Glycoconjugate Journal 18, 649–659, 2001

Terminal glycosylation in cystic fibrosis (CF): A review emphasizing the airway epithelial cell

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Altered terminal glycosylation, with increased fucosylation and decreased sialylation is a hallmark of the cystic fibrosis (CF) glycosylation phenotype. Oligosaccharides purified from the surface membrane glycoconjugates of CF airway epithelial cells have the Lewis x, selectin ligand in terminal positions. This review is focused on the investigations of the glycoconjugates of the CF airway epithelial cell surface. Two of the major bacterial pathogens in CF, *Pseudomonas aeruginosa* and *Haemophilus influenzae*, have binding proteins which recognize fucose in α-1,3 linkage and asialoglycoconjugates. Therefore, consideration has been given to the possibility that the altered terminal glycosylation of airway epithelial glycoproteins in CF contributes to both the chronic infection and the robust, but ineffective, inflammatory response in the CF lung. Since the glycosylation phenotype of CF airway epithelial cells have been modulated by the expression of wtCFTR, the hypotheses which have been proposed to relate altered function of CFTR to the regulation of the glycosyltransferases are discussed. Understanding the effects of mutant CFTR on glycosylation may provide further insight into the regulation of glycoconjugate processing as well as new approaches to the therapy of CF.

Keywords: **glycosylation, cystic fibrosis, airway epithelial cells, fucose, sialic acid, CFTR, CFTR transfection**

Abbreviations: **CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; wt, wild type; $\triangle$F508, CF gene mutation in which phenylalanine is substituted at position 508; TNFα, Tumor necrosis factor alpha; NMR, nuclear magnetic resonance; Fucα1,3/4GlcNAc, fucosyl residues in α1,3/4 position to antennary *N*-acetylglucosamine; FucT, fucosyltransferase; ST, sialyltransferase; TGN, trans Golgi network; WGA, wheat germ (*Triticum* vulgaris) agglutinin; SNA, *Sambucus nigra* agglutinin; MAA, *Maackia amurensis* agglutinin; PNA, peanut agglutinin; CTB, B subunit of cholera toxin.**

Introduction

The surface of the airway epithelial cell is an important and complex interface of the lung with the environment. This surface is marked by the interaction of secreted products endogenous to the host with a variety of extrinsic agents and organisms. The functions and responses of the airway epithelial cells serve to maintain normal pulmonary function. However, the cascade of events which result from mutations in the CFTR gene [1] underlie the pathogenesis of the chronic, progressive lung disease, which is the hallmark of CF [2]. A well-described feature of a variety of fractions of CF glycoconjugates has been an alteration in the ratios of monosaccharides which are in terminal positions on the branches of the oligosaccharides [3]. More recently, it has been demonstrated that this altered glycosylation phenotype, an increased ratio of Fucα1,3/4GlcNAc to sialic acid, is also present on the glycoproteins of the CF airway epithelial cells. Moreover, this CF phenotype of altered terminal glycosylation in the surface membrane glycopeptides from CF airway epithelial cells is directly related to the presence of either wt or mutant CFTR [4]. In this review, the relationship between the CF glycosylation phenotype and mutations in CFTR will be discussed. In addition, the potential therapeutic implications for the infection with specific bacterial pathogens and the robust, but ineffective, inflammatory response in the CF airway will be considered.

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CF glycosylation phenotype

The observation of altered glycosylation in CF has been documented by many different methods. Since the finding by Dische and colleagues that duodenal and pancreatic mucoprotein fractions had an increased ratio of fucose to sialic acid content [5], it has been widely reported that an alteration in glycosylation is a characteristic manifestation of the CF phenotype (Table 1).

Table 1. Glycosylation phenotype of CF airway epithelial cells

Tissue	*Cell type*	*Fraction examined*	*Method*	*Reference*
		Increased fucose		
Nasal epithelia	CF/T43[1]	Membrane glycoproteins	Lentil lectin binding	[16]
Nasal polyp	Primary cells[2]	Peripheral glycopept	Fucosidases	[4]
Trachea	Primary cells	Cilia	PA II fucose binding lectin	[59]
Lung	wtCFTR-/-mouse[3]	Lung sections	Lectin binding	[60]
Nasal epithelia	CF/T43	Membrane glycopeptides	Fucosidases	[4]
Trachea	CF/T1[4]	Membrane glycopeptides	Fucosidases	[4]
		Decreased sialic acid		
Nasal polyps	Primary cells	Secreted glycoprotein/lipid	Radioassay	[18]
Nasal epithelia	Primary cells	Cell surface	Flow cytometry	[19]
Nasal polyps	Primary cells	Cell surface	Lectin binding	[21]
Trachea	9/HTEo-[5]	Cell surface	Cholera toxin binding	[61]
Nasal epithelia	CF/T43	Peripheral glycoconjugates	Chemical	[4]
Nasal epithelia	Primary cells	Peripheral glycoconjugates	Chemical	[4]
Trachea	CFT/1	Peripheral glycoconjugates	Chemical	[4]

[1]Immortalized nasal polyps (ΔF508/ΔF508).
[2]Cells from tissue grown in primary culture.
[3]wtCFTR-/-mouse, wtCFTR knockout mouse.
[4]Immortalized tracheal epithelial cells (ΔF508/ΔF508).
[5]Immortalized non-CF human tracheal epithelial cells with constitutive expression of wtCFTR.

This body of work represents research on a number of different types of cells by a number of different methods. The data point to a characteristic decrease in sialic acid and an increase in fucose content of glycoconjugates on the surface of CF cells. The following is a selected review detailing the CF glycosylation phenotype with an emphasis on the airway epithelial cell. For a more comprehensive review, see [3]. It is important to note that the altered terminal glycosylation phenotype which has been confirmed many times in CF intestinal mucins is not characteristic of CF airway mucins [6]. Roussel and colleagues have implicated the inflammation and infection in the CF lung as an important factor in modifying the glycosylation of CF airway mucins. They report that TNFα increases the expression of specific glycosyltransferases and sulfotransferases in human bronchial mucosa [7]. For a review of CF airway mucin glycosylation, see Lamblin et al. [6].

Altered fucosylation in CF fibroblasts

Early studies on membrane glycoproteins employed CF fibroblasts as it was postulated that these cells were not subjected to the damaging pathophysiology of the disease [8]. The monosaccharide content of partially purified released membrane glycopeptides from a total of seven fibroblast cell lines obtained from CF patients and matched controls was analyzed by gas liquid chromatography [9,10]. These studies showed that the hydrolyzed CF fibroblast membrane glycoproteins had an increased ratio of fucose to the monosaccharides mannose and galactose as compared to matched controls. Additionally, analysis of a high molecular weight glycoprotein found in the fibroblast medium of both CF and control cultures found a significant difference in carbohydrate composition of the CF isolate as compared to controls—the mean fucose content of the CF glycopeptides versus normal controls was 2.33 ± 1.7 and 0.31 ± 0.05 nmol/10^6 cells, respectively [9].

Similar membrane glycoproteins and secreted fibronectin obtained from CF fibroblasts were further characterized by 500-MHz ^{1}H-NMR spectroscopy [11]. NMR analysis confirmed the aforementioned finding of increased fucosylation of CF membrane glycopeptides. Fucosyl residues were found in α1,3 linkage to branch GlcNAc in CF but not non-CF membrane glycopeptides. Fucose was found in α1,6 linkage to GlcNAc-1 in CF and non-CF oligosaccharides. However, the ratio of fucosyl residues in α-1,6 linkage to GlcNAc1 relative to NeuNAc was 1:5 versus the 1:15 ratio seen in oligosaccharides of non-CF glycopeptides. Further, this analysis revealed the presence of triantennary oligosaccharides in CF cell glycoprotein fractions. Interpretation of the findings of this analysis led to the proposal of the oligosaccharide structure derived from CF fibroblast glycopeptides (Figure 1).

Altered fucosylation in CF airway epithelial cells

As the site of the most lethal pathology in cystic fibrosis is the respiratory tract [2], research focused on the analysis of the airway epithelium (Table 1). Immortalized airway epithelial cells became available [12–14] and were studied. CF/T43, nasal epithelium from a CF patient homozygous for the ΔF508 mutation immortalized by the SV40 T-antigen [12], and BEAS-2B [15], bronchial epithelium immortalized by adeno/SV40 virus were particularly useful. These cells were incubated with [^{3}H]Fuc [16] and membrane glycoproteins were released and

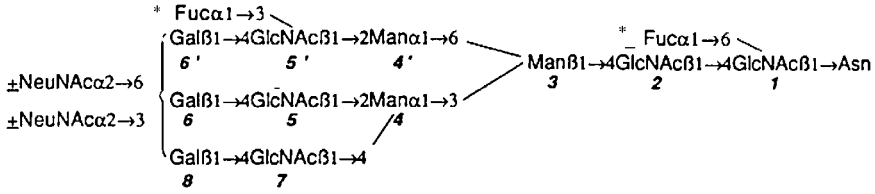

Figure 1. Oligosaccharide structure obtained from ^{1}H-NMR spectroscopy of glycopeptides isolated from membrane glycoproteins of CF fibroblasts. *Denote the fucosyl residues and ± denotes the decrease in sialic acid on one or more branches, representing the glycosylation phenotype of CF. α1,3 fucosyl residues can also occur on GlcNAc 5 or 7 [11].

partially purified as previously described [10]. Subsequent binding of CF and non-CF ^{3}H-glycopeptides to lentil lectin Sepharose displayed a result similar to that found from the fibroblast experiments—43% of glycoproteins from CF/T43 cells bound to the lentil lectin versus 23% of glycoproteins from BEAS-2B cells. These results confirmed the presence of more Fucα1,6GlcNAc on CF oligosaccharides than the non-CF cells. Table 1 summarizes additional studies demonstrating increased fucosylation in CF airway cells.

In another subsequent study [4], CF/T43 and BEAS-2B cells were incubated in culture with [^{3}H]Fuc, and the glycopeptides were exposed to almond α-1,3/4 fucosidase. Similar to prior studies, the amount of Fucα1,3/4GlcNAc was found to be increased 9-fold in CF cells compared to non-CF cells. Similar analysis yielded 9-fold more Fucα1,3/4GlcNAc in CF polyps than in non-CF polyps.

Specific fucosidases [17] were used to compare the content of Fucα1,3/4GlcNAc and Fucα1,2Gal of membrane glycopeptides in CF/T43 and BEAS-2B cells. An inverse relationship was demonstrated: CF cells had a high ratio of Fucα1,3/4GlcNAc to Fucα1,2Gal, whereas non-CF cells had a low ratio. This trend was also demonstrated in CF and non-CF primary cells. However, CF/T43 cells displayed 50% greater activity in α1,2 fucosyltransferase assays than BEAS-2B cells, which was consistent with the finding of 50% less α1,2 fucosyltransferase mRNA in BEAS-2B cells compared to that of CF/T43 cells. Thus, this reciprocal relationship of Fucα1,3GlcNAc and Fucα1,2Gal on the surface membranes in CF cells was not due to lack of biochemical machinery.

Altered sialylation of CF cells

Barasch et al. [18] examined the sialylation of SV40 immortalized airway epithelium from a CF nasal polyp and from non-CF trachea (Table 1). In their first experiment, these cells were incubated with [^{3}H]*N*-acetyl mannosamine overnight for 12 h and neuraminidase-treated transferrin for 4 h. The transferrin from the culture supernatant was isolated. Subsequent treatment with neuraminidase yielded 50% less sialic acid from the CF transferrin than non-CF transferrin. As the rate of incorporation of [^{3}H]*N*-acetyl mannosamine and [^{125}I]transferrin in the cytosolic fractions of CF and non-CF cells were equal, these results suggested that sialylation of secreted transferrin is decreased in the CF cells.

To further study the sialic acid content of all secreted glycoproteins, cells were incubated [18] with [^{3}H]*N*-acetyl mannosamine and [^{35}S]methionine for 18 h. The amount of [^{3}H]sialic acid incorporated into the secreted glycoproteins from the normal cells was significantly greater than from the CF cells. Additionally, the amount of cytosolic incorporated [^{35}S]methionine were similar. Hence, it was suggested that sialylation of most secreted glycopeptides is decreased in immortalized CF airway cells.

Finally, cells were incubated with [^{14}C]butyrate and [^{3}H]*N*-acetyl mannosamine to analyze glycolipids [18]. After cell extraction, gangliosides were analyzed by thin layer chromatography. Using appropriate standards, it was shown that CF cells had a higher ratio of asialoGM_1:sialoGM_1 compared to non-CF cells (2.8 versus 2.3, respectively).

Asialo GM_1 residues from CF and non-CF airway cells from primary culture were subsequently quantified [19]. Using flow cytometric analysis, they found that 12% of CF cells and only 2.9% of control cells contained surface asialo GM_1 residues ($P = 0.03$). It was also shown that binding to CF cells by [^{125}I]-labeled *Pseudomonas aeruginosa* derived pilin could be competitively inhibited by the addition of asialo GM_1. The investigators suggested that the phenotype of decreased sialylation in CF cells could lead to an increase in the amount of nonsialylated ganglioside residues, one of which, asialo GM_1, could play a role in *P. aeruginosa* adhesion. Hence, this was one of the first studies to relate pathophysiology, increased *P. aeruginosa* binding and infection of airways, to the phenotype of altered glycosylation in CF airways.

A number of investigators have attempted to characterize the sialylation of membrane glycoconjugates by lectin binding. An early report analyzing the composition of glycoconjugates of various types of skin cells with a panel of lectins yielded no difference in lectin-binding characteristics between CF and non-CF cells [20]. However, numerous studies have subsequently been conducted [3], and the consensus of these studies concluded that there is a difference in lectin binding between CF and non-CF cells.

To address the question of whether sialic acid specific lectin-binding characteristics are altered in primary CF nasal polyps, 47 non-CF and 14 CF nasal polyps were analyzed with a panel of biotinylated lectins [21]. Among the lectins employed were wheat germ (*Triticum* vulgaris) agglutinin (WGA) which binds GlcNAc and neuraminic acid residues (Neu5Ac); *Maackia*

amurensis agglutinin (MAA) which binds α-2,3 sialic acid—Galβ-1,4 GlcNAc; and *Sambucus nigra* agglutinin (SNA) which binds α-2,6 sialic acid—GalNAc. After quantitating lectin binding with computer assisted microscopy, it was found that there was a significant difference between CF and non-CF epithelium in the staining intensity of WGA bound to the glandular epithelium, SNA staining intensity in the glandular epithelium, and the number of cells with positive MAA staining in the surface epithelium.

While analysis of cell surface glycoconjugates by lectin-binding experiments serves to be a straightforward and efficient method, such experiments can be susceptible to variables leading to imprecision. For example, while affinities of certain lectins have been characterized, binding of lectins are not entirely specific [22]. Hence, it has been suggested that all lectin-binding experiments be interpreted with parallel binding inhibition studies employing the appropriate carbohydrate ligand [22]. Unfortunately, many of the published reports of lectin-binding experiments in CF cells lack appropriate inhibition studies. Additionally, there exists a limited number of lectins specific for sialic acid residues. The use of a wide panel of such lectins in binding experiments thus provides only a qualitative estimate of total sialic acid content on the cell surface.

To quantify the total sialic acid content of airway cell membrane glycoproteins, partially purified membrane glycopeptides were analyzed [4] by the thiobarbituric acid assay [23]. It was shown that CF/T43 cells had more than a three-fold decrease in sialic acid content compared to non-CF BEAS-2B cells. More importantly, similar analysis showed a significant two-fold decrease in sialic acid content in CF primary cells compared to non-CF primary cells. To date, this study is the most comprehensive analysis of total sialic acid content of CF and non-CF airway epithelial cells.

Fucosylation and sialylation are modulated by CFTR expression

Since the identification of the CF gene, numerous methods have been used to transfect CFTR cDNA into cells and subsequently express CFTR in cells [24]. Consequently, a number of investigators have used these techniques to probe the role that CFTR expression has in the alteration of terminal glycosylation in CF, as summarized in Table 2.

To address the role that mutated CFTR could play in the alteration of terminal glycosylation, C127 mouse mammary epithelial cells, which do not express CFTR, were transfected with ΔF508 CFTR cDNA or wtCFTR cDNA [25]. Sialylation of membrane glycoconjugates was characterized by WGA and SNA lectin binding and a fluorescent cholera toxin B probe, which is specific for the glycosphingolipid GM_1. C127 cells expressing wtCFTR had approximately the same level of sialylation as naïve C127 cells. However, C127 cells expressing ΔF508 had significantly decreased amounts of sialic acid compared to naïve and wtCFTR-expressing C127 cells. This was early evidence that exogenous expression of mutated CFTR could lead to an altered glycosylation phenotype.

The effect of the addition of ΔF508 CFTR expression on terminal glycosylation was further investigated [26]. Normal human tracheal epithelial cells, 9/HTEo$^-$, which endogenously express wtCFTR, were transfected with ΔF508 cDNA by a

Table 2. Modulation of CF terminal glycosylation phenotype by CFTR expression

Cell type[1]	*Transfection*[2]	*Compared with non-transfected cells*[3]	*Reference*
CF/T1	wtCFTR	↓ Fucα1,3/4GlcNAc; ↑ SA	[62]
	wtCFTR	↓ Fucα1,3/4GlcNAc; ↑ SA	[4]
	wtCFTR	↓ Fucα1,3/4GlcNAc; ↑ Fucα1,2Gal	[17]
	wtCFTR	↓ PNA[4]	[29]
IB3	wtCFTR	↓ PNA	[28]
	wtCFTR	↓ PNA	[29]
	wtCFTR	↓ asialo GM_1	[27]
CFPAC	wtCFTR	↓ asialo GM_1	[27]
9/HTEo$^-$	CFTR R-domain	↑ asialo GM_1	[63]
	ΔF508	↓ SNA[5]	[26]
	CFTR R-domain	↓ SNA	[26]
C127	ΔF508	↓ WGA[6]	[25]

[1]Cell line in which the experiments were conducted: CF/T1, immortalized CF tracheal epithelial cells (ΔF508/ΔF508); IB3, CF bronchial cells (ΔF508/W1282X); CFPAC, immortalized CF pancreatic epithelial cells; 9/HTEo$^-$, immortalized non-CF human tracheal epithelial cells with constitutive expression of wtCFTR; C127, mouse mammary epithelial cells.
[2]cDNA transfected into and expressed in the cell type denoted: wtCFTR, wild-type CFTR cDNA; ΔF508, ΔF508 CFTR cDNA; CFTR R-domain, cDNA of the R domain of wtCFTR.
[3]Result of experiments as compared to cells not expressing the denoted cDNA: ↓, decreased compared to non transfected cells; ↑, increased compared to non-transfected cells.
[4]Binding of PNA (reported specificity to galactose residues linked β 1 $\rightarrow$ 3 to *N*-acetyl-D-galactosamine in the terminal position).
[5]Binding of SNA (reported specificity to sialic acid linked α-2,6 to GalNAc).
[6]Binding of WGA (reported specificity to GlcNAc and sialic acid residues (Neu5Ac)).

liposomal vector. Quantitative fluorescence microscopy was then used to assess binding to several lectins and cholera toxin B. Their results suggested that the expression of ΔF508 CFTR decreased α2,6-sialylation even in the presence of endogenous wtCFTR. cAMP-stimulated [^{36}Cl]-efflux assays showed that 9/HTEo$^-$ cells expressing ΔF508 CFTR had significantly decreased chloride channel function compared to controls. Hence, it was posited that α2,6-sialylation could be dependent on normal wtCFTR function. To assess this hypothesis, the R-domain of wtCFTR was transfected by a liposomal vector into 9/HTEo$^-$ cells [26]. Overexpression of this R-domain had been shown to inhibit the chloride channel activity of wtCFTR and was confirmed again by chloride efflux assay. Their experiments supported the hypothesis that CFTR chloride channel function could play a role in the glycosylation phenotype.

To investigate if wtCFTR expression could reverse the defective sialylation of membrane gangliosides in CF cells, IB3 CF bronchial cells and CFPAC CF pancreatic cells were transfected with wtCFTR [27]. Asialo GM_1 residues were detected by immunofluorescence. In both bronchial and pancreatic cells, transfected progeny had significantly less anti-asialo GM_1 antibody binding than naïve CF cells. The authors suggest that expression of wtCFTR led to a change in sialylation of membrane gangliosides. These results correlated with experiments showing that significantly more *P. aeruginosa* PAO1 bound to non transfected CF cells. Further, it was shown that this binding could be inhibited in a dose-related manner with anti-asialo GM_1 antibody.

To characterize the terminal glycosylation of rescued immortalized CF airway cells, quantitative lectin-binding assays were performed with HRP-conjugated PNA and HRP-WGA. PNA has been reported to preferentially bind to galactose residues linked $\beta 1 \rightarrow 3$ to *N*-acetyl-D-galactosamine in the terminal position, commonly found on non-sialylated O-linked oligosaccharides. The IB3 cell line and two IB3 clonal cell lines stably transfected with wtCFTR, S9 [28] and C38 [29], were analyzed in two separate experiments. There was no difference appreciated in the binding of WGA to IB3 and S9 cells [28]. However, S9 bound 60% less PNA than IB3 cells at all concentrations and times [28,29]. C38 cells bound 70% less PNA than IB3 cells [29]. These results suggest that IB3 cells had more galactose residues in a terminal position than S9 or C38 cells. The authors suggest that this could be a reflection of a decrease in sialylation of membrane glycoconjugates. This conclusion was supported when the difference in PNA binding was abolished when IB3 cells were incubated with neuraminidase.

As was illustrated above, experiments using lectin-binding assays have produced a number of conflicting reports regarding whether or not the glycosylation phenotype is dependent on wtCFTR expression and the degree of decreased sialylation in CF cells. Hence, investigators sought to concretely relate the degree of altered sialylation and fucosylation with the expression of wtCFTR.

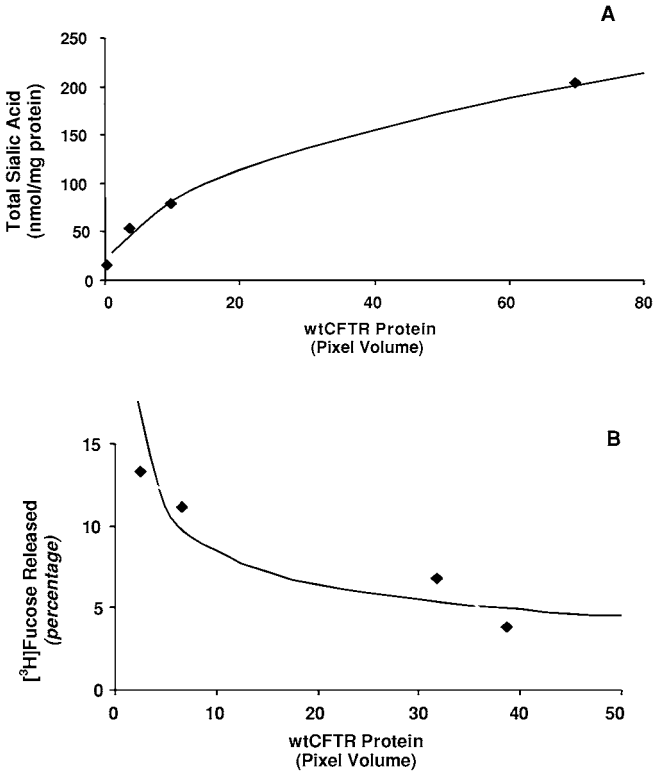

Figure 2. The dependence of sialic acid and Fucα1,3/4GlcNAc content of peripheral glycopeptides of airway epithelial cells on the expression of wtCFTR. Line represents the exponential equation generated characterizing this relationship. (A) The sialic acid content of CF/T1 cells is increased with the expression of wtCFTR where as (B) Fucα1,3GlcNAc is decreased [4].

A unique cell system was used where CF/T1 cells, immortalized airway epithelial cells from the trachea of a cystic fibrosis patient homozygous for the ΔF508 mutation, were transfected with wtCFTR cDNA [13]. These cells subsequently expressed high levels of functional wtCFTR at the cell membrane as verified by Western blot and patch-clamp analysis. The transfected cells were cultured for an extended time [4]. After the defined time, wtCFTR expression declines at a demonstrably predictable and calculable exponential rate, as verified by *in situ* hybridization of CFTR mRNA and Western analysis [4], before losing all detectable wtCFTR expression. Fucose and sialic acid content of glycoproteins released from these cells as CFTR expression declined were analyzed (Figure 2).

As in previous studies [16], cells were incubated in culture with [^{3}H]Fuc and subsequently exposed to almond α-1,3/4 fucosidase. Similar to prior results, the amount of Fucα1,3/4GlcNAc was found to be increased 3-fold in CF cells compared to the corrected CF cells. It was also shown that Fucα1,3/4GlcNAc significantly increased as wtCFTR expression decreased whereas nontransfected CF/T1 cells consistently displayed high levels of Fucα1,3/4GlcNAc. Further, the

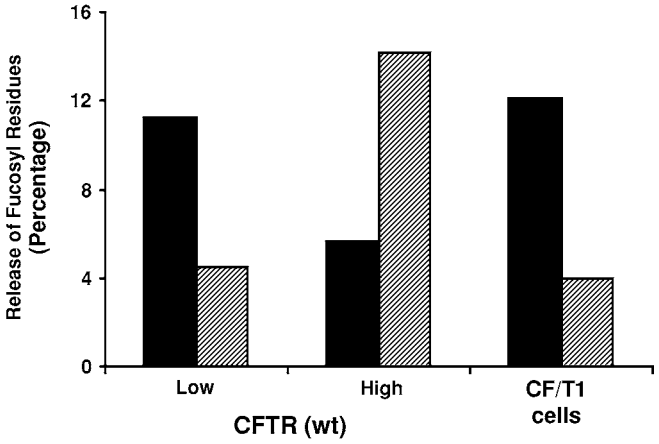

Figure 3. Comparison of Fucα1,3/4GlcNAc and Fucα1,2Gal from the membrane glycopeptides of CF/T1 cells. CF/T1 cells were transfected with wtCFTR and subsequently expressed high or low levels of wtCFTR as determined by Western blot analysis. Fucosyl residues were released by specific α-L-fucosidases. A reciprocal relationship is seen between Fucα1,3/4GlcNAc (black bar) and Fucα1,2Gal (striped bar) [17].

dependence of decreased α1,3/4 fucosylation on wtCFTR could be characterized as an exponential equation with high correlation (Figure 2B).

A subsequent study employing the same cell lines compared the content of Fucα1,3/4GlcNAc and Fucα1,2Gal in membrane glycopeptides with the use of specific fucosidases [17]. In transfected CF/T1 cells expressing low levels of wtCFTR, the ratio of Fucα1,3/4GlcNAc to Fucα1,2Gal was approximately 3, whereas those transfected cells expressing wtCFTR exhibited a ratio of approximately 0.33 (Figure 3). In nontransfected CF/T1 cells, the ratio was also approximately 3. The results obtained showed an actual alteration in the composition of membrane glycoproteins that was modulated by wtCFTR expression without a compensatory change in the mRNA expression and activity of fucosyltransferases.

To definitively quantitate the total sialic acid content of CF and rescued airway epithelial cells, the thiobarbituric acid assay [23] was used [4]. In a reciprocal relationship to Fucα1,3/4GlcNAc, total sialic acid was found to decrease exponentially as the transfected CF/T1 cells gradually lost expression of wtCFTR (Figure 2A). Total sialic acid could be expressed as an exponential function of the degree of wtCFTR expression with a high degree of correlation.

It was thus shown that the ratio of sialic acid to Fucα1,3/4GlcNAc in CF glycopeptides from airway cells was decreased similar to that reported in CF fibroblasts [11]. Transfection and expression of wtCFTR in CF cells reversed the phenotype, and, with subsequent regression of these transfected cells to the previous CF phenotype, the initial low ratio of sialic acid to fucose also returned.

This study unequivocally showed that the glycosylation phenotype correlated with degree of wtCFTR expression over an extended time. Further, this study provided distinct evidence that alterations in glycosylation in CF airway cells could be reversed with transfection of wtCFTR cDNA.

CFTR as a membrane glycoprotein

CFTR is a transmembrane glycoprotein, which functions as a Cl^- channel at the apical surface of epithelial cells [1]. It may also be involved in the regulation of other ion channels in the plasma membrane [30–32] and intravesicular trafficking and endosome fusion [33]. It is reported to function in the endoplasmic reticulum [34], clathrin-coated vesicles [35] and it has recently been proposed to play a role in the sorting and compartmentalization of the glycosyltransferases which are involved in terminal glycosylation in the Golgi [17,36]. It is reported that CFTR with the most common mutation, ΔF508, has a defect in processing which results in an inefficiently folded glycoprotein that has reduced delivery to its destination [3,33,37].

Cheng et al. [38] reported that the decrease in the size of oligosaccharides of CFTR is responsible for the synthesis of immature CFTR in CF, and it is accepted that the ΔF508 mutation belongs to this category [37]. It has been proposed that CFTR may utilize an atypical and highly regulated pathway for transit to the cell surface [39]. In other studies, ΔF508 was shown to interfere with a step in the maturation of immature CFTR from ER to the Golgi complex [40,41]. Others showed that the ER-retained immature form of CFTR was more susceptible to protease digestion [42,43]. Studies by Kopito et al. [44] suggested that up to 75% of wild-type CFTR is degraded prior to localization in the surface membrane. However it is important to remember that many of the studies of the processing of CFTR were performed on cells that were overexpressing CFTR and that they may not reflect the situation of airway epithelial cells expressing endogenous levels of CFTR [45].

The degradation of CFTR occurred at a faster rate than the synthesis in the presence of castanospermine, an inhibitor of glycosylation processing [46]. It was pointed out that the lack of the proper oligosaccharides on the mutant CFTR might contribute to its rapid degradation. The inhibitor-induced lack of glycosylation may be similar to the deglycosylated intermediary reported prior to degradation [47]. Thus although CFTR has only two potential glycosylation sites, it appears that the glycosylation may be the key to CFTR survival.

In a detailed report on the glycosylation of CFTR, O'Riordan et al. [48] have shown that the fully mature, glycosylated form of CFTR, expressed in CHO cells and in a mammary tumor cell line transfected with wtCFTR, contained a unique pattern of glycosylation which consisted of complex oligosaccharides containing *N*-acetyllactosamine repeating units. The finding may be significant taking into account the proposed role of these structures to protect the mature protein from proteolytic degradation [49]. O'Riordan et al. [48] stressed the fact that the elongation of polylactosamine in the Golgi at 21°C correlates with the fact that ΔF508 will traffic to the membrane

at low speed [50]. Thus as a result of the low temperature the ΔF508 CFTR may traffic through the Golgi slowly, and the polylactosamine structures are synthesized. In a discussion of the report by O'Riordan, it was acknowledged that this was the only critical analysis reported on the oligosaccharides of CFTR. However, it was pointed out that polylactosamine is a product of tumor cells and CHO cells, and the experiments should be repeated on endogenous CFTR in CF and non-CF airway cells [45]. Therefore it is not yet known if CFTR, which modulates the glycosylation phenotype of the cell membrane, is itself subject to the expression of the CF glycosylation phenotype.

Hypotheses proposed for the expression of the glycosylation phenotype

Several hypotheses have been proposed for the glycosylation phenotype in CF. Since the terminal glycosylation of the glycoproteins or glycolipids in the surface membrane is the area of specific change any hypothesis must involve Golgi and specifically the TGN. In the CF cells, sialic acid is decreased and Fucα1,3GlcNAc is increased when compared to non-CF cells (Table 1). Moreover, Fucα1,2Gal is decreased as recently shown [17].

Barasch et al. [18] reported defective acidification and a decrease in sialic acid of CF cells. They observed that the pH of the Golgi in CF cells was altered and hypothesized that the lack of acidity in the Golgi vesicles inhibited the action of the sialyltransferase and thus caused a decrease in sialylation of the surface glycoproteins and glycolipids. The CF TGN pH value of 6.8 compared to the non-CF TGN pH of 6.5 that they reported were hardly significant enough to inhibit the activity of sialyltransferases. As shown in Table 3, both fucosyl- and sialyltransferases usually have a broader pH range and not as sharp an optimum. Subsequently, others were not able to detect a difference in pH values between Golgi of CF and non-CF cells [51,52]. Radioimaging was used to detect hyperacidification (pH 6.0 to 6.2) in the TGN of immortalized epithelial cells from CF lungs. The acidification was correlated with PNA binding to the cell lines [29]. However, again, the pH values that they suggest would inhibit specific glycosyltransferase activity do not agree with the pH values reported for either the fucosyl- or sialyltransferases (Table 3). This contradicts the hypothesis of Barasch et al. [18] which evoked an effect of acidity on the sialyltransferases. Moreover, the experiments of Poschet et al. [29] directly duplicated those of Rhim et al. [4] which showed unequivocally by chemical assay the modulation of sialic acid by wtCFTR. Other observations on the modulation of glycosylation have been reviewed in Table 2 and [3]. Although others [52] could not show a relationship of CFTR to acidification they acknowledged a number of potential reasons for the disparity. These included the possibility of differences in membrane potential caused by CFTR.

Delmotte et al. [7] have hypothesized that the hypersialylation they observed in mucins of patients with severe lung disease was due to the action of cytokines which increased the action of sialyl and fucosyltransferases. They attribute TNFα to initiating the sialylation. In their case, sialylation was increased in CF mucins in comparison to normal mucins.

Recently, Scanlin and Glick [17,36] have proposed that wtCFTR acts on the TGN and aids in the function of the TGN in terms of terminal glycosylation processing. CFTR is proposed to act as an ion channel intracellularly as well as at the surface membrane where it serves as a Cl^- channel as has been described [53].

Rhim et al. [4] reported a reciprocal relationship for Fucα1,3GlcNAc and NeuAc in CF and non-CF airway cell membranes. Glick et al. [17] further expanded the terminal glycosylation to show a reciprocal relationship between Fucα1,3GlcNAc and Fucα1,2Gal. That is, a high amount of Fucα1,2Gal and sialic acid, and a low amount of Fucα1,3GlcNAc were found in surface membrane glycoconjugates of non-CF cells. In contrast, Fucα1,3GlcNAc was increased, and Fucα1,2Gal and NeuAc were decreased in the CF cell membrane glycoproteins. On the other hand, when the activity of α1,2fucosyltransferase was examined, significant activity was present and the mRNA expression for FucT-2 was present in both CF and non-CF cells to a significant extent. Thus it is puzzling why the non-CF cells have a large amount of Fucα1,2Gal on their surface when the CF cells do not.

Table 3. Range of optimum pH values of fucosyl- and sialyltransferases

	pH values[1]	*Reference*
Fucosyltransferases		
FUT2 (α2-FucT)	6.0–7.2	[64]
FUT3 (α3/4-FucT)	6.0–7.4	[65]
FUT4 (α3-FucT)	6.2–7.0	[66]
FUT5 (α3-FucT)	5.0–7.0	[67]
FUT6 (α3-FucT)	7.2–8.0	[66]
FUT7 (α3-FucT)	6.2–7.0	[68,69]
FUT8 (α6-FucT)	5.6,7.0	[70–72]
FUT9 (α3-FucT)	6.8–7.2	[73]
Sialyltransferases		
ST3Gal-I	6.0–6.5	[74,75]
ST3Gal-II	6.0–6.5	[76,77]
ST3Gal-III	6.0–6.5	[78,79]
ST3Gal-IV	6.0–6.8	[76,80]
ST3Gal-V	6.0–6.5	[81,82]
ST3Gal VI	6.0	[83]
ST6Gal-I	6.0–6.8	[84–87]
ST6GalNAc-I	6.0–6.5	[88–90]
ST6GalNAc-II	6.0–6.5	[89,91]
ST6GalNAc-III	6.0–6.5	[92,93]
ST6GalNAc-IV	6.0–6.5	[92,93]
ST6GalNAc-V	6.0–6.5	[94,95]
ST6GalNAc-VI	6.0	[96]

[1] May vary with the tissue source and buffer composition. These selected references and others are discussed extensively in [97].

Since these three terminal transferases act on the same substrates one can assume that if the order of action is altered and α1,3 FucT acts first in CF then NeuAcT or α1,2FucT could not act, therefore the glycosylation phenotype is expressed on the CF cells. We propose that wtCFTR aids in compartmentalization of the terminal glycosyltransferases in the Golgi, and mutant CFTR (ΔF508) does not perform this function properly, and actually interferes with compartmentalization.

As discussed, the role of CFTR in acidification of endosomes and Golgi, particularly the TGN has been addressed by several investigators after the report of Barasch et al. [18] that the pH values of the Golgi vesicle could be responsible for the alteration in sialic acid. It is generally agreed that wtCFTR recycles from the surface membrane through internal vesicles [33,46], but it is not agreed what role it has in protein sorting and glycosylation.

The hypothesis that we propose depends on the sorting of glycosyltransferases via autograde/retrograde transport of proteins through the Golgi as described by Allan and Balch [54] or Weiss and Nilsson [55]. We propose that wtCFTR functions in the TGN to maintain the proper compartmentalization of the sorting enzymes (specifically the terminal glycosyltransferases) as they pass through the Golgi, to and from the surface membrane carrying the glycoconjugates which require processing (Figure 4). Mutated CFTR does not have this function [17] even though it may reach the surface membrane and recycle to the TGN [46]. In the presence of mutant CFTR the terminal glycosyltransferases are not sorted to the proper compartments

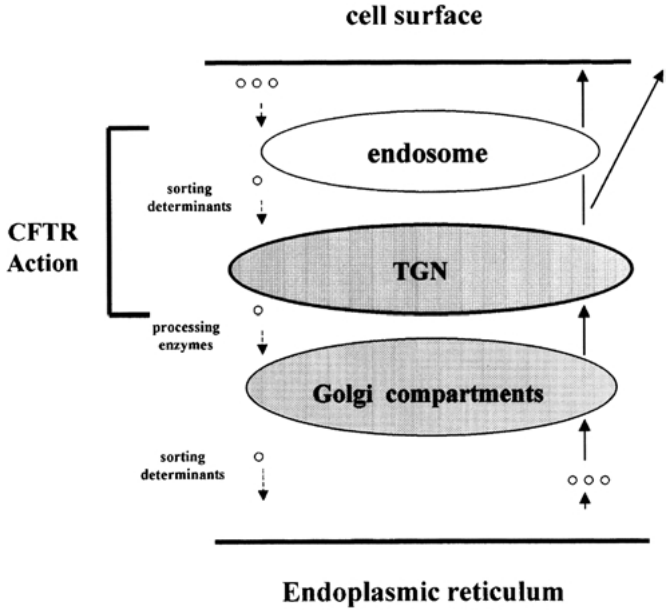

Figure 4. Schematic representation of protein sorting by directed maturation of the Golgi compartments proposed by Allan and Balch [54]. We hypothesize that CFTR acts on the sorting determinants in the TGN, as denoted. When CFTR is mutated, compartmentalization of terminal glycosyltransferases are altered resulting in the CF glycosylation phenotype [17,36].

and therefore act on the processing glycoproteins in an aberrant order. As discussed, if α1,3FucT acts first it will prevent the activity of the two other terminal glycosyltransferases, sialyltransferase, and α1,2FucT. These latter two enzymes will not act after the glycoconjugates are α1,3 fucosylated, yielding the glycosylation phenotype as reported for CF (Table 1). At this time we cannot predict if the role of wtCFTR in the TGN is based on its ion channel function, acidification, membrane potential, or other undescribed mechanisms.

Future directions

In recent years, there has been renewed interest in identifying modifier genes which could influence the severity of CF in individuals with a given set of mutations in CFTR [56]. The recent study by Bronsveld et al. [57] of siblings who are homozygous for the ΔF508 mutation provided strong support for the hypothesis that there is a genetic modifier of the severity of the clinical disease in CF as well as directly of the function of mutant CFTR. We propose that many of the enzymes, which regulate the biosynthesis and processing of surface membrane glycoproteins, are potentially important candidates for modifier genes in CF. In this review, we emphasized that for many of the glycosyltransferases which are involved in terminal glycosylation, subtle differences in pH alone would not explain the altered glycosylation phenotype which has been described in CF [4] (Table 3).

Another class of diseases, the congenital disorders of glycosylation, is an area of active investigation [58]. There have been several reports of CF serum glycoproteins that have glycosylation patterns that are similar to those seen in some patients with congenital disorders of glycosylation. It is proposed here, and is under active investigation, that mutations in some of the enzymes that are described to result in syndromes of congenital disorders of glycosylation could serve to modify the severity of CF. This hypothesis is currently under investigation (TF Scanlin and HH Freeze, unpublished).

In addition to providing potential therapeutic targets for the treatment of CF, detailed dissection of the pathways for processing and sorting surface membrane glycoproteins and glycolipids in CF airway epithelial cells may shed new light on the regulatory elements of these highly conserved and complex processes.

Acknowledgments

Supported in part by the Cystic Fibrosis Foundation (CFF SCANLI00Z0, CFF SCANLI00G0). The authors gratefully acknowledge Kearston Ingraham for assistance in preparing the manuscript and all of the authors on our papers cited here.

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Received 22 May 2002; accepted 28 June 2002