Epigenetics of Myotonic Muscular Dystrophy

RNA disease complexity demonstrates many levels of gene regulation

Myotonic muscular dystrophy affects approximately 1 in every 8000 adults. It is the most prevalent form of adult muscular dystrophy. This is an RNA disease that is affected by methylation, changes in the polyadenyl tail, trinucleotide repeat expansion, RNA processing errors, structural changes that affect function, developmental switches in genes, and antagonistic regulation of proteins. By teaching about this one disorder, most of the AP requirements of epigenetics in Big Idea 3 and 4 can be addressed. In this short unit, discussions about complex gene regulation topics, paper models of RNA processing, and play-doh models of the more complex interactions will be used to explain the epigenetics of this disease. A simulated assay will be used to demonstrate a potential diagnostic tool.

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This curriculum was developed in the laboratory of:

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The Berglund lab uses a broad range of approaches to study the molecular mechanisms of neurological diseases that are caused by microsatellite repeat expansions. For many of these diseases (myotonic dystrophy, ALS and ataxias), RNA processing (pre-mRNA splicing) pathways are negatively impacted with specific changes in pre-mRNA splicing proposed to lead to symptoms observed in affected individuals. We use biochemical, cellular and genomic assays to understand the mechanisms through which these diseases alter pre-mRNA splicing. The goal of our research is to use the results from these fundamental studies to identify innovative strategies to reduce or correct the improper pre-mRNA splicing that occurs in the disease state. For example, we have recently shown that small molecules can be used to rescue the mis-splicing in cell and mouse models of myotonic dystrophy.

TABLE OF CONTENTS

INTRODUCTION

Myotonic muscular dystrophy provides a focus for studying many epigenetic concepts. AP Bio students are expected to understand RNA processing and alternate RNA splicing, methylation, the effects of interactions between proteins, how transcription factors can affect gene expression, that genes are switched on or off during developmental stages, and the role of miRNAs. Students do not need to know great details on these topics but this disease can provide an example for the effect of each of these on human health.

My hope is that students will move through the unit and have their curiosity piqued. They will learn the basics of the disease and stop to consider how it might be treated. Students will take the abstract concepts and make them tangible through modeling activities. Then students will conduct a simulated assay test for the disease. There are many aspects of this disease that are still not understood and therapies still need to be developed. In addition to academic concepts, I hope that students will learn that there are many opportunities for careers in the field of research.

DNA and RNA structure should have been taught prior to this lesson. In addition, replication, transcription, and translation are concepts students should already be familiar with. Students will review transcription in this lesson but will be lost if it has not already been taught.

TIPS ABOUT THIS CURRICULUM

Lesson Plan Format: All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

KEY QUESTION(S): Identifies key questions the lesson will explore.
OVERALL TIME ESTIMATE: Indicates total amount of time needed for the lesson, including advanced preparation.
LEARNING STYLES: Visual, auditory, and/or kinesthetic.
VOCABULARY: Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.
LESSON SUMMARY: Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.
STUDENT LEARNING OBJECTIVES: Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.
STANDARDS: Specific AP standards addressed in the lesson. Also collected in one list.
MATERIALS: Items needed to complete the lesson. Number recommended for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.
BACKGROUND INFORMATION: Provides accurate, up-to-date information from reliable sources about the lesson topic.
ADVANCE PREPARATION: This section explains what needs to be done to get ready for the lesson.
PROCEDURE WITH TIME ESTIMATES: The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.
ASSESSMENT SUGGESTIONS: Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/post test) that can be given. Teachers should feel free to create additional formative and summative assessment pieces.
EXTENSIONS: (ACTIVITIES/LITERATURE) There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.
RESOURCES/REFERENCES: This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available. All references and resources are also collected in one list.
STUDENT PAGES: Worksheets and handouts to be copied and distributed to the students.
TEACHER MASTERS: Versions of the student pages with answers or the activity materials for preparation.

Implementation notes: This curriculum should be modified and adapted to suit the needs of the teacher and students. To help make implementation easier in this first draft, notes have been included in lessons as needed.

Science Subject: AP Biology and IB Biology

Grade and ability level: 11-12 grade students in advanced biology

Science concepts: proteins, RNA processing, alternate RNA splicing, polyadenylation, introns and exons, miRNAs, differentiation, and transcription factors

LESSON SUMMARIES

LESSON ONE: Myotonic muscular dystrophy and RNA processing
This is a presentation on the vocabulary and concepts that form the basis of gene regulation beyond the genome. The lesson starts with the DNA and how the repeat expansion can increase with each generation and continues with RNA processing. Transcription is reviewed but is considered pre-requisite knowledge. Modeling of the steps of RNA processing reinforces the learning.

LESSON TWO: Protein interactions and alternative RNA splicing
Students will work in pairs to demonstrate some of the concepts and complex interactions involved in protein interactions and alternate RNA splicing.

LESSON THREE: miRNAs and potential therapeutic treatments
Chromatin structure changes and the impact of miRNAs is introduced. The effects of miRNAs are modeled by students. This is followed by a short presentation of some of the ongoing research avenues being used to develop therapies for future use.

LESSON FOUR: A diagnostic assay
This is a simulation of a potential method by which patients may someday be tested for this disorder. This is not currently in practice as the details of synthesizing the markers to determine the size of the expansion is still in research stages.

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LESSON SEQUENCING GUIDE

Since the classroom teacher knows his or her students best, the teacher should decide the sequencing of lessons. Below is a suggested pacing guide that can be used when planning to use this curriculum.

50 minute periods

	Day 1	Day 2	Day 3	Day 4	Day 5
Week 1	Lesson 1 Intro and RNA processing (40 minutes)	Lesson 2 Protein interactions and alternative gene splicing (50 minutes)	Lesson 3 Chromatin structure, miRNAs, and potential therapeutics (30 minutes)	Lesson 4 The assay (40 minutes)	Assessment (50 minutes)

VOCABULARY

DNA: Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms.

RNA: Ribonucleic acid is one of the three major macromolecules (along with DNA and proteins) that are essential for all known forms of life. Like DNA, RNA is made up of a long chain of components called nucleotides. Each nucleotide consists of a nucleobase, a ribose sugar, and a phosphate group. RNA directs the synthesis of proteins.

Transcription: DNA → RNA; During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand. The RNA complement includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement.

Translation: RNA → Protein; In translation, messenger RNA (mRNA) produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or polypeptide, that will later fold into an active protein.

UTR: untranslated region

Exon: region of the DNA/RNA that can be translated into a string of amino acids

Intron: region of the DNA/RNA that is not translated into amino acids and may serve another purpose such as a regulation sequence or code for various types of RNA molecules

MBNL1: muscle-blind like protein 1; there are at least 3 variations of this protein and it has many functions in the body including a regulatory protein for alternative RNA splicing

Primary transcript: pre-mRNA; mRNA when it first comes off the RNA polymerase and is a compliment of all of the nucleotides that made up the gene including the introns

Mature mRNA: mRNA after RNA processing has taken place

Alternate RNA splicing: a process by which multiple different mature mRNAs can be produced from one gene

Epigenetics: translates to upon the genome; the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself.

Chromatin: is a complex of DNA and proteins that forms chromosomes within the nucleus of eukaryotic cells. Nuclear DNA does not appear in free linear strands; it is highly condensed and wrapped around nuclear proteins in order to fit inside the nucleus.

MicroRNA: (abbreviated miRNA) is a small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, that functions in RNA silencing and post-transcriptional regulation of gene expression.

Triplet expansion: a kind of mutation where trinucleotide repeats in certain genes exceed the normal, stable threshold, which differs per gene

Myotonia: inability to relax voluntary muscle after vigorous effort

RNA processing: The RNA copy of a protein encoding gene must be modified in several ways before it can be transported out of the nucleus and translated into protein.

RNA splicing: splicing is the editing of the nascent pre-messenger RNA (pre-mRNA) transcript. After splicing, introns are removed and exons are joined together (ligated)

5' cap: is a specially altered nucleotide on the 5’ end of some primary transcripts such as precursor messenger RNA

Poly-A tail: Polyadenylation is the addition of a poly(A) tail to a messenger RNA. The poly(A) tail consists of multiple adenosine monophosphates; in other words, it is a stretch of RNA that has only adenine bases. In eukaryotes, polyadenylation is part of the process that produces mature messenger RNA (mRNA) for translation.

Antagonistic: acting in opposition; opposing, especially mutually

Sequestered: isolated and hidden away

Spliceosomes: a large and complex molecular machine found primarily within the splicing speckles of the cell nucleus of eukaryotic cells. The spliceosome is assembled from snRNAs and protein complexes.
The spliceosome removes introns from a transcribed pre-mRNA, a kind of primary transcript.

Toxic RNA: Studies have found that they arise from repetitive non-coding RNA sequences, also known as toxic RNA, which inhibit RNA-binding proteins leading to pathogenic effects. The most studied RNA-dominant diseases include, but are not limited to, myotonic dystrophy and fragile X-associated tremor/ataxia syndrome

Nucleosome: a structural unit of a eukaryotic chromosome, consisting of a length of DNA coiled around a core of histones.

Methylation: a process by which methyl groups are added to DNA.

NEXT GENERATION SUNSHINE STATE STANDARDS – SCIENCE

	Lesson			
AP Standards	1	2	3	4
2.E.1: Timing and coordination of specific events are necessary for the normal development of an organism, and these events are regulated by a variety of mechanisms.		x	x	
2.E.1. a. Observable cell differentiation results from the expression of genes for tissue specific proteins.	x	x	x	
b. Induction of transcription factors during development results in sequential gene expression. *Evidence of student learning is a demonstrated understanding of each of the following:* 1. Embryonic induction in development results in the correct timing of events.		x	x	
2. Genetic mutations can result in abnormal development.	x			
3. Genetic transplantation experiments support the link between gene expression and normal development.		x	x	
4. Genetic regulation by microRNAs plays an important role in the development of organisms and the control of cellular functions			x	
In Eukayotic cells the mRNA transcript undergoes a series of enzyme-regulated modifications. *To foster student understanding of this concept, instructors can choose an illustrative example such as:* • addition of a poly-A tail • addition of a GTP cap • Excision of introns (Notes - all of these are included)	x			
4.A.3: Interactions between external stimuli and regulated gene expression result in specialization of cells, tissues and organs. a. Differentiation in development is due to external and internal cues		x	x	
4.B.1: Interactions between molecules affect their structure and function. a. Change in the structure of a molecular system may result in a change of the function of the system.	x	x	x	

IB Standards				
7.2 Transcription and gene expression				
Understandings: • Eukaryotic cells modify mRNA after transcription • Splicing of mRNA increases the number of different proteins an organism can produce. • Gene expression is regulated by proteins that bind to specific base sequences in DNA.	x	x		

BACKGROUND INFORMATION

General knowledge of DM1

Myotonic Dystrophy type 1 is the most common form of adult onset muscular dystrophy. It affects approximately 1 in 8000 individuals(Cho and Tapscott, 2006). This is a complex multi-systemic disorder caused by a CTG repeat expansion in the 3' untranslated (UTR) region of the DMPK gene. This gene is located on chromosome 19q13.3. There is another form of this disease called type 2 which is located on chromosome 3q21 but I will only be addressing type 1 in this lesson. Changes in alternative splicing, translation, localization, and mRNA stability due to sequestration of MBNL proteins and up-regulation of CELF1 are key to DM1 pathology (Chau and Kalsotra, 2014).

Myotonic dystrophy is one of over 30 known disorders that are due to extensions of tandem repeats above a critical size (Cho and Tapscott, 2006). It is inherited through the autosomal dominant pattern and affects many systems of the body. Some of the symptoms include muscle hyperexcitability (myotonia), progressive muscle wasting, cardiac arrhythmias, insulin resistance, gastrointestinal dysfunctions, posterior iridescent cataracts, and neuropsychiatric disturbances (Chau and Kalsotra, 2014). Normal individuals usually have from 5 to 35 repeats of CTG in the 3' UTR region of the DMPK gene. Symptoms have been seen in individuals with as few as 50 repeats. Individuals with large repeat sizes of (>1000) are typically associated with the worst form of the disease (Chau and Kalostra, 2014).

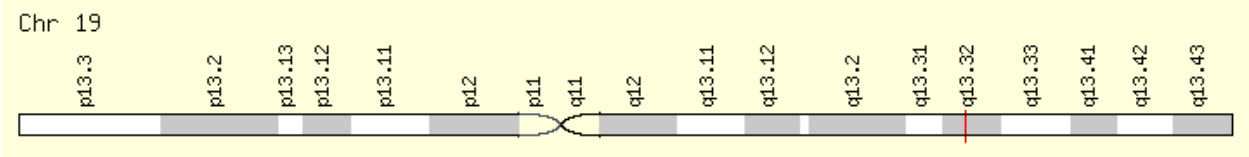


Image source: www.genecards.org

The image above shows chromosome 19 and the location of the DMPK gene marked with the red line.

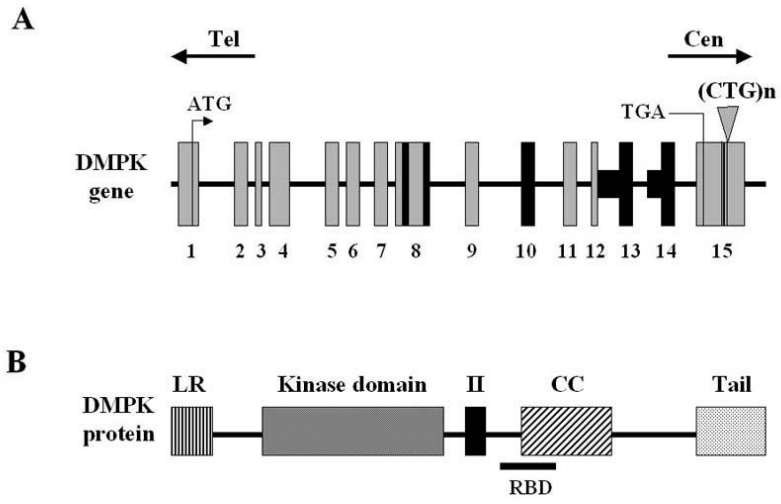


Image source: http://theses.ulaval.ca/archimede/fichiers/21404/ch02.html

The diagram above demonstrates the many introns and exons found in the DMPK DNA. The line you see through the middle is the introns and the various shaded rectangles are the exons. The TGA mark is the stop codon and after that is the 3'untranslated region that will be included in the mRNA. It is important to note that the sections labeled exons mean they are an exon in at least one RNA transcript formed from this section of the DNA but will not be included and expressed in all RNA transcripts. In DM the last rectangle is actually an untranslated region that is included in the RNA transcript but not used to code for amino acids. This is the region that includes the CTG repeat section.

Errors in Replication of DMPK gene

When the gene is replicated, there is slippage in the CTG repeat section that causes hairpin loops like shown below. The greater the repeat number the greater the slippage and number of loops. As the enzymes replicate the DNA, the loops are copied as well so that the repeats are copied. Over generations, the number of repeats gets progressively larger and so do the effects of this disorder.

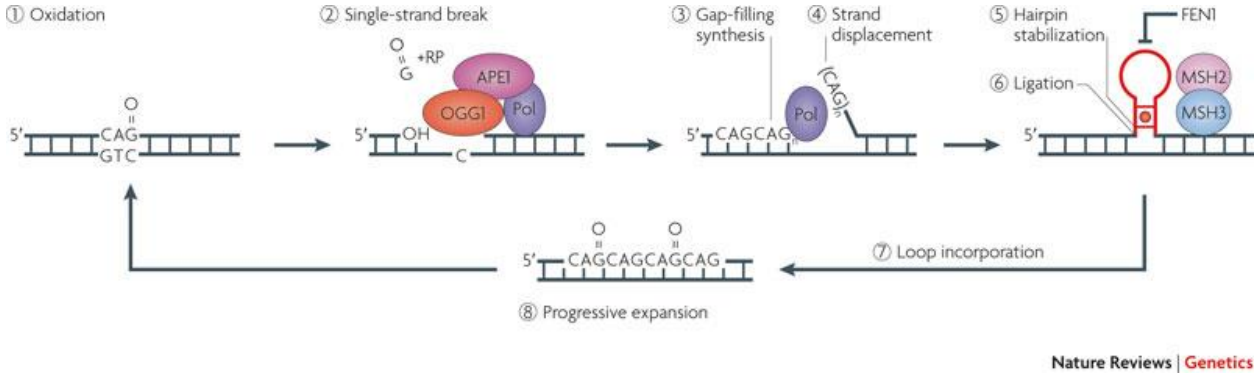


Image source: http://www.nature.com/nrg/journal/v11/n11/fig_tab/nrg2828_F2.html

This diagram is only included to demonstrate how the repeat expansion in the DNA gets copied during replication and how that expansion can increase in size during successive replication events. IB and AP students do not need to know the details of how this occurs. It will be interesting for them to understand that the error can increase over time and that it occurs in a non-coding region of the gene.

Transcription and RNA processing from the DMPK gene

The first step of transcription involves copying the DNA into RNA by RNA polymerase. Please stress that all nucleotides are copied into the pre-mRNA also called the primary transcript. After the stop code(for translation) in the DNA a section called the 3' UTR is also transcribed into this primary transcript even though it will not code for amino acids. Then the primary transcript undergoes RNA processing. The introns are removed along with any exons that are not specified for the function of this specific mature RNA. The 3' UTR region is included in the mature RNA and then the 5' cap and polyA tail is added. This means the final mature RNA includes a 5' cap, a number of exons, the 3' UTR region, and the polyA tail. Which exons are included and which are excluded is controlled by a number of proteins that function as regulatory proteins and splicing factors. The diagram below is a simplified model as this is actually a very large gene that contains 15 exons over 13 kb (Tiller, 2011). The model below shows only 3 exons.

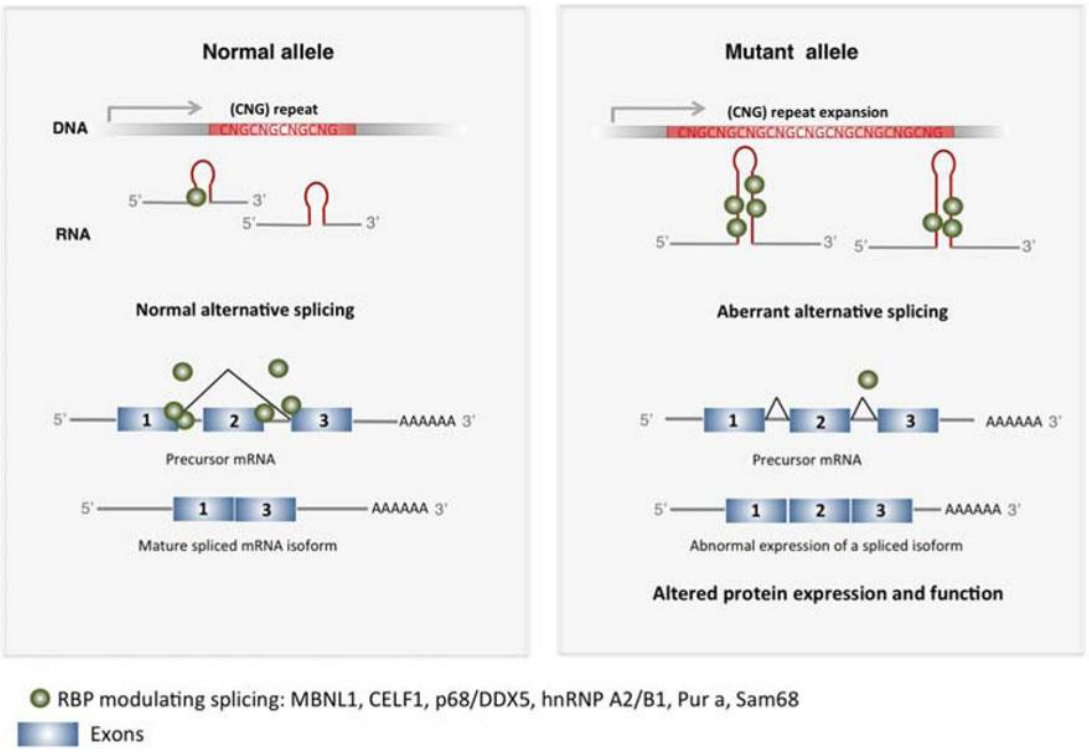


Image source: www.journal.frontiersin.org

In a healthy allele, there are a small number of repeats and splicing of introns and exons occurs normally resulting in a mRNA that will leave the nucleus, join up with a ribosome, and then be used to produce the protein kinase that will carry out its function correctly. In addition, a second protein made from a completely different gene will be free to carry out its function which is to control appropriate splicing of a number of RNA transcripts with various functions. This protein is called MBNL1 and will be discussed more in the next section.

In a mutated allele with 50 or more CTG repeats, the primary transcript will have 50 or more CUG repeats. This will go through RNA processing and the expanded section in the mRNA will form hairpin loops. These loops attract and bond to the MBNL1 protein which removes this protein from the cytoplasm and binds it up in the nucleoplasm so that it has decreased function in both places. In addition, these loops prevent this mRNA from leaving the nucleus.

Problems due to sequestering of the MBNL1 protein

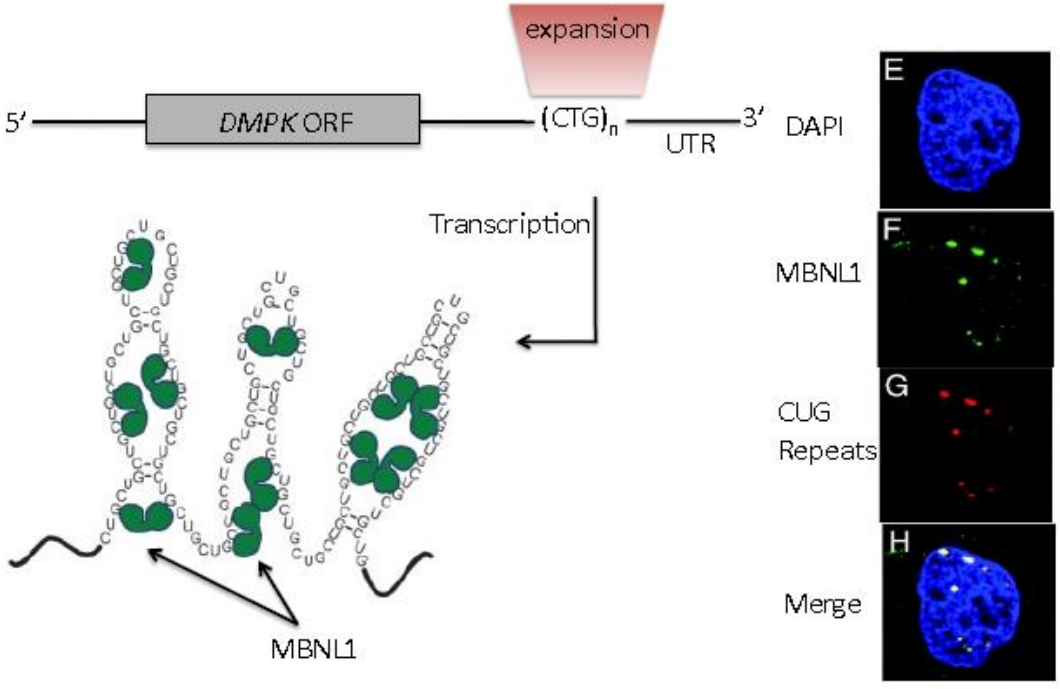
MBNL1 protein is sequestered in the hairpin loops of the DMPK mRNA. These loops attract the MBNL1 almost like a magnet. This removes the MBNL1 protein from the cytoplasm and prevents it from carrying out its normal duties. MBNL1 is an important regulator of alternate RNA splicing patterns. For example, MBNL1 regulates the splicing pattern of the CLCN1 mRNA which produces a protein involved in the muscle-specific chloride channel of the sarcolemma. This is a protein channel in the plasma membrane of muscle cells. In mice, a lack of MBNL1 caused the inclusion of exon 7a (which should have been spliced out) and that resulted in reduced function of the chloride channel (Chau and Kalsotra, 2014). This change in structure and function caused myotonia. Myotonia is defined as temporary rigidity of the muscles or delayed relaxation.

MBNL1 plays a role in the splicing of a number of gene transcripts. When MBNL1 is not available to participate in the splicing of a gene transcript called BIN1, exon 11 is skipped and the result is muscle weakness. This is another symptom of Myotonic dystropy (Chau and Kalsotra, 2014).

Significant research is still needed to understand how exactly MBNL1 regulates splicing of these and other proteins. There is room for our students to become research scientists and study diseases such as this. RNA disease is still a young and growing area for research.

Localization of the mRNA

The structural change of the hairpin loops in the mRNA causes the mRNA to be unable to leave the nucleus. Since we have two chromosomes for this gene, even in DM patients they are likely to have one copy that is normal. This chromosome will produce a correct transcript that can leave the nucleus and produce the dystrophin protein correctly. Once the mRNA leaves the nucleus, various proteins interact with it and eventually it will be degraded. This is one of the reasons why we must continually transcribe new mRNAs for the same gene. They just do not last forever. However, the mRNA with the hairpin loops cannot leave the nucleus and accumulates in the nucleoplasm where degradation of mRNA occurs much slower. In addition, the hairpin loops attract and sequester MBNL1 proteins which also take up space in the nucleus. The result is that the DMPK mRNA and MBNL1 protein interfere with normal nuclear function just by being in the way and the MBNL1 protein is not free to carry out its proper function.



Warf *et. al.* 2009

Protein interaction problems between MBNL1 and CELF1

MBNL1 and CELF1 proteins are antagonistic in nature. CELF1 is in higher concentration in cells during embryonic development. MBNL1 is in higher concentration after birth. Both affect muscle development at different stages of

growth. When MBNL1 is sequestered, the concentration of available MBNL1 is decreased in the cell. This causes up-regulation of CELF1 and activation of embryonic type muscle activity(Chau and Kalsotra, 2014). This type of muscle activity is not sufficient to support muscle activity and development after birth and contributes to the myotonia and muscle wasting. This is a great example of a developmental switch of gene activity.

Chromatin structure/methylation changes due to the repeat expansion

DMPK gene is located in a "gene-rich region" of chromosome 19. This means that other protein coding genes are close by. The CTG repeat expansion in the 3'UTR region of this gene causes chromatin structure changes as well as its own transcription and translation problems. There is evidence that suggests this normally 'open' chromatin region becomes 'closed' making it less accessible to DNA binding factors for gene regulation due to the repeat expansion (Cho and Tapscott, 2006). The gene directly downstream of DMPK is called SIX5. Mice with a SIX5 deletion developed premature cataracts which is also a symptom of Myotonic dystrophy (Cho and Tapscott, 2006). Therefore, some of the symptoms may be due to add on effects of the CTG expansion in the DMPK gene.

Impact of miRNAs

MicroRNAs are known to play a role in gene expression. Research is needed to work out all the mechanisms and impacts of miRNAs. In the case of DM1 patients, the CUG expansion in RNA causes a decrease in expression of a group of microRNAs that include miR-1. In human patients, is correlates with cardiac arrhythmias and fibrosis (Chau and Kalsotra, 2014). Due to mouse studies, it is thought that this is due to transcriptional defects. The downregulation of these microRNAs caused by the expansion seems to have an effect on skeletal muscle strength as well.

Works Cited

Chau, Anthony and Auinash Kalsotra. (2014) Developmental Insights Into the Pathology of and Therapeutic Strategies for DM1: Back to the Basics. *Developmental Dynamics, 244:377-390.*

Cho, Diane and Stephen Tapscott. (2006) Myotonic dystrophy: Emerging mechanisms for DM1 and DM2. *Science Direct Biochimica et Biophysica Acta 1772 (2007) 195-204.*

Tiller, G. E. (2011). *DYSTROPHIA MYOTONICA PROTEIN KINASE; DMPK*. Retrieved July 07, 2016, from OMIM: http://www.omim.org/entry/605377

LESSON ONE: MYOTONIC MUSCULAR DYSTROPHY AND RNA PROCESSING

KEY QUESTION(S): What is Myotonic Muscular Dystrophy? How does epigenetics apply to this disease?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 10 minutes to download the presentation and review it. Make sure to have copies of the paper models the students will work with in advance.
- Class time: 45 minutes for presentation, discussion, and modeling activities

LEARNING STYLES: Visual, auditory, and kinesthetic

VOCABULARY:

- UTR
- epigenetics
- chromatin
- microRNA
- deregulation
- triplet expansion
- myotonia
- primary transcript
- mature mRNA
- RNA processing
- RNA splicing
- exons
- introns
- 5' cap
- poly A tail

PRE-REQUISITE KNOWLEDGE:
Students should be well versed in DNA and RNA structure. Replication, transcription, and translation should have been taught prior to this unit.

LESSON SUMMARY: Presentation reviewing some concepts and introducing others is provided. There are short interactive components built in and short video links. I have included the presentation in two formats: SMART notebook and PowerPoint. If you have the SMART notebook program, you should use that file. Everything is built into it for all 4 lessons. If not, use the PowerPoint and download the other files individually.

Lesson 1 discusses the how this disease is due to errors in replication and how those errors are increased in successive generations. The transmission of this error from DNA to RNA is discussed and the steps of RNA processing to form the mature RNA is included. Students then model the steps of RNA processing.

STUDENT LEARNING OBJECTIVES:
The student will be able to...

- Describe one example of how errors in replication can be detrimental to an organism.
- explain how eukaryotic cells modify RNA after transcription

STANDARDS:

IB Standards

IB 7.2 Transcription and gene expression

Understandings:

- Eukaryotic cells modify mRNA after transcription
- Splicing of mRNA increases the number of different proteins an organism can produce.
- Gene expression is regulated by proteins that bind to specific base sequences in DNA.

AP Standards

In Eukayotic cells the mRNA transcript undergoes a series of enzyme-regulated modifications.

To foster student understanding of this concept, instructors can choose an illustrative example such as:

- addition of a poly-A tail
- addition of a GTP cap
- Excision of introns

MATERIALS:

- SMART notebook or PowerPoint presentation
- copies of the paper models (one for every pair of students)
- scissors per pair
- tape per pair

BACKGROUND INFORMATION: Teachers are encouraged to read the background information presented on earlier pages of this packet and to review the presentation slides before class starts.

ADVANCE PREPARATION:

1. Download the presentation
2. Review the presentation and background information
3. Have the copies made
4. Gather scissors and roles of tape

PROCEDURE WITH TIME ESTIMATES:

1. (35 minutes) The presentation will lead you and the students through the content with short video clips
2. (10 minutes) Follow the directions for the modeling activity
3. Circulate to answer questions and monitor for understanding during the modeling activities

ASSESSMENT SUGGESTIONS:

- Observations during practice time
- If you have clickers, you could write some simple questions just to assess level of knowledge after the presentation

EXTENSIONS:

- Case study from National Center for Case Studies called "mRNA processing: No Longer a Headache" http://sciencecases.lib.buffalo.edu/cs/collection/detail.asp?case_id=849&id=849

RESOURCES/REFERENCES:

Chau, Anthony and Auinash Kalsotra. (2014) Developmental Insights Into the Pathology of and Therapeutic Strategies for DM1: Back to the Basics. *Developmental Dynamics, 244:377-390.*

Cho, Diane and Stephen Tapscott. (2006) Myotonic dystrophy: Emerging mechanisms for DM1 and DM2. *Science Direct Biochimica et Biophysica Acta 1772 (2007) 195-204.*

Tiller, G. E. (2011). *DYSTROPHIA MYOTONICA PROTEIN KINASE; DMPK*. Retrieved July 07, 2016, from OMIM: http://www.omim.org/entry/605377

STUDENT PAGES: RNA MODELS OF DMPK

STUDENT PAGES: PROTEIN MODELS

LESSON TWO: PROTEIN INTERACTIONS AND RNA TOXICITY

KEY QUESTIONS: What is alternate RNA splicing? How can more than one protein be produced from the same gene? How can proteins regulate gene expression?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 10 minutes
- Student Procedure: 40 minutes

LEARNING STYLES: Visual, auditory, and kinesthetic.

VOCABULARY:

- alternate RNA splicing
- antagonistic
- sequestered
- spliceosomes
- toxic RNA

LESSON SUMMARY: A presentation of content relating protein interactions and RNA toxicity to DM1 is provided. Several modeling activities will be conducted to make these abstract concepts more concrete.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

- Demonstrate with models that differentiation in development is due to internal cues that trigger gene regulation by proteins.
- Describe how development is due to expression of genes specific to a particular tissue.
- Describe how change in the structure of a molecular system may result in change of the function of the system using a specific example.

STANDARDS: see table on pages 9-10

MATERIALS:

1. the presentation
2. copies of the paper models per pair of students
3. tape and scissors per pair of students
4. toilet paper core per pair of students
5. paperclips (2 per student group)

BACKGROUND INFORMATION: Background information needed for this assignment is at the beginning of this guide.

ADVANCE PREPARATION:

1. Make copies of the models for every 2 students
2. Gather tape, paper clips, and scissors for student use

PROCEDURE WITH TIME ESTIMATES:

1. (3 minutes) Present concepts of protein interactions and RNA toxicity
2. (20 minutes) Follow steps of modeling activities
3. (3 minutes) Present on RNA toxicity
4. (10 mintues) Follow steps of modeling activity

ASSESSMENT SUGGESTIONS:

- Monitor learning by watching modeling activity and asking students to explain why they are doing what they are doing

RESOURCES/REFERENCES:

- same as for lesson 1

STUDENT PAGE: CLCN1 MODEL (NEED TWO COPIES PER STUDENT PAIR)

LESSON THREE: CHROMATIN, MIRNAS, AND TREATMENT

KEY QUESTION(S): How can changes in chromatin structure affect gene expression? What is the function of miRNAs? Where is research heading with treatment protocols?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 10 minutes
- Student Procedure: 30 minutes

LEARNING STYLES: Visual, auditory, and kinesthetic

VOCABULARY:

- miRNA
- nucleosome
- methylation

STUDENT LEARNING OBJECTIVES:

The student will be able to...

- describe nucleosomes and how they relate to chromatin structure
- give an example of how methylation changes can affect phenotype
- describe miRNAs and their basic function

STANDARDS: See table on page 9-10.

MATERIALS:

- Presentation file
- models per student pair
- tape

BACKGROUND INFORMATION: see beginning of this guide

ADVANCE PREPARATION:

- make copies of the models for the students
- provide tape for student use

PROCEDURE WITH TIME ESTIMATES:

ASSESSMENT SUGGESTIONS:

- Circulate and monitor understanding during the modeling activity

RESOURCES/REFERENCES:

Same references as for the previous 2 lessons

STUDENT PAGE: MIRNA AND TRNA MODELS

LESSON FOUR: DIAGNOSTIC ASSAY

KEY QUESTION(S): What is an assay? How could a test of this type be applied to Myotonic Muscular Dystrophy?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure: 45 minutes

LEARNING STYLES: Visual and kinesthetic.

VOCABULARY:
no new vocabulary

STUDENT LEARNING OBJECTIVES:
The student will be able to...

1. Apply the understanding of trinucleotide repeats to patient degree of disease

STANDARDS: See table on page 9-10.

MATERIALS:

BACKGROUND INFORMATION:
see slide on the assay

ADVANCE PREPARATION:

- Prepare solutions Use
- gather materials

PROCEDURE:

- Have students work lab teams (groups of 2 or 3 are recommended)
-

ASSESSMENT SUGGESTIONS:

1. Answer questions that accompany the lab

EXTENSIONS:

- You could purchase an ELISA kit from companies such as Edvotek, BioRad, or even Ward's scientific to extend the assay concept

RESOURCES/REFERENCES:
Chau, Anthony and Auinash Kalsotra. (2014) Developmental Insights Into the Pathology of and Therapeutic Strategies for DM1: Back to the Basics. *Developmental Dynamics, 244:377-390.*

Cho, Diane and Stephen Tapscott. (2006) Myotonic dystrophy: Emerging mechanisms for DM1 and DM2. *Science Direct Biochimica et Biophysica Acta 1772 (2007) 195-204.*

Tiller, G. E. (2011). *DYSTROPHIA MYOTONICA PROTEIN KINASE; DMPK*. Retrieved July 07, 2016, from OMIM: http://www.omim.org/entry/605377

Student Handout: Lab Directions

REFERENCES

Chau, Anthony and Auinash Kalsotra. (2014) Developmental Insights Into the Pathology of and Therapeutic Strategies for DM1: Back to the Basics. *Developmental Dynamics, 244:377-390.*

Cho, Diane and Stephen Tapscott. (2006) Myotonic dystrophy: Emerging mechanisms for DM1 and DM2. *Science Direct Biochimica et Biophysica Acta 1772 (2007) 195-204.*

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STUDENT PAGES: CONTENT ASSESSMENT

Student name: _____ Date: _____

Circle One: Pre-test Post-test

TEACHER ANSWER KEY: CONTENT ASSESSMEN