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# Cystic Fibrosis Transmembrane Conductance Regulator in Human and Mouse Red Blood Cell Membranes and Its Interaction With Ecto-Apyrase

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**Abstract** Elevated blood ATP and increased red blood cell (RBC) ATP transport is associated with cystic fibrosis (CF). In this report, we demonstrate the presence of the wild-type and the ΔF508 mutant form of the CF transmembrane conductance regulator protein in RBC membranes and its putative interaction with ecto-apyrase, an ATP hydrolyzing enzyme also present in the RBC membrane. RBC membranes of control and ΔF508 individuals and of wild-type and CF transmembrane conductance regulator-knockout mice were examined by immunoblot using several antibodies directed against different epitopes of this protein. These experiments indicated that human RBC membranes contain comparable amounts of the wild-type CF transmembrane conductance regulator protein and the ΔF508 mutant form of the protein, respectively. CF transmembrane conductance regulator protein was also detected in wild-type mouse RBC membranes but not in the gene knockout mouse RBC membranes. Antibodies directed against ecto-apyrase co-immunoprecipitated CF transmembrane conductance regulator protein of human RBC membranes indicating a physical interaction between these two membrane proteins consistent with ATP transport and extracellular hydrolysis. We conclude that RBCs are a significant repository of CF transmembrane conductance regulator protein and should provide a novel system for evaluating its expression and function. J. Cell. Biochem. 91: 1174–1182, 2004. 

**Key words:** CFTR; ΔF508 CFTR; CD39; ecto-apyrase; ATP transport; cystic fibrosis

Cystic fibrosis transmembrane conductance regulator (CFTR) is a member of the ATP-binding cassette (ABC) superfamily of membrane proteins [Hyde et al., 1990]. In a variety of cell lines, CFTR has been shown to be associated with transmembrane ATP transport [Cantiello et al., 1994; Reisin et al., 1994; Schwiebert et al., 1995; Abraham et al., 1997]. Mutations in the CFTR result in the disease cystic fibrosis (CF) [Cheng et al., 1990; Welsh and Smith, 1993]. An estimated half or more of newly synthesized wild-type CFTR protein is degraded in the endoplasmic reticulum (ER) and hence does not mature and become integrated into the plasma membranes of polarized epithelial cells [Lukacs et al., 1994; Ward and Kopito, 1994]. ER-associated degradation of the CFTR and ΔF508-CFTR by the ubiquitin-proteasome pathway has been shown [Sato et al., 1998; Xiong et al., 1999; Fuller and Cuthbert, 2000]. The ΔF508-CFTR mutation, responsible for approximately 70% of CF diagnoses, is thought to result in abnormal folding of the protein and increased degradation in the ER compared to wild-type CFTR [Cheng et al., 1990; Sheppard et al., 1995]. Thus, the presence of CFTR and ΔF508-CFTR in the red blood cell (RBC) membrane is of considerable importance with respect to its role in ATP transport.

RBCs act as transporters of oxygen and carbon dioxide and participate in the maintenance of plasma ATP levels by releasing stored

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intracellular ATP. Jenkins et al. [1993] first demonstrated increased total RBC ATP content in CF subjects compared to controls. CFTR knockout mice were shown to have increased blood plasma ATP concentrations and total RBC ATP content compared to blood from wild-type mice [Abraham et al., 1996]. Lader et al. [2000] have observed elevated plasma ATP in CF individuals, which is consistent with our observation that CFTR knockout mice have elevated plasma ATP levels compared to wild-type controls [Abraham et al., 1996]. Further data from our laboratory [Abraham et al., 2001] show that mutation of CFTR or gene knockout results in increased ATP release and elevation of plasma ATP levels indicating that wild-type CFTR downregulates ATP release. Additional evidence for the involvement of CFTR in RBC ATP transport comes from the report by Sprague et al. [1998] indicating that deformation-induced ATP release from RBCs was dependent on the presence of wild-type CFTR-like activity. This deduction was based on pharmacological data from RBCs using the inhibitors glibenclamide or niflumic acid. However, ATP transport function of other ABC proteins in the RBC cannot be excluded based on this pharmacological evidence alone. An example of an ABC protein other than CFTR present in the RBC is the multidrug-associated protein (MRP) [Pulaski et al., 1996].

It is proposed that ATP release occurs through a membrane complex [Abraham et al., 2001]. Support for the view comes from the findings that ecto-apyrase (CD39) is found in plasma membranes associated with ABC proteins. Postulated functions of CD39 among others [Yeung et al., 2000] include regulation of purinergic signaling.

The level of CD39 expression appears to be correlated with ABC protein expression [Abraham et al., 2001]. CD39 hydrolyzes extracellular ATP to ADP that is subsequently hydrolyzed to AMP. The extracellular ATPase activity of CD39 is greatest when this membrane glycoprotein is in a tetrameric form [Wang and Guidotti, 1996]. This property of CD39 is in concert with a putative hetero-oligomeric structure existing between CD39 and CFTR that can positively affect ATP transport and extracellular ATP hydrolysis [Abraham et al., 2001].

We present data confirming the presence of CFTR proteins in human and mouse RBC membranes. CFTR protein in the RBC membrane indicates a re-evaluation of CFTR function in the RBC membrane. We have presented co-immunoprecipitation data that CFTR and ΔF508-CFTR are associated with CD39 (ecto-apyrase). In particular, CFTR may function as a heteromeric structure through protein–protein interaction with other RBC ABC proteins and/or with the ATP metabolizing enzyme, ecto-apyrase (CD39).

## MATERIALS AND METHODS

### Blood Collection

Three milliliter aliquots of blood were collected by venous puncture using Vacutainers® containing ethylenediamine tetra-acetic acid (EDTA) from genotyped volunteers. Mouse blood was collected from the tail vein. Plasma, RBC, and buffy coat (white blood cells) fractions were separated by centrifugation at 2,500 rpm. Plasma and buffy coat were removed by aspiration. The remaining, packed RBCs were washed four to five times in ice-cold phosphate-buffered saline (PBS) containing protease inhibitor cocktail (1 mM EDTA, 20 μg/ml phenylmethylsulfonyl fluoride (PMFS), 20 μg/ml aprotinin, 5 μg/ml pepstatin A, and 5 μg/ml leupeptin). After each wash and centrifugation step, the top layer was aspirated to remove residual white blood cells. An aliquot of the packed, washed RBCs was subjected to Coulter analysis in the Dartmouth-Hitchcock Medical Center clinical laboratories. White blood cell contamination was limited to one white blood cell in 100,000 RBCs.

### Genotyping

Specimen collection for genotyping was done with a Cheekbrush DNA Specimen Collection Kit from genzyme GENETICS (Framingham, MA). Samples were shipped overnight to Genzyme GENTICS for analysis.

### Recombinant CFTR

Dr. Peter Maloney (Johns Hopkins University) provided purified, recombinant human CFTR from *Spodoptera frugiperda* insect cells (Sf9 cells).

### Knockout Mice

Exon 10 CFTR knockout mice (Cftrm1UNC) were obtained from Jackson Laboratory, Bar Harbor, ME and as a gift from Dr. Beverly Koller, University of North Carolina, Chapel

Hill, NC [Snouwaert et al., 1995]. Exon 1 CFTR knockout mouse blood was a gift from Dr. Lap-Chee Tsui and Dr. Richard Rozmahel of the University of Toronto [Rozmahel et al., 1997].

### Human Colon Adenocarcinoma T84 Cells

T84 cells (American Type Culture Collection, Manassas, VA) were maintained in DMEM/F-12 media (Life Technology, Rockville, MD) containing L-glutamine supplemented with 10% fetal bovine serum (FBS) and antibiotics (Life Technology).

### RBC Ghost Membranes Preparation

Washed RBCs were lysed in ice-cold, 5 mM sodium phosphate, pH 8.0 containing protease inhibitor cocktail for 10 min on ice, and extensively washed and sedimented by centrifugation at 27,000*g* for 20 min at 4°C according to the method of Casey et al. [1989]. The purified RBC membranes were dissolved using 1% sodium dodecylsulfate (SDS) or 50 mM tris-(hydroxymethyl)amino-methane (Tris)-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, and 1% Igepal (NP-40) plus protease inhibitor cocktail. Total protein was determined by the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL).

### Electrophoresis

RBC ghost membrane and protein was combined with an equal volume of two times SDS-gel loading buffer (5% SDS, 125 mM Tris-HCl, pH 6.8, 25% glycerol, 0.0025% bromophenol blue, 2-mercaptoethanol or dithiothreitol) and separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE), using a 6% polyacrylamide gel by the method of Laemmli [1970].

### Protein Transfer

The SDS–PAGE-separated proteins were transferred to polyvinylidene difluoride membranes (PVDF) for 20 min at 75 mA in 10 mM 3-[Cyclohexylamino]-1-propane-sulfonic acid (CAPS) pH 11.0.

### Immunoblot Analysis

The PVDF membranes with transferred protein were blocked with 5% milk, 1% normal serum corresponding to the species of HRP-conjugated second antibody used, Tris-buffered saline (TBS) or PBS, followed by incubation with anti-CFTR antibodies diluted 1:200 to 1:1,000 in 5% milk, TBS or PBS overnight at 4°C. After further washing, the membrane was incubated with the appropriate secondary antibody conjugated to horseradish peroxidase (HRP) (Pierce, Rockford, IL) in 5% milk for 1 h, washed and the protein signal visualized using the Pierce SuperSignal West Pico Chemiluminescent Substrate kit (Pierce, Rockford, IL) and exposure to film (Kodak, X-OMAT AR). Antibodies used for immunoblotting were anti-human CFTR, N-terminal specific, rabbit polyclonal antibody, A2 (kindly donated by Dr. William Skach, University of Oregon Health Science Center), anti-human CFTR, C-terminal specific, rabbit polyclonal antibody (kindly donated by Dr. Andrew Mulberg, University of Pennsylvania), anti-human R-domain specific, rabbit polyclonal antibody, (kindly donated by Dr. Catherine Fuller and Dr. Dale Benos, University of Alabama, Birmingham), anti-human CFTR, first extracellular loop specific, mouse monoclonal antibody [Walker et al., 1995] (Affinity BioReagents, Golden, CO), anti-human, C-terminal specific, mouse monoclonal antibody, (R&D Systems, Minneapolis, MN). Goat anti-mouse CFTR, C-terminus specific was purchased from Research Diagnostics (Flanders, NJ). Duplicate blots were processed with the corresponding normal IgG (e.g., normal rabbit IgG) and HRP-conjugated second antibody or second antibody alone to identify non-specific signals.

### Enzymatic Degradation of the Fully Glycosylated, CFTR "C" Form to the Unglycosylated "A" Form in Control T84 Cells and RBC Ghosts

T84 cells were washed with ice-cold PBS. The washed cells and human erythrocyte membranes prepared as described above were dissolved in 50 mM tris(hydroxymethyl)amino-methane (Tris)-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, and 1% Igepal (NP-40) plus protease inhibitor cocktail. One hundred micrograms total protein from the T84 or erythrocyte ghost 10,000*g* supernatant was used for digestion of the samples with N-glycosidase F or Endoglycosidase H (New England Biolabs, Beverly, MA). The sample volumes were increased 20-fold with 50 mM sodium phosphate (pH 7.5) (N-glycosidase F) or 50 mM sodium citrate (pH 5.5) (Endoglycosidase H). Either N-glycosidase F or Endoglycosidase H (5,000 NEB units) were added to the samples and incubated for 18 h at 37°C. Control samples received an

equal volume of the appropriate buffer instead of enzyme. Samples were concentrated using Microcon® centrifugal filters (Millipore Corp., Bedford, MA) for subsequent separation by SDS–PAGE. The signals for the T84 cells were obtained after a 5-min exposure and the RBC ghost signals were obtained after a 45-min exposure.

### Co-Immunoprecipitation of CFTR and CD39 From Control T84 Cells and RBCs

A non-denaturing lysate buffer to prevent non-specific membrane protein aggregation was used for immunoprecipitation. T84 cells were lysed by sonication on ice in 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Igepal CA-630 (Sigma, St. Louis, MO), 0.5% sodium deoxycholate with phenylmethylsulfonylfluoride (PMSF), aprotinin, sodium orthovanadate, and leupeptin to inhibit proteases. The purified RBC membranes were dissolved using the same non-denaturing buffer. Total protein was determined by the bicinchoninic acid BCA assay (Pierce, Rockford, IL).

Five hundred micrograms of T84 whole cell lysate or dissolved RBC ghosts were incubated with monoclonal, anti-CD39 antibody with gentle rocking overnight at 4°C. The reaction mixture was reacted with protein A agarose beads (20 μl of 50% bead slurry) for 3 h at 4°C. The reaction mixture was then centrifuged for 30 s at 4°C. The pellet was washed three times with 1 ml phosphate buffered saline. Subsequently, the eluted proteins from the protein A agarose were separated by SDS–PAGE, transferred to PVDF membrane, and reacted with CFTR antibody (rabbit anti-human CFTR).

## RESULTS

Purified human and mouse RBC membranes were analyzed by immunoblot for expression of CFTR and ΔF508-CFTR using a number of polyclonal and monoclonal anti-human CFTR and anti-mouse CFTR antibodies. Figure 1, lanes 1–9, shows CFTR signals from human RBC membranes obtained from nine wild-type CFTR individuals. A rabbit polyclonal anti-human CFTR (R-Domain-specific) antibody was used to detect CFTR in Figure 1. The characteristic immunoblot pattern for epithelial cell derived CFTR consists of the "A" (unglycosylated), "B" (core glycosylated), and "C" (fully glycosylated or mature) forms [Gregory et al., 1991; Morris et al., 1993; Chang et al., 1994]. Fully glycosylated CFTR protein, "C" form, has a molecular weight of approximately 170–190 kDa and has the highest apparent molecular weight compared to the "A" and "B" forms of the protein. The "A" form signal from RBC membranes was very weak. The putative "B" band co-migrated with the purified recombinant CFTR from Sf9 cells (data not shown). This conclusion is based on the report by Ramjeesingh et al. [1997] that purified, recombinant CFTR from Sf9 cells gives a single band when separated by SDS–PAGE and subsequent identification by immunoblot that is consistent with the "core glyclosylated" or "B" form of CFTR. The major CFTR bands observable in Figure 1 are consistent with the fully glycosylated "C" form of CFTR protein. The "C" form appears to predominate in the mature, terminally differentiated RBC membranes.

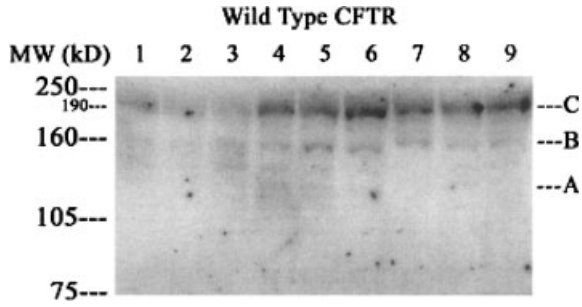


**Fig. 1.** Wild-type CFTR expression in human red blood cell membranes. **Lanes 1–9**: Twenty-five micrograms each of purified human red blood cell membrane protein from nine wild-type CFTR subjects. An affinity purified, rabbit polyclonal, anti-human CFTR (R-domain specific) antibody was used to detect both purified Sf9 human recombinant CFTR and human red blood cell (RBC) CFTR. The mature (fully glycosylated) form of CFTR is indicated by "**C**." The "co-reglycosylated" form of CFTR is indicated by "**B**." **Lanes 4** and **5** show faint signals for the putative "unglycosylated" form of CFTR indicated by an "**A**."

Figure 2 compares expression of wild-type CFTR to ΔF508-CFTR in the human RBC membranes using four other specific antibodies. Two rabbit polyclonal anti-human CFTR antibodies (N-terminus (Panel A) and C-terminus specific (Panel B), respectively and two mouse monoclonal anti-human CFTR antibodies (C-terminus (Panel C) and first extracellular loop specific (Panel D), respectively) were used. After stripping, the blot was used for each different antibody tested. The Band 3 protein level in each sample was determined by immunoblot using an anti-human Band 3 rabbit polyclonal antibody (Panel E). The Band 3 signal for each sample was used for normalization of total

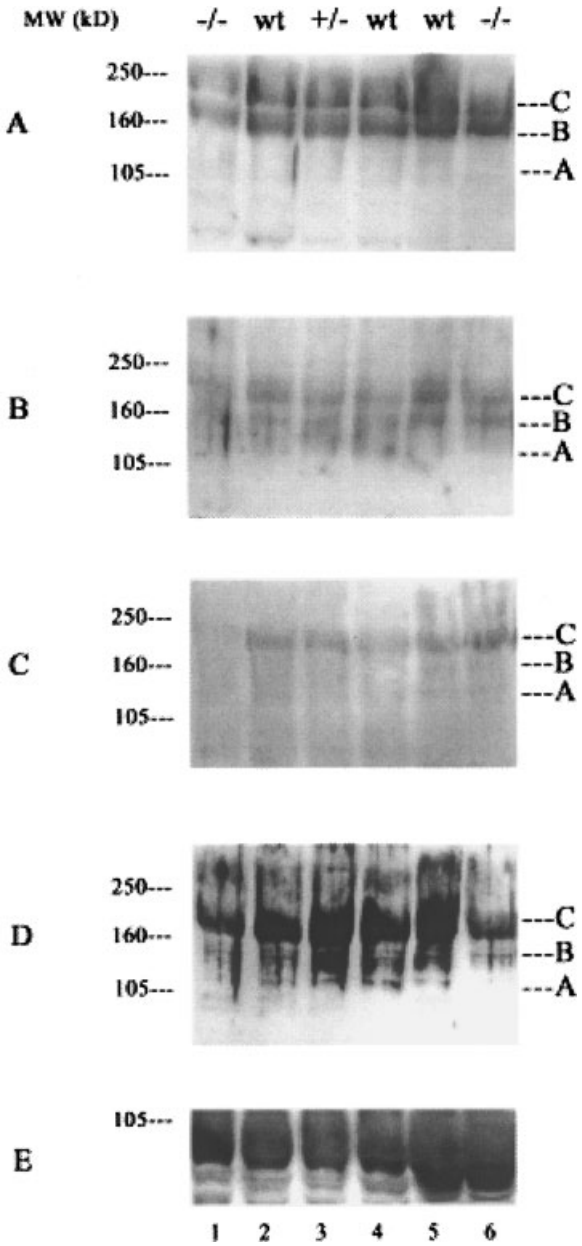


**Fig. 2.** CFTR expression in wild-type and ΔF508-CFTR homozygous and heterozygous human red blood cell (RBC) membranes as determined by four different anti-human CFTR antibodies. **Panel A**: CFTR expression from RBC membranes determined by reaction with rabbit polyclonal anti-human CFTR (N-terminal-specific). **Lanes 1** and **7**; 50 μg of RBC membrane protein from homozygous, ΔF508-CFTR individuals. **Lanes 2**, **4**, and **5**; 50 μg of RBC membrane protein from wild-type individuals. **Lane 3**; 50 μg of RBC membrane protein from a ΔF508-CFTR heterozygous individual. **Panel B**: Same blot stripped and reprobed with a second rabbit polyclonal anti-human CFTR (C-terminal specific). **Panel C**: Same blot stripped and reprobed with a mouse monoclonal anti-human CFTR (C-terminal-specific). **Panel D**: Same blot, stripped and reacted with mouse monoclonal anti-human CFTR, first extracellular loop-specific antibody (41). **Panel E**: The same blot stripped and reprobed with rabbit polyclonal anti-human Band 3.

protein loaded. The ratio of the "C" band signal intensity to Band 3 signal intensity for each sample from Figure 2 is shown in Table I. The analysis indicates that expression of the mature forms of either CFTR or ΔF508-CFTR is comparable in human RBC membranes.

Figure 3 indicates that the "C" form (fully glycosylated or mature CFTR) from T84 cells is shifted to the "A" form (increased band intensity indicated by an asterisk) upon addition of N-glycosidase F (Lane 2). There is no band shift for T84 cell CFTR "C" form after addition of endoglycosidase H (Lane 4). Human RBC CFTR "C" form is also shifted to the "A" form upon addition of N-glycosidase F (Lane 6, indicated by and asterisk) and no band shift upon addition of endoglycosidase H (Lane 8).

Additional compelling immunoblot evidence for CFTR expression in RBC membranes is presented in Figure 4. The CFTR signal was absent from RBC membranes prepared from homozygous CFTR exon 10 knockout mice (UNC mice) (Fig. 4). The CFTR signal is present in the wild-type and the heterozygous mouse RBC membranes. The RBC CFTR signal from the heterozygous UNC mouse (±, 19.3 density units) was less intense than that of the wild-type mouse (+/+, 161.0 density units) (Fig. 4). The immunoblot in Figure 4 was also reacted with a goat-anti mouse CFTR polyclonal antibody and showed similar CFTR signals (+/+, 106.9, ±, 77.4, and −/−, 0.0 density units). The RBC CFTR signal was also absent in the homozygous (−/−) CFTR exon 1 knockout mouse (Toronto), while the wild-type mouse RBCs expressed CFTR (50.6 density units) (data not shown).

Figure 5 shows that CFTR from control T84 cells co-immunoprecipitated with anti-CD39 (Panel A, Lane 2). CFTR from ΔF508 and wild-type individuals' RBC membranes was also co-immunoprecipitated with anti-CD39 antibody (Panel B, Lanes 1 and 2).

## DISCUSSION

The CF phenotype manifests itself in many epithelial structures including airway, gastrointestinal tract, and sweat ducts. Historically, most investigation has centered on the trafficking and function of CFTR and mutant CFTR in these epithelial structures. Boucher et al. [1984] investigated the possibility that the CF chloride defect extended to the non-epithelial, RBC.

**TABLE I. Human Red Blood Cell Wild-Type CFTR and +/−, −/− ΔF508CFTR/Band 3 Signal Ratios From Signal Densities**

| Antibody | +/+ | +/+ | +/+ | +/+ (mean/SD) | −/− | −/− | −/− (mean) | +/− |
|---|---|---|---|---|---|---|---|---|
| N-Terminus (P)[a] | 0.63 | 0.69 | 0.79 | 0.70/0.08 | 0.45 | 0.84 | 0.65 | 0.65 |
| C-Terminus (P) | 0.53 | 0.49 | 0.58 | 0.53/0.05 | 0.50 | 0.57 | 0.54 | 0.50 |
| C-Terminus (M)[b] | 0.50 | 0.38 | 0.43 | 0.44/0.06 | 0.43 | 0.56 | 0.50 | 0.48 |
| 1st extracellular loop (M) | 1.12 | 1.17 | 1.12 | 1.14/0.03 | 0.95 | 0.93 | 0.94 | 1.31 |

CFTR "C" band and Band 3 signal densities (arbitrary units) were determined using NIH Image software.
[a]P, rabbit polyclonal anti-CFTR.
[b]M, mouse monclonal anti-CFTR.

This investigation examined RBC chloride ion transport, and found no significant differences between control and phenotypic CF subjects. Recent findings of CF-associated (i.e., ΔF508-CFTR homozygous individuals) elevation of blood ATP levels observed in our laboratory and by others [Jenkins et al., 1993; Abraham et al., 1996] led to our examination of RBCs for CFTR and ΔF508-CFTR expression. The presence of CFTR and ΔF508-CFTR in the RBC membrane is important with respect to ATP and chloride ion transport functions of blood.

In polarized epithelial cells, subcellular organelles mediate CFTR processing and degradation. Normal epithelial trafficking of CFTR involves its integration into membranes of the endoplasmic reticulum (ER) and its interaction with Hsc70/Hsp40, Hsp90, and calnexin [Yang et al., 1993; Pind et al., 1994; Loo et al., 1998; Meacham et al., 1999]. Epithelial CFTR maturation includes glycosylation [Morris et al., 1993; Ernst et al., 1994; Wei et al., 1996; Jiang et al., 1997] and its integration into the plasma membrane [Rommens et al., 1991; Greger et al., 1996]. Degradation of CFTR has been reported to involve ubiquintination and association of CFTR with the endoplasmic reticulum [Xiong et al., 1999]. In contrast to epithelial cells, the

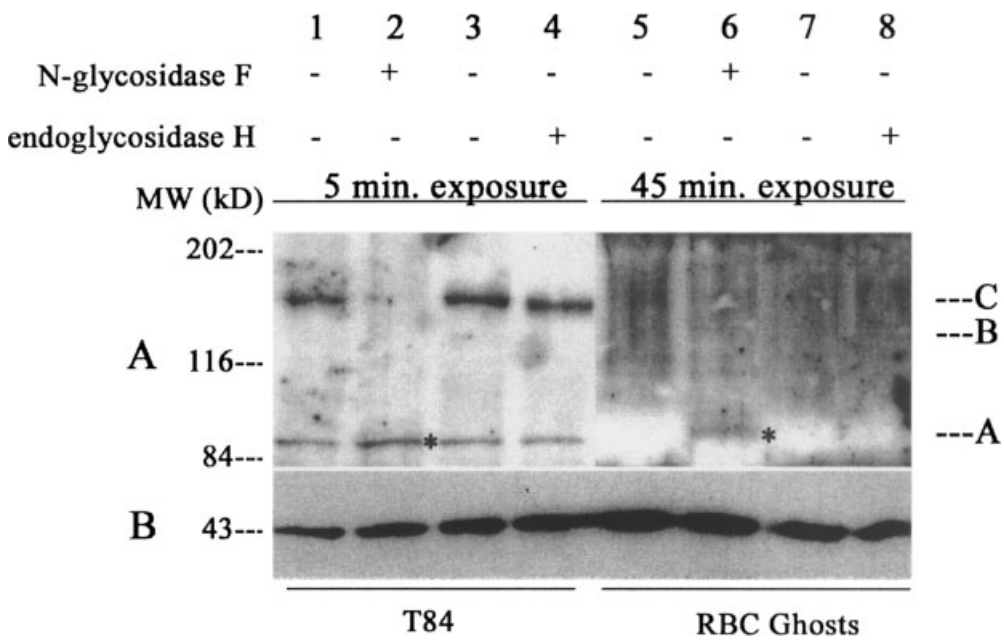


**Fig. 3.** Enzymatic degradation of the fully glycosylated, CFTR "**C**" form to the unglycosylated "**A**" form in control T84 cells and red blood cell (RBC) ghosts. **Panel A**: **Lane 1** and **3**, 100 μg total protein from T84 cells (control lanes: no enzymes added). **Lane 2**, 100 μg total protein from T84 cells treated with N-glycosidase F. The increased signal intensity of the "**A**" form of CFTR is noted by an asterisk (*). **Lane 5**, 100 μg total protein from T84 cells treated with Endoglycosidase H. **Lanes 5–8**, 100 μg of RBC ghost protein treated in parallel with either N-glycosidase F or Endoglycosidase H as for the T84 cell protein. "**C**" indicates fully glycosylated CFTR. "**B**" indicates the "core" form of CFTR. "**A**" indicates the unglycosylated form of CFTR. **Panel B**: The blot was stripped and reprobed with rabbit anti-actin as a control for gel loading error and/or transfer uniformity.

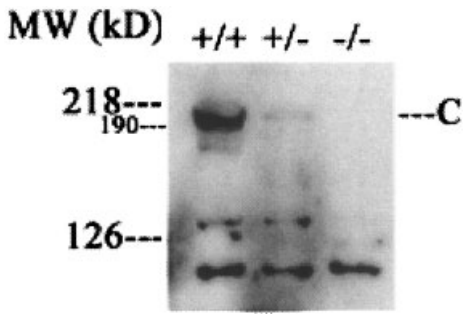


**Fig. 4.** Mouse red blood cell (RBC) CFTR. Fifty micrograms each of wild-type (+/+), heterozygous (±), and homozygous (–/–) UNC, CFTR exon 10 knockout mouse, RBC membrane protein was separated and transferred to membrane and probed with a mouse monoclonal anti-CFTR (first extracellular loop specific) that reacts with both human and mouse CFTR.

matured RBC has no nucleus or subcellular organelles [Goldwasser, 1981 Tong and Goldwasser, 1981]. The absence of ER and associated subcellular organelles involved in protein recycling in mature RBCs may contribute to stability of CFTR and ΔF508-CFTR.

Kalin et al. [1999] reported that the mutated form of CFTR, ΔF508-CFTR was detectable in respiratory and intestinal tracts in amounts and localization comparable to that of wild-type CFTR. This report indicated that trafficking of ΔF508-CFTR in certain cell types resulted in normal integration into the plasma membrane. Our finding of ΔF508-CFTR in the RBC membrane is compatible with this observation and indicates that ΔF508-CFTR and wild-type CFTR undergo comparable trafficking processes in the developing RBC.

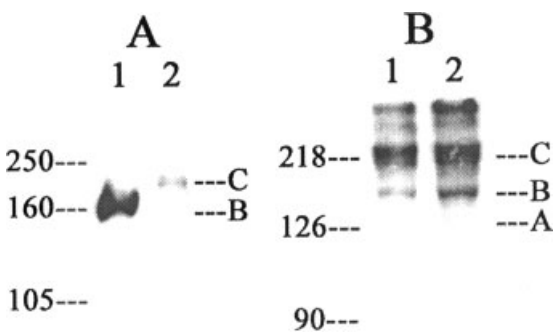


**Fig. 5.** Co-immunoprecipitation of CFTR and CD39 from control T84 cells and red blood cells (RBCs). **Panel A**: **Lane 1**, 10 ng of recombinant wild-type CFTR purified from Sf9 insect cells. **Lane 2**: CFTR from T84 cells co-immunoprecipitated with anti-CD39 anti-body. **Panel B**: **Lane 1**, CFTR co-immunoprecipitated from RBC ghosts of a ΔF508-CFTR human subject with anti-CD39 anti-body. **Lane 2**: CFTR co-immunoprecipitated from RBC ghosts of a wild-type human subject with anti-CD39 anti-body. "**C**" indicates the fully glycosylated form of CFTR.

The RBC and its precursors are readily accessible for investigation of CFTR processing, expression, transport function(s), and protein–protein interaction with other RBC ABC proteins and/or membrane proteins such as CD39 [Abraham et al., 2001].

CFTR is also believed to participate in transmembrane ATP release in RBC membranes [Abraham et al., 1997]. From this perspective, the presence of purinergic receptors on the apical and basolateral surfaces of epithelial cells are consistent with the importance of extracellular ATP and purines at both these cellular surfaces [Hwang et al., 1996, 2000; McCoy et al., 1999]. Elevation of systemic ATP levels in CF may be a compensatory mechanism mediated by CFTR or ΔF508-CFTR in association with other RBC membrane proteins. We have presented co-immunoprecipitation data indicating that CFTR and ΔF508-CFTR are associated with CD39 (ecto-apyrase). Increased systemic ATP levels may also contribute to CF pathophysiology. The presence of CFTR in RBCs, its role in chloride ion transport, interaction with other membrane proteins, along with its role in ATP release make the RBC in CF an important and accessible system for further investigation.

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