RESEARCH COMMUNICATION

A novel procedure for the efficient purification of the cystic fibrosis transmembrane conductance regulator (CFTR)

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This report describes a novel, single-step strategy for the purification of the cystic fibrosis transmembrane conductance regulator from Sf9 cells, which will facilitate studies of the structure–function relationships of this clinically important molecule. The

new method combines the use of the novel detergent sodium pentadecafluoro-octanoate with metal-affinity chromatography to produce a high yield of purified protein which can be functionally reconstituted as a chloride channel and an ATPase.

INTRODUCTION

It is well known that membrane proteins are difficult to extract from their lipid environment. However, purification of proteins to homogeneity is required for assessment of their intrinsic activity and study by powerful structural techniques including electron microscopy and image analysis. Furthermore, meaningful structural data requires that purified membrane proteins be reconstituted into model membranes in their native conformation. The cystic fibrosis transmembrane conductance regulator (CFTR) is a clinically significant membrane protein. Its absence or dysfunction in the apical membrane of epithelial cells causes the disease cystic fibrosis [1]. Therefore the development of experimental methods for the purification and functional reconstitution of CFTR will not only contribute to our understanding of the mechanism of action of this molecule, but could also lead to the invention of specific therapeutic tools for the treatment of this disease.

Our previously published strategy for the purification and functional reconstitution of CFTR from Sf9 cells provided sufficient material for initial determination of its intrinsic channel and catalytic activity [2,3]. As a result of these studies, we now have direct evidence that CFTR forms a regulated chloride channel once it has been phosphorylated by protein kinase A (PKA) [2,3]. Furthermore, we have direct evidence to confirm an hypothesis which exists in the literature [4–6], that CFTR regulates its channel activity by hydrolysing ATP and coupling this function to a gate which opens and closes the pore through which chloride ions move [7]. However, the progress of our studies of the structural basis for the ATPase and channel functions of CFTR have been limited because the original method for CFTR purification was very time consuming. This purification protocol involves multiple steps and is very time consuming (requiring 8 days). Recently, Loo and Clarke [8] utilized metal-affinity chromatography to purify P-glycoprotein, a protein which is closely related to CFTR, in a single step. They reported that expression of P-glycoprotein (engineered to possess

a polyhistidine tag), purification to 80% homogeneity and functional assay could be completed within 2 days. In the past, application of this approach to the purification of CFTR expressed in Sf9 cells was hampered by the poor solubility of Sf9 CFTR in detergents other than SDS, a strongly ionic detergent which effectively prevented the binding of His-tagged proteins to the metal (nickel) resin [8]. In the present report we describe a new, rapid scheme for the purification from Sf9 cells of CFTR possessing a C-terminal polyhistidine tag (CFTR-His), in which we have used the novel, weakly ionic detergent, sodium pentadecafluorooctanoate (NaPFO) [9] in conjunction with metal-affinity chromatography. This one-step purification procedure can be completed within 1–2 days and leads to the effective purification (95%) of CFTR-His. Furthermore, purified CFTR-His can be functionally reconstituted by a single-step reconstitution procedure (1–2 days). CFTR-His-containing proteoliposomes exhibit ATPase activity and electrogenic chloride uptake capacity, which are comparable with or greater than those activities previously determined for CFTR purified using the conventional method. Therefore this new method for CFTR-His purification should be readily applicable in most laboratories to expedite efforts toward the understanding of the structure–function relationships of this protein.

EXPERIMENTAL

Generation of CFTR-His tag construct

The CFTR open reading frame (ORF) in pBlueBac4 (Invitrogen) was modified by PCR to encode ten histidine residues at the C-terminus of the protein. A PCR product, encoding the C-terminus of CFTR plus the polyhistidine tag, was ligated into a baculovirus transfer vector containing the CFTR ORF so that the wild-type 3' end of the ORF was replaced with the His-tagged 3' end. The reverse oligonucleotide: 5'-CTGACGGTACCACTAGTGATGATGATGATGATGATGATGATGATGATGATGAAGCCTTGATCTTGCACC-3' was used in conjunction with the upstream oligonucleotide: 5'-ATGGT-

Abbreviations used: CFTR, cystic fibrosis transmembrane conductance regulator; CFTR-His, CFTR possessing a C-terminal polyhistidine tag; mAb, monoclonal antibody; NaPFO, sodium pentadecafluorooctanoate; ORF, open reading frame; PKA, protein kinase A; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine.

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GTGTCTTGGGATTCA-3', to create this product and was then subcloned back into the parent construct. The entire amplified region was confirmed by sequencing. Whereas the C-terminus of CFTR is QDTRL* (* = stop codon), the C-terminus of CFTR-His is QDTRL(H)10*.

CFTR expression and purification

Recombinant CFTR-His baculovirus was produced in Sf9 cells by co-transfection of the baculoviral transfer vector pBlueBac4, encoding the CFTR-His ORF, with linear wild-type viral DNA (Bac-N-Blue DNA; Invitrogen). The viral supernatant generated from this transfection was used to infect cells, and recombinant events were detected as blue plaques. Following plaque purification, working stocks of recombinant virus were produced and titred as described previously [2].

The starting material for purification was an Sf9 cell pellet produced from a 1 litre suspension of cells which had been infected with recombinant baculovirus containing the CFTR-His ORF, over a 44 h time period. The cells (approx. 10^9) were resuspended in 100 ml of PBS containing 2% (v/v) Triton X-100, a cocktail of protease inhibitors (10 μ g/ml leupeptin, 10 μ g/ml aprotinin, 10 μ M E64, 1 mM benzamidine, 2 mM dithiothreitol and 5 mM MgCl_2) and DNase I (20 units/ml). The suspension was shaken at room temperature on a nutator for 1 h, and then centrifuged at 100 000 *g* for 2 h at 4 °C. The pellet was solubilized in 100 ml of 8% (w/v) NaPFO [prepared by titrating pentadecafluoro-octanoic acid (Fluorochem) in 25 mM phosphate buffer to pH 8.0, with NaOH] on a magnetic stirrer for 17 h at room temperature. Undissolved residues were sedimented at 60 000 *g* for 1 h and the supernatant was filtered (0.22- μ m filter) before application to a freshly regenerated, 25-ml nickel column at a rate of 1 ml/min and at room temperature. Immobilized Ni-nitrilotriacetate agarose resin was obtained from Qiagen. The column was washed with 60 ml of 20 mM phosphate buffer (pH 7.8) containing 100 mM NaCl and 4% (w/v) NaPFO. A pH gradient (pH 7.8–4.0) was applied to the column using an FPLC (Pharmacia) in a 100 ml volume by titrating the phosphate buffers described above. Fractions (3 ml) were collected and analysed by SDS/PAGE and Western blotting.

CFTR-His reconstitution

CFTR-His protein (approx. 50 μ g) in 4 ml of phosphate buffer (described above) containing 4% (w/v) NaPFO, pH 6.8, was concentrated to 600 μ l in a Centricon concentrator (molecular mass cut-off, 50 kDa). The protein sample was then diluted 10-fold with 8 mM Hepes/0.5 mM EGTA, pH 7.2, and re-concentrated to 600 μ l to reduce the final NaPFO concentration to 0.4%. This volume of CFTR-His-containing buffer was added to 300 μ l of liposomes containing 3 mg of phospholipids, phosphatidylethanolamine (PE)/phosphatidylserine (PS)/phosphatidylcholine (PC) (Avanti Polar Lipids)/ergosterol (Sigma) (5:2:1:1, by weight), for CFTR-His reconstitution. The mixture was dialysed twice (Spectrapor, molecular mass cut-off, 50 kD), with and without 0.025% (w/v) sodium azide, for 18 h against 4 litres of 8 mM Hepes/0.5 mM EGTA, pH 7.2.

Protein detection

Purified CFTR-His protein was subjected to SDS/PAGE (6% acrylamide) and protein was detected with silver stain as described previously [10,11]. For immunoblotting, the protein was transferred to nitrocellulose and probed with an anti-CFTR mono-

clonal antibody (mAb), M3A7 [7,8]. Immunoreactive bands were visualized by enhanced chemiluminescence (Amersham).

Phosphorylation of CFTR-His

CFTR-His was phosphorylated in liposomes by the catalytic subunit of PKA, as described previously [7]. Protein-free liposomes were similarly treated, and used as a control. In order to remove PKA after the phosphorylation reaction, CFTR-His proteoliposomes and protein-free liposomes were centrifuged (Airfuge) at 100 000 *g* for 30 min, washed twice by sonication with buffer and pelleted in the Airfuge centrifuge. Pellets were resuspended in the appropriate buffer for ATPase measurements. For bilayer studies of channel function, CFTR-His was phosphorylated as described above and PKA was separated from the proteoliposomes by micro-spin chromatography using Sephadex G-50.

Assay of CFTR-His ATPase activity

ATPase activity was measured as the production of [α - 32 P]ADP from [α - 32 P]ATP by purified, reconstituted CFTR-His as described previously [7]. The nucleotides were separated by poly(ethyleneimine)-cellulose chromatography. Unless otherwise stated, the assay was carried out in a reaction mixture (15 μ l) containing 100 ng of CFTR-His in phospholipid liposomes, 50 mM Tris/HCl, 50 mM NaCl, 2 mM MgCl_2 , and 8 μ Ci of [α - 32 P]ATP (3000 Ci/mmol) and unlabelled ATP, pH 7.5. Reaction mixtures were incubated at 32 °C for 4 h and the reaction was terminated by the addition of 5 μ l of 10% (w/v) SDS. Samples (1 μ l) were applied to a poly(ethyleneimine)-cellulose plate and developed in 1 M formic acid/0.5 M LiCl. The location and quantification of radiolabelled ATP and ADP were determined with a PhosphorImager (Molecular Dynamics). The data were analysed using the ImageQuant software package (Molecular Dynamics).

Assays of CFTR-His activity as a chloride channel

Concentrative tracer-uptake assay for study of CFTR-His function

A concentrative tracer-uptake assay, developed by Garty et al. [12] and modified by Goldberg and Miller [13], was used to characterize the chloride conductance properties of reconstituted CFTR-His. Proteoliposomes were preloaded with 150 mM KCl and centrifuged through Sephadex G-50 columns equilibrated with glutamate salts (125 mM K-glutamate, 25 mM Na-glutamate, 10 mM glutamic acid, 20 mM Tris/glutamate, pH 7.6) to replace external chloride. Uptake was initiated and quantified by the addition of 1.0 μ Ci/ml of $^{36}\text{Cl}^-$. Intravesicular $^{36}\text{Cl}^-$ was assayed at various time points following separation of liposomes from the external media using a mini anion-exchange column (Dowex 1) [13].

Planar bilayer studies of liposomes containing purified CFTR-His

Planar lipid bilayers were formed by painting a 10 mg/ml solution of phospholipid [PE/PS (1:1, by weight)] in *n*-decane over a 200- μ m aperture in a bilayer chamber. Typically, the *cis* compartment of the bilayer chamber (the compartment to which liposomes were added) contained 300 mM KCl and the *trans* compartment, connected to ground, contained 50 mM KCl. As in our previous studies, proteoliposome fusion with planar lipid bilayers was facilitated and detected by the introduction of nystatin (120 μ g/ml), a technique originally described by Woodbury and Miller [14]. Single-channel currents were detected with a bilayer

amplifier (custom made by M. Shen, Physics Laboratory, University of Alabama, AL, U.S.A.). Data were recorded and analysed using pCLAMP 6.0.2 software (Axon Instruments Inc.). Before analysis of dwell times, single-channel data were digitally filtered at 100 Hz.

Histogram analysis

Open- and closed-time histograms were created with a logarithmic x -axis with 10 bins/decade and a lower limit of 10 ms. The maximum-likelihood method was used to fit the data with one or two exponentials (pCLAMP 6.0 software; Axon Instruments Inc.). The goodness-of-fit was assessed using the log-likelihood ratio test.

Statistics

Results are expressed as means $\pm$ S.D. or S.E.M. Statistical significance was assessed using the log-likelihood ratio test or Student's t test.

RESULTS

Solubilization of CFTR-His in NaPFO

The ability of NaPFO to solubilize CFTR-His from a crude membrane preparation was compared with various detergents, including SDS (Figure 1). Whereas Triton X-100, CHAPS and dodecylmaltoside were ineffective in solubilizing CFTR-His, NaPFO was almost as effective as SDS for CFTR-His extraction. The solubilization efficiency of NaPFO was most effective in buffers with low salt concentrations and was optimal at a detergent:protein ratio exceeding 7.5 (Figure 1).

Purification and reconstitution of CFTR-His by nickel-affinity chromatography

Unlike the strongly ionic detergent SDS, NaPFO did not prevent the binding of CFTR-His to the nickel column. However, NaPFO did appear to reduce the nickel affinity of all proteins, including CFTR-His. Whereas most protocols for nickel-affinity chromat-

Figure 1 Solubilization of CFTR-His expressed in Sf9 cells by NaPFO

Left panel: 500 μ g of total membrane protein prepared from CFTR-His-expressing Sf9 cells was treated with various detergents (% w/v) in a final protein concentration of 7.5 mg/ml in 20 mM phosphate buffer, pH 8.0. High-salt solutions contained 500 mM NaCl and low-salt solutions, 100 mM NaCl. Samples were shaken for 1 h on a nutator and then spun in an airfuge at 100000 g for 30 min. Solubilized protein (25 μ l) was subjected to SDS/PAGE and Western blotting. The blot was probed with the anti-CFTR mAb, M3A7. Right panel: CFTR-His solubilized by NaPFO at various concentrations was expressed as a percentage of the SDS-solubilized CFTR-His protein. Relative band intensities were quantified using National Institutes of Health image software (v1.61).

Figure 2 Immobilized metal-ion affinity chromatography of Sf9 CFTR in NaPFO

Upper panel: elution profile with the pH gradient indicated, generated from 25 mM phosphate buffers, pH 7.4 and pH 4.0, containing 100 mM NaCl and 4% (w/v) NaPFO. Lower panel: silver-stained protein bands after SDS/PAGE (6% acrylamide) of crude NaPFO membrane extract and the fractions shown. Lane 5, shows the immunoreactivity of the protein from lane 4 with the CFTR mAb M3A7.

ography call for elution of contaminating proteins at pH 6.0, we found that contaminating proteins solubilized in NaPFO could be effectively eluted at pH 7.4 and NaPFO-solubilized CFTR-His was eluted at pH 6.8 (Figure 2).

Assessment of fractions containing CFTR-His by SDS/PAGE (Figure 2) revealed a single, silver-stained band migrating at a molecular mass expected for core glycosylated CFTR, 135 kDa [11]. We confirmed that CFTR-His was purified to near homogeneity (approx. 90%) by densitometric analysis of the silver-stained gels corresponding to three different purifications. Identity of the purification product as CFTR was confirmed by immunoblotting using the mAb M3A7 and quantitative amino-acid analysis. The yield of purified CFTR-His protein obtained from 1 litre of Sf9 cells (5×10^9 cells) was approx. 700 mg, a higher yield than the 500 mg reported for our previous method for CFTR purification [2]. CFTR-His was readily reconstituted from NaPFO micelles into phospholipid vesicles composed of PE/PS/PC/ergosterol (5:2:1:1, by weight) through two successive dialysis steps as described previously [2,7].

ATPase activity of CFTR-His

As in our previous studies of Sf9-CFTR purified in SDS [7], we determined that CFTR-His purified using NaPFO exhibited a slow rate of ATP hydrolysis which was enhanced by pre-

Figure 3 ATPase activity of CFTR-His

Left panel: a representative thin-layer chromatographic separation of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ (lower arrow) and $[\alpha\text{-}^{32}\text{P}]\text{ADP}$ (upper arrow). The left lane indicates lack of $[\alpha\text{-}^{32}\text{P}]\text{ADP}$ production (—) by liposomes alone incubated with $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ for 4 h at 32 °C, and the right lane indicates $[\alpha\text{-}^{32}\text{P}]\text{ADP}$ production by liposomes containing CFTR-His (100 ng) (+). Right panel: ATP dependence of ATP hydrolysis by PKA-phosphorylated CFTR-His (○) or untreated CFTR-His (□) (each 100 ng protein). The means $\pm$ S.D. are shown for experiments performed in duplicate. Data were analysed according to the Hill equation, $v = S^h V_{\max} / S^h + K'$, where v and $V_{\max}$ are the initial and maximal rates of hydrolysis respectively, S is the ATP concentration, h is the Hill coefficient and the constant K' is related to K_m by the equation; $K' = (K_m)^h$ to yield the following parameters for phosphorylated CFTR-His and partially phosphorylated CFTR-His: K_m , 128 μM (○) and 2.9 mM (□); $V_{\max}$, 1.56 nmol/4 h (○) and 1.75 nmol/4 h (□); h , 1.23 (○) and 0.93 (□) respectively.

phosphorylation with the catalytic subunit of PKA (Figure 3). The mean rate of ATP hydrolysis by phosphorylated CFTR-His preparations at 1 mM Mg-ATP, 1.69 ± 0.21 nmol/4 h (S.E., $n = 5$), tended to exceed that determined previously for the same mass (100 ng) of phosphorylated CFTR purified using the original procedure, 1.16 ± 0.34 nmol/4 h (S.E.) [7], although the difference was not statistically significant. Furthermore, as in our previous analyses of SDS-purified CFTR protein, PKA phosphorylation increased the ATPase activity of CFTR-His by increasing its affinity for Mg-ATP (Figure 3).

Channel activity of CFTR-His

The overall chloride channel-forming capacity of purified, reconstituted CFTR-His was assessed by measuring electrogenic $^{36}\text{Cl}^-$ uptake by proteoliposomes. As in our previous studies of purified CFTR, proteoliposomes containing purified, phosphorylated CFTR-His possessed the ability to accumulate $^{36}\text{Cl}^-$ in response to an inwardly-directed electrical potential difference [15]. We found that the maximum $^{36}\text{Cl}^-$ uptake by proteoliposomes containing phosphorylated CFTR-His exceeded that determined for phosphorylated CFTR purified using the conventional method, 4.2 ± 0.9 nmol/h per μg (S.D., $n = 14$) versus

2.4 ± 0.7 nmol/h per μg (S.D., $n = 4$, $P < 0.05$) respectively. This observation suggests that either there is an increase in the proportion of CFTR molecules which are functionally active following this purification protocol or each individual molecule exhibits an increase in channel activity.

Single-channel analyses indicate that individual molecules of CFTR-His exhibit similar biophysical properties to individual molecules of CFTR. First, as expected, PKA phosphorylation and the addition of Mg-ATP were required for activation of CFTR-His-channel function (Figure 4) [1–7]. Secondly, the unitary conductance of CFTR-His channels reconstituted in planar lipid bilayers, i.e. 11.8 ± 0.4 ps ($n = 5$) was similar to that determined for CFTR, approx. 11 ps [2], in the presence of the same electrical (–10 to –50 mV) and chemical (300 versus 50 mM KCl, *cis* versus *trans*) driving forces. Thirdly, CFTR-His possesses high selectivity for anions relative to the cations. In the presence of the asymmetrical KCl solutions described above, single channel current reversal occurred at +50 mV, a value close to that predicted for an ideally chloride selective channel, i.e. +45 mV (results not shown). The relative cation impermeability was supported by the lack of effect of substituting 300 mM KCl with 300 mM *N*-methyl-D-glucosamine chloride solutions (Figure 4). Finally, as predicted for a chloride-selective pore, single-channel currents were abrogated upon exchange of KCl with K-glutamate-containing solutions.

Similarly, we found that the gating of the channel activity of CFTR-His was comparable with that which we had reported previously for purified, reconstituted CFTR [2,7]. Following activation by PKA phosphorylation and addition of 1 mM Mg-ATP, CFTR-His single channels exhibited the slow gating normally associated with CFTR function in native membranes as well as in planar lipid bilayers (Figure 5) [1–7,16]. Dwell-time histograms (Figure 5), generated to quantify mean channel open and closed times, revealed that the kinetics of purified CFTR-His channel opening and closing were similar to those reported for purified CFTR [7]. As in our studies of purified CFTR, we found that a single exponential distribution described the log open-time histogram, whereas the log closed-time histogram was best described using two distributions, representing a short- and a long-duration closed time, typical for bursting channel behaviour. For subsequent burst analysis, we determined the burst delimiter, τ_c , to be 50 ms, according to the method described by Winter et al. [17]. The mean interburst dwell time and burst dwell times for CFTR-His in the presence of 1 mM Mg-ATP were used to evaluate the rate of opening to a burst and the rate of closing from a burst respectively. As for CFTR, the rate of closure of CFTR-His channels, i.e. 1/s, was slower than the bursting rate of 4/s and was limiting to the overall channel transition rate. The

Figure 4 Chloride-channel function of CFTR-His

This panel shows single-channel activity of PKA-phosphorylated CFTR-His in the presence of 1 mM Mg-ATP at the holding potential of –50 mV. The ionic composition of bilayer compartments is shown in the adjacent cartoons. NMDG, *N*-methyl-D-glucosamine.

Figure 5 Planar lipid bilayer studies of CFTR-His channel gating

Upper panel: record of single-channel activity of PKA-phosphorylated CFTR-His in the presence of 1 mM Mg-ATP. Lower panel: open- and closed-time histograms determined from CFTR-His channel activity (over 7 min), as shown above. The channel open-time histogram (left) was described by a single exponential, indicating the presence of a single open time (53.6 ± 0.1 ms), and the closed-time histogram (right) was described by two exponential distributions, indicating the presence of both short- and long-duration closed times (10.0 ± 0.1 and 163.38 ± 0.01 ms respectively). The mean burst and interburst durations, 858.6 ± 0.3 and 275.6 ± 0.1 ms, were determined using $\tau_c = 50$ ms as the burst delimiter.

overall transition rate for CFTR-His channel gating was comparable with that determined for purified CFTR, namely 1–2/s. Therefore the improvement in transport capacity measured in the electrogenic $^{36}\text{Cl}^-$ -flux assay of CFTR-His-containing liposomes most likely represents an increase in the proportion of channel-competent molecules using the new purification strategy.

DISCUSSION

The novel purification scheme for CFTR presented here offers several advantages over our previous methods [2,3]. First, whereas CFTR purification from Sf9 cells was achieved by solubilization using the harsh detergent, SDS, we now show that CFTR can be purified under milder conditions using the novel detergent, NaPFO. Secondly, because this detergent is compatible with metal-affinity chromatography, purification of CFTR-His can be achieved rapidly in a single step. Preliminary experiments with other ion-channel proteins suggests that the strategy may be generally applicable to this class of proteins (M. Ramjeesingh, E. Garami, K. Galley and C. E. Bear, unpublished work).

Now that we have developed a rapid and efficient method for CFTR purification, we have overcome a major impediment to our future studies of the structure–function relationships of CFTR. For example, the ATPase activity of intact CFTR can be measured only in preparations of purified, active CFTR. Hence, this novel purification method will facilitate the detailed analysis of the ATP hydrolysis cycle through production of mutant proteins containing amino-acid substitutions within consensus sites for ATPases. Finally, the ease of purification of CFTR has significantly improved, which results in the production of milligram quantities of CFTR protein so that direct examination of the structural features of CFTR by electron microscopy and image analysis is now feasible [18].

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REFERENCES

- Welsh, M., Tsui, L.-C., Boat, T. F. and Beaudet, A. L. (1995) in *The Metabolic and Molecular Basis of Inherited Disease*, 7th edn., (Scriver, C. R., Beaudet, A. L., Sly, W. S. and Valle, D., eds.), pp. 3799–3876, McGraw-Hill, New York
- Bear, C. E., Li, C., Kartner, N., Bridges, R., Jensen, T., Ramjeesingh, M. and Riordan, J. (1992) *Cell* **68**, 809–818
- O'Riordan, C. R., Erickson, A., Bear, C. E., Li, C., Manavalan, P., Wang, K. X., Marshall, J., Scheule, R. K., McPherson, J. M., Cheng, S. H. and Smith, A. E. (1995) *J. Biol. Chem.* **270**, 17033–17043
- Anderson, M., Berger, H., Rich, D., Gregory, R., Smith, A. E. and Welsh, M. (1991) *Cell* **67**, 775–784
- Baukowitz, T., Wang, T., Nairn, A. and Gadsby, D. (1994) *Neuron* **12**, 473–482
- Gunderson, K. L. and Kopito, R. R. (1995) *Cell* **82**, 231–239
- Li, C., Ramjeesingh, M., Wang, W., Garami, E., Hewryk, M., Lee, D., Rommens, J. M., Galley, K. and Bear, C. E. (1996) *J. Biol. Chem.* **271**, 28463–28468
- Loo, T. W. and Clarke, D. M. (1995) *J. Biol. Chem.* **270**, 21449–21452
- Shepherd, F. H. and Holzenburg, A. (1995) *Anal. Biochem.* **224**, 21–27
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Kartner, N., Hanrahan, J., Jensen, T., Naismith, L., Sun, S., Ackerley, C., Reyes, E., Tsui, L.-C., Rommens, J., Bear, C. and Riordan, J. (1991) *Cell* **64**, 681–691
- Garty, H., Rudy, B. and Karlish, S. J. D. (1983) *J. Biol. Chem.* **258**, 13094–13099
- Goldberg, A. F. X. and Miller, C. (1991) *J. Membr. Biol.* **124**, 199–206
- Woodbury, D. and Miller, C. (1990) *Biophys. J.* **58**, 833–839
- Li, C., Ramjeesingh, M. and Bear, C. E. (1996) *J. Biol. Chem.* **271**, 11623–11626
- Tabcharani, J., Lowe, W., Elie, D. and Hanrahan, J. (1990) *FEBS Lett.* **270**, 157–164
- Winter, M., Sheppard, D., Carson, M. and Welsh, M. J. (1994) *Biophys. J.* **66**, 1398–1403
- Rosenburg, M. F., Callaghan, R., Ford, R. C. and Higgins, C. F. (1997) *J. Biol. Chem.* **272**, 10685–10694