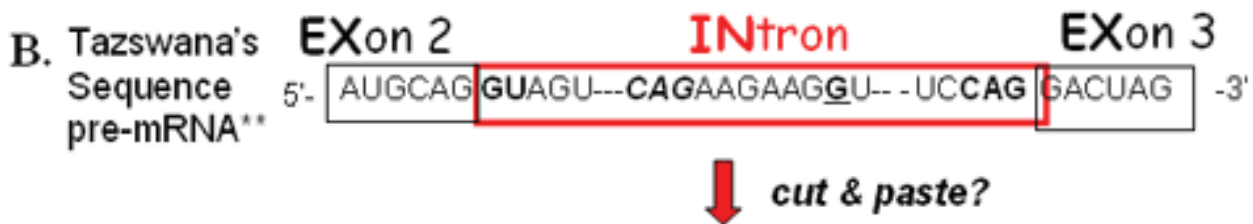
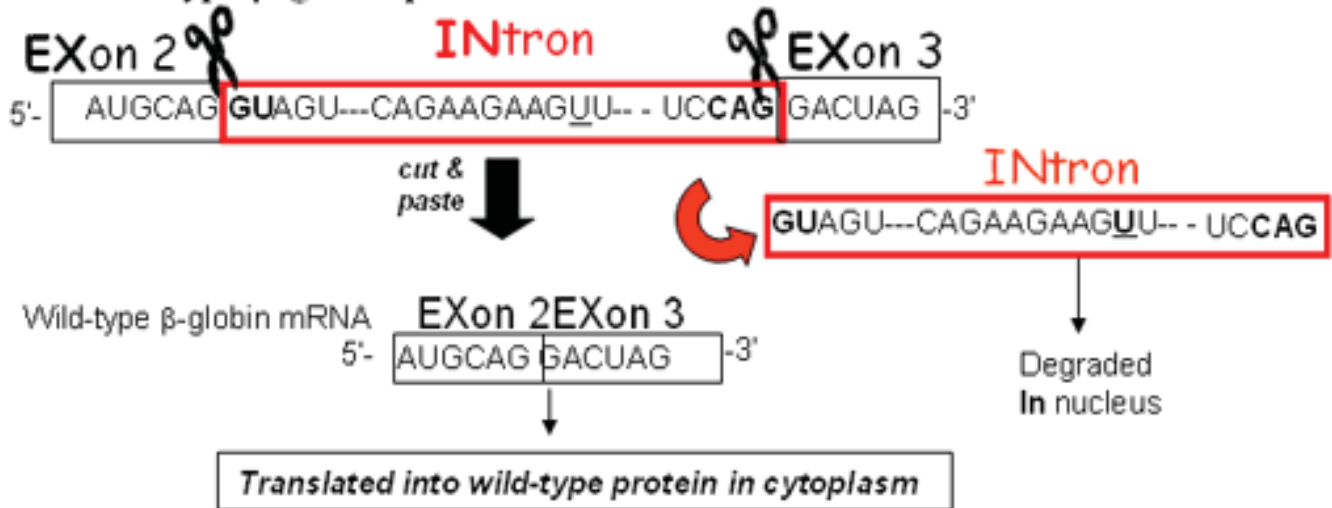
**Figure 6-1.** RNA Hybridization Analysis of Tazswana’s RNA. The grey triangle indicates relative size of nucleic acids: larger RNA molecules migrating higher in the gel than smaller RNA molecules. Lane 1, Mr. William’s RNA sample (father). Lane 2, Mrs. William’s RNA sample. Lane 3, Tazswana’s RNA (note the two bands, one is correct size, but is also defective). Lane 4, Tazswana’s Grandmother (see Handout 2: Beta-Globin Inheritance). Lane 5, control patient with two wild-type alleles (lacking any thalassemia phenotypes). [Credit: This figure has been modified from Figure 4 in Antonarakis, S.E., et al., *β-Thalassemia in American Blacks: Novel mutations in the “TATA” box and an acceptor splice site.* *Proc Natl Acad Sci USA*, 1984. 81(4): p. 1154–8.]

### Questions

1. Tazswana’s mRNA sample (extracted from her reticulocytes) has been run in Lane 3.
  - a. Compare Lane 3 to Lane 5. In terms of pre-mRNA processing, explain why Tazswana has an extra band. Include drawings of both the mRNAs shown in Lane 3 versus the one band shown in Lane 5 (symbolize the two exons as rectangles, and the one intron as a single line).
  - b. Predict how the size(s) of her mRNA bands will affect the size and amino acid content of her  $\beta$ -globin protein.
  - c. Tazswana has one normal sized  $\beta$ -globin mRNA. Explain why she has severe symptoms and from whom she has inherited the intron mutation (you may wish to refer to Handouts 2 and 4).
  - d. Based on these results, discuss Tazswana’s treatment options.
2. (Optional) Assume the  $\beta$ -globin mRNA in Lane 4 is from Tazswana’s grandmother. How is it different from the normal mRNA shown in Lane 5? Discuss the cause of this difference in terms of the mutation and why Tazswana’s grandmother is symptom free.

## Handout 7—Analyzing Tazswana’s Intron

### A. Wild-type $\beta$ -globin pre-mRNA



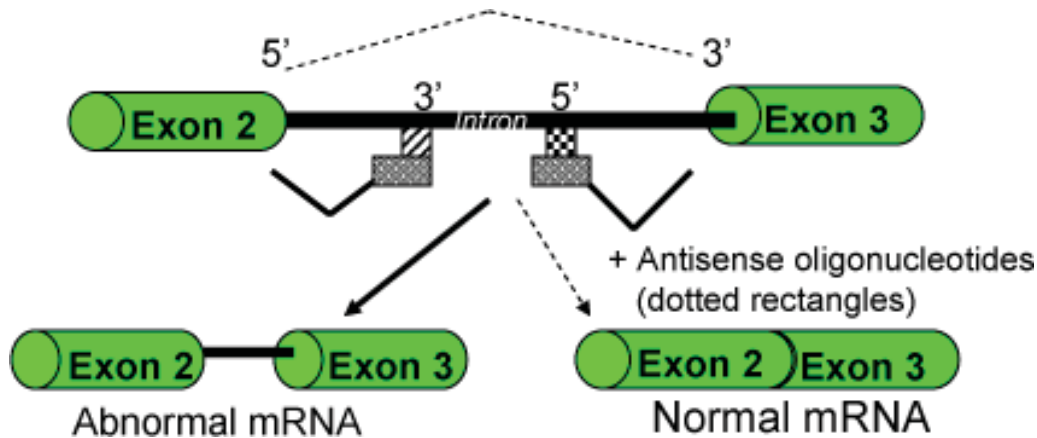
**Figure 7-1.** Analyzing Tazswana’s intron. In certain  $\beta$ -thalassemias, a base substitution occurs at position 705 in the second intervening sequence (IVS2, intron 2) with a transition from T to G occurring in the DNA. The transition converts the U (underlined, Panel A) in the wild-type IVS2 pre-mRNA to a G (underlined and in boldface, Panel B) in Tazswana’s pre-mRNA. This results in a GU 5’ splice site, called an aberrant splice site, appearing in the middle of intron 2. As a result, another intron sequence that was already present, CAG, will now be used (bolded and italicized in Panel B). Because the site was there, but not recognized, it is called a cryptic splice site.

### Questions

- Comparing and contrasting Tazswana’s pre-mRNA intron to the normal intron, answer the following questions (use terms such as 5’ splice site, 3’ splice site, etc.):
  - How is Tazswana’s intron *similar* to the normal  $\beta$ -globin pre-mRNA?
  - How is Tazswana’s intron *different* from the normal  $\beta$ -globin pre-mRNA?
- Assuming that the cellular splicing machinery will recognize sets of “GU” and “CAG” together in the right context as marking the beginning and end of an intron, and that an extra “GU” will be grouped with the next downstream “CAG” as a “complete set,” predict how Tazswana’s pre-mRNA will be processed into mRNA. Show the resulting mRNA sequence from this process.
- If the doctors could somehow trick the spliceosome (the cell’s machinery that splices pre-mRNAs into mRNAs) into ignoring Tazswana’s extra splicing sequence, could she have a normal  $\beta$ -globin mRNA? Explain why or why not.



## Handout 8—Pre-mRNA/DNA Gene Therapy for Tazswana



**Figure 8-1.** Gene Therapy for Tazswana. Alternative splice patterns are shown. Wild-type splicing (dashed line and arrow) results in exon 2 juxtaposed to exon 3. The alternative splice pattern (solid lines and arrow) results in retention of a portion of the intron between exons 2 and 3. Oligonucleotides (10 nt long) can affect alternative splicing of the  $\beta$ -globin pre-mRNA. The  $\beta$ -globin pre-mRNA (top of figure) that causes Tazswana's  $\beta$ -thalassemia is depicted. The mutation causing the aberrant 5' splicing sequence is represented by a checkered-box. The cryptic 3' splicing sequence in the intron is represented by a striped box. The antisense oligonucleotides are represented by polka dot, grey rectangles.

Figure modified from "Therapeutic potential of antisense oligonucleotides as modulators of alternative splicing" Sazani, P. and R. Kole. (2003) *The Journal of Clinical Investigation* 112(4)481-486.

### Questions

- What is "alternative splicing" and how does it apply to Tazswana's case?
- Design Tazswana's gene therapy to force the splicesome into the "normal" alternative splice pattern.
  - Propose a complementary DNA sequence to interact (hybridize) with Tazswana's pre-mRNA. Draw the 10 nt DNA oligonucleotide that will complementary base-pair to the pre-mRNA to specifically "mask" the extra 5' splice site (underlined). Don't mask the normal splice sites! Assume her intron sequence for the pre-mRNA is as shown here:  
 5'GUAAGUCUAAAUGUCCCCUCAGAAGAAGGUCACUCUAAACGACUCUUCAG3'
  - Discuss the type of cell the oligonucleotides need to be delivered to in order to affect alternative splicing of the  $\beta$ -globin pre-mRNA.
- Recall that splicesomes are composed of snRNA and protein. Explain how hybridization (complementary base-pairing) of the DNA oligonucleotides to the cryptic and aberrant pre-mRNA sequences would correct the splicing defect.
- If Tazswana's 5' splice site at the correct location (exon2/intron2 boundary) had a point mutation from G to U so that the original 5' splice site read "UU" instead of "GU," would the gene therapy correct her  $\beta$ -thalassemia? Explain your answer.
- (Optional) Since the oligonucleotides target splice site consensus sequences that are common to all introns, wouldn't they potentially cause side-effects in many cell types? Discuss within your group and come up with:
  - Potential side-effects
  - Plan to limit side-effects.
- If you were in Tazswana's family, would you want Tazswana to undergo gene therapy treatment? Explain why or why not.