balance, these studies demonstrate that the apically mislocated EGFR in cpk collecting tubular cells binds EGF, and functions as a tyrosine kinase. Further, altered quantitative and qualitative EGFR expression correlated directly with hyperresponsiveness to the mitogenic actions in vivo.

Such data have relevance to treatment of cyst formation and progressive enlargement in PKD. In vitro application of specific blocking antiEGFR antibodies or tyrphostins block the cystogenic effects of EGF and TGFα. It is also possible that manipulation of the EGFR promoter activity could lead to the development of sophisticated delivery systems which might target such activities to cystic renal epithelium.

References

II. ABNORMAL TRANSPORT BY EPITHELIAL CELLS

CFTR: Cloning, mutations and functions
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CFTR, the product of the gene which when mutated causes cystic fibrosis, is involved in both chloride secretion and absorption in some epithelia, for example, in the shark rectal gland which is a prototype of the former process, and in the reabsorptive duct of the sweat gland, which is representative of the latter. The major disease-causing mutation (ΔF508) was initially identified in that tissue. The use of N-glycosylation sites inserted at various positions in the sequence by in vitro mutagenesis has recently confirmed the original topological model in which there are six membrane-spanning segments in each half of the protein. This, together with mutagenesis of specific residues in many of these segments, strongly suggest that they assemble in some manner to form the ion pore. CFTR is a substrate for multiple protein kinases, phosphatases and eleven protein kinase A (PKA) phosphorylation sites. These account for the activation in excised membrane patches by ATP and PKA. This level of regulation is integrated with another level involving interactions of ATP at the plasma membrane with a time course similar to that observed for inhibition of endocytosis. CFTR function in intracellular membranes has been implicated in the degree of acidification of various intracellular compartments of the protein processing and secretory pathways. This represents a potential mechanism for alterations in macromolecular secretory products or other transport events.

Issues surrounding CFTR trafficking require a specific and sensitive means of detecting the protein in cells and tissues. To address this problem, we chose to epitope tag the CFTR protein on one of the extracellular loops and at the C-terminus, with the M2 epitope as a means of detecting the recombinant protein when it was expressed. The externally tagged CFTR was detected in the surface membrane of non-permeabilized cells, and the percent of cells stained by M2 epitope antibody correlated with their halide permeability response to cAMP. Antibody labeling experiments showed that surface CFTR was rapidly endocytosed under non-stimulated conditions. cAMP inhibited CFTR endocytosis and increased the plasma membrane content of CFTR about three-fold. CFTR tagged at the C-terminus was expressed in Xenopus oocytes. Frozen sections were prepared and stained using the M2 antibody. cAMP caused a condensation of fluorescence at the plasma membrane with a time course similar to that observed for stimulation of transmembrane Cl currents. These findings indicate that cAMP-dependent membrane trafficking of CFTR contributes to the chloride conductance response evoked by secretory agonists. These tagged CFTRs should be useful for further studies of CFTR trafficking and CFTR-protein interactions.

In cultured cells that express CFTR endogenously or after its expression by gene transfer, CFTR has been localized to the plasma membrane and shown to function as a cAMP-activated Cl channel. In primary cultures or cell lines that form an epithelial monolayer, CFTR can be identified in the apical membrane domain. This location fits with its role in transepithelial Cl secretion. Small linear Cl channels are also found in the apical

Solute and fluid secretion mechanisms in intestinal and airway epithelial cells
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Since the cloning of the gene responsible for cystic fibrosis, tremendous progress has been made in understanding its function as a cAMP-regulated Cl channel. In concert with other transport mechanisms, this apical membrane Cl channel mediates CAMP-dependent salt and water secretion across the epithelial cells of airway and intestinal tissues. In CF patients who carry the ΔF508 mutation, the mutant protein is retained intracellularly and is not targeted to the plasma membrane. Previous studies have shown that the mutant protein is functional, thus approaches designed to facilitate plasma membrane targeting of ΔF508 CFTR would benefit the majority of CF patients. The mechanisms responsible for retention of mutant protein within the ER are unknown. Wild-type CFTR is involved in cAMP-dependent regulation of vesicle recycling at the plasma membrane. Cells that express wild-type CFTR show CAMP-induced activation of exocytosis and inhibition of endocytosis. CFTR function in intracellular membranes has been implicated in the degree of acidification of various intracellular compartments of the protein processing and secretory pathways. This represents a potential mechanism for alterations in macromolecular secretory products or other transport events.
membranes of confluent monolayers. However, recent studies suggest that in epithelial cells, the expression of CFTR at the apical membrane depends on the level of cellular differentiation and the acquisition of cell polarity.

Colonial cells that have not formed polarized monolayers fail to target CFTR to the plasma membrane. These un-polarized cells retain CFTR in a perinuclear location and show no activation of plasma membrane anion conductance in response to cAMP. Iodide efflux assays show that cAMP enhances anion efflux from polarized HT29 cells, but fails to activate either the parental un-polarized cell line or the polarizing cells prior to confluence. After polarization, these cells show apical membrane CFTR staining and cAMP-activated Cl secretion, detected as a transepithelial Cl current. Expression studies show that both the polarized and un-polarized HT29 cells express equivalent amounts of CFTR mRNA and protein. In addition, CFTR is expressed in the non-polarized cells as the fully processed glycosylated protein so that in these cells, CFTR has encountered the Golgi glyco-processing enzymes. Yet, CFTR is retained in a post-Golgi intracellular compartment, perhaps in vesicles of the trans-Golgi network, until the cells form tight junctions and discrete apical and basolateral membrane domains. This apical targeting mechanism is shared by some other apically-directed glycoproteins, such as proteases and hydrolases and differs from that observed for the Na/K-ATPase, which is targeted to and retained at the basolateral membranes. The signals responsible for intracellular CFTR retention in non-polarized cells are as yet unknown. Nevertheless, these studies demonstrate that the expression of this component of the Cl secretory machinery depends on cellular differentiation and may, therefore, be similar to the intermediate stage of epithelial differentiation thought to occur in polycystic kidney disease. The CFTR retention signals characteristic of non-polarized epithelial cells are apparently not expressed in non-epithelial cells, which target CFTR to the plasma membrane.

It will be interesting to determine why these epithelial targeting signals influence the cellular location of mutant CFTRs.

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Maturation of solute transport mechanisms in pulmonary epithelia

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Recent studies have indicated that the epithelium lining the distal (alveolar) regions of the fetal and perinatal lung do not merely represent a passive barrier to the movement of fluid and solutes, but rather actively transports ions. In utero, the fetal lung is normally filled and distended with fluid which results from the active secretion of Cl (~5 ml/kg/hr of fluid) by the epithelium. The presence of this fetal lung liquid is critical for the normal development of the lung. At birth, the lung must become air filled and become a net fluid absorbing organ. During labor there is a rapid switch from fluid (Cl) secretion to fluid (Na) absorption which arises, at least in part, from the increase in circulating endogenous hormones with β2 agonist properties. The active Na transport by the distal lung epithelium plays a critical role in the clearance of lung liquid from the newborn's air spaces; impaired Na transport resulted in respiratory distress, hypoxemia, and elevated lung water contents in otherwise healthy newborn animals. Na transport is equally important in the postnatal lung, as it plays a critical role in the clearance of pulmonary edema. Recent studies have shown that the distal lung regions of the human lung are similar to the lungs of lower animals and absorb fluid by active Na transport.

As in other Na transporting tissues, there is a Na/K-ATPase located on the interstitial (basolateral) side of the epithelial monolayer which creates an electrochemical gradient for Na movement across the cell's membrane and an apical (alveolar side) entry pathway for Na. Although some Na absorbing epithelia may utilize Na exchangers or symports, these plasma membrane proteins do not play a role in fluid absorption from the perinatal lung's air spaces. Rather, Na transport by both the perinatal and adult alveolar epithelium utilizes Na channels.

It is now known that there are a family of epithelial Na channels that have no structural, biochemical, or nucleotide similarity to the voltage-regulated Na channel found in excitable cells. Since polyclonal antibodies which recognize the putative amiloride-binding subunit of the renal epithelial Na channel label fetal and adult alveolar epithelial apical membranes and recognize polypeptides in alveolar epithelial membrane vesicles, it is likely that alveolar epithelia have at least one type of of Na channel which is antigenically similar to the renal epithelial Na channel. There is biochemical and physiologic evidence for more than one type of Na channel in rat alveolar epithelium. Patch clamp studies have identified a 25 pS nonselective cation channel (NSC), along with 12 pS and 4 pS highly selective Na channels in membrane vesicles, which are permeant ion channels are all amiloride-sensitive. Membrane vesicle, Ussing chamber and whole cell patch clamp studies also indicate that there are both H-type and L-type (EIPA) sensitive Na channels on the apical membrane of the alveolar epithelium. These Na permeant ion channels are all amiloride-sensitive. Membrane vesicles, Ussing chamber and whole cell patch clamp studies also indicate that there are both H-type and L-type (EIPA) sensitive Na channels on the apical membrane of the alveolar epithelium. These Na permeant ion channels are all amiloride-sensitive. Membrane vesicles, Ussing chamber and whole cell patch clamp studies also indicate that there are both H-type and L-type (EIPA) sensitive Na channels on the apical membrane of the alveolar epithelium. Whether these different alveolar epithelial Na channels are in different alveolar epithelial cell subtypes, and whether they arise from the same or different genes is unknown.

A recent major breakthrough has been made using functional expression cloning techniques to determine the primary structures of the putative pore forming subunit of the rat epithelial Na channel (αENaC) and two highly homologous subunits (β and γENaC) of the rat epithelial Na channel. The latter two subunits markedly increase the Na channel activity when their cRNA is co-expressed with αENaC cRNA in Xenopus laevis oocytes. All three subunits have only two transmembrane spanning regions