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Control of Cystic Fibrosis Transmembrane Conductance Regulator Expression by BAP31*

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Expression of the cystic fibrosis transmembrane conductance regulator (CFTR) is stringently controlled by molecular chaperones participating in formation of the quality control system. It has been shown that about 75% of all CFTR protein and close to 100% of the [ΔPhe^{508}] CFTR variant are rapidly degraded before leaving the endoplasmic reticulum (ER). B cell antigen receptor-associated proteins (BAPs) are ubiquitously expressed integral membrane proteins that may control association with the cytoskeleton, vesicular transport, or retrograde transport from the *cis* Golgi to the ER. The present study delivers evidence for cytosolic co-localization of both BAP31 and CFTR and for the control of expression of recombinant CFTR in Chinese hamster ovary (CHO) cells and *Xenopus* oocytes by BAP31. Antisense inhibition of BAP31 in various cell types increased expression of both wild-type CFTR and [ΔPhe^{508}]CFTR and enabled cAMP-activated Cl$^-$ currents in [ΔPhe^{508}]CFTR-expressing CHO cells. Coexpression of CFTR together with BAP31 attenuated cAMP-activated Cl$^-$ currents in *Xenopus* oocytes. These data therefore suggest association of BAP31 with CFTR that may control maturation or trafficking of CFTR and thus expression in the plasma membrane.

Epithelial Cl$^-$ transport is controlled by the cystic fibrosis transmembrane conductance regulator (CFTR),[1] which is a Cl$^-$ channel and a regulator of other ion channels (1). Cl$^-$ transport and regulation of ion channels is defective in cystic fibrosis (CF), because of more than 900 different CFTR mutations. The most common mutation, [ΔPhe^{508}]CFTR, leads to a defect in maturation and processing of the CFTR protein and thus does not allow for expression of sufficient amounts of CFTR in plasma membranes of epithelial cells and non-epithelial cells that express recombinant CFTR (2, 3). It has been shown that because of incomplete folding, [ΔPhe^{508}]CFTR is not capable of leaving the ER and thus is not processed to a more mature and glycosylated form. Therefore, it does not become protease-resistant, is retained in the ER, and will not undergo complex glycosylation (4). It is therefore detected as a band of lower molecular weight when analyzed on an SDS gel (5, 6). Several proteolytic systems, including proteasomes, contribute to degradation of wild-type CFTR (wtCFTR) and [ΔPhe^{508}]CFTR (3, 7). The enzymes and compartments responsible for degradation of CFTR are part of the ER quality control system of secretory proteins (8). Binding of CFTR to the ER membrane chaperone calnexin (9) and the cytosolic chaperone Hsp70 or Hsp90 (10, 11) has been demonstrated. Small amounts of [ΔPhe^{508}]CFTR that escape proteolysis is trafficked to the cell membrane, where it functions as cAMP-regulated Cl$^-$ channel (12, 13). This is usually 1% or less of the amount of wtCFTR present in the native tissue and is probably higher in *Xenopus* oocytes (2, 14).

Recently, several proteins have been identified which bind to B cell antigen receptors (BCR) (15). These BCR-associated proteins (BAPs) were identified initially in mouse B cells and have been shown to be expressed ubiquitously in various types of cells, including CHO cells (16). BAP31 and other members of this family of proteins are present in the ER where they exist as integral membrane proteins associated with membrane immunoglobulin D in B cells (15, 16). It has been suggested that BAP31 and related proteins play a role in vesicular transport and control anterograde transport of certain membrane proteins such as cellubrevin (16, 17). We therefore examined in the present study whether BAP31 also affects expression of CFTR. We were particularly interested to see whether expression of [ΔPhe^{508}]CFTR can be influenced by manipulation of the expression of BAP31. The data presented here demonstrate that BAP31 inhibits both expression of wtCFTR as well as [ΔPhe^{508}]CFTR and suggest that BAP31 is participating in formation of the quality control system.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection with BAP31 Antisense Oligonucleotides—CHO cells expressing either wtCFTR or [ΔPhe^{508}]CFTR (kindly provided by Dr. X.-B. Chang and Prof. J. Riordan, Mayo Clinic Scottsdale, Scottsdale, AZ) were cultured in α-minimum essential medium supplemented with 8% fetal bovine serum, 50 μmol/liter methotrexate, penicillin (100 units/ml) and streptomycin (100 μg/ml) (Life Technologies, Germany) in a humidified atmosphere with 5% CO_2 (18). Human bronchial epithelial cells (16HBE14o−) and mouse epithelial-collecting duct cells (M1) were cultured as described previously (19, 20). About 5×10^6 CHO cells expressing [ΔPhe^{508}]CFTR were electroporated in the presence of 12 nmol/liter (40 μg/ml) of a plasmid encoding the enhanced green fluorescent protein (pEGFP-c1; CLONTECH, Germany) and about 0.5 μmol/liter (3.5 μg/ml) phosphorothioated (stabilized) oligonucleotides antisense to the first 20 bases of the BAP31 coding sequence. Control cells were transfected with EGFP and missense oligonucleo-

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[1] The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; CHO, chinese hamster ovary cells; GST, glutathione *S*-transferase; IBMX, 3-isobutyl-1-methylxanthine; EGFP, enhanced green fluorescent protein; BAP, B cell antigen receptor-associated protein; wt, wild-type; HBE, human bronchial epithelial cells; M1, mouse epithelial-collecting duct cells.

tides only. Cells were electroporated (Bio-Rad) at 400 V/500 μF and subsequently kept on ice for 10 min. Following, cells were resuspended in culture medium and plated on culture dishes. Alternatively, cells were transfected using 1 μmol/liter antisense DNA and the transfection reagent DOTAP (Roche Molecular Biochemicals) according to the manufacturer's protocol.

Western Blotting of CFTR and BAP31—Cells were lysed in sample buffer containing 10% SDS and 100 mmol/liter dithiothreitol. Protein concentration was determined according to a modified Laury method, and the lysates containing 20 μg of protein were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting as described previously (21). Proteins were separated by 7% (CFTR) and 12% (BAP31) SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes (Bio-Rad), and bound antibodies were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech) (22). Using the different protocols, antisense transfection and Western blot analysis were performed three to seven times for each cell line. A mouse monoclonal antibody (M3A7) was used for detection of CFTR (kindly provided by Prof. Dr. J. Riordan, Mayo Clinic Scottsdale) (23). Specific rabbit anti-BAP31 serum has been raised against the GST fusion protein carrying the C-terminal half of BAP31 (16). Horseradish peroxidase-conjugated goat anti-mouse antibodies were obtained from Southern Biotechnology Associates. The goat anti-mouse IgG (H+L)-DTAF and goat anti-rabbit IgG (H+L)-Texas Red secondary antibody conjugates were from Jackson Laboratories.

$^{36}Cl^-$ Efflux—CHO cells grown on 35-mm culture dishes were analyzed for forskolin (10 μmol/liter) activated $^{36}Cl^-$ efflux. Electroporated CHO cells were seeded on 35-mm culture dishes and were allowed to grow to subconfluence for 72 h. After rinsing the culture dishes with efflux buffer (all mmol/liter) 140 NaCl, 3.3 KH_2PO_4, 0.83 K_2HPO_4, 1 $CaSO_4$, 1 $MgSO_4$, 5 HEPES, 10 glucose, pH 7.4, 37 °C) fresh efflux buffer (1 ml) containing 2 μCi/ml $^{36}Cl^-$ was added to each dish. Cultures were incubated for 2 h at 37 °C and then washed three times for 10 s with efflux buffer. 1 ml of efflux buffer was applied, removed in 1-min intervals, and replaced by fresh buffer. At 3 min, forskolin (10 μmol/liter) was added to the buffer. Cells were extracted overnight at 4 °C, remaining $^{36}Cl^-$ was determined, and samples were counted in scintillation mixture. The percent efflux/min was calculated according to Ref. 24.

Patch Clamp Analysis and GFP Fluorescence—Cell culture dishes were mounted on the stage of an inverted microscope (IM35, Zeiss, Oberkochen, Germany) and kept at 37 °C. The bath was continuously perfused with Ringer solution at a rate of about 20 ml/min. EGFP fluorescence was observed at 480-nm excitation and 520-nm emission using fluorescence microscopy (Zeiss, Oberkochen, Germany). Only cells demonstrating EGFP fluorescence were used for patch clamp analysis. During the experiments, 115 mmol/liter Cl^- was replaced by equimolar concentrations of the impermeable anion gluconate ($30Cl^-$). Patch clamp analysis was performed in the fast whole cell configuration according to (25). The patch pipettes had an input resistance of 2–4 megohms and were filled with a solution containing (mmol/liter) KCl 30, potassium gluconate 95, NaH_2PO_4 1.2, Na_2HPO_4 4.8, EGTA 1, $CaCl_2$ 0.726, $MgCl_2$ 1.034, D-glucose 5, ATP 1. The pH was adjusted to 7.2, the Ca^{2+} activity of this solution was 0.1 μmol/liter. The access conductance was measured continuously and was between 30 and 120 nanosiemens. Currents (voltage clamp) and voltages (current clamp) were recorded using a patch clamp amplifier (List Medical Electronic, Darmstadt, Germany) and were displayed continuously on a pen recorder (Gould Instruments). In regular intervals, membrane voltages (V_c) were clamped in steps of 10 mV to ± 40 mV. G_m was calculated according to Ohm's law from the measured I and V_c values (25). CFTR Cl^- currents were activated by 3-isobutyl-1-methylxanthine (IBMX; 100 μM) and forskolin (10 μM) (Sigma).

Immunofluorescence—CHO cells were grown on coverslips, washed with ice-cold phosphate-buffered saline containing 0.01% azide and were fixed with 2% paraformaldehyde in phosphate-buffered saline for 15 min at room temperature and washed three times in phosphate-buffered saline. Cell were permeabilized with blocking buffer containing 1% fetal calf serum, 0.01% azide and 0.03% saponin (Sigma-Aldrich) for 15 min. Cells were incubated with antibodies diluted in blocking buffer for 15 min at room temperature. Unbound antibodies were removed by washing with blocking buffer. After incubation with secondary antibodies, cells were washed three times in blocking buffer, air dried, and mounted in 10 μl of Fluoromount-G (Southern Biotechnology Inc.) on a microscope slide. Fluorescence microscopy was conducted using a LEIKA TCS confocal laser scanning microscope (Leitz, Germany).

cDNA and in Vitro Transcription of cRNA—To generate the cDNA encoding BAP31 missing the C-terminal KK*XX* motif (BAP31-KK*XX*), the BAP31-containing plasmid was digested with *Sma*I/*Bsa*AI and religated. The resulting cDNA was missing the last 26 nucleotides encoding the C-terminal 8 amino acids. cDNA sequences encoding wtCFTR, [ΔF508]CFTR, BAP31, BAP31-KK*XX*, BAP29, and E3/19k were subcloned into pBluescript SK+ (Stratagene). For *in vitro* transcription using either T7 or T3 promoters, plasmids were linearized with *Kpn*I, *Bam*HI, *Sal*I, and *Not*I, respectively. *In vitro* transcription was performed using T7 and T3 polymerase, respectively, and a cap analog (mCAP RNA capping kit, Stratagene).

Double Electrode Voltage Clamp in Oocytes from Xenopus laevis—Oocytes were obtained from adult *X. laevis* frogs, defolliculated by 1-h treatment with collagenase (type A, Roche Molecular Biochemicals) and subsequently injected with 10–30 ng of RNA encoding the individual proteins. Recordings were taken 2–3 days after injection. During two electrode voltage clamp recordings, i/v curves were obtained every 20 s by voltage clamping the oocyte from −80 to +40 mV in steps of 20 mV. Conductances were calculated according to Ohm's law. Oocytes were continuously perfused with amphibian Ringer containing 96 mM NaCl, 2 mM KCl, 1.8 mM Ca^{2+}, 5 mM HEPES, 1 mM Mg^{2+} at pH 7.55. CFTR Cl^- currents were activated by IBMX (1 mM) and forskolin (10 μM).

Materials and Statistical Analysis—All used compounds were of highest available grade of purity. Dimethyl sulfoxide (Me_2SO) was from Merck (Darmstadt, Germany). IBMX and forskolin were from Sigma. The data are shown as original recordings or as mean values ± S.E.; *n* refers to the number of experiments. Statistical analysis was performed according to paired or unpaired Student's *t* test. $p < 0.05$ were accepted to indicate statistical significance (*).

RESULTS

BAP31 Controls Expression of CFTR and Is Colocalized with CFTR in CHO Cells—[ΔPhe^{508}]CFTR does not mature properly and is therefore excluded from the glycosylation pathway. Thus, [ΔPhe^{508}]CFTR appears as a lower molecular weight band on an SDS gel when compared with wtCFTR. We analyzed expression of wtCFTR and [ΔPhe^{508}]CFTR in CHO cells lines stably transfected with either wtCFTR or [ΔPhe^{508}]CFTR (4). Mature and fully glycosylated CFTR of molecular weight of about 180 kDa is detected in wtCFTR-expressing cells whereas [ΔPhe^{508}]CFTR appears as a immature precursor of a lower molecular weight of about 150 kDa (Fig. 1*A*). CHO cells do also show expression of BAP31 as indicated by Western blot analysis (Fig. 1*A*). Both wtCFTR- and [ΔPhe^{508}]CFTR-expressing cells were transfected with BAP31 antisense oligonucleotides (+ AS), which largely reduced expression of BAP31 when compared with control cells (−AS). Parallel to the reduced expression of BAP31, we detected an increase in both expression of wtCFTR as well as [ΔPhe^{508}]CFTR, suggesting that expression of CFTR is controlled by BAP31. Experiments were carried out at least in triplicates but generally 5–7 times. Expression of BAP31 was also detected in various cultured cell types from pancreas (CFPAC), collecting duct (M1), colon (HT_{29} and T_{84}) and airways (CFDE, 16HBE14o−, 9HTE), although levels of BAP31 expression varied significantly between the different cell lines. In all theses cell lines, CFTR is expressed endogenously (data not shown). In mouse collecting duct cells (M1) and human bronchial epithelial cell lines (16HBE14o−; HBE), expression of BAP31 was blocked by antisense treatment. Reduced expression of BAP31 was paralleled by enhanced expression of CFTR (Fig. 1*B*). These data suggest that BAP31 also controls expression of endogenous CFTR in airways and collecting duct of the kidney.

Because Western blot analysis suggested that BAP31 is involved in the control of synthesis and maturation of CFTR, we performed immunofluorescence stain of both CFTR and BAP31 (Fig. 2). The cells showed a pronounced staining for BAP31 (Texas red fluorescence) and were also significantly stained for wtCFTR (DTAF green fluorescence). The overlay (yellow fluorescence) of both pictures shows co-staining of the two proteins except for areas close to the plasma membrane (Fig. 2). These data suggest that both CFTR and BAP31 are colocalized in the ER of CHO cells and thus support the idea of BAP31 controlling

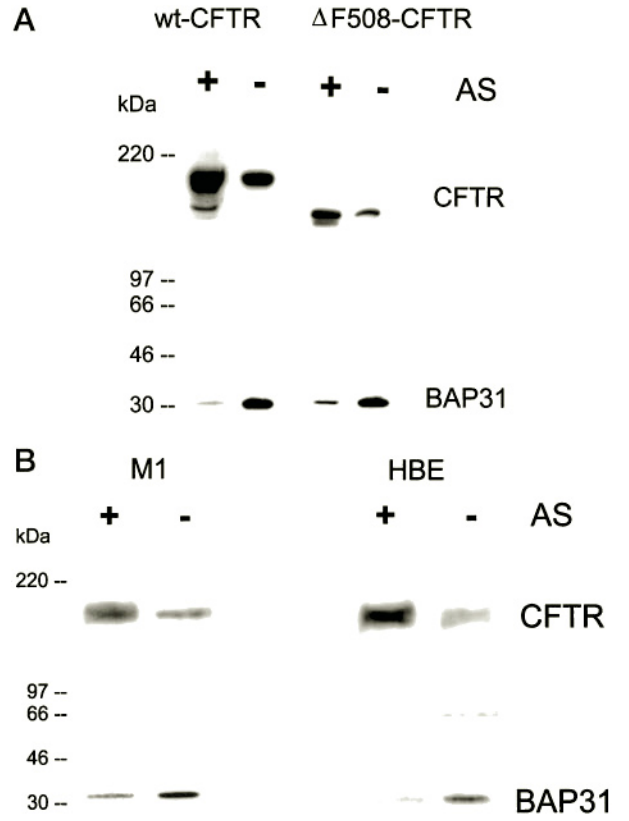

FIG. 1. **Western blot analysis of the expression of wtCFTR, [ΔF508]CFTR and BAP31 in CHO, M1, and HBE cells.** Equal amounts of protein (20 μg) were isolated from CHO cells, grown in the presence of a BAP31 antisense oligonucleotide (+*AS*) or missense oligonucleotide (−*AS*), and from M1 and HBE cells growing in either the presence or absence of BAP31-antisense, and were separated on SDS gels. When grown in the presence of antisense nucleotides, expression of BAP31 was largely reduced in CHO cells (*A*) and the epithelial cell lines M1 and HBE (*B*). The decrease in BAP31 expression was paralleled by an increase in CFTR expression in CHO cells, expressing CFTR exogenously (*A*), and the epithelial cell lines M1 and HBE, expressing CFTR endogenously (*B*). Individual experiments were carried out 3–7 times.

maturation or trafficking of CFTR.

Inhibition of BAP31 Expression Enhances Cl^- Conductance and Recovers Cl^- Channel Activity in Cells Expressing [ΔPhe^{508}]CFTR—To examine whether BAP31 also affects functional expression of CFTR, we analyzed cells transfected with BAP31 antisense as well as control cells in a $^{36}Cl^-$ efflux assay. To that end, electroporated CHO cells were grown on 35 mm-culture dishes and loaded with $^{36}Cl^-$. Subsequently, $^{36}Cl^-$ efflux was measured in the absence or presence of forskolin (10 μmol/liter). As shown in Fig. 3, $^{36}Cl^-$ efflux was continuously declining in the absence of forskolin (*dashed lines*). In wtCFTR-expressing CHO cells, stimulation with forskolin largely enhanced $^{36}Cl^-$ efflux, indicating activation of a CFTR Cl^- conductance, whereas no $^{36}Cl^-$ efflux was activated in [ΔPhe^{508}]CFTR-expressing CHO cells. However, in BAP31 antisense-treated cells expressing [ΔPhe^{508}]CFTR, forskolin was able to induce a small but significant $^{36}Cl^-$ efflux, and $^{36}Cl^-$ efflux in wtCFTR-expressing cells was augmented (*solid lines, filled circles*). These data suggest that blocking of BAP31 expression enhances CFTR expression and leads to a residual Cl^- channel activity in [ΔPhe^{508}]CFTR-expressing CHO cells.

We further cotransfected wtCFTR or [ΔPhe^{508}]CFTR-expressing CHO cells with a 40-fold molar excess of BAP31 antisense oligonucleotides and the expression plasmid for green fluorescent protein (pEGFP-C1). Control cells were transfected with pEGFP-c1 only. EGFP fluorescence was monitored during subsequent patch clamp experiments and was used as an indicator for successful transfection. Only fluorescent-labeled cells were used for patch clamp experiments. Activation of wtCFTR was studied initially in wtCFTR-expressing cells. Upon stimulation with IBMX (100 μmol/liter) and forskolin (10 μmol/liter), a large whole cell conductance was activated and the cell membrane voltage (V_m) was depolarized (Fig. 4, *A* and *B*, *upper traces*). Partial replacement of extracellular Cl^- by impermeable gluconate (30Cl−) partially blocked the activated whole cell conductance and further depolarized V_m, indicating activation of a whole cell Cl^- conductance. In the absence of BAP31 antisense, [ΔPhe^{508}]CFTR-expressing cells did not show a response to any of the above maneuvers (Fig. 4, *A* and *B, middle trace*). However, in BAP31 antisense-incubated cells, forskolin and IBMX were able to activate a residual Cl^- conductance as indicated by changes in whole cell conductance and V_m (Fig. 4, *A* and *B, lower trace*). Fig. 5 summarizes the effects of IBMX and forskolin and $30Cl^-$ on whole cell conductances and membrane voltages and clearly indicates a significant cAMP-activated whole cell Cl^- conductance in CHO cells expressing [ΔF508]CFTR after blocking expression of BAP31.

Coexpression of BAP31 and CFTR in Xenopus Oocytes—We further examined how coexpression of BAP31 affects activation of wtCFTR in oocytes of *X. laevis*. Fig. 6*A* shows whole cell recordings obtained from oocytes expressing only wtCFTR or coexpressing wtCFTR together with BAP31. The corresponding I/V curves are shown in Fig. 6*B*. The tracings, I/V curves, and the summary (Fig. 6*C*) of this series of experiments clearly show that coexpression of BAP31 attenuates significantly the activation of a CFTR Cl^- conductance in *Xenopus* oocytes. Injection of a non-translated missense-cRNA did not affect CFTR currents, suggesting that decrease in CFTR is caused by expression of BAP31 rather than nonspecific effects because of additional cRNA injection (data not shown).

To further elucidate the impact of BAP expression on CFTR Cl^- conductance, we coexpressed a BAP31 variant lacking the last C-terminal 24 nucleotides. This truncated BAP31 version lacks the C-terminal KKEE amino acid motif, which is known as an ER retrieval sequence (consensus sequence KK*XX*) and which may also have a function as a transport or internalization signal (26, 27). The ability to inhibit CFTR expression in *Xenopus* oocytes seems to rely on the presence of the KK*XX* motif, because BAP31-KK*XX* was unable to decrease cAMP-activated CFTR Cl^- conductance (Fig. 7). Transfection with BAP31 carrying the KK*XX* may cause an ER overload and may thus cause stress to the cells (28). To exclude artificial effects on CFTR Cl^- conductance caused by possible ER stress, we coexpressed CFTR together with the adenovirus protein E3/19K. E3/19K has been demonstrated to accumulate in ER membranes and to cause cells stress, thereby activating the transcription factor NFκB (28). However, ER stress does not seem the cause for reduced CFTR expression in *Xenopus* oocytes, because E3/19K exerted no inhibitory effects on CFTR Cl^- conductance (Fig. 7). Finally, BAP29, a protein homologous to BAP31 was coexpressed together with CFTR and slightly but not significantly attenuated activation of CFTR upon increase in intracellular cAMP. When coexpressed together with BAP31, heterodimerization of BAP29 and BAP31 is likely to occur (16). The putative BAP31/BAP29 heterodimer was also able to inhibit expression of CFTR Cl^- conductance, as shown in Fig. 7. Taken together, these data strongly suggest regulation of CFTR expression by BAP31, which might be part of the quality control system.

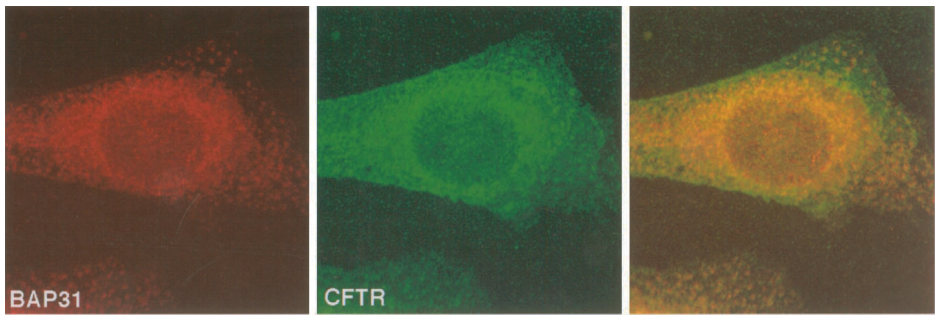

FIG. 2. **Co-localization of BAP31 and CFTR in CHO cells.** Immunofluorescence analysis of CFTR and BAB31 in CHO cells expressing wtCFTR. CFTR and BAP31 were labeled using respective primary antibodies and goat anti-mouse IgG (H+L)-DTAF (CFTR, *green fluorescence*) and goat anti-rabbit IgG (H+L)-Texas Red (BAP31, *red fluorescence*) secondary antibody conjugates. The overlay (*yellow fluorescence*) of both pictures shows co-staining of the two proteins except areas around the plasma membrane.

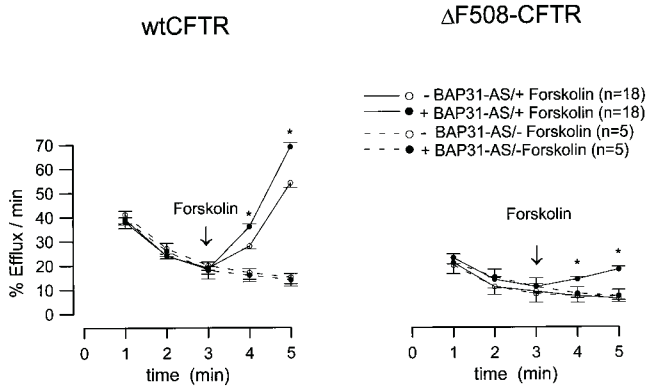

FIG. 3. **Cl^- ion transport measured by $^{36}Cl^-$ efflux in CHO cells expressing wtCFTR or [ΔF508]CFTR.** Cells were grown in the presence of BAP31 antisense oligonucleotides (+*BAP31-AS*) or missense oligonucleotides (−*BAP31-AS*). Forskolin (10 μmol/liter) was added after 3 min, and efflux was determined for another 2 min (*solid line*). *Dashed lines* indicate time course of non-stimulated cells. *Asterisk* indicates significant differences between −BAP31-AS- and +BAP31-AS-treated cells (unpaired Student's *t* test).

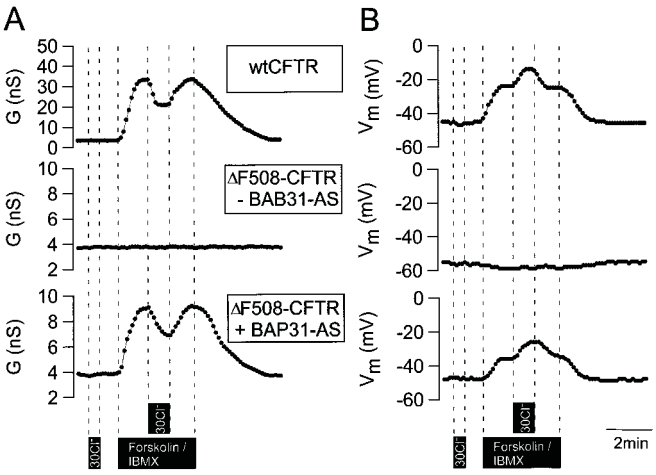

FIG. 4. **Patch clamp analysis of CHO cells expressing wtCFTR or [ΔF508]CFTR grown in the presence of BAP31 antisense oligonucleotide (+*BAP31-AS*) or missense oligonucleotide (−*BAP31-AS*).** Recordings of the whole cell membrane conductance (*A*) and membrane voltage (*B*). Effects of stimulation with forskolin/IBMX (10 μmol/liter and 100 μmol/liter) and partial replacement of extracellular Cl^- by gluconate ($30Cl^-$).

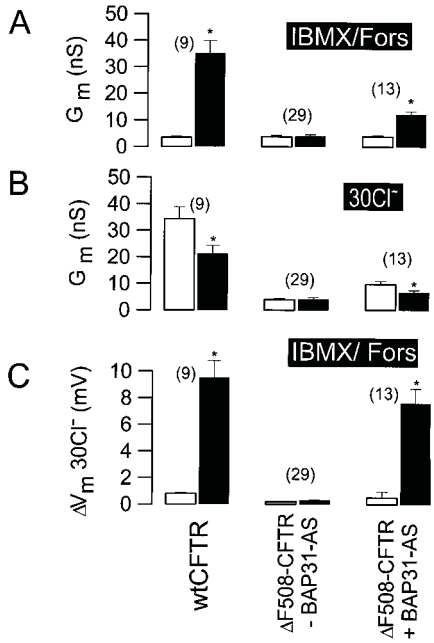

FIG. 5. **Summary of the patch clamp experiments shown in Fig. 4.** *A,* whole cell currents were activated by IBMX and forskolin in CHO cells expressing wtCFTR and CHO cells expressing [ΔPhe^{508}]CFTR when grown in the presence of BAP31 antisense oligonucleotides. *B,* impact of partial replacement of extracellular Cl^- by gluconate ($30Cl^-$) on whole cell Cl^- conductance activated by forskolin/IBMX. The whole cell conductance is inhibited by $30Cl^-$ in CHO cells expressing wtCFTR and CHO cells expressing [ΔPhe^{508}]CFTR when grown in the presence of BAP31 antisense oligonucleotides. *C,* depolarization of the membrane voltage (V_m) by $30Cl^-$ before and after stimulation with IBMX/forskolin. V_m is depolarized by $30Cl^-$ after stimulation with forskolin/IBMX of CHO cells expressing wtCFTR and CHO cells expressing [ΔPhe^{508}]CFTR when grown in the presence of BAP31 antisense oligonucleotides. (), number of experiments; *, significant difference from control (paired Student's *t* test).

DISCUSSION

The present data suggest that the putative integral membrane protein BAP31 interferes with expression of CFTR in heterologous expression systems. BAP31 was identified initially in the murine myeloma cell line J558L and was shown later to be expressed in various cell types (15). In the present study, we detected expression of BAP31 in CHO cells and also in various types of human epithelial cells lines derived from colon, pancreas, kidney collecting duct, and airways (data not shown). Both BAP29 and BAP31 contain three stretches of hydrophobic amino acids, suggesting that these proteins are multiple spanning transmembrane proteins (16). When BAP31 expression was blocked by antisense oligonucleotides in CHO cells, expression of both wtCFTR or [ΔPhe^{508}]CFTR seemed to be enhanced. The amount of mature wtCFTR protein was enhanced when expression of BAP31 was inhibited in CHO cells, expressing exogenous wtCFTR. Our data show that this effect is not limited to cells overexpressing CFTR. In airway epithelial and collecting duct cells, inhibition of BAP31 expression induced enhanced expression of endogenous CFTR. Expression of mature [ΔPhe^{508}]CFTR in CHO cells after BAP31-antisense

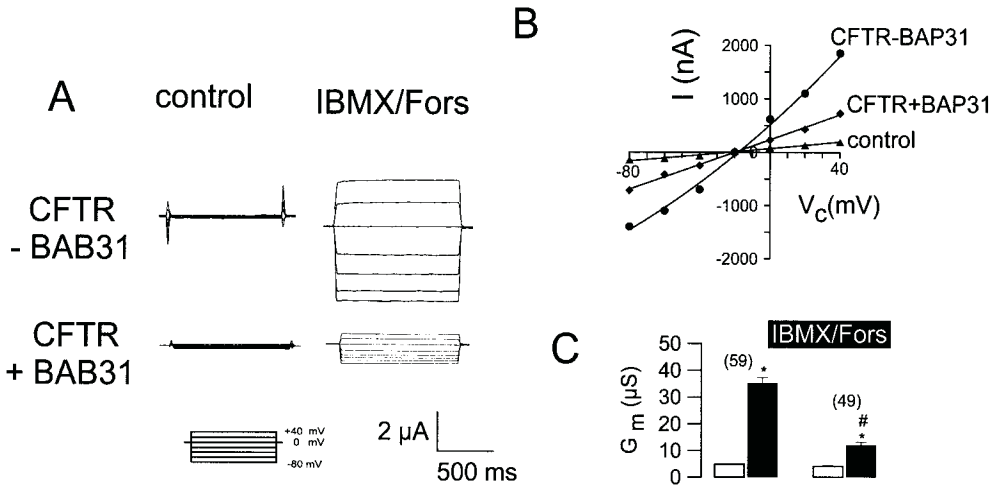

FIG. 6. **Impact of BAP31 on the activation of CFTR Cl^- conductance in *Xenopus* oocytes.** *A,* whole cell currents activated by IBMX and forskolin (1 mmol/liter and 2 μmol/liter) in CFTR expressing oocytes and impact of the coexpression of BAP31. *B,* I/V curves for the IBMX- and forskolin-activated whole cell currents in water-injected control oocytes and oocytes expressing CFTR (*CFTR-BAP31*) or coexpressing CFTR and BAP31 (*CFTR+BAP31*). *C,* summary of the whole cell conductances activated by IBMX and forskolin in oocytes expressing CFTR or coexpressing CFTR and BAP31. (), number of experiments; *, significant difference from control (paired Student's *t* test); #, significant difference from CFTR-BAP31 (unpaired Student's *t* test).

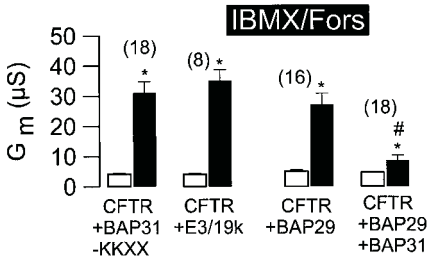

FIG. 7. **Summary of whole cell conductances activated by IBMX/forskolin in wtCFTR-expressing oocytes.** Oocytes were coinjected with cRNA encoding (i) BAP31 lacking the C-terminal KK*XX* motif (+*BAP31-KKXX*), (ii) the ER-localized transmembrane protein E3/19k, (iii) BAP29, and (iv) BAP29 together with BAP31. *, significant differences (Student's paired *t* test); #, significant difference when compared with whole cell conductance measured in oocytes expressing CFTR only; (), number of experiments.

treatment was not detected by Western blotting. However, the immature form of the protein was more abundant in cells treated with BAP31-antisense. Moreover, functional assays clearly suggest expression of [ΔPhe^{508}]CFTR protein in the plasma membranes of antisense-treated cells. Thus, mutant CFTR protein may be located in plasma membranes in either an immature unglycosylated form or the small amounts of mature [ΔPhe^{508}]CFTR protein cannot be detected by standard Western blotting. Even longer exposures of the x-ray films did not show a CFTR protein band of high molecular weight. However, it is well known from previous studies that only small amounts of CFTR are required for functional expression in epithelial cells (12, 29).

The experiments performed in *Xenopus* oocytes supply further evidence for the impact of BAP31 on processing and expression of CFTR. Interestingly, coexpression of both BAP29 and BAP31 was even more efficient in inhibiting CFTR expression, compared with the effects of solely expression of BAP31. Thus, CFTR may interact with BAP31 homodimers or heterodimers of BAP31 and BAP29, which have been reported previously (16). The present data further stress the importance of the KK*XX* motif at the C-terminal end of BAP31, because no inhibition of CFTR conductance was observed in the absence of this motif. The KK*XX* motif is an ER retention signal for residual proteins of the ER, which cycle between ER and the Golgi (16, 17). It has also been demonstrated to function as a transport signal (30). KK*XX*-carrying proteins bind to COP proteins and are involved in the retrograde transport from the *cis* Golgi to the ER (31, 32). In addition, the KK*XX* motif may also serve as an internalization and endocytosis signal (27). The KK*XX* motif seems to be crucial for the inhibitory effects of BAP31 on CFTR. As mentioned above, the KK*XX* represents an ER retrieval signal and thus ER overload by overexpressing BAP31 may lead to cell stress, activation of NFκB, and eventually cell death by apoptosis (28, 33). In fact, BAP31 probably takes part in the control of programmed cell death (34, 35). Although we cannot completely rule out a possible nonspecific effect of BAP31 on CFTR expression, we were able to show that expression of the protein E3/19k that binds very tightly to the ER and induces ER overload (28) does not interfere with expression of CFTR in *Xenopus* oocytes.

Interestingly, it has been shown recently that virus maturation in the ER is controlled by an KK*XX* ER retrieval signal and that viruses lacking the envelope glycoprotein encoding the KK*XX* motif have a higher chance for budding at the plasma membrane (36). The present results suggest that trafficking or maturation of a variety of transmembrane proteins might be controlled by BAP31, including CFTR. However, it is unlikely that BAP31 affects expression of all membrane proteins, because we did not detect an increased expression for the α-subunit of the Na^+/K^+-ATPase in BAP31-AS treated HBE or M1 cells (data not shown). One function of BAP31 could be that it participates in formation of the quality control mechanism of membrane protein synthesis, which was postulated for CFTR (37). It is now well known that biosynthesis of CFTR is strictly controlled by this mechanism, resulting in degradation of most of the wtCFTR and basically all of [ΔPhe^{508}]CFTR (3). Because [ΔPhe^{508}]CFTR is by far the most frequent mutation causing cystic fibrosis, interfering with the expression or function of BAP31 in epithelial cells could be a new way to circumvent the Cl^- channel defect in cystic fibrosis.

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