Article

Cholesterol Modulates CFTR Confinement in the Plasma Membrane of Primary Epithelial Cells

Asmahan Abu-Arish,[1] Elvis Pandzic,[2] Julie Goepp,[3] Elizabeth Matthes,[3] John W. Hanrahan,[3] and Paul W. Wiseman[4,*]
[1]Physics and Physiology, [2]Physics, [3]Physiology, and [4]Chemistry & Physics, McGill University, Montreal, Quebec, Canada

ABSTRACT The cystic fibrosis transmembrane conductance regulator (CFTR) is a plasma-membrane anion channel that, when mutated, causes the disease cystic fibrosis. Although CFTR has been detected in a detergent-resistant membrane fraction prepared from airway epithelial cells, suggesting that it may partition into cholesterol-rich membrane microdomains (lipid rafts), its compartmentalization has not been demonstrated in intact cells and the influence of microdomains on CFTR lateral mobility is unknown. We used live-cell imaging, spatial image correlation spectroscopy, and k-space image correlation spectroscopy to examine the aggregation state of CFTR and its dynamics both within and outside microdomains in the plasma membrane of primary human bronchial epithelial cells. These studies were also performed during treatments that augment or deplete membrane cholesterol. We found two populations of CFTR molecules that were distinguishable based on their dynamics at the cell surface. One population showed confinement and had slow dynamics that were highly cholesterol dependent. The other, more abundant population was less confined and diffused more rapidly. Treatments that deplete the membrane of cholesterol caused the confined fraction and average number of CFTR molecules per cluster to decrease. Elevating cholesterol had the opposite effect, increasing channel aggregation and the fraction of channels displaying confinement, consistent with CFTR recruitment into cholesterol-rich microdomains with dimensions below the optical resolution limit. Viral infection caused the nanoscale microdomains to fuse into large platforms and reduced CFTR mobility. To our knowledge, these results provide the first biophysical evidence for multiple CFTR populations and have implications for regulation of their surface expression and channel function.

INTRODUCTION

The cystic fibrosis transmembrane conductance regulator (CFTR) is a tightly regulated anion channel expressed at the apical surface of epithelial cells (1,2). Anion flux through the CFTR drives transepithelial fluid secretion and is required for efficient mucociliary clearance of inhaled bacteria and other particles from the lung (reviewed by Frizzell and Hanrahan (3)). Mutations in the *cftr* gene cause cystic fibrosis (CF), an autosomal recessive disease characterized by diminished salt and fluid secretion, accumulation of viscid mucus, and recurring cycles of airway infection by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and other pathogens. Most morbidity and mortality in CF results from chronic inflammation of the airways and the resulting gradual decline in lung function.

The CFTR associates with other proteins in a macromolecular complex, and its channel activity and surface expression are regulated by local signaling and recycling through endosomal compartments, respectively. These processes are expected to depend on the compartmentalization and lateral mobility of CFTR and its interactome, although CFTR dynamics at the cell surface are not well understood. Most studies have focused on protein-protein interactions, but lipids may also play a role in compartmentalizing CFTR. About half of the CFTR in Calu-3, an airway epithelial cell line, is found in a detergent-resistant membrane (DRM) fraction hypothesized to include lipid rafts (4). Rafts are hypothesized nanoscale microdomains in the plasma membrane that are enriched in cholesterol and glycosphingolipids (5,6) and are thought to cluster receptors and intracellular signaling molecules including G protein coupled receptors (7), tumor necrosis factor receptor (8), and kinases, notably Src (8,9) and protein kinase C (10,11).

Activation of the tumor necrosis factor receptor and inhibition of Src kinase increases the association of these signaling molecules with lipid raft components and is accompanied by recruitment of the CFTR to the DRM fraction (8), suggesting that the CFTR may partition into microdomains during inflammation. This partitioning may be due to lipid interactions with CFTR itself or with the scaffold protein NHERF1, which links the CFTR to the actin cytoskeleton (12) and also binds to cholesterol at the plasma membrane (13). Colocalization of fluorescently labeled CFTR with the lipid raft markers cholera toxin B or caveolin-1 has also been observed during infection by *Pseudomonas aeruginosa* (14–16), and the CFTR has been proposed to bind and internalize bacteria to help clear them from the lumen and to initiate lipid-raft-dependent epithelial cytokine and chemokine release (8,14–17). The

Submitted March 9, 2015, and accepted for publication April 23, 2015.
*Correspondence: paul.wiseman@mcgill.ca
Editor: Amitabha Chattopadhyay.

0006-3495/15/07/0085/10 $2.00
http://dx.doi.org/10.1016/j.bpj.2015.04.042

presence of a CFTR population in the DRM fraction implies heterogeneity among CFTR molecules. Only one homogeneous population of CFTR channels was detected based on single-particle tracking (18), so it remains uncertain whether some CFTR channels are present in lipid rafts under resting conditions and whether this distribution influences their lateral mobility.

In this work, we quantified the distribution of CFTR in primary human bronchial epithelial cells using spatial image correlation spectroscopy (ICS (19)) and studied CFTR dynamics using a modified version of k-space ICS (kICS (20,21)). To identify the CFTR population that is in cholesterol-rich membrane microdomains, transport and partitioning dynamics were compared under control conditions and after treatment with cholesterol oxidase or cholesterol esterase to deplete or augment cholesterol, respectively. kICS analysis revealed two dynamically distinct populations of CFTR on the plasma membrane, one of which had large (relative to the focal-spot dimensions)-spatial-scale transport dynamics indicative of CFTR mobility outside of microdomains. The other population exhibited small-spatial-scale dynamics and displayed confinement consistent with nanoscale membrane domains. Cholesterol depletion reduced CFTR confinement and the fraction of CFTR in the confined population, whereas these were both increased by cholesterol insertion. The results show a clear dependence of CFTR distribution and dynamics on cholesterol and suggest that a fraction of CFTR exists in lipid rafts under physiological conditions. Lipid-raft fusion into large platforms during infection may enhance the regulation of CFTR channel activity or modulate stability of the channel at the cell surface.

MATERIALS AND METHODS

Cell culture and adenovirus infection

Primary human bronchial epithelial (HBE) cells were obtained at first passage from the Cystic Fibrosis Canada Primary Airway Cell Bank at McGill University (see the Supporting Material for details). Cells were seeded on vitrogen-coated (PureCol, Advanced BioMatrix, San Diego, CA), glass-bottom FluoroDishes (World Precision Instruments, Sarasota, FL) and maintained in bronchial epithelial cell growth medium (22) at 37°C. When they reached 80% confluence, they were infected with adenovirus particles directing the expression of green-fluorescent-protein (GFP)-labeled CFTR (GFP-CFTR) (23) at a multiplicity of infection of 100 colony-forming units per cell in OptiMEM medium supplemented with 100 nM vitamin D3 (Calbiochem, Billerica, MA) for 2 days. The cells were rinsed with phosphate-buffered saline and kept in fresh OptiMEM for 2 days, then imaged in OptiMEM with 5% CO_2 at 37°C.

Treatments

All treatments were performed acutely at 37°C. To reduce cholesterol and disrupt lipid rafts, cells were incubated with 1 unit/mL cholesterol oxidase (COase, Sigma, St. Louis, MO) for 30 min before and during imaging. COase was used because in preliminary experiments it was found to cause less cell damage during extended imaging periods compared to methyl-β-cyclodextrin (MβCD). To increase cholesterol production and raft stability, cells were treated with 0.1 unit/mL cholesterol esterase (CEase, Sigma) for 30 min before and during imaging. To examine the acute effects of adenoviral infection on CFTR distribution and dynamics, HBE cells that had already been transduced with GFP-CFTR were exposed to GFP-CFTR adenovirus particles at a multiplicity of infection of 100 for 30 min and then imaged.

Confocal microscopy

Live cell imaging was performed in the Advanced BioImaging Facility of the McGill Life Sciences Complex using an LSM-710 confocal microscope (Zeiss, Jena, Germany), which was equipped with a multiline argon laser (488 nm, 25 mW) and a 561 nm line laser (15 mW). Time-series images were collected from flat regions of the plasma membrane near the coverslip using 0.5% laser power to minimize fluorescence photobleaching. Under these conditions, fluorescence photobleaching was measured to be $<10\%$ of the average fluorescence intensity of the first frame of the image series. EGFP was excited using the 488 nm laser line and Alexa-594 was excited at 561 nm. Imaging was performed using a Plan-Apochromat 63× (NA 1.40) oil immersion objective, a confocal pinhole of 1 Airy unit, and a digital gain setting of 900. The experiments were performed on subconfluent, unpolarized HBE cells expressing GFP-CFTR in a humidified cell incubator on the microscope stage maintained at 37°C with 5% CO_2 (Live Cell Instrument, Seoul, South Korea).

The time series consisted of 800 images (256 × 256 pixels) collected from a single cell and focused on an area of membrane in contact with the glass coverslip. Image series were collected at a frame rate of 6.5 Hz, a pixel diameter of 0.06 μm, and a pixel dwell time of 1 μs. For the analysis of confined dynamics, 59–136 image series were analyzed per stimulation condition. For the purpose of visually showing CFTR distribution in the plasma membrane, individual confocal images of a 1024 × 1024 pixel area were collected at 0.2 Hz, with a pixel diameter of 0.13 μm, a dwell time of 6 μs, and laser power of 5% to enhance the signal/noise ratio.

Spatial ICS

ICS was used to measure the degree of aggregation and cluster density for CFTR in cells exposed to different stimuli. The ICS technique has been described in detail elsewhere (19). Briefly, the degree of aggregation (DA) was calculated as

$$DA = \frac{\langle i \rangle}{CD} \tag{1}$$

and the cluster density (CD) as

$$CD = \frac{\langle n_p \rangle}{\pi \omega_0^2}, \tag{2}$$

where CD is the average number of independent fluorescent entities per unit area, $\langle i \rangle$ is the spatial average pixel intensity of the image region of interest (ROI), $\langle n_p \rangle$ is the average number of particles per beam focus, and ω_0 is the e^{-2} beam radius. $\langle n_p \rangle$ is the reciprocal of the zero spatial lags amplitude of the spatial correlation function of the image, which is obtained from a nonlinear least-squares fit of a Gaussian function. Details of the spatial image cross-correlation spectroscopy technique can be found elsewhere (24,25) and in the Supporting Material.

kICS: Theory and analysis

CFTR dynamics were studied using kICS analysis (20,21). Briefly, for this analysis, the k-space time correlation function, $\Phi(\vec{k};\tau)$, is obtained by calculating the temporal correlation function of the k-space image series