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The Cystic Fibrosis Gene

S.J. Louise Smith

Abstract

It's now well over a year since the cystic fibrosis gene was cloned and there is still much to be done before its localisation can be translated into an improvement in health care for affected people. I'm not going to go into any details on how the gene was located, for this information (which is rather technical) see ref. 1. However to put it rather bluntly, despite the fact that the gene has been localised and sequenced has been sequenced, no-one really knows what it does. The cystic fibrosis gene has been named the CFTR gene (cystic fibrosis transmembrane conductance regulator). It is located on the long arm of chromosome 7 and is composed of 27 exons which code for 1,400 amino-acid residues. There seem to be several different final products of the CFTR gene which result from the removal of exons from the first nucleotide binding fold. The functional significance of these products is not known.

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Table III. Advantages and Disadvantages of amniocentesis**Advantages**

- All pregnancies with a 1 in 4 risk of cystic fibrosis can be assessed.
- No prior family workup is required
- Assays may be completed within 1 hour of receipt of the sample

Disadvantages

- Decreased accuracy of diagnosis (cf.CVS). Increased false positives and false negatives
- If termination is required then it will have to be a late termination (18-19 weeks).

major complication of advanced cystic fibrosis. Initially patients had a poor response to transplantation but success rates are now improving. There have been no reports of the epithelial transport defects occurring in the new lung, although the defects are still present above the site of the anastomosis.

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The Cystic Fibrosis Gene

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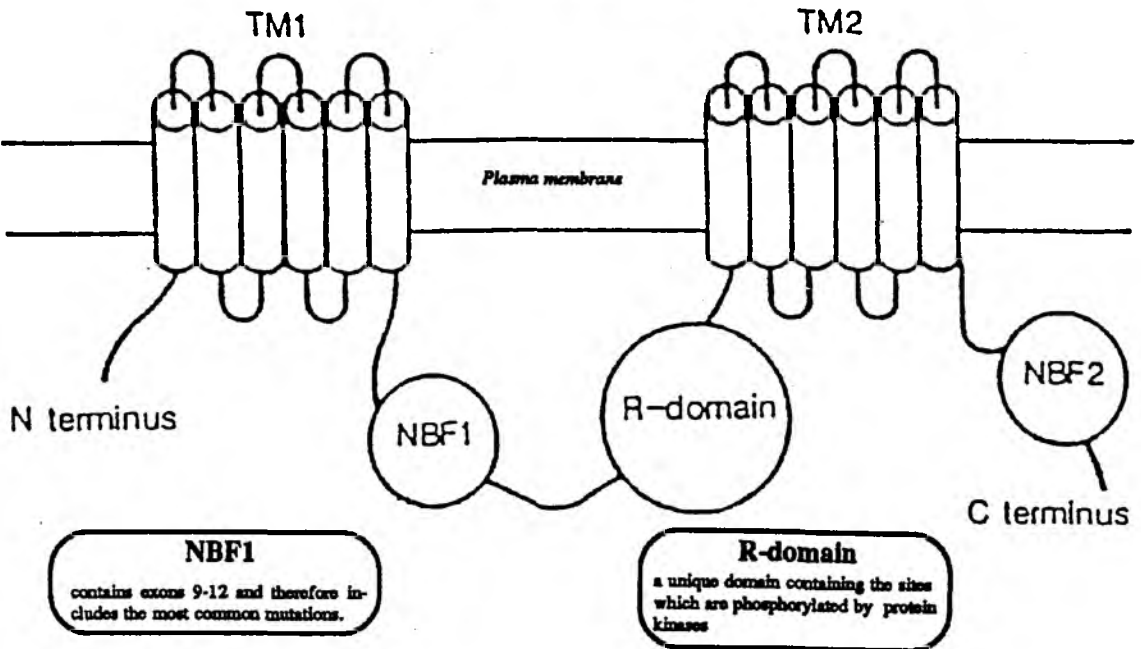


Fig.1. Schematic model of the Cystic Fibrosis gene product (CFTR). TM =Transmembrane region. NBF =nucleotide binding folds.

As I have already stated, the actual protein, and its function in man, is not known, however the sequence of the amino-acids forming the gene is known and this can be used. (directly and in comparison to other genes) to predict a structure for the protein. Using this information it is thought that the gene codes for a large trans-membrane protein which is very similar to several transport proteins all of which have in common the coupling of ATP hydrolysis to the pumping of molecule into or out of the cell. A basic diagram of the suggested structure is shown (Fig.1). These findings agree with what was already known about the pathogenesis of cystic fibrosis; that is that the disease seemed to be caused by defective transport of chloride ions across epithelia. Unfortunately the story is not that simple, it is thought that the CFTR gene product is *not* the ion-channel involved. There are several reasons for this;

1) The CFTR-protein is coupled to ATP-hydrolysis whereas the normal ion channels are not.

2) Normal ion channels allow ions to flow across them in both directions while the CFTR-protein has already been shown to be very like a family of transport proteins which only allow active transport in one direction.

3) Chloride channels seem to be different in different tissues but the mutations found in all C.F. patients so far have been defects in a single gene.

4) The CFTR-protein seems to, quite simply, be too big to be a ion channel.

So, what does the protein actually do? It has been suggested that the protein might transport a molecule which regulates the chloride channel; another explanation is that the protein handles a substrate which

Cystic Fibrosis mutations

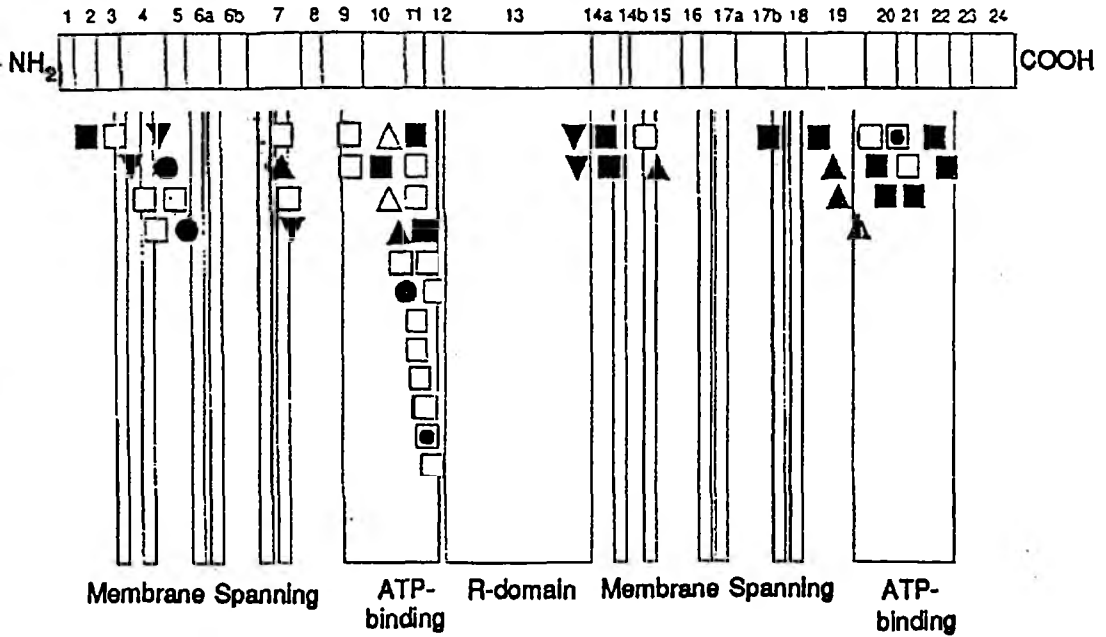


Fig.2. The CFTR gene exons. Each symbol shows the location of one identified mutation.

acts as a regulator on the chloride channel⁽²⁾. No doubt more information will be discovered in the near future since this question seems to be central to the whole field of cystic fibrosis.

The finding of the CFTR gene has meant that the exact location of this protein in the tissues of the body can now be determined. This has been done by raising antibodies to the CFTR protein and examining where these antibodies attach to tissues. Preliminary results show that the antibodies attach to sweat glands and to a subset of T-cells but not, as would be expected bearing in mind the clinical observations, to the pancreatic cells. Furthermore it is thought that the antibodies bind specifically to the reabsorptive and not the secretory side of the sweat glands. Further studies are, of course,

needed to confirm the exact location of CFTR and these surprising results need to be explained with respect to the clinical disease.

A lot of work has been going on all over the world in an attempt to explain what has gone wrong with the CFTR gene in patients with cystic fibrosis; in others words the identification of the mutations in the gene. This has been found to be more complicated than would have been wished, at present well over 60 different mutations have been found in the gene. The most common of these is the deletion of a phenylalanine residue in about 70% of all patients. In Scotland this is the most common mutation also, and accounts for about 73% of the mutations in this group of patients; the next two commonest mutations between them account

for a further 11.4% of patients. These two mutations both involve a glycine residue, in the first instance glycine is changed to an aspartine residue, in the second mutation the code for glycine is changed to a "STOP" code which terminates the synthesis of the protein. The information on all mutations when it is collected together shows a rather unusual pattern: it seems the the largest number of mutations are clustered together in the area of the first nuclear binding fold (NBF₁). (Fig.2.) The reason for this, again, is not really known but the second NBF has certainly been examined for mutations exhaustively. It has also been observed that there are no large deletions or rearrangements anywhere in the gene which is surprising given that the CFTR gene is very large.

Attempts have been made to match the mutations in the gene (remember that it is a recessive disorder so there are two mutant copies) to the observed clinical situation in the patient, for example the most common mutation described as a "severe" mutation while other mutations found in the exons 1,9 and 12 are "mild". Surprisingly two patients with both genes containing the common and severe mutation have been found to have pancreatic sufficiency which confounds the above hypothesis. Such attempts to match the patient's symptoms to the genetic cause of the disease will probably only be successful once the molecular basis of the disease is actually understood.

All that I've written so far seems rather negative however some progress has been made; one of the most exciting developments in the last year has been the success of correcting abnormal chloride channel permeability in cultured CF cell by getting them to express normal CFTR⁽³⁾. This

sounds promising but it must be remembered that there is a huge jump from gene insertion into the genome of a cultured cell *in vitro* to possible gene therapy in patients with cystic fibrosis. This is the situation in all aspects of gene therapy, for all different diseases despite the optimism of many people. It has been suggested that it could be possible to deliver a normal CFTR gene to the airway epithelial cells by use of an aerosol delivery, this kind of treatment depends on the use of retroviruses to get the new gene inserted into the patient's DNA. These techniques of retroviral therapy *in vivo* are sadly not yet adequately developed, but who knows what will happen in the future?

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