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Word Count: 2443Introduction: Cystic fibrosis is an inherited autosomal recessive disease that exerts its main effects on the digestive system and the lungs. This disease is the most common genetic disorder amongst Caucasians.

Cystic fibrosis affects about one in 2, 500 people, with one in twenty five being a heterozygote. With the use of antibiotics, the life span of a person afflicted with CF can be extended up to thirty years however, most die before the age of thirteen. 1 Since so many people are affected by this disease, it’s no wonder that CF was the first human genetic disease to be cloned by geneticists. In this paper, I will be focusing on how the cystic fibrosis gene was discovered while at the same time, discussing the protein defect in the CF gene, the bio-chemical defect associated with CF, and possible treatments of the disease. Finding : The classical genetic approach to finding the gene that is responsible for causing a genetic disease has been to first characterize the bio-chemical defect within the gene, then to identify the mutated protein in the gene of interest, and finally to locate the actual gene. However, this classical approach proved to be impractical when searching for the CF gene. To find the gene responsible for CF, the principle of “ reverse genetics” was applied.

Scientists accomplished this by linking the disease to a specific chromosome. After this linkage, they isolated the gene of interest on the chromosome and then tested its product. 2Before the disease could be linked to a specific chromosome, a marker needed to be found that would always travel with the disease. This marker is known as a Restriction Fragment Length

Polymorphism or RFLP for short. RFLP’s are varying base sequences of DNA in different individuals which are known to travel with genetic disorders.

3 The RFLP for cystic fibrosis was discovered through the techniques of Somatic Cell Hybridization and through Southern Blot Electrophoresis (gel separation of DNA). By using these techniques, three RFLP’s were discovered for CF; Doc RI, J3. 11, and Met.

Utilizing in situ hybridization, scientists discovered the CF gene to be located on the long arm of chromosome number seven. Soon after identifying these markers, another marker was discovered that segregated more frequently with CF than the other markers. This meant the new marker was closer to the CF gene. At this time, two scientists named Lap-Chu Tsui and Francis Collins were able to isolate probes from the CF interval. They were now able to utilize to powerful technique of chromosome jumping to speed up the time required to isolate the CF gene much faster than if they were to use conventional genetic techniques. 3In order to determine the exact location of the CF gene, probes were taken from the nucleotide sequence obtained from chromosome jumping.

To get these probes, DNA from a horse, a cow, a chicken, and a mouse were separated using Southern Blot electrophoresis. Four probes were found to bind to all of the vertebrate’s DNA. This meant that the base pairs within the probes discovered contained important information, possibly even the gene. Two of the four probes were ruled out as possibilities because they did not contain open reading frames which are segments of DNA that produce the mRNA responsible for genes. The Northern Blot electrophoresis technique

was then used to distinguish between the two probes still remaining in order to find out which one actually contained the CF gene.

This could be accomplished because Northern Blot electrophoresis utilizes RNA instead of DNA. The RNA of cell types affected with CF, along with the RNA of unaffected cell types were placed on a gel. Probe number two bound to the RNA of affected cell types in the pancreas, colon, and nose, but did not bind to the RNA from non-affected cell types like those of the brain and heart. Probe number one did not bind exclusively to cell types from CF affected areas like probe number two did.

From this evidence, it was determined that probe number two contained the CF gene. While isolating the CF gene and screening the genetic library made from mRNA (cDNA library), it was discovered that probe number two did not hybridize. The chances for hybridization may have been decreased because of the low levels of the CF gene present within the probe. Hybridization chances could also have been decreased because the cDNA used was not made from the correct cell type affected with CF. The solution to this lack of hybridization was to produce a cDNA library made exclusively from CF affected cells. This new library was isolated from cells in sweat glands. By using this new cDNA library, probe number two was found to hybridize excessively.

It was theorized that this success was due to the large amount of the CF gene present in the sweat glands, or the gene itself could have been involved in a large protein family. Nevertheless, the binding of the probe proved the CF gene was present in the specific sequence of nucleotide bases

being analyzed. The isolated gene was proven to be responsible for causing CF by comparing its base pair sequence to the base pair sequence of the same sequence in a non-affected cell. The entire CF cDNA sequence is approximately 6, 000 nucleotides long. In those 6, 000 n.

t.’s, three base pairs were found to be missing in affected cells, all three were in exon #10. This deletion results in the loss of a phenylalanine residue and it accounts for seventy percent of the CF mutations. In addition to this three base pair deletion pattern, up to 200 different mutations have been discovered in the gene accounting for CF, all to varying degrees. The Protein Defect: The Cystic Fibrosis gene is located at 7q31-32 on chromosome number seven and spans about 280 kilo base pairs of genomic DNA. It contains twenty four exons.

4 This gene codes for a protein involved in trans-membrane ion transport called the Cystic Fibrosis Transmembrane Conductance Regulator or CFTR. The 1, 480 amino acid protein structure of CFTR closely resembles the protein structure of the ABC-transporter super family. It is made up of similar halves, each containing a nucleotide-binding fold (NBF), or an ATP-binding complex, and a membrane spanning domain (MSD).

The MSD makes up the transmembrane Cl- channels. There is also a Regulatory Domain (R-Domain) that is located mid-protein which separates both halves of the channels. The R-Domain is unique to CFTR and is not found in any other ABC-transporter. It contains multiple predicted binding sites for protein kinase A and protein Kinase C. 4 Mutations in the first MDS

are mainly found in exon #4 and exon #7. These types of mutations have been predicted to alter the selectivity of the chloride ion channels.

4 Mutations that are in the first NBF are predominant in CFTR. As previously mentioned, 70 percent of the mutations arising in CF cases are deletions of three base pairs in exon #10. These three base pairs give rise to phenylalanine and a mutation at this site is referred to as DF508.

5 Such a mutation appears not to interfere with R-Domain phosphorylation and has even been reported to transport chloride ions. 6; 7 There are five other frequent mutations that occur in the first NBF. The first is a deletion of an isoleucine residue, DF507. The second is a substitution of glycine or amino acid #551 by aspartic acid/F551D. The third involves stop mutations at arginine #553 and glycine #542.

The fourth is substitutions of serine #549 by various other residues. The fifth is a predicted splicing mutation at the start of exon #11. 7Mutations within the R-Domain are extremely rare. The only reason they do occur is because of frameshifts. Frameshifts are mutations occurring due to the starting of the reading frame one or two nucleotides later than in the normal gene translation. 4Mutations in the second membrane spanning domain of the CFTR are also very rare and have only been detected in exon #17b. These have no relevance to mutations occurring in the first membrane spanning domain. They apparently do not have a significant impact on the Cystic Fibrosis Transmembrane Conductance Regulator either.

4Mutations in the second nucleotide-binding fold occur frequently in exon #19 and exon #20 by the deletion of a stop signal at amino acid number

1282. Exon #21 is sometimes mutated by the substitution of asparagine #1303 with lysine #N1303K. 4The Bio-Chemical Defect: Studies of the chloride channels on epithelial cells lining the lungs, sweat glands, and pancreas have shown a consensus in that the activation of chloride secretion in response to cAMP (adenosine 3′, 5′-monophosphate) is impaired in cystic fibrosis cases. Another affected, independently regulated chloride channel that has been discovered is activated by calcium-dependent protein kinases. Sodium ions have also been noted to be increasingly absorbed by apical sodium channels. 8 Therefore, the lack of regulated chloride ion transport across the apical membranes and apical absorption of sodium ions, impedes the extracellular presence of water.

Water will diffuse osmotically into cells and will thus cause the dehydration of the sol (5- mm fluid layer of the cell membrane) and the gel (blanket of mucus) produced by epithelial cells. 9 As a result of this diffusion of water, airways become blocked and pancreatic proteins turn inactive. An Account of the Absorption and Secretion of Cl-, Na+, and Proteins: An inward, electrochemical Na+ gradient is generated by the Na+, K+-ATPase pump located in the basolateral membrane (the cell side facing the organ it is lining). A basolateral co-transporter then uses the Na+ gradient to transport Cl- into the cell against its own gradient. This is done in such a way that when the apical Cl- channels within the membrane spanning domain open, Cl- diffuse passively with their gradient through the cell membrane. 4In pancreatic duct cells, a Na+, H+-ATPase pump is used and a bicarbonate secretion is exchanged for Cl- uptake in the apical membrane. Chloride ions then diffuse passively when the Cl- channels are opened.

Such secretions also allow for the exocytosis of proteins in the pancreas which will later be taken into the small intestines for the breaking down of carbohydrates. 4In addition to the pump-driven gradients and secretions, there exists autonomic neurotransmitter secretions from epithelial cells and exocrine glands. Fluid secretion, including Cl-, is stimulated predominately by cholinergic, a-adrenergic mechanisms, and the b-adrenergic actions. 4 Such chemical messengers cannot enter the cell, they can only bind to specific receptors on the cell surface and transmit messages to and through an intracellular messenger such as Ca2+ and cAMP by increasing their concentration. The intracellular message is transmitted across the cell by either diffusion or by a direct cascade. One example of a directed cascade is the following: Possible Treatments For Cystic Fibrosis: One suggested treatment for CF has been to provide the missing chemicals to the epithelial cells. This can be accomplished by the addition of adenosine 3′, 5′-monophosphate (cAMP) or the addition of the nucleotide triphosphates ATP or UTP to cultures of nasal and tracheal epithelia.

This has been proven to alter the rate of Cl- secretion by removing the 5-mmeter sol layer of fluid in the respiratory tract. 9 Moreover, luminal application of the compound amiloride, which inhibits active Na+ absorption by blocking Na+ conductance in the apical membrane, reduced cell secretion and absorption to a steady state value. Another treatment that has been suggested is to squirt solutions of genetically engineered cold viruses in an aerosol form into the nasal passages and into the lungs of people infected with CF. This is done in hopes that the virus will transport corrected copies of

the mutated gene into the affected person’s airways so it can replace the mutated nucleotides.

10 This form of treatment is known as gene therapy. A different approach taken in an attempt to cure cystic fibrosis involves correcting the disease while the affected “ person” is still an embryo. Test tube fertilization (in vitro fertilization) and diagnosis of F508 during embryonic development can be accomplished through a biopsy of a cleavage-stage embryo, and amplification of DNA from single embryonic cells.

5 After this treatment, only unaffected embryos would be selected for implantation into the uterus. Affected embryo’s would be discarded. Conclusion: Chloride conductance channels have dramatic potentials. One channel can conduct from 1×106 to 1×108 ions per second. 8 This is particularly impressive when you consider the fact that there are not many channels present on cells to perform the required tasks. As a result of this, a mutation of one channel or even a partial mutation of a channel, that causes a decrease in the percentage of channel openings, can exert a major effect. Even the mildest of cures altering the Cystic Fibrosis Conductance Regulator in CF afflicted people would lead to significant improvements in that individuals health. Since cystic fibrosis is the most common genetic disorder, particularly amongst Caucasians, in today’s society, intense research efforts towards its cure would be invaluable.

When will cystic fibrosis be completely cured? No one can say for sure but, strong steps have already been taken towards reaching this goal.