

On overview of Duchenne and Becker Muscular Dystrophy highlighting one promising treatment approach.

Bill Tillier,  
Calgary

December 2005

- This talk will review **information** on Duchenne and Becker Muscular Dystrophy in a simple and understandable way.
- One potential treatment approach will be highlighted giving a **summary** of the basic ideas behind it.
- 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 **simple** English.

# Knowledge Translation.

- This talk is an example of **knowledge translation** (KT).
- KT is an important **new tool** in medical research.
- KT involves making a **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.
  - Health care providers and researchers can better understand patients and their needs.

# Who Was Duchenne?



- A French neurologist, born September 17, 1806; died September 15, 1875.
- Discovered that external electrical stimulation could cause muscle movements.
- Probably the first to do a biopsy to get tissue from a living patient for microscopic exam.
- Helped develop the neurological examination – he is generally seen as one of the founding fathers of neurology.
- In 1861, he described a boy with the form of muscular dystrophy we now call Duchenne.

- Dr. Guillaume-Benjamin Duchenne de Boulogne.
- Duchenne [DO-shin] Dystrophy [DIS-trow-fee]

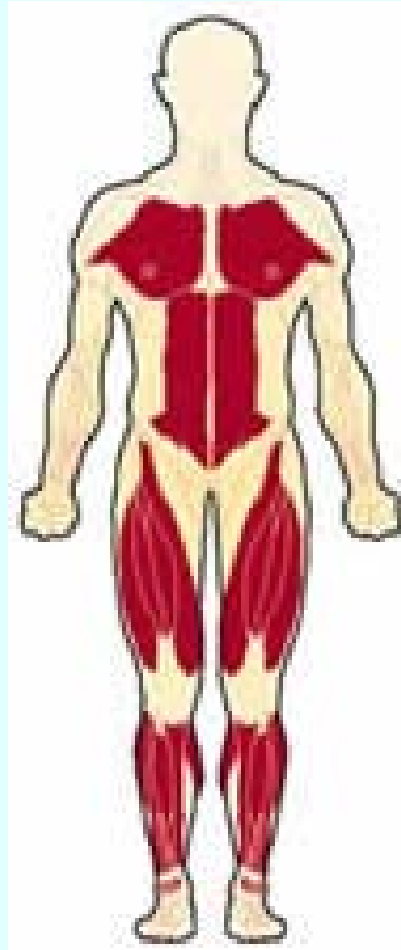
# How Common Are They?

- Duchenne muscular dystrophy (DMD) is the most common type of muscular dystrophy (MD).
- Duchenne affects 1 in 3,500 male births and is thought to have an estimated overall prevalence of 63 per million people in the population.
- Becker MD (BMD), named after the German doctor Peter Emil Becker, who first described this variant of Duchenne dystrophy in the 1950s, affects about 1 in 5,000 male births and has an estimated overall prevalence of 24 per million population.

# Common Symptoms.

- DMD affects boys and very rarely, can affect girls. DMD typically appears between 1 and 6 years of age with weakness in the pelvis and upper limbs, resulting in clumsiness, frequent falling, an unusual walk and general weakness. 30% of patients display mild mental retardation. As DMD progresses, weakness spreads and skeletal deformities may contribute to breathing disorders. Most patients die in their early twenties because of muscle-based breathing and heart problems.
- Becker symptoms are similar to Duchenne's but are milder and tend to be more variable.

# Common Symptoms.

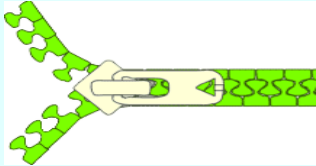


- From: [http://www.hndp.chb-genomics.org/pat\\_fam-dmd\\_bmd.php](http://www.hndp.chb-genomics.org/pat_fam-dmd_bmd.php).

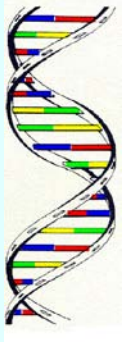
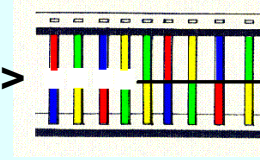
- The cause of Duchenne and Becker MD is **genetic**.
- We will present a brief overview of information on the **basic** genetic code and then discuss the problem.
- The **internet** contains a great deal of background information on all of these aspects.
- Again, it is the **basic ideas** that are important 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]

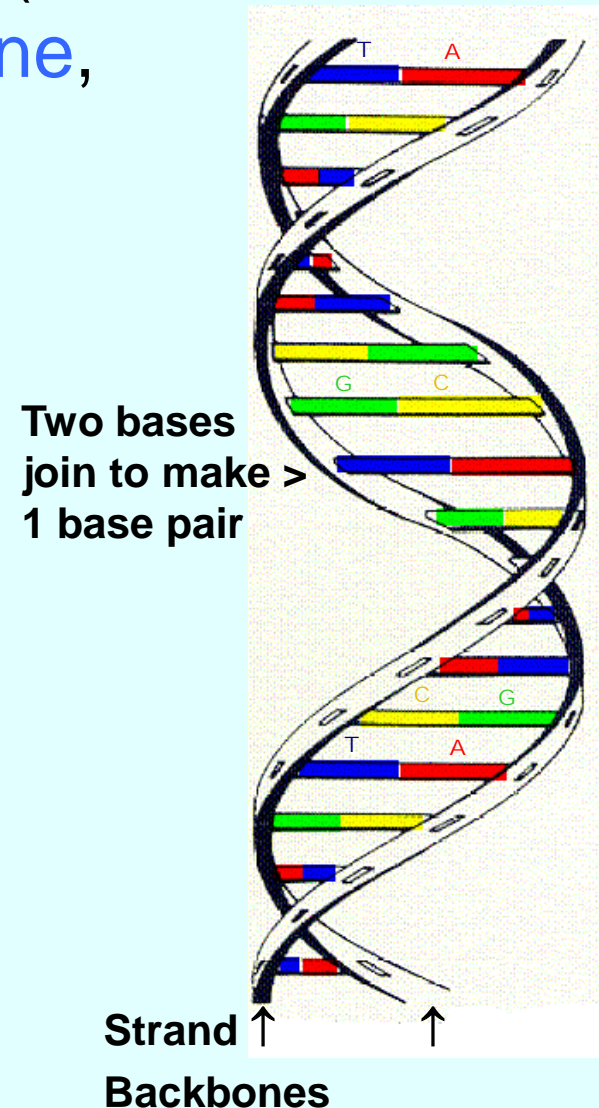
**Helix** ↑

- 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.

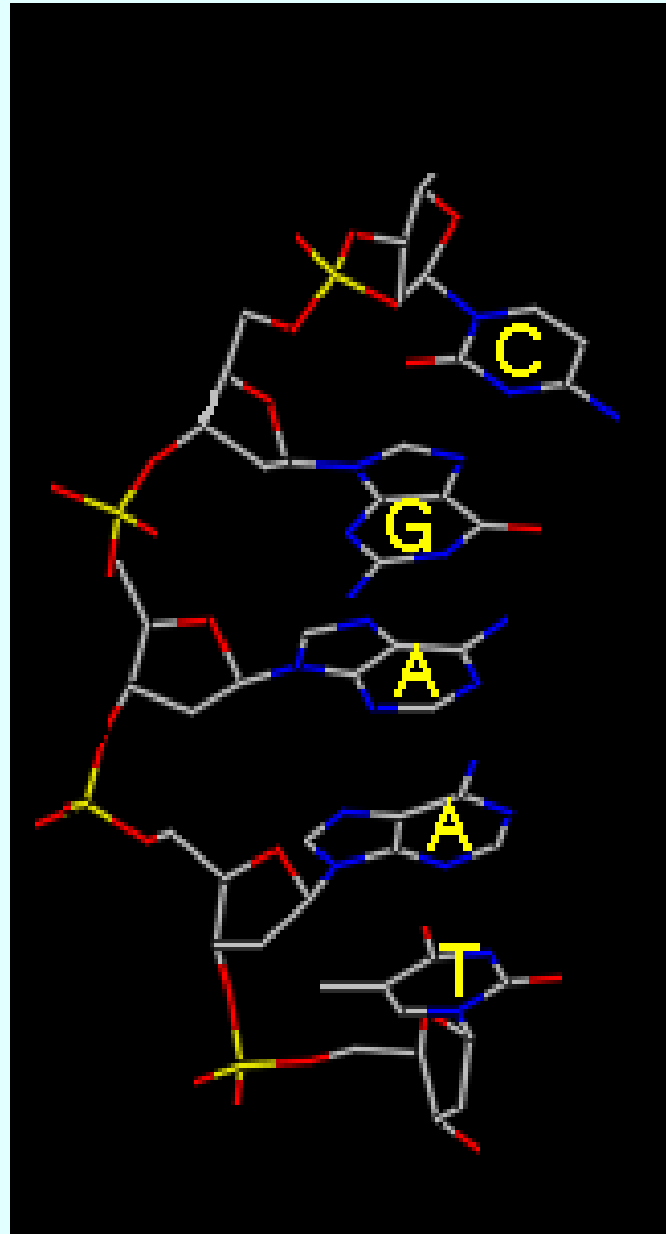
# DNA Helix.

- 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.

## DNA Helix.



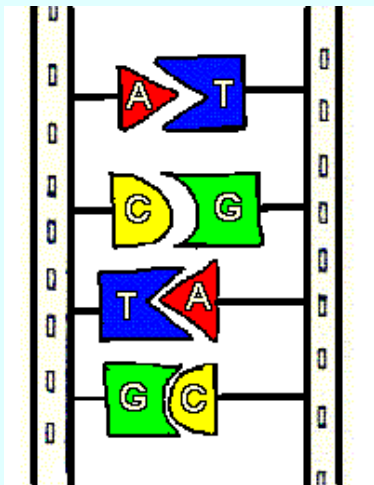
- On the left, we see the DNA **backbone** of one strand, shown in red, yellow and grey (it is mostly made out of sugar and phosphate molecules).



- 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.

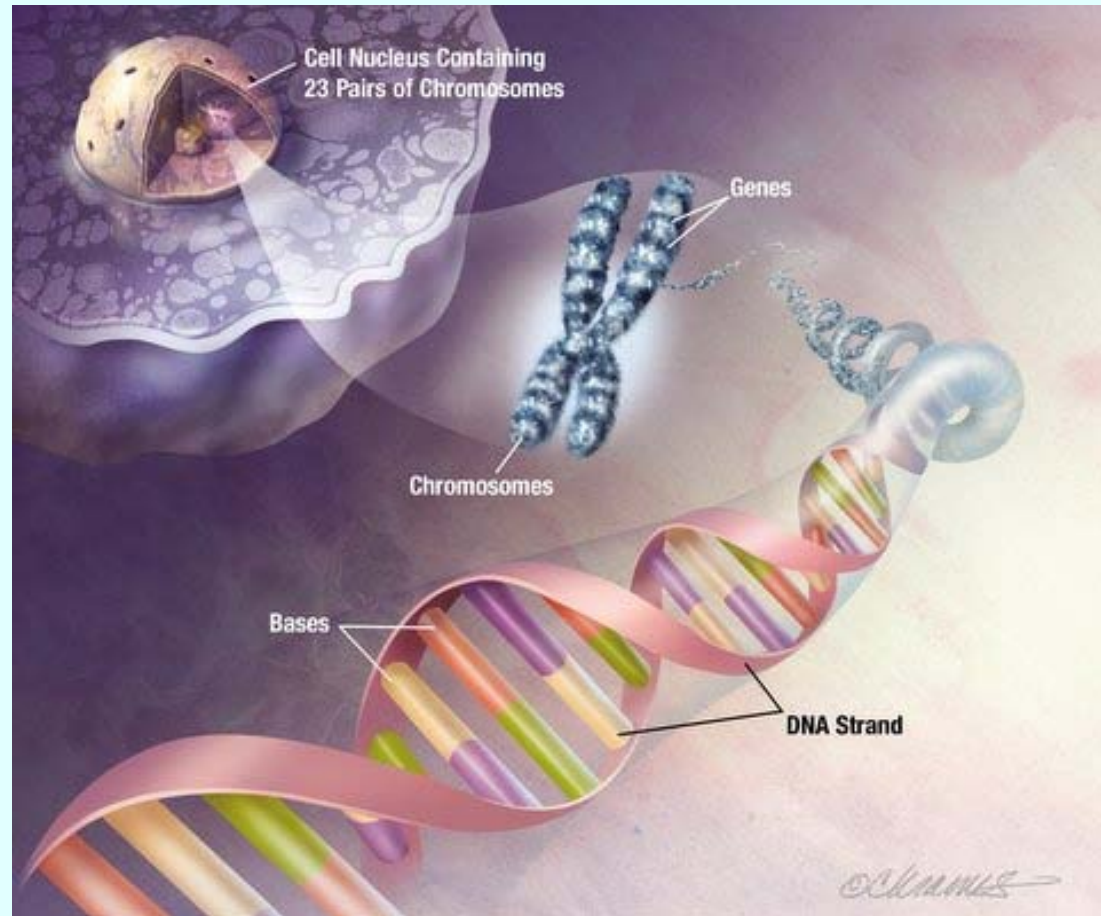
Examples:

**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](http://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:

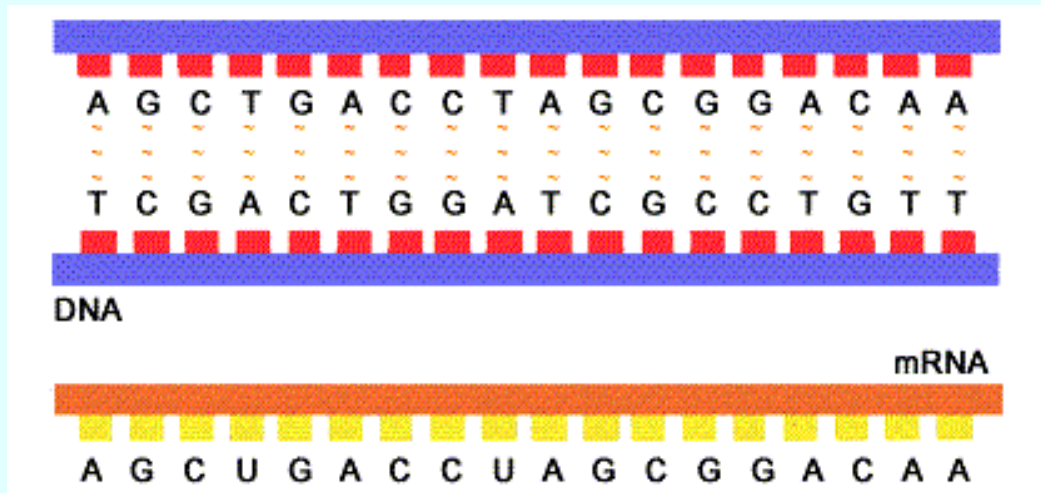
- **DNA - transcription--> RNA - translation-->Protein.**

# 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 Myotonic Dystrophy, the genetic defect is 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.

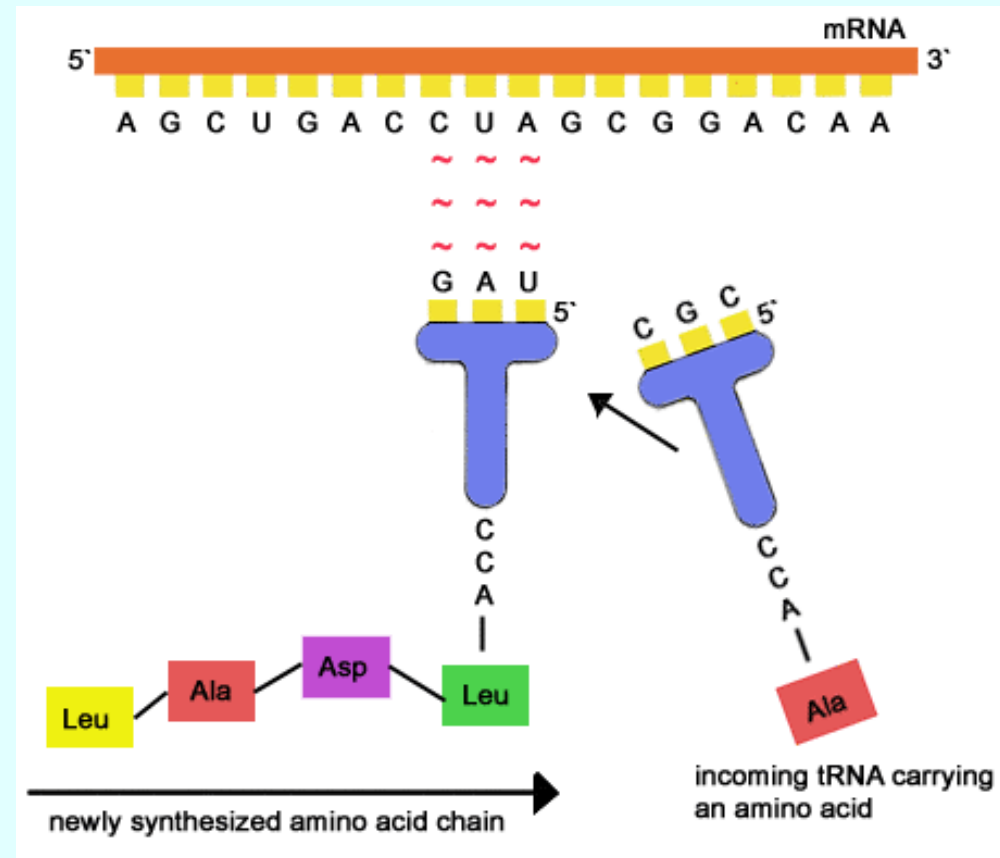
- To put a genetic message into action:
  - The DNA helix unzips into one sense strand (not used) and one template, antisense strand.
  - An RNA strand forms by moving along the template DNA strand and adding new bases corresponding to the sequence it finds.
  - When done, the 2 DNA strands zip back 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](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.

# Dystrophin is the Problem.

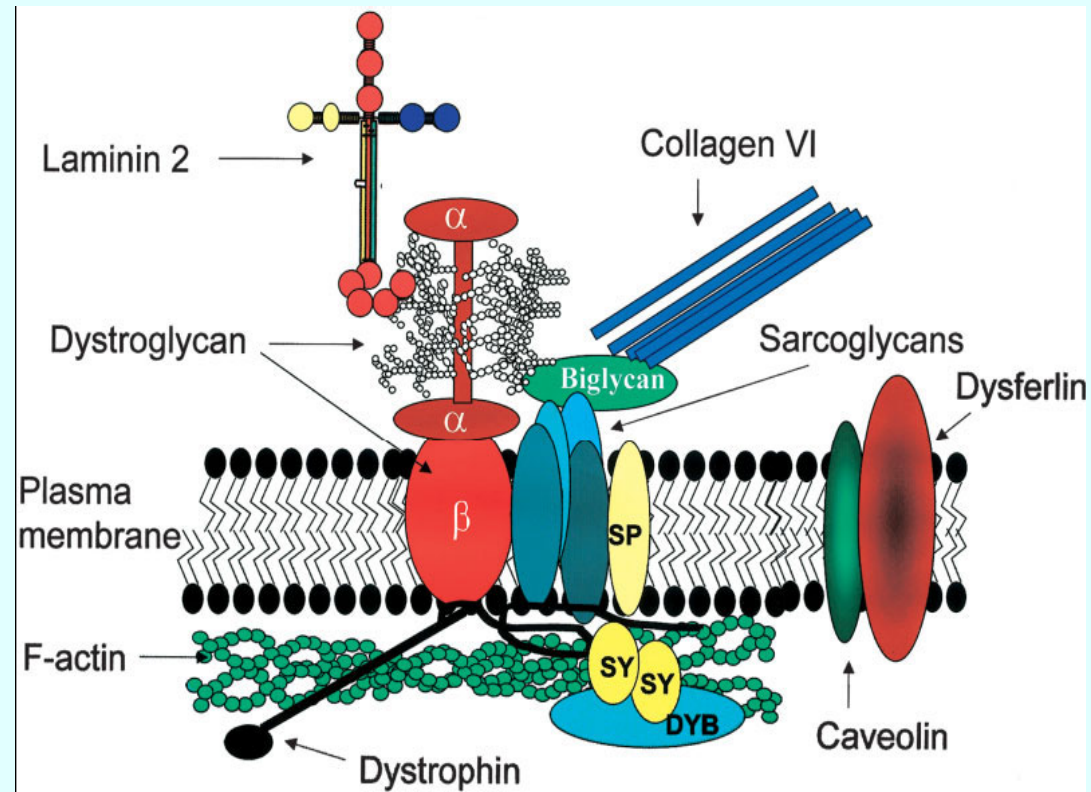
- Duchenne and Becker MD are caused by problems in the gene that makes a protein called **dystrophin**.
- The gene makes several slightly different **forms** of dystrophin [DIS-trow-fin] protein and some of these play different roles in different tissues in the body:
  - For example, dystrophin is needed in the muscles, in the brain and in the retina of the eye.
- Dystrophin is especially important for long term **muscle function**. If there is not enough dystrophin, the muscle cells breakdown and eventually die.
- Duchenne and Becker are essentially two versions of the **same disorder**: Duchenne reflects more serious dystrophin depletions, Becker, milder forms.

# The Dystrophin Gene.

- The dystrophin gene is **unusually large** – it contains 79 exons, coding for 3685 amino acids. (By comparison, the gene for insulin protein contains just 3 exons coding for only 51 amino acids).
  - All of these 79 exons have to be present, lined up and spaced correctly for dystrophin protein to be made perfectly.
- **Mutations** (deletions) cause deficiencies in 1 or more of these exons leading to abnormal, decreased or absent dystrophin in cells – this causes symptoms.
- Duchenne and Becker are types of **dystrophinopathy** – [DIS-trow-fin-OP-path-ee] – a pathology of dystrophin.

# Dystrophin in Muscle.

- Muscle is made up out of many different proteins, all interacting with each other – think of the many gears working together in an old clock.



From: *Muscle Nerve* 32: 577–588, 2005 (p. 578).

- Dystrophin plays a critical role inside muscle cells, acting as a “shock absorber” and anchor, “holding the muscle cell together.”

# 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. Most people (96%) with Duchenne MD have frameshift **mutations**. (numbers from: Neuromuscular Disease Center, Washington University, St. Louis, MO <http://www.neuro.wustl.edu/neuromuscular/index.html>).
- This type of mutation often prevents any functional dystrophin production and people with this type of mutation often show severely reduced or zero levels of dystrophin.
- Prenatal testing, (amniocentesis), for pregnancies at risk is possible if the DMD disease-causing mutation has been identified in a family member.

# Nonsense Mutation.

- Recall that the mRNA 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 mRNA 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|

# Duchenne versus Becker.

- People with Duchenne or Becker MD will display symptoms reflecting how much functional dystrophin protein they are actually getting.
- As explained, people with **Duchenne** MD usually have the more serious **frameshift** mutations that may prevent any functional dystrophin production.
- In **Becker** MD, the mutations are usually in frame (the message makes sense) but deletions in the DNA make the protein too short – **nonsense** mutations.
- People with Becker usually have partially functioning dystrophin – this leads to less severe symptoms and slower progression compared to Duchenne MD.

# 23 Pairs of Genes.

- Our 46 **chromosomes** carry the 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.
- The sex chromosomes determine gender: boys have an X and a Y; X inherited from Mom, Y from Dad and girls have an XX; one X from Mom, one X from Dad.
- The dystrophin gene is located on the X chromosome and is inherited in a **recessive** manner.

# DMD and BMD are X Linked.

- Boys only have 1 X chrm (inherited from Mom), so they only have 1 copy of the genes on the X. If an X gene has a mutation, the boy will have the disorder.
- Girls are **usually** protected if they inherit one defective X because they inherit a second copy of the X. In very early development, one X in each cell is shut off. **Usually**, this is a random process and the girl is left with 50% mutated and 50% healthy Xs – usually enough to compensate for the mutated version.
- In rare cases, more of the healthy Xs are shut off and the girls develop symptoms – “**manifesting carriers**,” in some instances, as severe as male cases.

- Females with a mutation on one of their X genes are called “**carriers**.” Each son of a carrier mother has a 50% chance of inheriting DMD or BMD through the X Mom gives him. Each daughter has a 50% chance of again being a carrier through the X Mom gives her.
- A male with DMD or BMD passes his one mutated X chromosome to each of his daughters, so each daughter will become a carrier.
- A male with DMD or BMD only passes his Y chromosome to each son, so none of his sons will get his defective X and none will have the condition or be a carrier (unless the mother is also a carrier).

- Most boys with DMD or BMD inherit the mutation from their mother (2/3 of cases).
- However, the dystrophin gene is quite prone to **new mutations** because of its huge size. In about 30% of cases, the mother is not a carrier – a new mutation occurred in the gene as it was copied in the egg that produced the child.
  - In these cases, it is unlikely that **this Mom** will have another child with DMD or BMD.
  - The male child with the new mutation may develop DMD or BMD. Both males and females may pass on the mutated X to subsequent children (see 39).

# 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 this DNA lead to altered protein function and are the **usual** culprits in most genetic disorders.
- In Duchenne and Becker MD, a mutation in an exon of the **dystrophin gene** leads to problems with the function of **dystrophin protein**, more severe in Duchenne, less so in Becker.
- The dystrophin gene is carried on the **X chromosome**, so boys who inherit it from Mom are always affected.
- In some **rare** cases, females can also be affected.

# Today's Goal: Symptom Reduction.

- If Doctors could make the defective dystrophin more functional they may be able to “turn severe Duchenne symptoms into milder Becker symptoms.”
- The person will not be “cured” but, with the medication, he or she may have enough dystrophin available to live a more normal lifestyle and have a more normal lifespan.
- We will now highlight one approach, called **antisense therapy**, that tries to do this.
- Many research groups are currently working on this or similar approaches.

# Antisense Therapy.

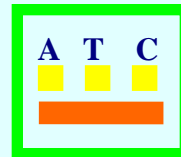
- If a piece of DNA is faulty, the RNA will copy the mistake & pass it on to the protein production phase.
- If doctors can see the DNA mistake, they can easily figure out the sequence of the corresponding final sense strand of mRNA that the cell makes.
- Doctors can create a piece of **antisense mRNA** 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.

# Antisense Blockage.



Here is a sense strand of mRNA. In this example, let's say the mRNA has copied a mutated section of DNA code – UAG – in the red box.

The antisense code is the opposite of the sense.

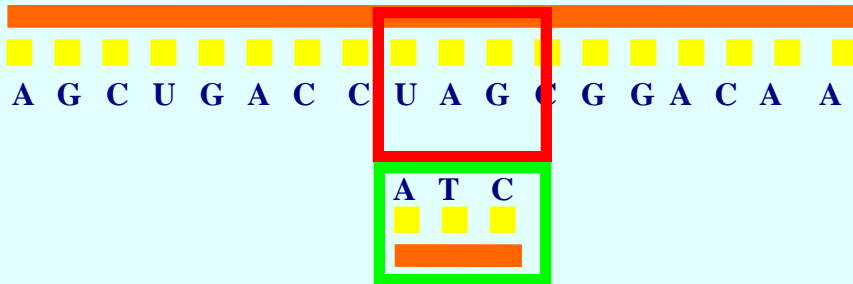


U = A,  
A = T,  
G = C

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

# Antisense Blockage.

- The mistake is in the red box:



- A short section of RNA (green box) is introduced, blocking out the mistake.
- If the mistake (like an extra section of repeats) can be blocked, maybe the problem can be helped or solved 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 & the needle jumps over it to finish the song. The song may not be perfect, but may be close enough to get the job done.

# Exon Skipping.

- As we explained, the problem is in a section of DNA called an exon. This technique skips over the defect, therefore it is sometimes called **exon skipping**.
- It appears that exon skipping will someday be used to treat both nonsense and reading frame mutations.
- This technique could help the **majority** of people with Duchenne MD – but not everyone – as there are other types of mutations as well, especially in Becker MD. Other types of therapies will have to be developed for these types.

- “Systemic delivery of antisense oligoribonucleotide restores dystrophin expression in body-wide skeletal muscles.”
- This work, done by Qu Long Lu’s group in London, and published in January 2005, was done on **mice** that have been bred to have a disease closely resembling DMD.
- Reference: The Proceedings of the National Academy of Sciences, *102*, #1, 198-203, January 4, 2005.

- This research was able to discover a premature stop sign and create a small piece of corresponding antisense mRNA.
- The piece of antisense mRNA was injected into the mice, binding onto the mutation and effectively covering it up, thereby letting the mRNA “skip” over the mutation.
- “Near-normal” dystrophin levels were expressed in the mice and their muscle function greatly improved.

- Progress is **ALWAYS ONE SMALL STEP AT A TIME**:
  - This approach is a step in the right direction, however, many **CRITICAL** questions remain:
    - For some reason, in this study by Lu, the treatment did not work on the mouse's cardiac muscle.
    - This research is looking at only **ONE** very specific mutation.
    - The authors conclude that their research shows “a practical and genuine hope that we may be able to move the approach from the [laboratory] bench to the bedside.”

- To work widely, this approach needs to address each specific type of mutation involved.
  - This approach could be seen as a personalized treatment.
- Only about 15% of Duchenne cases will be helped by nonsense mutation suppression, about 70-80% may be helped by exon skipping to restore frame reading.
- Much more research (years) will be needed to make these practical therapies.
- These treatments will not “cure” the person, but hopefully would lead to a reduction of the severity of the symptoms of Duchenne – a big step.

- These treatments are **not** gene therapy as the original dystrophin gene is not affected (the DNA mutation is not fixed).
  - The therapy modifies the *result* of the mutation in the mRNA.
  - If the person stopped taking the medicine, his or her symptoms would return.
- Perhaps someday, Doctors will be able to repair the actual DNA sequence that is faulty and create a lifelong cure for the problem.

# Another Approach Also Shows Promise. <sup>53</sup>

- Dr. Jacques Tremblay and his team of the Centre Hospital of the University of Laval (CHUL), are leading research in transplanting cells (myoblasts) to treat muscular dystrophy.
- Immature, muscle-derived stem cells (*myoblasts*) are transplanted from fathers into their Duchenne sons.
- In a 2004 study, the boys began producing normal dystrophin in a small percentage of their injected muscles and they didn't reject the donated cells. (MOLECULAR THERAPY Vol. 9, No. 3, March 2004)
- Dr. Tremblay says he believes that this method will be part of the overall solution to treating Duchenne MD.