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Terminal glycosylation and disease: Influence on cancer and cystic fibrosis

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Terminal glycosylation has been a recurring theme of the laboratory. In cystic fibrosis (CF), decreased sialic acid and increased fucosyl residues in α1,3 position to antennary *N*-acetyl glucosamine is the CF glycosylation phenotype. The glycosylation phenotype is reversed by transfection of CF airway cells with wtCFTR. In neuronal cells, polymers of α2,8sialyl residues are prominent in oligodendrocytes and human neuroblastoma. These findings are discussed in relationship to early studies in our laboratories and those of other investigators. The potential extension of these concepts to future clinical therapeutics is presented.

Keywords: **α1,3fucose, α1,6fucose, sialic acid, polyα2,8sialic acid, surface membrane glycoforms, cystic fibrosis, lactosylated polylysine gene therapy vector**

Introduction

The article is divided into two sections. The first section concerns early studies of sialic acid and mammalian surface membranes leading to the description of the terminal glycosylation of membrane glycoforms of cancer cells. It is pointed out how these investigations are relevant today since they provided the foundation to devise experiments in other laboratories, in addition to our own.

The second section summarizes some of our studies on cystic fibrosis (CF), the most common lethal genetic disease of Caucasians. CF has a glycophenotype, which is expressed by altered terminal glycosylation. Background and current concepts are woven into this section. Taking into consideration the altered terminal glycosylation phenotype, the biosynthetic machinery necessary, and current concepts of cell biology, a hypothesis is proposed to account for the glycosylation phenotype in CF. Our work with a synthetic glycoconjugate, lactosylated polylysine, is part of a thrust to diverse genetic and molecular strategies for the future treatment of CF.

This is not a comprehensive review, but the reader will find references to review articles. The focus is on our first hand information, although many other laboratories have advanced the concepts. Whenever possible, we have attempted to illustrate the role of our studies in the current understanding of glycobiology in the year 2000 and beyond.

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Monosaccharide to polymer

Sialic acid

Sialic acid represents a perfect carbohydrate with which to look back and yet go forward into the new millennium. It is hard to imagine that sialic acid broke into the biological scene only 40 years ago. The early history of sialic acid has been reviewed [1] and therefore only a few comments are necessary to stress the excitement of participating in the characterization of *N*-acetyl neuraminic acid [2]. Over the years numerous modifications of the simple sialic acid were found [3,4] and the carbohydrate must be a 'citation classic'. For example, the early investigations were stimulated by the binding of influenza virus to cells by a sialylated mechanism. Gottschalk [5] showed that sialic acid was removed by a viral sialidase. In spite of the many reports which followed [3] including the X-ray crystal structure demonstrating the binding of the virus to cell-surface receptors [6] and cloning of the sialyl transferase genes [7], many questions remain. In fact, a recent report of Spray et al. [8] clearly demonstrated that influenza virus will bind and infect desialylated MDCK cells. This provacative report reopens the role of sialylation in viral attachment to cells. For this reason and many others [9,10], sialic acid will remain a major topic for the future.

Polysialic acid

Currently many biological phenomenon are attributed to sialic acid mostly based on the charge effect [9]. The most highly charged are homopolymers of α2,8 sialyl residues—termed polysialic acid. The function of the sialic acid polymers on the neural cell adhesion molecule, N-CAM, first described by Edelman [11] has been studied extensively [12]. The polymer has been shown to be longer or shorter influencing the migration of cells from the neural crest. Recent information [13] can be used to suggest that polysialic acid is present on other molecules in addition to N-CAM and the Na^+ channel [14]. Indeed, the metastatic potential (simulating migration) of human neuroblastoma was attributed to the presence of polysialic acid which was found on neuroblastoma metastatic to the bone marrow [15]. A similar situation was found in small cell lung carcinoma [16] and other tumor cells [17].

Several genes have now been cloned for polyα2,8sialyltransferases, which synthesize longer polymers of α2,8 sialic acid. STX, now called ST8SiaII, was cloned by Livingston and Paulson in 1993 [18]. It was not until several years hence that the identity of the gene product was shown to be a polyα2,8sialyltransferase [19]. Other groups cloned a polyα2,8sialyltransferase from hamsters [20] and mice [21] and humans [19,22]. The different characteristics of the cDNAs and the enzymes were used to show that two different polyα2,8sialyltransferases exist, ST8SiaII (STX) and ST8SiaIV (PST) both of which sialylate N-CAM, albeit differently [23]. A third enzyme ST8SiaIII has been cloned but the transferase has not yet been fully described [24] although it has been shown to sialylate N-CAM and other substrates [25].

In spite of many investigations, the biosynthesis of polysialic acid is not fully established in mammalian cells even though the original studies on colominic acid in bacteria appeared decades ago [26]. One of the issues yet to be answered is whether or not one ST8 enzyme can synthesize more than one linkage, which would be necessary if the same enzyme that synthesized the polymer could add the initial α2,8 sialyl residue to an α2,3 or α2,6 sialyl residue on an existing glycoprotein. In many cases it appears that only one enzyme is necessary [25] but others provide evidence for an initiating enzyme [27,28]. In addition, a recent elegant study expands the existence of multiple sizes of oligomers and dimers in pig brain suggesting that multiple polyα2,8sialyltransferases exist [13]. These investigations open the field for additional studies.

Human neuroblastoma was used to show the presence of polysialic acid in mammalian tumors and greater than 55 sialyl residues were found in the polymer [29]. Neuroblastoma was also a source of investigations of the neurotoxin responsive Na^+ channel. The glycoprotein nature of the channel [30] as well as the influence of terminal glycosylation on the activity [31,32] were expanded by others [14] to show that polysialic acid was present on the purified channel.

A role for a specific polyα2,8sialyltransferase has been proposed during the maturation of oligodendrocytes, the myelin forming cells of the CNS [33]. Examination of the biosynthesis and mRNA expression of polysialic acid has led to the conclusion that shorter oligomers exist in addition to polymers of 10 sialyl residues and larger. Moreover, the length of the polymers of α2,8 sialic acid is related to the maturation of oligodendrocytes and thus, their ability to myelinate. The larger sialyl polymers are present when the oligodendrocytes are migrating and shorter oligomers are synthesized when the cells mature and begin to form myelin [34]. Finne and colleagues have recently described the network made possible by polymers of α2,8 sialyl residues. They suggested that this filament bundle network may be responsible for neural migration [35]. These reports open new and potentially important areas for the control of neural functions. Terminal glycosylation with sialic acid homopolymers remains a vibrant area for the new millennium.

Surface membranes

The isolation of surface membranes from mammalian cells was pioneered by several groups in the early 1960s. The most popular methods involved exploding the cells followed by density gradient separation of the 'outer' membranes [36–38]. Our laboratory devised several methods to isolate intact surface membranes from cells in culture providing the first proof that the surface membrane existed as an organelle which was independent from internal membranes [39]. Figure 1 shows a photomicrograph of a preparation of surface membranes [40]. Note that the membranes are morphologically identifiable.

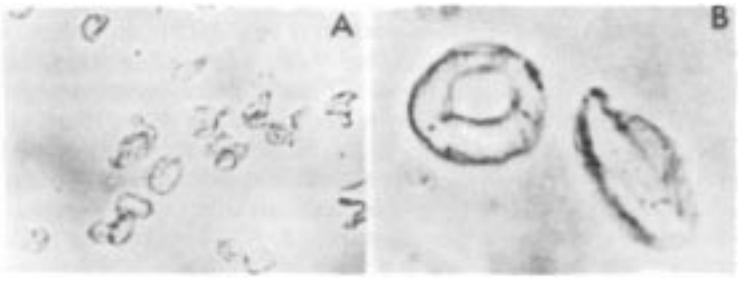

Figure 1. Surface membranes isolated from L cells by the zinc ion procedure. Phase contrast. (A) ×350, (B) ×1400 (40).

A variety of methods were devised and these early investigations have been reviewed [41,42]. Defining the surface membrane as a distinct and interactive organelle provided the foundation for the elucidation of the many phenomena such as locomotion, adhesion, signal transduction, endocytosis, and channel activity, which are now known as cell membrane functions.

Surface membrane glycoforms in cancer

Multi-antennary oligosaccharides

Glycoforms of normal and transformed cells were proposed in 1970 based on the size characteristics of glycopeptides by gel filtration [43–45]. Further extension of the identity of the glycoforms based not only on size but also charge and sialic acid analysis showed that multi-antennary oligosaccharides were found on the surface membrane glycoproteins after viral transformation. A schematic representation of these studies was presented [46] and is shown in Figure 2. Although the complex forms are valid even today, they do look primitive in comparison to today's structural information. Twenty or more investigations by our laboratory [47] and others followed, confirming that the difference in membrane glycopeptides was due to more highly branched oligosaccharides after transformation. Some of these are reviewed and termed the Warren/Glick effect [45]. Studies which added support to the structure were performed using 500 MHz ^{1}H-NMR spectroscopy [48] and demonstrated sialic acid was a requirement for transformation. At that time the tedious efforts of sequencing oligosaccharides by enzyme degradation [49] were solved by the NMR spectrum [48]. The laboratories of Schachter [50] worked out many of the enzymatic pathways for much of oligosaccharide synthesis, which along with the findings of the multi-antennary oligosaccharides (Warren/Glick effect), lead to the proposal that GlcNAcTV was the responsible glycosyltransferase [51]. The development of GlcNAcTV null mice and the potential commercialization of this finding was then explored (Reviewed in 52).

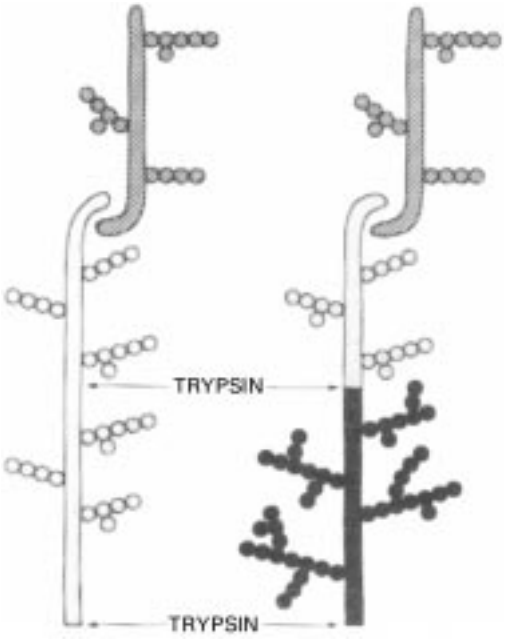

Figure 2. Simplified arrangement of membrane glycoproteins. The circles represent monosaccharides attached to a polypeptide backbone, each unit representing a Pronase fragment. The trypsin fragments are indicated. The black areas are the glycopeptides characteristic of virus transformed cells. The open and clear areas represent glycopeptides found in the normal cells. The hatched areas are loosely associated glycoproteins. Note the branching of the oligosaccharides [46].

Although GlcNAcTV is necessary for branching, it was shown that immortalized cells (NIH3T3, for example) had membrane glycoproteins which were highly branched and that the difference after transformation with an oncogene was in terminal glycosylation [48]. As shown by high resolution ^{1}H-NMR spectroscopy, the multi-antennae were capped with α-Gal residues before transformation. After transformation, the α-Gal residues were replaced by sialic acid. Nevertheless, as pointed out above, other laboratories [52] have pursued the concept that GlcNAcTV is the key enzyme for the glycosylation observed in malignant transformation. We proposed that higher branching may come with immortalization or as a first step in transformation. The final step of transformation involving the glycoform would be sialylation [48].

The direct effect of the glycoforms of the surface membrane on the oncogenic properties of the N-myc transformed cells was shown with the use of swainsonine (SN), an inhibitor of α-manosidase II. SN, which prevents the synthesis of matured branched oligosaccharides, reversed the ability of the malignant cells to form colonies in soft agar [53]. These studies published in 1987 have since developed into clinical trials [52,54]. It will be interesting to learn how these early results complement the recent report that overexpression of bamacan/SMC3 causes transformation [55].

Fucosyl residues αl,6 to core GlcNAc

The original membrane glycoforms of transformed cells were defined with glycopeptides made radioactive by metabolic labeling with L-[^{3}H]fucose. This allowed the definition of the fucosylated glycoforms with multi-antennae, which were shown to be related to malignancy [56] as well as many types of transformation as discussed above [45,47,57]. It has been our experience that the majority of the membrane glycoproteins of cultured cells have α1,6fucosyl residues at the core GlcNAc. However, the study of α1,6fucosyltransferase has been difficult due to the substrate requirements of a biantennary oligosaccharide with terminal GlcNAc residues [58,59]. More recently, Taneguchi and his colleagues have pursued the fucosylation of the core GlcNAc in relationship to certain tumors. They have cloned the enzyme [60] so it is expected that the significance of α1,6FucT, which adds a

terminal Fuc on the core GlcNAc, will be unraveled in the immediate future [61]. On this note, Stubbs et al. [62], showed that α1,6fucosylation at the core GlcNAc altered the configuration of a biantennary oligosaccharide.

Fucosyl residues α1,3 to antennary GlcNAc

In studying the glycoforms of human neuroblastoma cells, significant amounts of terminal fucosyl residues in α1,3 linkage to antennary GlcNAc (Fucα1,3/4GlcNAc) were found [63]. The use of a specific α1,3/4fucosidase from almonds, which was originally reported by Kobata and his group [64], made this possible. The presence of Fucαl,3GlcNAc was further confirmed by ^{1}H-NMR spectroscopy [65], which unequivocally demonstrated fucosyl residues in α1,3 position on human neuroblastoma surface glycopeptides. The enzyme, α1,3fucosyltransferase was purified from human neuroblastoma [66] with the intent to clone the gene for the enzyme. About that time John Lowe and his colleagues began the cloning of a series of α1,3fucosyltransferases [67] and were generous with making their clones available. Although it has never been shown using molecular biology procedures, the α1,3FucT from human neuroblastomas, had the substrate characteristics [66], that led to the proposal that the neuroblastoma enzyme is FucTIV [68]. To date, cDNAs of six human α1,3FucTs have been cloned and characterized. Indeed, at the same time there was an increase in the number of laboratories investigating molecules containing this terminal glycosylation in connection with the selectin ligands [69]. The biotech industry has pursued this subject aggressively because of the extravasation of leukocytes into areas of inflammation [70,71]. Terminal glycosylation with fucosyl residues in α1,3 linkage to GlcNAc which was virtually unknown in 1980 as a cell-reactive carbohydrate [63] has become a major topic for the new millennium.

Surface membrane glycoforms in cystic fibrosis

Early observations of Dische and his colleagues [72] showed that sialic acid was deficient and fucose was increased in CF duodenal fluid. A summary of the literature has recently lead to the accumulation of more than 40 references confirming decreased sialylation and/or increased fucosylation in CF glycoconjugates [73]. CF, a common lethal genetic disease [74], has the phenotype of altered terminal glycosylation.

Skin fibroblasts

In early studies, skin fibroblasts could be obtained from CF patients and grown in culture. This allowed sufficient cells to examine α-L-fucosidase [75,76] and to perform GLC analyses [77] and eventually ^{1}H-NMR analysis [78] on partially-purified membrane glycopeptides. The analyses revealed that CF membrane glycoconjugates had terminal glycosylation differences when compared with those obtained from normal age, race, and sex matched fibroblasts. The structure, given in Figure 3, marks the glycosylation phenotype in CF, increased fucosylation and decreased sialylation. The increase in α1,6 fucosylation lead to the purification of this enzyme from the fibroblasts [79]. The enzyme was cloned by Taniguchi and his colleagues [60], who are also studying the potential importance of the enzyme as discussed in the previous section.

The presence of α1,3fucosylation on the antennary GlcNAc of the oligosaccharide (Figure 3) is another example of information obtained by ^{1}H-NMR analysis. The importance of Fucα1,3GlcNAc has been shown in CF airway epithelial cells and will be discussed further under *Airway Epithelial Cells*.

The decrease in sialic acid of the CF fibroblast oligosaccharides supported a reciprocal relationship of sialyl and fucosyltransferases [80]. The cloned cDNA for the α1,3FucTs' [67] and NeuAcTs' [7] will allow further study of these terminal glycosylation changes in CF fibroblasts.

CFTR

The gene, CFTR (cystic fibrosis transmembrane regulator), which when mutated causes CF, was cloned in 1989 [81] therefore little attention has been given by investigators to the glycosylation phenotype in the past decade. Although CFTR has been shown to be an apical Cl^- channel, other roles for this transmembrane glycoprotein, have been proposed and in the past several years a number of investigators have addressed the intracellular location of CFTR [82].

To define the roles for CFTR in addition to its function as a Cl^- channel, various intracellular compartments have been implicated as responding to CFTR. These include roles in vesicle trafficking [83] and acidification [84] although both

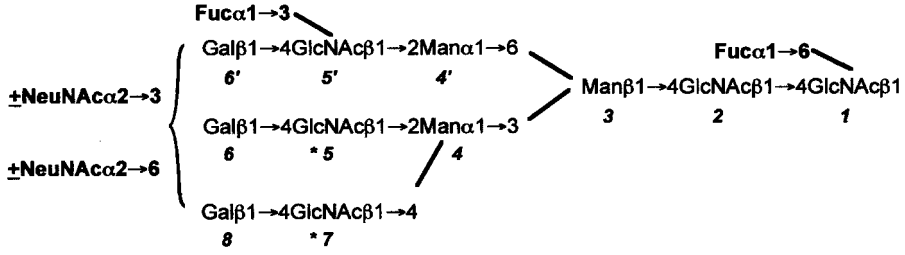

Figure 3. Oligosaccharide structure obtained from ^{1}H-NMR spectroscopy of glycopeptides isolated from membrane glycoproteins of CF fibroblasts. The monosaccharides shown in bold represent the glycosylation phenotype of CF, * potential α1,3fucosylation site [78].

areas have generated positive and negative results. In general, it was proposed that ΔF508 CFTR did not reach the surface membrane and thus could not function as the Cl^- channel [85]. In studies to determine whether or not mutant CFTR was present in the surface membrane preparations we isolated surface membranes from airway epithelial cells as shown in Figure 1. With the use of antibodies to CFTR, we showed that mutant CFTR was localized in morphologically identifiable surface membranes. The turnover ratio of surface membrane CFTR was lower than the CFTR of internal membranes. Moreover, mutant CFTR was more stable in the surface membrane fractions. Although the experiments could not distinguish between a pool of CFTR in or near the surface membrane, these studies suggest that a recycling pathway is defective in CF [86]. A comprehensive description of some of the many studies of intracellular CFTR and the potential consequences of the localization has been recently published [87]. However, no mechanisms are as yet forthcoming to substantiate the various hypotheses. It does however seem valid to attribute roles to CFTR in addition to that of an apical Cl^- channel.

Airway epithelial cells

The lungs are the site of morbidity and mortality of CF patients due to leukocyte infiltration and bacterial infection. Although surface membranes were isolated from the CF airway cells [88] and shown to contain CFTR, very little was known about the membrane glycoproteins of CF airway cells. We first showed an increase in a α1,6fucosylation by the binding of the CF airway cell membrane glycoproteins to lentil lectin-Sepharose [89]. Subsequently it was reported that the other monosaccharides of the glycosylation phenotype, decreased sialic acid and increased Fucα1,3GlcNAc (Figure 3) were observed among CF membrane glycopeptides from airway epithelial cells [90]. Importantly using a CF airway epithelial cell line transfected with wtCFTR, it was shown that the glycosylation phenotype was reversed by the expression of wtCFTR [91]. That is, when wtCFTR was expressed in CF airway cells, the Cl^- channel function was restored and the terminal glycosylation of the membrane glycoproteins returned to that of normal airway cells. Thus, the reciprocal relationship of NeuAc to Fucα1,3GlcNAc has been shown to be modulated with the expression of wtCFTR in the airway cells. Figure 4 gives an example of the fucosylation of the airway epithelial cells under the influence of ΔF508 CFTR. As the expression of wtCFTR decreases, terminal fucosylation increases leading to the glycosylation phenotype of CF.

Terminal glycosylation in the form of α1,2fucosylation was also shown in reciprocal relationship to α1,3fucosylation [92]. In the case of α1,2 fucosylation, the expression of mRNA and enzyme activity of FucTII was demonstrated in the CF airway cells. The biosynthetic machinery was present but did not reflect the surface expression of terminal glycosylation. The fucosyl residues in α1,2 linkage to Gal were not present on CF

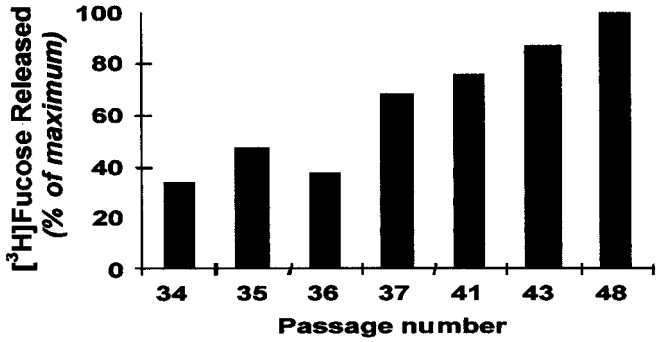

Figure 4. Modulation of Fucα1,3/4GlcNAc with the expression of wtCFTR. Fucose was released by an α1,3/4fucosidase [108]. After passage 40, wtCFTR was decreased. The amount of wtCFTR as detected by Western blot analysis decreased from 40 to 5 pixels ($\times 10^6$) at P34 and P48, respectively. See Rhim et al. [91] for details.

airway cell glycoproteins although they were present on the non-CF airway cell glycoproteins. Due to this finding as well as our earlier studies [86,89], we proposed the hypothesis that another function of wtCFTR is to aid in sorting terminal glycosyltransferases to the proper compartments within the transGolgi network (TGN). Mutant CFTR would disrupt Golgi compartmentalization, placing α1,3FucT in a position prior to NeuAcT or α1,2FucT. Since all three enzymes compete for the same substrate, when α1,3FucT acts, the other two are silent, giving the CF glycosylation phenotype. This is represented in Figure 5 using a scheme from Allan and Balch [93], which depicts protein sorting by directed maturation of Golgi compartments. The bracket shows where CFTR could potentially effect glycosylation. Our hypothesis is different from that proposed by Barasch *et al.* [84], who reported that the acidification of the Golgi was responsible for the effect on glycosyltransferases. Our hypothesis was presented [92] and experiments are underway to test the validity of an additional role for CFTR. It should be noted that this new role for CFTR could also relate to the sulfation changes observed in CF glycoconjugates [94].

Functions of terminal glycosylation in CF

Terminal glycosylation may have a functional consequence in the pathology of the CF lung. Soon after birth, the lung becomes colonized with bacteria and leukocytes infiltrate. It has even been proposed that in the CF airway leukocytes are present prior to bacterial infection [95]. The presence of terminal glycosylation, decreased sialic acid, and increase Fucα1,3GlcNAc on the CF airway cells may provide the initial target for both the bacteria and the leukocytes.

We have recently shown that *Haemophilus influenzea*, which colonizes the CF lung, binds more avidly to CF airway epithelial cells than to non-CF airway cells. Moreover, the binding was inhibited by low concentrations of fucoidan (Figure 6) suggesting a fucose-related receptor for *H. influenzae* [96].

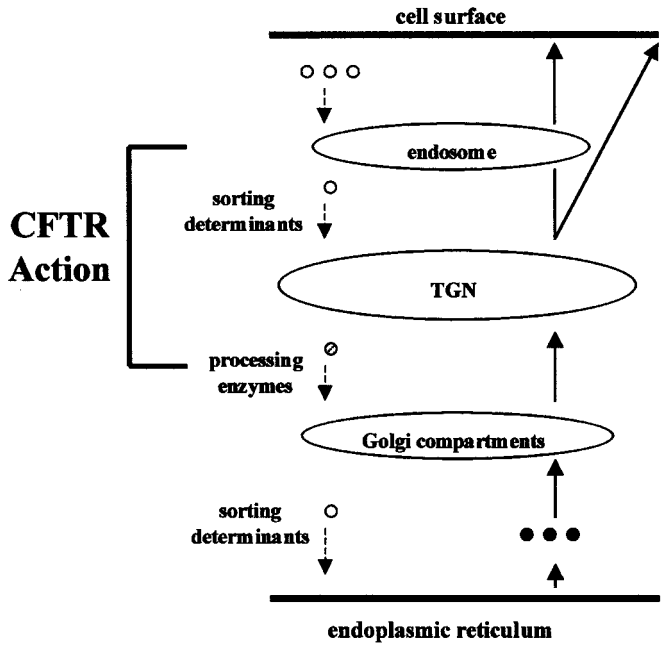

Figure 5. Schematic representation of protein sorting by directed maturation of the Golgi compartments proposed by Allen and Batch [93]. We hypothesize that CFTR acts on the sorting determinants in the TGN shown in brackets (redrawn from reference 93). When mutated, CFTR alters the compartmentalization of glycosyltransferases, the CF glycosylation phenotype results [92].

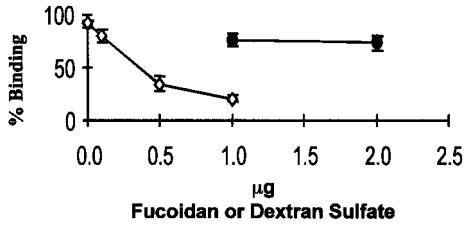

Figure 6. Inhibition of the binding of *H. influenzae* to CF airway cells. Fucoidan (◇) or dextran sulfate (●) were added directly to *H. influenzae* for 1 h at 5°C. Bacteria (10^7) containing the noted concentrations of fucoidan or dextran sulfate were inoculated directly onto CF/T43 cells (10^5) grown on a coverslip. After 90 min the coverslips were fixed and stained. The bacteria were counted (per 100 cells) in a microscope [96].

The binding of *Pseudomonas*, which is also a major pathogen in the CF airway [74], has been shown to have an asialo-substrate [97] and the Fuc binding protein of *Pseudomonas*, PAII, inhibits ciliary binding in CF tracheal explants and the inhibition is reversed by Fuc [98].

The adherence of neutrophils to CF cells showed a similar phenomenon. That is, after stimulation, neutrophils have greater adherence to the CF airway cells. This adherence is inhibited by fucoidan. Stimulated with phorbol esters, TNFα, or fMLP, neutrophils have lesser adherence to non-CF cells and no inhibition with fucoidan. CF and non-CF cells in primary culture give the same neutrophil adherence as the cell lines [99].

Terminal glycosylation has thus figured in laboratory studies of CF since the original observations by Dische in 1959 [72] and is currently an active area. The field has been recently reviewed [73]. The coordination of information on sialylation and fucosylation allowed the proposal of a hypothesis for the action of CFTR (Figure 5) on glycosylation [92]. At the time that human milk oligosaccharides were being fractionated on huge columns of charcoal (Personal experience MCG), it was not at all evident that biotech companies could be started on this theme—that is the inhibition of bacteria or neutrophil extravasation by glycomimetics [70]. Now after having defined the glycophenotype of CF airway cells it is of utmost importance to examine the role of the altered terminal glycosylation in the infection and inflammation which have the hallmark of CF lung disease.

Glycosylated vectors for gene therapy

Gene therapy has great potential for the treatment of genetic diseases such as CF. Since the lungs are the major site of morbidity in CF, it has been proposed that a vector containing the cDNA for wtCFTR would provide the most direct approach. Therefore, much experimentation has focused on carrying the CFTR gene with viral vectors into the lungs. The viral vectors have proved to be ineffective for CF gene therapy due to their immunogenicity as well as other factors [100,101].

The use of molecular conjugates to deliver genes has developed as an alternative approach [102]. Particularly successful has been the use of lactosylated polylysine, which has been shown to have highly efficient targeting to the surface of airway epithelial cell *in vitro* [103] and possibly to the nuclear membrane [104]. Figure 7A is a schematic representation of the transfection process. The cartoon is enhanced by the visualization of the vector/plasmid complex by electron microscopy (Figure 7B), and internalization of the complex into the nucleus of CF/T43 cells (Figure 7C). Poly-L-lysine having 40% of the ε amino groups substituted with lactosyl residues is thus a powerful vector to transfer reporter genes [103,105] or the CFTR gene [106] into airway cells in culture. This vector is easily prepared and is neither immunogenic nor toxic following carbohydrate substitution [105,107]. The vector alone is readily sprayed into mouse lungs and taken up by the airway cells. It is anticipated that with further refinement, lactosylated polylysine will be a successful vehicle for gene transfer in CF.

Closing comments

Structural analyses related to function were performed at many stages of our investigations utilizing the current or most applicable methodology at that time. Beginning investigations with one uncharacterized molecule of sialic acid and proceeding to biological functions such as metastasis and the

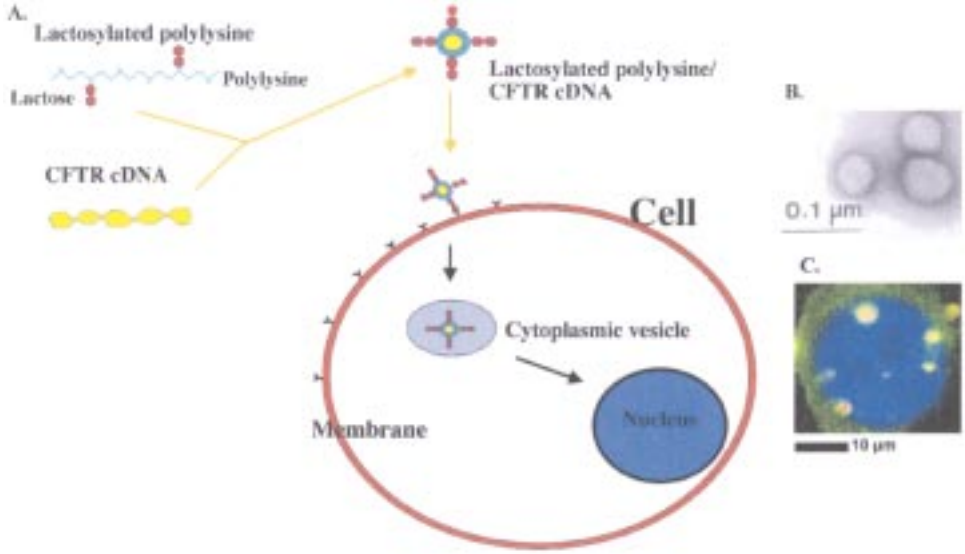

Figure 7. Schematic representation of transfection of CF cells using lactosylated polylysine as vector. A) Scheme depicting the preparation and entry into the cell of lactosylated polylysine/plasmid complex, which is visualized by photomicrographs of the actual process: B) electron micrograph of the lactosylated polylysine/pCMV*LacZ* complex; and C) confocal microscopy showing a nucleus (blue) of CF/T43 cells containing the internalized lactosylated polylysine/pCMV*LacZ* complex. The reporter gene was labeled with rhodamine and the lactosylated polylysine was labeled with FITC, therefore a yellow signal represents co-localization of the vector/plasmid complex in the cell nucleus [104]. See details [105,109].

description of polymers of α2,8 sialyl residues in oligodendrocytes covers a span of many years. Antennary fucosyl residues have taken a similar course. Throughout the investigations, when the biochemical analyses were supplemented with ^{1}H-NMR spectroscopy in collaboration with J.F.G. Vliegenthart, information on the terminal glycosylation was obtained. It is clear that surface membrane glycoforms from transformed/tumor cells and from CF fibroblasts/airway epithelial cells have altered terminal glycosylation. The influence of the altered glycosylation on function has been shown whenever possible. The recurring theme for these two disparate diseases is sialylation and fucosylation. When one or both of these terminal glycosylations were altered, function was disturbed.

Oligosaccharides, which have been studied extensively in the past, may be developed as effective glycomimetics for the treatment of the infection and inflammation in the CF lung. The influence of terminal glycosylation of glycoforms has proved to have direct relevance to human disease. We are optimistic that in the future this knowledge will lead to the development of effective gene and molecular therapy.

Acknowledgments

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