

An Overview of Myotonic Muscular Dystrophy

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Our Goal.

- Our goal is to give a **general overview** of Myotonic Muscular Dystrophy.
- This talk is a **summary** of basic ideas.
- This talk will give a fair amount of **information** and background but in a simple and understandable way.
- The **basic** ideas are really pretty straightforward and we can get a pretty good sense of what its all about in just a few minutes.
- Scientific articles are full of very complex language. As much as possible, this talk is in **plain** English.

Knowledge Translation.

- This talk is an example of **knowledge translation** (KT).
- This is an important **new tool** in medical research.
- KT involves making a two way **bridge** of understanding between researchers, Doctors and patients.
- Complex messages are lost if they are not easily understood. Our goal is to present information in a way that is **understandable** to all interested parties.
 - Patients can better understand research findings and judge information pertaining to them.
 - Doctors and researchers can better understand patients and their needs.

What is Myotonic Dystrophy (DM)?

- Usually called **myotonic dystrophy** (the DM comes from: *Dystrophia Myotonica*) although some call it myotonic muscular dystrophy (MMD).
- First described by Hans Steinert in 1904, there are now two types recognized (**DM1** and **DM2**).
- In 1992, a **genetic mutation** was discovered to cause DM1 – also called myotonic dystrophy of Steinert, or Steinert's dystrophy.
- In 1994, a **second mutation** was found and DM2 was differentiated – also called proximal myotonic myopathy (PROMM).

Myotonic Overview.

- DM is a **complex genetic disorder** that may affect **multiple** body systems (muscle, the eye, heart, brain, hormones, gastrointestinal tract, uterus and skin):
 - Core features: weakness in the finger flexors, neck and face, tongue and lips, ankles, myotonia (can't loosen grip), cataracts.
 - A wide range of other symptoms may appear.
- DM is the **second** most common muscular dystrophy (MD) overall (behind Duchenne) but the most common **adult** form of MD. Affects about 1 in 8,000 people worldwide but it also occurs in “pockets.”

Myotonia in Quebec.

- DM1 is disproportionately common in the **Saguenay-Lac-Saint-Jean** region of Quebec with about 1 in 500 people affected.
- This high rate has been traced back to one couple who carried DM1 and settled in New France in 1657.
- This phenomena is called a “**founder effect.**”
 - Founder effects are largely behind differences seen in the frequency and sometimes in the exact nature of genetic characteristics in different populations of people living in different areas.
 - Due to a founder effect, it is possible to see small geographic locations with very high (or very low) rates of a given genetic disorder.

Some Myotonia Symptoms.

- **Myotonia** means abnormally long muscle contractions or slowed relaxation after a muscle contraction.
- A person with DM often has difficulty **relaxing** his or her grip, especially in the cold. DM causes general weakness, usually beginning in the muscles of the hands, feet, neck, or face. It may slowly progress to other muscles, including the **heart**. It also may affect the muscles of the digestive system, causing constipation and other digestive problems.
- DM may affect a **wide variety** of other organ systems, may adversely affect intellectual abilities, often increases sleep needs & decreases motivation and may have other impacts on personality and behavior.

High Variability.

- Myotonic Dystrophy is an **extremely variable** condition:
 - In members of the same family:
 - Symptoms can vary widely in severity,
 - It can vary in the systems of the body it affects,
 - It can vary in the age of onset.
 - Even within an individual: the genetic problem in DNA samples – repeat lengths – may vary from tissue to tissue.
 - Sometimes, one generation will show very mild symptoms, making it seem like the disorder has “skipped” a generation.

Classifications of DM.

- 1). DM can be classified as type 1 or type 2.
- 2). Symptom severity forms the basis for a common classification of DM:
 - 1). “Mild DM” (adult onset): People with “mild DM” often lead active lives and may even be unaware that they have the disorder.
 - 2). “Classical DM” (adult onset): People commonly have muscle weakness and wasting, myotonia, hand and wrist weakness and/or foot drop.
 - 3). “Congenital Myotonic Dystrophy” (CMD): A very severe form of DM1, often fatal in young children (not seen in DM2).
- 3). DM can be classified as adult onset or childhood onset (congenital DM1).

A Wide Range of Symptoms.

- Symptom severity differentiates 3 types of DM cases:
 - 1). “**Mild DM**”: People with “mild DM” often lead active lives and may even be unaware that they have the disorder.
 - 50% of people with the disorder show some visible signs by about 20, but many do not develop clear symptoms until after 50.
 - If people feel muscle weakness or mild myotonia, they may attribute it to “stiffness” or arthritis.
 - People are often tested and diagnosed because they have a relative with a more obvious case of DM.

- 2). “**Classical DM**”: People commonly have muscle weakness and wasting, myotonia and foot drop (affects the ability to raise the foot at the ankle – affects walking).
 - Heart abnormalities are a common concern.
 - The individual’s behaviour may be affected.
 - Age of onset is typically in the second or third decade of life, but a mild mask-like appearance of the face (“myotonic facies”) and myotonia may be observed in childhood.
 - Adults may become physically disabled and may have a shortened life span.

The Most Severe Type.

- 3). “**Congenital Myotonic Dystrophy**”(CMyD): A far more severe but rare form of DM1 can occur in second or third generations and can be fatal for affected infants. Infants with CMyD have muscle weakness and poor respiratory function, often leading to death. Motor function gradually improves in surviving children and they are usually able to walk. However, they will develop the clinical signs of classical DM later in life. Cognitive disabilities are present in 50-60% of these individuals.
 - CMyD involves a more severe mutation of the same type that causes DM1 (not seen in DM2).

DM1 Versus DM2.

- DM1 & DM2 have different genetic causes.
- Congenital Myotonic Dystrophy is not seen in DM2.
- DM2 may show milder symptoms than seen in DM1.
- DM1: the size of the mutation is related to how soon symptoms appear and how severe they are; apparently this is not as closely related in DM2.
- DM1 often involves intellectual and personality impacts that are apparently less common in DM2.
- In DM2, the face is less affected and weakness often starts in the thighs and hips (in DM1 it's more in the face, and starts in the hands and feet / ankles).

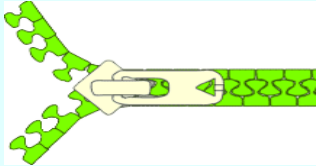
Incidence of DM1 and DM2.

- Research is contradictory in terms of how common each type is, some studies say that 98 percent of people with myotonic dystrophy have DM1; other sources suggest that the prevalence of DM1 and DM2 are about equal.
- Due to founder effects, DM is not evenly distributed in populations and pockets of both disorders are seen.
- Research suggests that DM2 is more common in Northern Europeans and their descendents:
 - In Germany, DM2 may be as common as DM1.

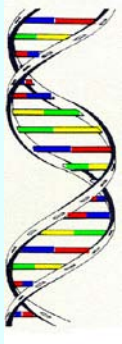
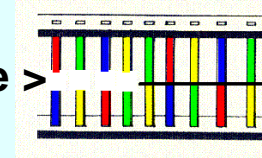
- The cause of myotonic dystrophy is **genetic**.
- We will present some information on the **basic** genetic code and then discuss the problem that causes DM.
- The **internet** contains a great deal of background information on all of these aspects.
- Again, it is the **basic ideas** that are important here, not the complex details.

Basic DNA.

- Our genetic information is carried as two long strands of chemicals – they join together like a long zipper.



Chemicals on each strand join in the middle >



- The two strands twist to form a **helix** of “DNA.”
- DNA**: deoxyribonucleic acid.

[dee-**O**X-see-rye-bow-noo-**C**lay-ick]

Helix ↑

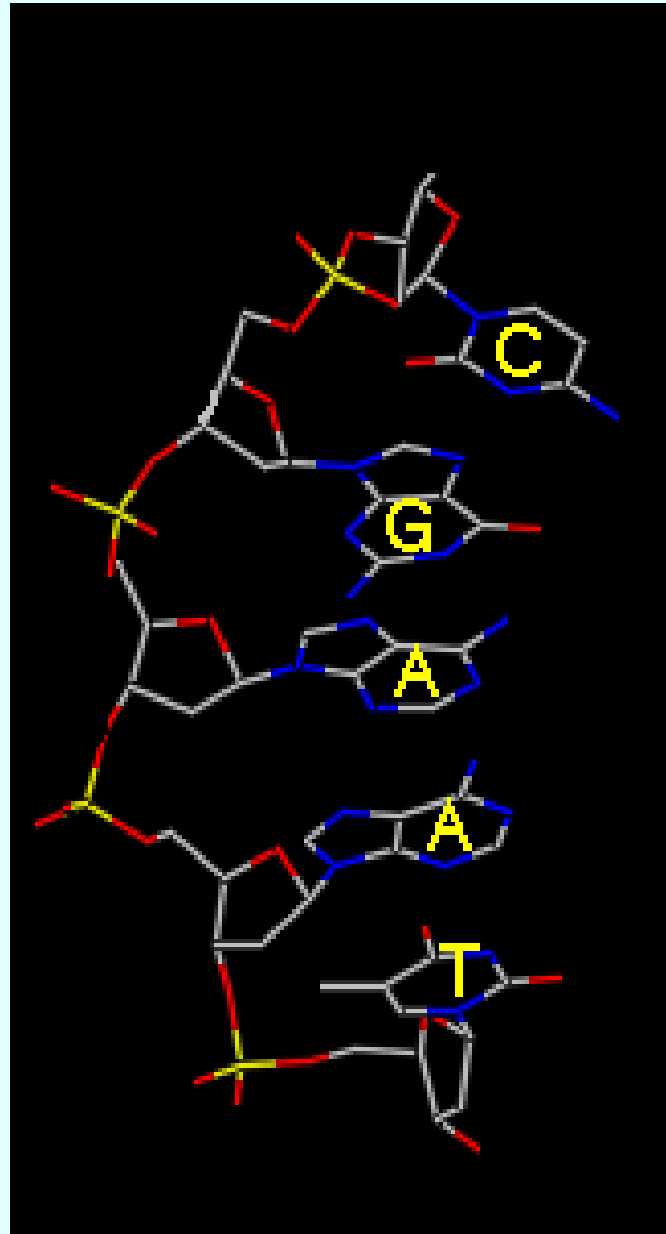
- Only **FOUR** different chemicals are used to make this message – think of each tooth in the zipper as one of these four chemicals.
- The genetic message is the **sequence** (order) of the chemicals in the zipper.

DNA Helix.



- The 4 chemicals are called “**bases**” (or **nucleotides**) **A = adenine**, **T = thymine**, **C = cytosine**, **G = guanine**
- Part of the **complexity** comes from the sheer sizes involved:
 - One piece of DNA can have **millions** of bases (chemical teeth) in the zipper.
 - The largest single message yet found, the gene involved in causing **Duchenne MD**, has 2,220,223 bases on each side.

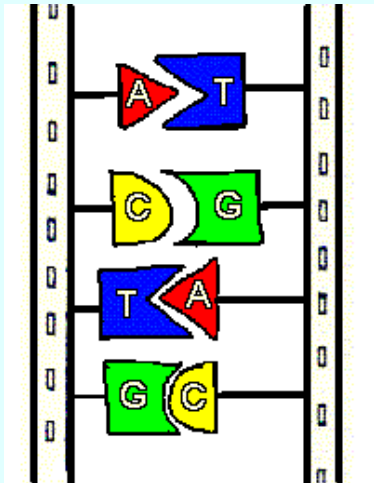
- On the left, we see the DNA **backbone** of one strand, shown in red, yellow and grey (it is mostly made out of sugar and phosphate molecules).



- On the right, we can see five **bases** attached in **sequence** (**C G A A T**)

Certain Bases Always Pair Up.

- Due to their chemical properties, certain bases on one strand **pair up** with certain bases on the other strand:
 - **A** always pairs with **T** and **T** always pairs with **A**
 - **C** always pairs with **G** and **G** always pairs with **C**.



- This pairing makes it easy: once Doctors know the chemicals on one side of the zipper, they will know exactly what is on the opposite side of the zipper.

Genetic Code – Codons.

- The 4 letters in the DNA code – **A** **T** **C** and **G** – make up 3 letter “words” (called **codons**) that spell out the genetic messages.

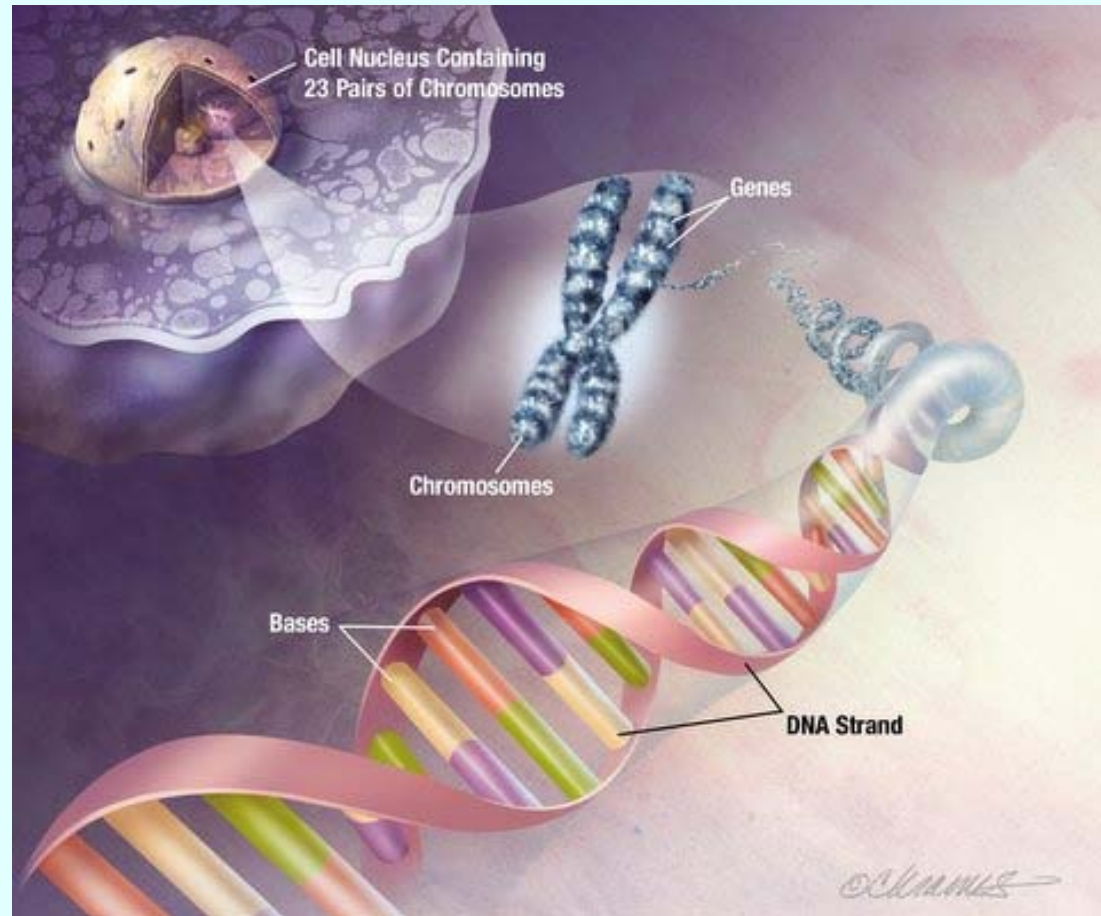
Examples:

G G G **G G C** **A G T**

- There are **64** different combinations possible.
- The entire genetic code is made up of series of these 64 codons, presented in different combinations to form “sentences” – these are what we commonly refer to when we talk about **genes**.
- Each gene is a “sentence” of code spelling out the formula for one or more **proteins** in the body.

DNA Forms Chromosomes.

- Each very long helix of DNA is tightly packed to form one **chromosome**.
- We inherit 23 from Dad & 23 from Mom. Each set of 23 contains about 25,000 genes with about 3.2 billion base pairs.
- The chromosomes (Chrome-muh-soams) are found in the center of the cell – the “**nucleus**.”



From: www.alzheimers.org/.../IMAGES/HIGH/Dna_high.jpg
National Institute on Aging, National Institutes of Health

DNA Summary.

- DNA is made up of a two long strings of sugar (**deoxyribose**) and phosphate links (molecules) that form the outside backbone of each strand.
- The four base chemicals (also called **nucleotides**) attach along the inside of the backbone strands.
- Bases on opposite strands bond to each other in the middle, zipping the strands together into a **helix**.
- **Sequences of bases** form our 25 to 30,000 **genes**.
- DNA forms **chromosomes**: we get 23 from each parent, these are then copied for the rest of our lives.
- Chemicals in the cell can unzip and re-zip the helix as the genetic messages are needed by the cells.

Basic RNA.

- **RNA** stands for ribonucleic acid.
[rye-bow-noo-**Clay**-ick]
- RNA is similar to DNA, except:
 - RNA has the same bases as DNA, except one – instead of **T = thymine** there is a **U = uracil**.
 - In RNA, the chemical backbone of the strand has a different type of **sugar** – it has a **ribose** instead of a deoxyribose.
 - RNA is usually found as just a **single strand** – it usually does not form into a two stranded helix.
 - **Many** kinds of RNA have been identified, some are well understood, others, not at all.

The Message Has To Be Copied: 1.

- The sequences on the DNA are **not** directly used to make proteins.
- DNA is in the center of the cell (the **nucleus**) and it never leaves.
- **Proteins** are made in the body of the cell.
- RNA acts as a **messenger**, making a copy of the sequence needed from the DNA and carrying it out of the cell's center into the body of the cell where it is used to make protein.

Here is an overall summary:

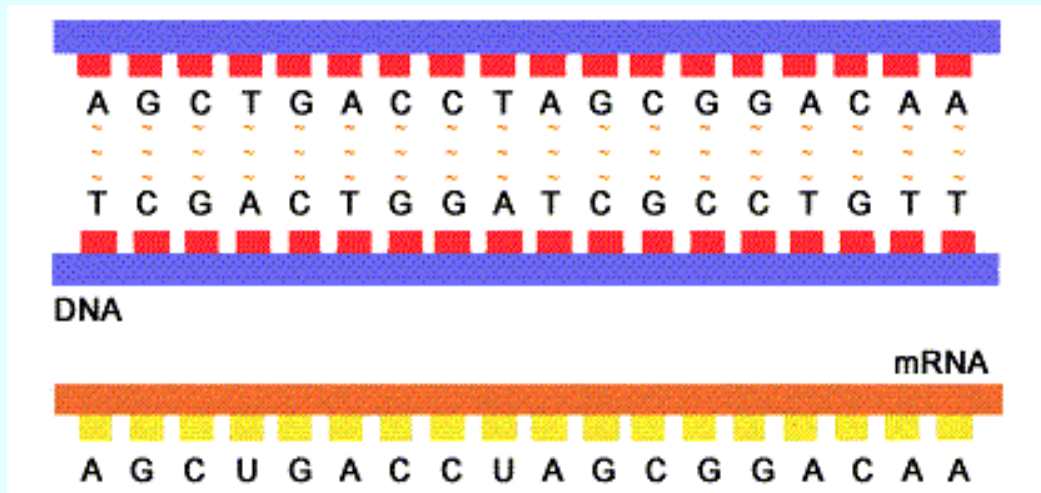
- **DNA - transcription--> RNA - translation-->Protein.**

The Message Has To Be Copied: 2.

- DNA to RNA **transcription** has four basic steps:
 - Step 1: The 2 strands of DNA unwind and **unzip**.
 - Step 2: Primary RNA “sees” a **start sign** (“promoter”) on the DNA strand and it joins on, copying the sequence of the four bases – the **reading frame** – until it hits a **stop signal**.
 - Step 3: This primary RNA carries a complete copy of the base sequence of the DNA – it is now **edited** into **messenger RNA (mRNA)**.
 - Step 4: The final mRNA moves into the body of the cell and acts as a template for **protein synthesis**.

Copying The Message.

- The two unwrapped DNA strands are shown in blue. The top strip – called “**sense**” (coding) DNA – is not used in the copying (“**transcription**”) process.



- This yellow strip is the newly formed primary RNA strand. Notice it comes out as an **exact copy** of the top DNA strip (but with **U** for **T**).

← The second blue strip is the **template strand** of DNA used to make the copy (this is the opposite side of the sense DNA strip, so it is called “**antisense**” (non-coding) DNA).

Genetic Messages.

- Sections of base sequence are read as 3 letter words – **codons**, to form “sentences” – the gene’s message.
- In the next few slides, I am going to “cheat” a bit and use some common 3 letter English words to illustrate how the triplet genetic code makes sense and how mutations create problems.
- Remember, in “real life” there are just 4 letters in the genetic alphabet – **A T G** and **C** and the three letter codons they form don’t make much sense to us (but they **do** make sense to the protein factory in the cell).

The Sequence Contains the Message.

- The DNA sequence of bases contains the messages needed by the cell but not all of the DNA is used:
 - It is estimated that only about **3 percent** of the DNA consists of coding sequences used to make proteins – it is not clear what the rest does, but there is a growing appreciation that it probably plays important roles.
- Recall, the whole DNA sequence in the gene is initially **transcribed** into **primary RNA**:
 - Primary RNA is then **edited**, some parts are kept (the actual **coding sequences** called **exons**) and the other parts (**untranslated regions** called **introns**) are removed from the final mRNA message.

The Sequence is Edited.

- An illustration:

- Sequence:

exons	↓	introns	↓
dek		THEdke	OLDute
CAT	yji	WAS	khyFAT
	ert		

- The sequence is now **edited** – the introns are removed to yield the **final mRNA message**:

|THE OLD CAT WAS FAT|

This message is now **translated** into a protein.

- In the illustration above, the start and the end of the **reading frame** is shown as **| |**

- The **sequence of exons** between these bookends is the critical message used to make a protein.

Introns Play A Role?

- **Intron code** – untranslated regions (UTR) – are **not** kept in the mRNA & are not used to make proteins.
- Until recently, this untranslated intron code was thought to play no role and was called “**junk DNA.**”
- The **majority** of DNA (97%) is made up of intron code.
- Recently, it has been shown that some of this material plays a **crucial role** in switching on and off genes.
- Another clue to the importance of introns is found in **disorders**, e. g., in DM, the genetic defect occurs in an untranslated section of the intron code.
- Overall, the role of this untranslated code and how it operates is **not well understood** yet.

Summary From DNA to mRNA.

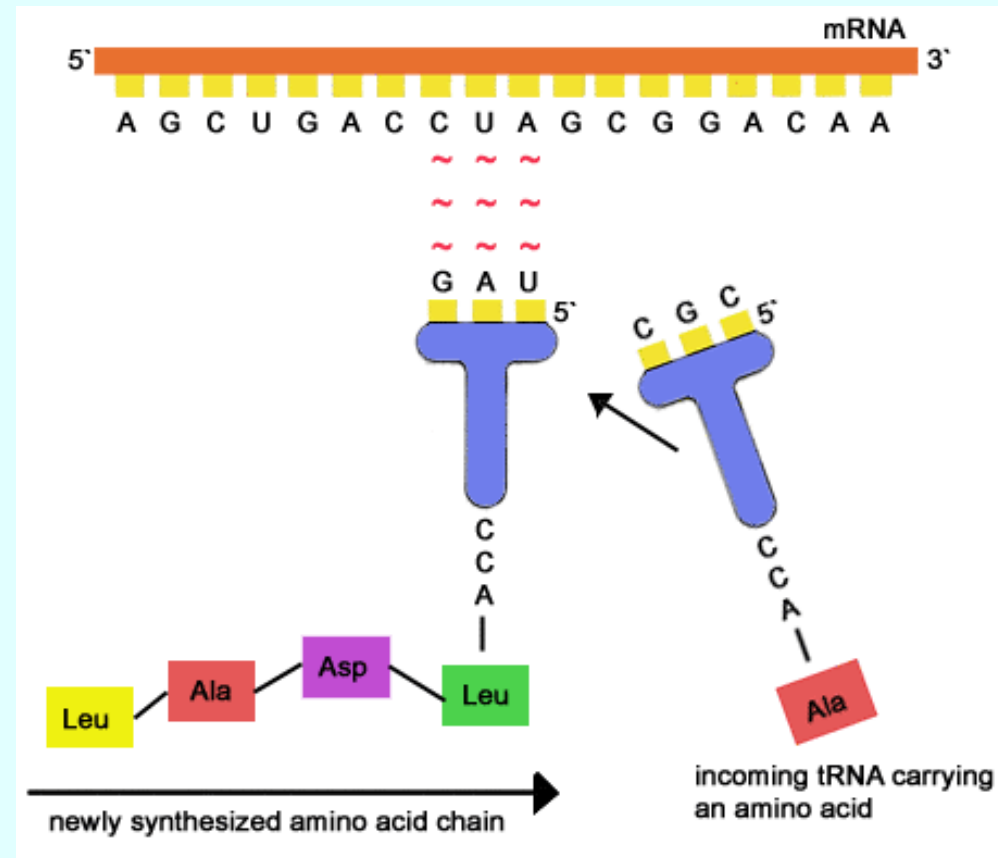
- To put a genetic message into action:
 - The DNA helix unzips into one sense strand (not used) and one template, antisense strand.
 - An RNA strand forms by moving along the template DNA strand and adding new bases corresponding to the sequence it finds.
 - When done, the 2 DNA strands zip back together.
 - The RNA is edited and introns are removed before it moves into the body of the cell. Differences in editing allow one gene to make several different mRNAs and thus make several different proteins.
- Proteins are made according to the sequences of code carried by the mRNAs that move into the cell.

Protein is Made.

- The normal operation of proteins is critical to the function of every system in the body.
- Estimates are there are >100,000 proteins in humans.
- Each **edited** mRNA sequence spells out a protein.
- **Proteins** are made up out of chains of chemicals called **amino acids (AA)**.
- Proteins are made by “factories” in the cell (**ribosomes**) that “read” the mRNA base sequence.
- Ribosomes assemble the different amino acids into the new chain according to the mRNA sequence.
- There are **64** three “letter” **codons**: 61 represent different amino acids, 3 stand for stop signs.

Translation.

- There is some overlap as 61 codons specify just **20** different amino acids.
- As the mRNA is read, the code tells the factory what amino acid to add next in the new **protein chain**: this is called **translation**.
- Proteins usually contain from tens to a few thousand amino acids.
- The largest protein yet found is Titin, a muscle protein with 26926 amino acids strung together in one long single chain.



- The **sequence** of amino acids defines the protein.

<http://library.thinkquest.org/C004535/media/translation.gif>

Finished Protein.

- One or more chains of AAs are used to form a protein. In a complex series of steps, the cell gathers the strand(s) into a final **folded, 3-D shape**.
- This shape is critical to the protein's ability to function.
- It does not take much of a mistake to cause trouble.
- Mutations in DNA leading to altered protein function are the **usual** culprit in most genetic disorders.



- Example: a diagram of dystrophin protein.

http://imbs.massey.ac.nz/Staff_images/dystrophin-web1.gif Dr Andrew Sutherland-Smith

Synopsis.

- DNA carries the genetic code in sequences of chemicals that form genetic “messages.”
- A message is read and used as a template to make a unique sequence of amino acids.
- Amino acid sequences form into proteins.
- Proteins form into complex structures that are the basis of living matter.
- The code is not always final, some modifications can be made along the way that alter the final product and how it will function.
- To devise genetic treatments, doctors will have to understand this whole process in great detail.

Code is Always Being Copied.

- Most of the body's cells carry a complete copy of all of an individual's DNA.
- As each cell is formed, the DNA in the parent cell is **copied** and put into the daughter cell:
 - DNA is carried on as cells die and are replaced.
 - This replenishment process goes on at an extremely **high rate** in many types of cells:
 - Examples: in skin, in the reproductive system and in the gastrointestinal tract.
- This process is extremely **reliable** and there are mechanisms to check for accuracy but only one mistake in code reproduction can be disastrous.

Code is “Dumb” But Crucial.

- A sequence of code is like a phone number:
 - The code (number) **represents** some other **target**:
 - 911: represents the police, fire and ambulance
 - 411: represents directory assistance
 - Just as a phone number leads us to our target, DNA specifies the cell’s targets – the **proteins** that the cell produces and that are so critical to life.
 - We can see that a difference in just one letter can change the meaning significantly:
 - If we use 411 instead of 911 we get the wrong target.

Common Mutations.

- Changes in the normal sequence are called **mutations**.
- Mutations **usually** affect the structure of proteins, altering their function & usually leading to disorders.
- Example: A mutation that adds or deletes even one letter can shift the triplet reading frame and garble the message – a **frameshift mutation**:
 - Using our example: |THE OLD CAT WAS FAT|
If we delete the T, the frame shifts right, giving:
|HEO LDC ATW ASF AT|
- The protein factory in the cell makes the protein spelled out by this odd message and the protein does not work the way it should, leading to a disorder.

Frameshift: A Severe Mutation.

- Because a frameshift mutation usually garbles the message so badly, it is commonly a very **devastating** type of mutation with severe consequences.
- The protein produced is often misshaped and cannot function or interact with other proteins (think of the example of the gears in a clock needing to all work together).
- This usually leads to serious physical symptoms.
- An example is Duchenne muscular dystrophy.

Nonsense Mutation.

- Recall that the RNA will continue to copy the DNA code until it sees a stop signal.
- Some mutations can create an “accidental” stop sign in the middle of the message and the RNA stops copying too soon.
- This type of mutation is called a **nonsense mutation**. The result is that only part of the correct message is made and, in turn, only part of the protein is made:
 - From our example: |THE OLD CAT WAS FAT|
The nonsense mutation product code looks like:
|THE OLD CAT|

Base Pair Repeats.

- It is **normal** to see triplets or quadruplets – 3 or 4 bases together – repeated a number of times in a row, either within a gene or in the intronic code.
- When 3 chemicals are repeated, it's called a **trinucleotide repeat**:
Example: **CTG CTG CTG CTG CTG CTG CTG**
- There is normally some **variation** between different people, re: how many repeats are present and the number is **stable** over generations (children have roughly the same number as their parents).
- If the number of repeats is within “**normal**” **limits**, they do not cause problems.

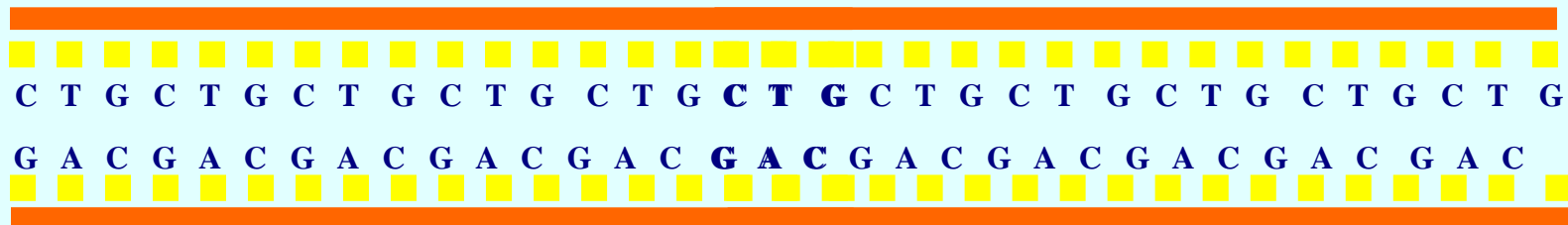
The Mutation in DM1.

- Sometimes a mutation can occur that **deletes** repeats or that creates **extra** repeats. If a section of repeats becomes too small or too large, it leads to problems.
 - In DM1, a **CTG** repeat is **expanded** in an intron – untranslated region of the dystrophia myotonica protein kinase gene (DMPK) on chromosome #19.
 - Normally, there are < 50 repeats and no problems.
 - People with DM1 may have from 50 to 2000 repeats in this section.
 - People with the very serious Congenital Myotonic Dystrophy may have up to 4000 or more repeats.
(In the medical research, these numbers vary a bit.)

Illustration.



Normal: 5 to 30 repeats.



DM1: 50 to 2000 repeats.

CMyD: up to 4000 or more repeats.

Diagnostic tests of the DNA can accurately detect the expansion repeats in DM1.

The Mutation in DM2.

- DM2 is caused by a the same type of mutation but involving the expansion of a **different repeat** section on a **different chromosome**.
 - In DM2, the expansion is found in an intron – untranslated section of the zinc finger protein 9 gene (ZNF9), found on chromosome #3.
 - DM2 involves an expansion repeat of **CCTG**, with from 75 - 11,000 repeats (5000 on average).
- DNA tests for DM2 are also now available.

Other Repeat Disorders Include:

- Fragile X syndrome: the most common cause of inherited mental retardation (IQ ~ 35-70).
 - Caused by a **C G G** repeat in an exon on the X chromosome.
- Huntington disease is caused by extra **C A G** repeats in an exon in the Huntington gene on chromosome 4.
- Friedreich ataxia is a neurodegenerative disorder caused by extra **G A A** repeats, located in an untranslated region of code on chromosome 9.
- Facioscapulohumeral Muscular Dystrophy (FSHD) involves a **deletion** of repeats in a section called D4Z4 found on chromosome #4.

- With each generation, the number of extra repeats often **increases**.
 - This phenomena is called **genetic anticipation**:
 - As the size of the repeated section increases, successive generations commonly show symptoms at an **earlier age** and / or may show **more severe** symptoms.
- Some cases show **mosaicism**. This happens when a **new mutation** occurs in a single cell very early in development (the rest are OK). As this cell divides, its daughter cells carry on the mutation. Thus, only some cells in the body have the mutation. The effect of DM mosaicism varies widely in each person.

Autosomes.

- Our 46 **chromosomes** carry our DNA code.
- The 46 chromosomes are found in 22 pairs called **autosomes** and one pair of sex chromosomes.
- Different genes are located on different chromosomes.
- Each pair of autosomes holds **two copies** of a given gene, one inherited from Dad, the other from Mom.
- We are focused on 2 pairs of autosomes, those involving chromosome # 19 for DM1 and # 3 for DM2.
- [The composition of pair # 23 determines sex: boys have an XY; X from Mom, Y from Dad and girls have an XX; one X from Mom and one X from Dad.]

Autosomal Dominant Inheritance.

- Myotonic dystrophy is inherited as an **autosomal dominant** condition; this means that only one copy of the gene with the genetic mutation (inherited from **either** Mom or Dad) is necessary for DM to occur in a person (the other gene copy is usually healthy but it is “overridden” by the dominant copy with the mutation and thus cannot help out).
- Because the disorder is genetically dominant, for each pregnancy, a parent with a mutation usually has a **50%** chance of transmitting the mutation to a child – in other words, each child has a 50% chance of inheriting the defect if a parent has the mutation.

Spontaneous Mutations.

- In some DM cases, the mutation is new.
- **Spontaneous** (new) mutations, are not inherited from either Mom or Dad – the mutation arises in one of the early cells that divide to form the embryo.
- In these cases, there is usually no prior family history of DM.
- A person with a spontaneous mutation in an egg or sperm cell will subsequently pass the mutation on to any children 50% of the time.

Summary: What We Need to Know.

- The DNA code in exons ends up being used as a template for making different **proteins** in the body.
- Mutations in DNA often lead to altered protein function and are the **usual** culprits in most genetic disorders.
- The DNA carried in the untranslated intron code is not used directly to make proteins.
 - It is not clear exactly what this DNA code does.
- The mutations in DM are in sections of intron code.
 - These mutations somehow disrupt normal gene function leading to the symptoms we see in DM1 and DM2.

- Although the repeat mutations do not directly create abnormal protein, there is recent evidence that the repeats do cause trouble in the **mRNA** of the cell.
- It appears that the extra mRNA is **blocked** from leaving the center of the cell and it **accumulates**. Myotonic dystrophy was the first disorder associated with a defect in RNA and not directly associated with a protein malformation as the result of a DNA mutation.
 - The **built-up mRNA** appears to interfere with the processing of proteins, leading to symptoms.

- Dr. Robert **Korneluk** at the Children's Hospital of Eastern Ontario has spent years studying how the DM mutation causes illness. His team also studies the effects of cell death and the factors that control it, including the role of cell death in DM.
- Dr. Jean **Mathieu** at the Centre Hospital of the University of Montréal is studying the well-being and social participation of DM patients to see what factors determine their quality of life.

- Dr. Jack **Puymirat**, director of the Human Genetics Research Department of the Centre Hospital of the U. of Laval (CHUL), focuses on the development of gene therapies for the treatment of myotonic dystrophy. His team are researching several methods to try to treat DM.
- Dr. Jacques **Tremblay**, also of the CHUL, along with his team (and with Dr. Puymirat), are leading research in transplanting muscle cells (myoblasts) to treat muscular dystrophy, an approach that may be used someday for DM.

Possible Therapies.

- Therapies for DM are at a very early stage.
- One idea is that Doctors need to somehow **block the extra repeats** and to cancel their effects while at the same time allowing the normal genes to function.
- Another approach is to try to activate specific chemicals (**enzymes**) inside the cell's center to target and destroy the abnormal built-up mRNA.
 - Dr. Puymirat is a leader in this research.

More Possible Therapies.

- In experiments with **mice**, in 2003, Dr. Puymirat, has been able to **neutralize** the abnormal DM gene, preserve the healthy gene and restore the muscle cell's normal function.
- Another Canadian approach developed by Dr. Jacques Tremblay, uses **transplanted muscle cells** to try to help restore proper muscle function.
- It is not clear which of these therapies may **someday** lead to a viable treatment of Myotonic Dystrophy.

- In May 2007, MDC, the Canadian Institutes of Health Research – Institute of Musculoskeletal Health and Arthritis (CIHR-IMHA) and The Rachel Fund announced that Dr. M. Mahadevan had received the first research award granted by The Rachel Fund.
- Dr. Mahadevan developed a strain of mice with extra CTG repeats and found that they display DM1. When he “turned off” the extra repeats, the damage in affected cells reversed and most function returned.
- Reversing the effect of the built-up mRNA restored the majority of normal function. The extra RNA is somehow toxic and if researchers can neutralize it, they can essentially restore normal function.

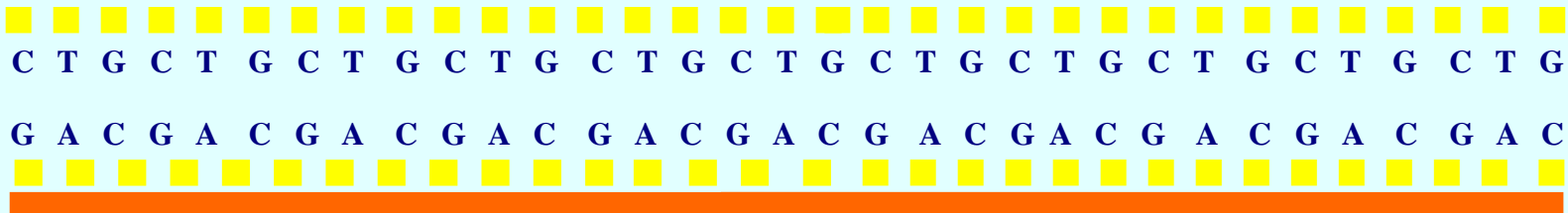
- John Day and Laura Ranum of the University of Minnesota have several recent articles on DM.
 - Day, J. W. and Ranum, L. P. W.. Genetics and Molecular Pathogenesis of the Myotonic Dystrophies. *Current Neurology and Neuroscience Reports* 2005, 5:55–59.
 - Day, J. W. and Ranum, L. P. W.. Review: RNA pathogenesis of the myotonic dystrophies. *Neuromuscular Disorders* 15 (2005) 5–16.
- Machuca-Tzili, L., Brook, D., and Hilton-Jones, D. Clinical and molecular aspects of the myotonic dystrophies: A review. *Muscle Nerve* 32: 1–18, 2005.
- **Also:** <http://www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?cmd=entry&id=160900>

- Following are a few slides that detail the basic idea of how **antisense therapy** may someday be used to block problem areas of DNA and specifically block the toxic effects of the expanded atrepeat sections in myotonic dystrophy.

Antisense Therapy.

- If a piece of DNA is faulty, the RNA will copy the mistake & pass it on to the protein production phase.
- If doctors can see the DNA mistake, they can easily figure out the sequence of the corresponding final sense strand of mRNA that the cell makes.
- Doctors can create a piece of **antisense mRNA** in the lab to “mirror” this mutated section of sense mRNA.
 - This piece of antisense mRNA can be inserted into the cell where it will lock onto to the corresponding section of mRNA and “cover up” the faulty section.
 - This allows the mRNA to “skip” the mistake and prevent passing the mistake on from the DNA to the protein production phase.

DM 1 Repeat

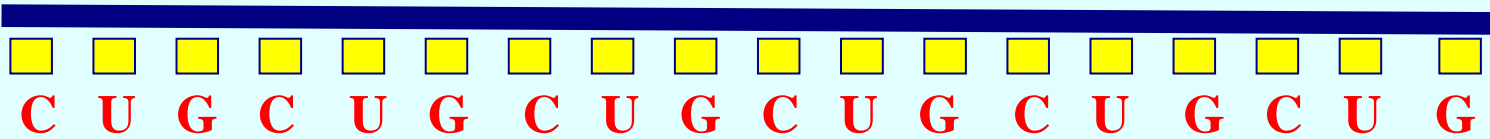


- Double stranded mutated DNA code
(more than about 50 repeats)



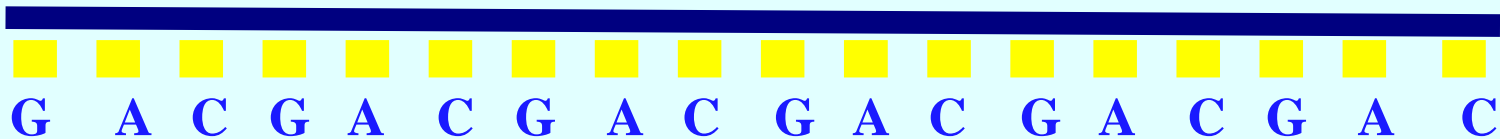
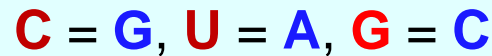
- Single strand RNA code appears to somehow get stuck inside the center of the cell becoming toxic (by tying up chemicals that should be doing other jobs?) thus interfering with the normal production of proteins and leading to the effects of myotonic dystrophy.

Antisense Blockage of RNA.



Above is the expanded “toxic” strand of mRNA.

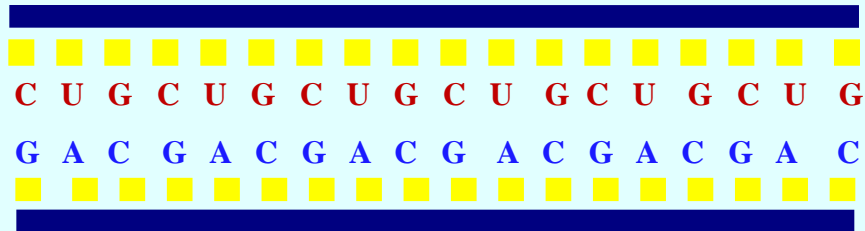
The blocking code will be
the opposite of the code.



Doctors make this piece (blue code) of blocking **antisense oligonucleotide** (AON) in the laboratory and put it into the cell – it attaches onto the sense RNA.

Antisense Blockage.

- Mutation is on the top line:



- A section of RNA (blue code) is introduced, it links onto the RNA code blocking the repeat.
- If the mistake (like the extra section of repeats) can be blocked, it looks hopeful the problem can be helped and the correct proteins restored.

- Think of a scratch on an old LP record. The needle comes to it and can't play the rest of the song. Here, doctors cover up the scratch & blocks its effects on the song. With the scratch blocked, the song plays, or, in our case, the normal proteins return.

- Although DM 2 involves a slightly different repeat and research advances will have to be tailored specifically to address DM 2 but it certainly looks like the basic principles described here should also apply.