

Dystonia and Endoplasmic Reticulum Associated Degradation

When my brother was eight, he was diagnosed with early onset Dystonia. Dystonia has had a long genetic history in my family. My grandfather is a carrier and shows some symptoms of Dystonia. My grandfather's brother had severe Dystonia during his life. My father is a carrier of the gene but, luckily, did not pass it on to me. Genetic testing can be done to see if the gene for Dystonia is present in a certain person's genome. In my brother's case of Dystonia the disease was hereditary. The disease can be contracted by other factors such as birth-related or physical trauma, infection, poisoning, or reaction to pharmaceutical drugs (Dystonia Risk Factors). Dystonia can range from acute contraction of the disease, localized to certain body parts, to generalized contraction of the disease, as my brother has. Dystonia is characterized by abnormal positioning, pain, cramping, and muscle spasms due to involuntary muscle movements. There is a common thought that the presence of anxiety, stress, and fatigue can worsen the symptoms of the disease. Some diagnoses of Dystonia consist of examining medical history and performing a physical evaluation. Dystonia can also be diagnosed by the use of a DNA sample in order to look for a mutation present in Dystonia (Dystonia Medical Research Foundation).

The immediate causes of Dystonia are not known presently and are thought to be caused by the pathology of the central nervous system and in particular the Basal Ganglia. The Basal Ganglia is a region in the bottom of the brain that also plays a role in Parkinson's disease and Huntington's disease (Definition of the Basal Ganglia). Dystonia is typically classified according to age of onset, distribution of symptoms in relation to the body, and cause of disease. The onset of the disease can be either during adulthood or during childhood. Dystonia can be focal, only affecting an acute portion of the body, or generalized, affecting the entire body. Primary Dystonia is when the disease is by itself a solitary symptom and not associated with any other diseases. Secondary Dystonia is characterized by Dystonia that is the result of another health condition. Dystonia is known to be the third most common

movement disorder in the United States, and known to affect about two hundred and fifty thousand people. The most common form of Dystonia is known as Torticollis, which is a symptom of cervical Dystonia that involves twisting of the head and neck area of the body (Neurological Movement Disorders).

Dystonia can be treated in a variety of ways even though a cure has not been found yet. Common Dystonia treatments include physical therapy, medication, and surgery. Common injections are from botulinum toxin and have to be repeated every four months. These injections such as Botox and Myobloc help to hinder some of the brain to body communication problems that cause Dystonia (Dystonia Treatment).

Recently a new treatment that is derived from botulinum toxin type a, which is a form of botox, was approved under the name of Xeomin®. Xeomin® is intended for patients with cervical Dystonia, which is Dystonia focused in the neck, to alleviate abnormal head position and neck pain. Cervical Dystonia is commonly rated on a Torticollis rating scale which shows how serious the condition is. The average change in rating of patients before using Xeomin® and after using Xeomin® was minus seven and a half points which is a great amount considering the maximum rating on the scale is thirty five (Xeomin Benefits).

Phenol or alcohol injections also may be used which are less expensive and last for about six months. When medications are used patients usually start on low dosages. The medications affect the neurotransmitters in the nervous system that control movement. Common medications that are prescribed to treat Dystonia include Anticholinergics, Benzodiazepines, Baclofen, and Dopamine agonists. Even though in some cases beneficial, these medications may cause dizziness, drowsiness, fatigue, nausea, lack of coordination and balance, and vomiting. If ultimately medications and all other treatments are unsuccessful, surgery is an option (Dystonia Treatment).

One surgery option for Dystonia patients is called ablative surgery. In this surgery the ablates,

the region of the brain that causes the abnormal movements, are located, targeted, and destroyed.

During the surgery the patient remains awake while a heated probe or electrode is inserted into the area that is targeted. This surgery is particularly severe for Dystonia cases in which the neck is affected. This surgery can be very risky because of the targeted areas' location in relation to other parts of the brain.

After the surgery the patient may have a speech impediment or other adverse affect. Another surgery

for Dystonia patients is called deep brain stimulation. This is a surgery in which an electrode, or small metal wire, is inserted through a hole in the skull into the region of the brain that controls movement.

This lead is then passed under the skin to the chest where it is then connected to two pacemakers which act as neurostimulators. A neurostimulator is a device that provides electrical stimulation to the nerves (neurostimulator). The pacemakers then send an electrical pulse to the leads that block nerve signals

that cause abnormal movement. When my brother's symptoms were at their worst point in the

progression of the disease, my parents decided the best option for him was to undergo deep brain

stimulation surgery. After many months and years of programming and tweaking, his surgery has

proved to be a successful endeavor. Even though the surgery ended up in ultimate success, his

hardships during the surgery furthered my desire to find in a cure for Dystonia. After seeing him in

intense pain after surgery, I wanted to be a part of eliminating surgery as an option for Dystonia and

supplementing it with a cure (Dystonia Treatment).

When I was younger, my intellectual capacity limited my ability to help cure Dystonia to solely doing fund raising. After I took my first year of biology in high school, I knew I wanted to contribute to

curing the disease in a research environment. Because of my extensive fund raising and my father's

involvement with Bachman Strauss Dystonia and Parkinson's Foundation, I applied and was accepted

to an internship in Dystonia research at Harvard Medical School/ Massachusetts General Hospital.

During my internship I worked for over three weeks on Dystonia research in the lab of Xandra

Breakefield, Ph.D.

Dr. Breakefield's research is centered on the understanding and treatment of diseases of the nervous system. The lab uses techniques of molecular genetics to try to uncover the workings of neurological disorders and uses vectors which have the ability to deliver genes to the nervous system (Neuroscience Faculty Member). A vector is a plasmid or viral chromosome into whose genome, or full genetic sequence, a fragment of foreign DNA is inserted. A plasmid is a circular loop of double stranded DNA that replicates independently of the chromosomes and is found in bacteria and Protozoa (Biological Vector). This laboratory identified the gene responsible for a severe form of Dystonia starting in childhood, characterized by a responsible protein called torsinA (Neuroscience Center at MGH). When I was present in the lab, there were two teams that conducted Dystonia research. The first team was a clinical trials team that investigated the use of drugs on Dystonia. The second team was a team that investigated the causes of Dystonia. I was part of the team that was investigating the causes of Dystonia.

I was teamed with a mentor and my job centered around helping to find the key causes of Dystonia on a molecular level. Most Dystonias on a molecular level have a GAG deletion in a gene encoding a protein called torsinA. In order to understand how a mutation in one's DNA causes a condition such as Dystonia, one must understand how DNA codes for proteins. This deletion is a mutation in the DNA or coding sequence of our genomes. DNA is made up of four fundamental base pairs which combine together to make a sequence that codes for particular genes. RNA's role in translation will be elaborated on later. When the sequence of the DNA within the gene is changed, the mRNA which the gene codes for is changed. When the gene within the cell is destined to become a protein the first part of its journey is transcription. This is a process in which RNA polymerase, an enzyme, produces an mRNA, or messenger RNA transcript from DNA. This process occurs within the nucleus of the cell where the DNA is located. The nucleus of the cell is at the center and is enclosed by its own membrane called the nuclear envelope. When the mRNA is made from the DNA it passes

through the nuclear envelope and then goes into the cytoplasm. The cytoplasm is the basic substance that fills the cell outside of the nucleus. Once the mRNA is exported into the cytoplasm the next destination is to begin a process called translation (Campbell).

Translation is a process in which the mRNA transcript is read and then turned into a protein product. This is the process in which the wrong functional mRNA that the Dystonia gene codes for gets translated into a protein that does not function properly which is thought to cause the symptoms of Dystonia. The process begins when the two parts of a ribosome latch onto the mRNA transcript and begin to read the bases. The nitrogenous bases of mRNA are the specific sequences that code for the proteins that the ribosomes translate. A ribosome is a cellular organelle that aids in the translation process and is made up of proteins and ribosomal RNA. The ribosome reads the bases of the mRNA in triplet pairs called codons. Each codon codes for a particular amino acid that then combines with other amino acids. When there is a mutation such as a deletion in the sequence of the DNA as is in Dystonia, the triplet reading frame of the polypeptides produced may be disrupted. This combination of amino acids coded for by each codon is in the mRNA sequence. Amino acids are the singular units of polypeptides, or units of proteins. The polypeptides coded for then are linked together until the transcript ends and then the polypeptide chain releases from the ribosome. The polypeptide chain then starts an elaborate process of folding in order to distinguish its three dimensional shape. This shape then dictates the function of the protein in the cell. The polypeptide chain is now a functional protein that is then utilized by the cell (Campbell).

In Dystonia, the base pair deletion in the gene that codes for a particular protein called torsinA causes the mRNA transcript not to be correct. As a result of the deletion, the codons differ and provide considerable difference when the mRNA transcript arrives at the ribosomes to be translated. When the mRNA is ready to be translated, since the codons are not correct, the right polypeptides are not formed. Since the wrong polypeptides are formed they can not combine to form a completely normal functional

protein. The mutant gene causes the formation of a mutant protein which is thought to be the cause of Dystonia. The cause of Dystonia is thought to be rooted in the function of certain proteins in the protein degradation pathway. In order to understand the root causes of Dystonia, we must first understand the protein degradation pathway (Nery, Flavia).

The protein degradation pathway in the cell consists of a pathway that is used to degrade proteins that are mutant, or proteins that are short lived (Protein degradation definition). Many of these proteins are tagged with a small protein called Ubiquitin that signals to the cell that these proteins are destined for degradation. Protein Degradation is a cellular function in which proteins that are misfolded or misformed are digested by the cell because they can not be used to the effectiveness of the original correct protein. The degradation pathway that was most involved in my research was the pathway associated with the Endoplasmic Reticulum. When proteins are at first transcribed from DNA into mRNA and then later translated from DNA to mRNA the proteins exit the ribosome and enter into Endoplasmic Reticulum space. The Endoplasmic Reticulum is an organelle that is attached to the nucleus of the cell. The Endoplasmic Reticulum is composed of two parts: the rough ER and the smooth ER. The rough ER is the outermost, or farthest section of the ER away from the nucleus. The rough ER is called rough because it has ribosomes attached to it. When the ribosomes on the surface of the membrane of the ER read an mRNA transcript, they produce a protein that then enters into the intermembrane space in the ER. The ribosomes bound to the outside membrane of the rough ER are constantly bound and released from the membrane. The ribosome first starts to bind to the ER once it is ready to synthesize a protein destined for a particular secretory pathway. A secretory pathway is a series of steps the cell uses to export proteins out of a cell. The rough ER is responsible also for key protein processing once proteins are in the cisternal space of the ER. The cisternal space of the ER is the space between both membranes of the ER. The next key job of the rough ER is to get the proteins ready to transport them where they are needed. The other component of the ER is smooth endoplasmic

reticulum. This is the inner part of the ER that is closest to the nucleus of the cell and does not contain ribosomes, therefore it is called smooth. One job of the smooth ER is calcium storage. The smooth ER stores calcium in muscle cells and releases it during contraction in order to trigger movement of the cell (The Endoplasmic Reticulum Organelle).

Understanding the ER's role in protein degradation is thought to be a key to understanding the molecular basis for Dystonia. Once the protein is in the ER and triggered to be degraded by the presence of ubiquitin tags, the next step in the degradation process is the traveling of the protein to a cellular organelle called the Proteasome. This organelle is the site where protein degradation occurs and is located in the cytosol or the fluid filled region outside of the nucleus of the cell. The Proteasome uses enzymes called proteases that degrade the misfolded or misformed protein by breaking them down in to their individual units called amino acids. Each protein is made up of a long sequence of amino acids. The specific order of these amino acids determines the function and shape of the protein that is going to be degraded. When an amino acid such as glutamic acid, as in the case with DYT1 Dystonia, is mutant, the shape and the function of the protein are modified. The modified protein in this case is a protein called torsinA. These proteases utilize a process called proteolysis in which they degrade the misfolded proteins into their constituent parts. Once the protein is fully degraded into its individual amino acids, the amino acids can then be reused and recycled (Protein Degradation).

The main problem that I helped in research was Dystonia's relation to TorsinA and protein degradation. TorsinA is a protein that is found in the lumen of the endoplasmic reticulum and nuclear envelope. The nuclear envelope is the two layered membrane that bounds a cell's nucleus (Videopedia). A main way to study the role of a protein such as TorsinA is to observe the actions of the protein in bacterial cells.

In order to obtain the means for quantifying the proteins involved in Dystonia, specific bacteria must be made that incorporate Dystonic DNA in to their genomes. The genome of the bacteria is the

whole DNA database that the organism implores. The first step in creating the bacteria whose proteins are going to be analyzed is called a double digestion. A double digestion is a process that uses restriction enzymes, which are enzymes that cut DNA at a specific site, to cut both the vector DNA and the Dystonic DNA. The vector used in the bacteria is a plasmid, which is a circular ring of DNA that can be incorporated into the bacteria's genome through electroporation (Campbell). Electroporation is the process of shocking the bacteria so that the vector can enter into the bacteria and then be expressed (Protocol for Electroporation). The vector has a certain promoter on it which ensures that the DNA inserted into the plasmid through the double digestion and ligation will be expressed by the bacteria once it is inserted back into its genome. In the study on Dystonia, an expression vector called PcDNA 3.0 was used because of its compatibility of restriction sites that could be used with specific restriction enzymes. The first step in the digestion was to use a specific restriction enzyme on both the DNA and the vector. These DNA samples are placed in two separate tubes where buffer, deionized water, and enzyme is added. The reaction is then allowed to proceed in a temperature controlled environment provided by an incubator. Once the two inserts are cut by the restriction enzymes they are then run out on a gel to ensure their size is right (Nery, Flavia). The process of gel electrophoresis is over-viewed later in the method of western blotting .

Once the digestion is complete, it is now time to join the Dystonic DNA with the newly cut open vector. This joining is done through a process called a ligation. A ligation is a reaction that uses an enzyme called Ligase to join the vector and the Dystonia DNA (Campbell). First, both DNA samples are mixed in a tube with a buffer, dionized water, and ligase (Nery, Flavia). The vector and Dystonic DNA are added in a specific ratio that ensures that the Dystonic DNA has a good chance at securing a place in the vector. The ligation then proceeds in an incubator to reach a specific temperature. Once the process is done, the contents of the DNA can be run on a gel once again to ensure that the reaction produced a single vector that includes the Dystonic DNA insert.

Once the vector is produced that contains the Dystonic DNA and the other DNA incorporated on the PcDNA vector it must be inserted in to the bacteria so that proteins can be later harvested for analysis. The next step in preparing the bacteria for protein analysis is a process called electroporation. This is a process in which the bacteria are shocked so that their membranes momentarily have holes big enough for the new Dystonic plasmid to enter the cell. Once the plasmid is in the cell, the bacteria can start producing the proteins required for analysis. The process begins by adding DNA, competent cells or cells ready to take the plasmid up, and other suspension components. Once the suspension is mixed, it is added to a cuvette. A cuvette is a small glass container that has two electrodes on either side that hook up to an electroporator. When the cuvette is plugged in to the electroporator, a metal current runs through the bacteria by means of the two electrodes, which allows them to take up the plasmid. Once the bacteria have taken up the plasmid they have to be cultured (protocol for electroporation).

To make sure that the bacteria have taken up the plasmid, so that the right proteins can be produced for later analysis, some of the bacteria undergo a process known as a miniprep. This procedure is used on the newly made bacteria to ensure that they contain the right DNA. Once the bacteria are obtained they are centrifuged in broth to obtain a bacteria pellet. This is the pellet from which the DNA will later be extracted. A centrifuge is a machine which spins the solution at high enough speeds that the more massive components of the solution go to the bottom of the container and then further separate depending on mass. After the bacteria is pelleted, a sodium hydroxide solution is added to the centrifuge tube. Then a lysis buffer is added to the tube in order to break open the bacteria so that their contents can be extracted. After the solution is mixed by inversion it then becomes clear. The tube is then incubated on ice for a period of time so that the DNA can precipitate. After the incubation is completed, the tube is then spun in the centrifuge once again. After the tube is done running on the centrifuge the clear supernatant liquid on the top of the solution is removed with a vacuum and the remaining part of the solution is transferred to a new tube. Isopropanol is then added to

the new tube. The new tube then undergoes another process of being centrifuged and the supernatant liquid is once again extracted by a vacuum. The tube is then inverted so that the pellet without any supernatant liquid can remain in the bottom of the tube. Once the pellet is obtained it is dissolved by a specific enzyme which is the first step in enabling it to be run on a gel by gel electrophoresis. The newly isolated DNA from the bacteria must also be dissolved by a restriction enzyme before being run on the gel to ensure that two pieces, the vector and the dystonic DNA insert, are correctly taken up by the cells. Once the gel has run, each slot in the gel specific to each corresponding colony of bacteria should show two lines in the gel that correspond to the plasmid backbone and the dystonic DNA. If these two bands are present, the correct DNA is expressed in the cell which allows bacteria to be cultured (Miniprep Procedure for Plasmids).

Bacteria are cultured so that they are able to multiply in an exponential manner, which produces an identifiable amount of protein that can later be analyzed. The bacteria are cultured on an agar plate, which provides the bacteria with all the essential nutrients the bacteria need to survive. The first step in the culture is to make the agar gel that occupies the plate that the bacteria grow on. Agar powder is first mixed with a nutrient medium and then warmed by means of a microwave. Once the agar is hot enough that it can be poured into plates, an antibiotic such as penicillin or streptomycin is added to the agar. These antibiotics have a crucial role in insuring only the desired bacteria that have the Dystonia plasmid present will grow on the plate. All other bacteria will die because of their lack of antibiotic resistance to the antibiotic. Because a specific antibiotic resistance plasmid was used when ligation occurred, only the Dystonic bacteria should grow on the agar plates. Once the agar contains the antibiotic, it is added to petri dishes where it is then left to solidify. Once the agar is solid, bacteria and culture media are then added. The bacteria plates are then put into an incubator where heat helps the bacteria to multiply exponentially. Once the bacteria are produced, they have multiplied copies of the Dystonia plasmid as well as multiplied as a whole colony. Because of this, bacteria can be frozen for

later use in experiments in case the plasmid needs to be recovered or the bacteria need to be cultured again (Nery, Flavia). Once the bacteria have produced proteins for analysis, an analytical method called western blotting is used in the lab.

Western blotting is a common technique used in the lab to show levels of torsinA within a given cell is called Western blotting. Western blotting is also commonly referred to as immunoblotting because a specific antibody is used specifically for its corresponding antigen. An antibody is a specialized immune protein and the antigen is the substance that is capable of causing the production of an antibody. The nature of the antibody and antigen reaction that occurs during the Western Blotting process can produce qualitative data about a given protein. The process of Western Blotting consists of many steps, the first of which is gel electrophoresis (Proteomics- Overview of Western Blotting).

Gel electrophoresis is a lab technique that separates charged molecules or proteins based on charge or mass. These charged molecules or proteins are forced through a gel by an electric current that runs throughout the gel. The specific form of gel used in the lab was called a polyacrylamide gel. This specific type of gel separates proteins by size. Once the gel is obtained, it has precast wells at the top of it in to which samples of proteins are added. These proteins are obtained from a specific cell to which they can be traced back. When the samples are ready to be loaded, they are loaded with a micropipet. A micropipet is a measuring instrument that measures precise volumes of media for the transfer to the wells. Once the gel is loaded it is put into a dock and then loaded with a running buffer. A running buffer is a liquid that carries the charge through both ends of the gel tank, which in turn helps to move the current through the gel. When the current is applied the proteins move down through the gel and create bands that can be compared to each other. The position of the band within the lane in respect to how far down the band has moved from the well indicates the size of the protein. When the proteins are later stained by a protein stain, the thickness of the protein band indicates the abundance of the protein which can be compared to the other samples that were initially loaded. After the sample of proteins is

run through a gel a water wash is used to wash any remaining buffers from the gel matrix. After this an acid or alcohol wash is then used to fix the gel which limits the further movement of the protein bands within the gel matrix. Next, the gel is treated with a stain reagent that allows the dye to diffuse into the gel and then bind with the proteins. The last step is destaining to remove the excess dye from the gel matrix so the proteins are left stained (Overview of Gel Electrophoresis).

Once gel electrophoresis is completed, the next step in completing a western blot is the transfer of proteins out of the gel to the membrane. Now that the gel electrophoresis step is completed, the gel contains proteins that need to be extracted in order to gain quantitative data about the experiment. In this case, protein has to be transferred out of the gel to compare the levels of protein accumulation in normal compared to Dystonic cells. The process in which the proteins are transferred from the gel to the membrane is called electroelution. Electroelution is the most efficient way to transfer the proteins out of the gel to the membrane because of its speed and accuracy. In this process, the protein containing gel is put in direct contact with a nitrocellulose membrane. The nitrocellulose membrane binds the proteins once they are pulled out of the gel by the flow of electricity that will push the proteins towards it. The gel and nitrocellulose membrane are put together to form a sandwich and then are submerged in a conducting solution called a buffer. The sandwich and buffer are contained in a running tank which has electrodes at both ends. Once the electricity is turned on, the proteins move from the gel into the nitrocellulose membrane and become tightly fixed. Once the process is over with, the result is a copy of the protein pattern from the gel on the nitrocellulose membrane. The membrane is then stained with a solution such as Ponceau S which is a staining solution to show the presence of proteins. The left over gel may also be stained to make sure all of the proteins have fully moved out of the gel. After the membrane is stained, it goes through a series of washing steps that remove unbound agents from the membrane. This washing is said to increase the signal-to-noise ratio. The noise is the data in the membrane that is irrelevant because of the incomplete or irregular binding of nonspecific proteins. An

ideal blot would have a high signal-to-noise ratio because the noise would be minimized and not interfere with the normal binding of proteins. This lack of interference would in turn make the blot more efficient and reliable. After the blot is washed, it is probed by primary and secondary antibodies. These antibodies bind to the proteins that are bound to the membrane (antibody definition). The antibody binds to the protein which is called the antigen (antigen definition). There are two common types of antibodies that are used. In my research we used monoclonal antibodies which are derived from mice because they bind specifically to the protein torsinA. The other type of antibodies commonly used are called polyclonal and are derived from rabbits. Once the primary antibodies are bound to the proteins and once the secondary antibodies are bound to primary antibodies, the membrane is ready to be subjected to detection methods. Either chemiluminescent or fluorescent substrates are then bound to the secondary antibodies on the membrane. The fluorescent or chemiluminescent substrates let the sites where the protein is present emit light. The blot is first covered with a solution to prevent it from touching the film that it is enclosed in. The blot is then exposed to X-ray film in a casing which lets only the chemiluminescent or fluorescent portions of the membrane emit photons, or small packets of light, which expose the film. This process is done in a dark room so other light will not distort or show up on the film. The film is then put into an imaging machine which prints pictures of the blot in order to quantify the data (Proteomics- Overview of Western Blotting).

In the blots that I helped conduct, a variety of proteins were traced in various blots. One main protein that was traced was called GFP-CFTR. GFP is a protein tag called green fluorescent protein which can be traced in specific cells. This protein tag allows the CFTR protein to be traced in a given cell. CFTR is a Cystic Fibrosis membrane protein that can be traced in the lumen of the endoplasmic reticulum of cells. The main purpose of doing western blots was to tell the difference in the accumulation of the GFP-CFTR proteins in the cell between the Dystonic cells and the wild type or normal cells. When the blots were analyzed the mutant torsinA or Dystonic cells showed an increase in

the accumulation of GFP-CFTR in the western blots. This increase in proteins suggests that they were not being degraded normally by the cell and their accumulation was shown by their abundance in the western blots. The abundance in the western blots was shown as a more distinct bold line than the normal wild type cells. The wild type cells did not show the level of accumulation of protein that mutant torsinA cells showed. This research seems to suggest one of torsinA's possible roles in Dystonia is in the degradation of proteins. Dystonia may be caused by a build up of proteins in the ER because of mutant torsinA (Nery, Flavia).

Another protein that was traced in the Dystonic and wild type cells was a protein called BiP. BiP stands for binding immunoglobulin protein and serves as an endoplasmic reticulum stress sensor (BiP is feed-back). This means that when BiP is present in high amount the cell is more stressed (ER, a specialized set of). The presence of BiP in various cells was compared with the accumulation of GFP-CFTR by western blot analysis. When the cell was stressed the BiP level rose, and as a result the cells Dystonic or mutant torsinA cells showed a higher accumulation of GFP-CFTR than the non-stressed cells. A stressed cell is a cell that is subjected to a change in environment, such as temperature. While I was present in the lab, the research on the effect of stress on the accumulation of proteins causing problems in the degradation pathway was not a central focus, but it was something that was investigated towards the end of my stay (Nery, Flavia).

Another way the accumulation of proteins was traced in cells was confocal microscopy. In order to use a confocal microscope on cells we had to first infect fibroblasts in a cell culture. We used a specific virus that contained Dystonic DNA, to inject the Dystonic DNA into the fibroblasts, or skin cells, to then be expressed. Once the fibroblasts were infected, they started to exhibit the characteristics in the culture of typical cells that were affected by Dystonia. The characteristics of typical Dystonia cells include the accumulation of proteins in the ER and retention of Dystonic DNA. Once they started to do this, they had to be plated out. The process of plating out cells consists of taking a few starting

cells and introducing them to their own plates that have a growth media on them. The growth media on the cells allows the cells to multiply quickly and provides all of the essential nutrients for the cells to grow and flourish. Once the cells are grown to their correct density they are taken to the confocal microscope (Nery, Flavia).

This microscopy is intended to detect co-localization of specific locations of proteins in the cell. Each protein was dyed with a corresponding fluorescent protein tag. These fluorescent protein tags allow the protein to be shown in its current location in the cell, due to its association with proteins that are characteristic of Dystonia. The fluorescent protein tags are incorporated into the genomes or full DNA database of cells through the use of separate vectors such as viruses that transfect the cells. A viral transfection is when a virus permeates a cell and incorporates its viral DNA into the host cell's DNA. This transfection is performed in a cell culture with Dystonic fibroblasts or patient skin cells. These cells are harvested from patients for the purpose of research and are kept alive on cell lines which establish these cells as a source for researchers of Dystonic cells for a long time. Once the cells are harvested, they are transfected in culture which provides the protein tags later needed for confocal microscopy (Gene Therapy: Techniques of). Cells are grown in a medium that provides all the essential nutrients required for the cells to grow. The environment has to stay sterile or bacteria and fungus will colonize the culture and ruin the cells. Each time the cells are taken out of storage, they must be examined under a microscope to check to see if they are infected by bacteria or fungus. If they are infected, the cells must be thrown out and the process must be completely restarted. Once the cells are grown to the correct volume on the cell culture plate, they must be harvested for investigation under a confocal microscope. Cells can then be removed from the culture plate and made available to be plated onto a slide. They can then be examined under a confocal microscope (Tissue Culture Methods).

Once the cells are plated, they are taken to a confocal microscope which uses high energy light to make proteins tagged with fluorescent protein tags show up as a picture (How does a confocal). The

protein to be degraded was tagged with another fluorescence protein called GFP or green fluorescence protein. When light was emitted by the confocal microscope the proteins in the cell tagged with GFP were shown in a specific location. This process was used on both the natural state cells as well as the Dystonic cells. The purpose of this confocal microscopy was to determine whether a specific protein was being degraded in a Dystonic or a natural state cell. The cells that contracted Dystonia were shown to have problems in the protein degradation pathway that was clearly visible due to the use of the confocal microscope. Once the desired image is acquired, a picture can be taken for use as a figure in the final paper. For the figure to be valid, the experiment has to be repeated three times with results that agree with each other. Figures from earlier blots and gels will also be added to the final paper by means of using a scanner. The same rule is valid for repetition for each aspect of the experiment. When I left my summer internship, the paper confirming Dystonia's role in the protein degradation pathway and in endoplasmic reticulum associated degradation was being finalized. Researchers confirmed that there was an accumulation of protein in the endoplasmic reticulum that they think contributed to Dystonia's symptoms. When I left, the repetition of experiments still needed to be completed before the paper was published. Current research includes continuing exploring the role of stress on a cellular level in Dystonia. Proteins in the cells were tagged with a stress protein called BiP that could be traced when the cell is under stress such as temperature or other stress. The prevailing thought is that when a Dystonic cell is under a particular stress it can trigger the symptoms of the disease. This is shown when cells tagged with BiP are subjected to a stimulus that stresses the cell. Proteins are then traced throughout the cell, in hope of accumulation in the ER degradation pathway to show increased symptoms of Dystonia when the cell is under stress. Similar tracing of proteins and brain abnormalities can be done by examining mouse models that are specifically produced for their help in the study of Dystonia (Nery, Flavia).

Recently, researchers have been attempting to develop mouse models that have Dystonia. A

mouse model is a mouse that acts as a model for a human case of Dystonia. This model would show the same symptoms, have the same brain abnormalities, and have the same protein interactions as a human case of Dystonia. These mouse models exhibit the TorsinA protein as well as show the symptoms of Dystonia. These mouse models are also a key part in the practice and implementation of medical devices that can alleviate the symptoms of Dystonia. One of the key questions that remains unanswered in Dystonia is the role of Dystonia in a carrier for a person who is a mutant for the Torsin1A gene. When an individual is a mutant for the Torsin1A gene, the physical symptoms of the disease have a low degree of onset, approximately thirty percent. Many researchers are working to learn why certain people may have the mutation characteristic of Dystonia but show no visible symptoms of the disease, where a person with the same mutation might be bed ridden with convulsions. This problem has led researchers to develop specific mice models that are used in conjunction with brain imaging techniques to analyze what is happening in the brain in manifesting cases of Dystonia versus cases that show no symptoms in Torsin1A mutant cases. These mice models can be better analyzed to understand disease mechanisms than previous subjects that can not be controlled in a lab setting. When these mouse models were analyzed, the DYT1 carriers, or the carriers for the Torsin1A mutation, were shown to have increased metabolic activity in certain parts of the brain. They also analyzed these mice during sleep which ended up showing an increase in the same amount of metabolic activity in the brain suggesting it is a genetic problem. These mice were also subjected to MRI scans in which their brains were analyzed. The results of the imaging showed specific abnormalities that caused reduced integrity in motor pathways. They also found in the non-manifesting cases of Dystonia in the mice that there is another brain pathway abnormality that might cause the symptoms of the disease not to manifest in these cases. New methods of animal imaging had to be developed in order to find these innovations in Dystonia research. Research in mouse models will continue, as they are a valuable asset. Even though these mouse models are helping to uncover the basis for Dystonia, the research has not yet promised a

cure (Ulig, Aziz).

Because we can not cure Dystonia yet, the best treatment for the disease is to treat its symptoms. Right now, the best treatment for Dystonia called deep brain stimulation. Deep brain stimulation is the surgery mentioned earlier in this paper that my brother had. It incorporates pacemakers implemented in the chest to metal leads that connect to the brain to intercept false brain signals that cause Dystonia. This surgery was first an experimental treatment for Dystonia, but now is being more widely prescribed to many patients (Going Global). The effectiveness of the surgery is constantly being improved by using better pacemakers or other methods but it is never one hundred percent effective. This surgery is also circumstantial depending on each case so the results of the surgery many vary. Since this surgery targets a specific part of the brain for treatment there is also a prevailing thought that there may be ways to target the same part of the brain with pharmacological agents in order to restore normal function to a given person (Dystonia Treatment).

Scientists have recently been trying to find torsinA effector molecules that act to alleviate the effects of Dystonia. As mentioned earlier, the secretion pathway of Dystonia cells is impaired compared to the normal wild type cells. In this research fluorescent proteins were traced through patient fibroblasts that have the torsinA mutation in order to see if certain antibiotics are successful in improving cell secretion. The thought is that if antibiotics can enhance the amount of secreted protein from the cell secretion pathway in Dystonia patient fibroblasts, the cause of the disease may be able to be treated with antibiotics. When patient Dystonia fibroblasts treated with an antibiotic called ampicillin were compared with normal wild type cells, the patient ampicillin treated fibroblasts were able to secrete the same amount of protein as the normal wild type cells suggesting that ampicillin may help to enhance the secretion pathway in Dystonic cells. If this research confirms that ampicillin can alleviate the lack of cell secretion in Dystonic cells, the next step will incorporate the implementation of mouse models to test antibiotic treatment of the disease in the mouse models. The application of

antibiotics to Dystonia seems to be a promising sector of research and will provide an alternate to a mechanical surgery such as deep brain stimulation. Besides treatment of Dystonia, there has been an effort in science to directly attempt to modify the mutant genes that cause Dystonia (Cao Songsong).

On a molecular level, scientists are currently trying to figure out the genetics of Dystonia. In the field of genetics there has been an effort of genome sequencing of humans. Genome sequencing is when a human's entire genetic sequence of DNA is decoded to its composition of bases which can then tell us what the role of specific genes is. Scientists in the field of Dystonia are attempting to screen the whole genome in order to look for genes associated with Dystonia. If we can find these genes, there may later be promise in coming up with molecular methods of regulating them or silencing them in order to treat the causes of Dystonia on a molecular level. In specific cells only certain regions of DNA are expressed and the other regions of the DNA that are not expressed are silenced by histone acetylation or DNA methylation. These methods ensure that the DNA that is not supposed to be accessed in a certain cell is not accessed. This lack of access constitutes what makes a skin cell a skin cell and not an eye cell or any other type of cell in the body. Since it is possible to silence certain genes, there is a thought that there may be a way to silence mutant genes to alleviate the cause of disease. Even though much of the current research in Dystonia shows signs of a possible cure, there is only current treatment available to help patients with the disease (Campbell).

In my brother's case, his results from this surgery are what has motivated me to pursue biomedical engineering in college. Biomedical engineering is a field that aims to apply mechanical engineering and other engineering concepts to systems in biology. Biomedical engineering produced the pacemakers as well as the surgery that was pivotal in my brother's surgery. I want to be a part of this movement in the future after college. I hope to establish my own lab that will attempt to come up with medical devices that will help my brother and other people like him. In college, I will also try to continue research on Dystonia or possibly research on other diseases. My lab work has given me a first

hand experience of researching a disease that is close to me that has directly fueled my interest in biology in college. Now, I am more insightful to the many aspects of research and how hard it is to actually find a cure or treatment for a specific disease. Because of this motivation, in the future I hope to work in the same lab or similar labs to continue to research Dystonia and ultimately help to find a cure for this disease.

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