Rescue of functional ΔF508-CFTR channels by co-expression with truncated CFTR constructs in COS-1 cells

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Abstract The most frequent mutant variant of the cystic fibrosis transmembrane conductance regulator (CFTR), ΔF508-CFTR, is misprocessed and subsequently degraded in the endoplasmic reticulum. Using the patch-clamp technique, we showed that co-expressions of ΔF508-CFTR with the N-terminal CFTR truncates containing bi-arginine (RXR) retention/retrieval motifs result in a functional rescue of the ΔF508-CFTR mutant channel in COS-1 cells. This ΔF508-CFTR rescue process was strongly impaired when truncated CFTR constructs possessed either the ΔF508 mutation or arginine-to-lysine mutations in RXRs. In conclusions, our data demonstrated that expression of truncated CFTR constructs could be a novel promising approach to improve maturation of ΔF508-CFTR channels.

Key words: Cystic fibrosis; Cystic fibrosis transmembrane conductance regulator; ΔF508-CFTR mutant; Channel activity; Co-expression; Electrophysiology

1. Introduction

Cystic fibrosis (CF) is a fatal genetic disorder that results from mutations in a gene encoding the cAMP-activated chloride channel known as the cystic fibrosis transmembrane conductance regulator (CFTR) [1–3]. The most frequent mutation identified in CF patients is a deletion of the phenylalanine codon at position 508 (ΔF508) that causes misprocessing and subsequent degradation of the mutant protein in the endoplasmic reticulum (ER) [4–7]. Although some of the ER chaperones that are involved in CFTR processing have been identified [8–12], it is still unknown how the ER quality control mechanism discriminates between correct and mutated CFTR. Conditions that affect protein folding such as high glycerol concentration or reduced temperature result in rescue of the ΔF508-CFTR mutant, which can reach the cell surface and forms a functional, cAMP-activated chloride channel [13–15]. The processing defect of ΔF508-CFTR can also be partially suppressed by second-site mutations in the nucleotide binding domain 1 (NBD1) [16–18], however, the molecular mechanism of this intragenic suppression still remains unclear.

Four putative ER retention/retrieval signals have been identified in the first half of CFTR [19]. These tripeptide RXR motifs are composed of two arginines (R) that are separated by any amino acid (X) and are homologues of the RKR motif responsible for the ER retention/retrieval in the K_{ATP} channel assembly [20]. The RXR motifs of CFTR are localized in the N-terminal tail ($R^{29}Q^{30}R^{31}$), in NBD1 ($R^{516}Y^{517}R^{518}$ and $R^{553}A^{554}R^{555}$) and the regulatory (R) domain ($R^{764}R^{765}R^{766}$) (Fig. 1A). Arginine-to-lysine mutations in RXRs of ΔF508-CFTR result in the appearance of a functional channel in the plasma membrane, suggesting that these motifs are responsible for interaction of the nascent chain with as yet unidentified components of the ER quality control system [19].

Here we show that transient co-expression of ΔF508-CFTR with truncated CFTR polypeptides in COS-1 cells results in a remarkable increase of cAMP-activated chloride current, indicating a functional rescue of ΔF508-CFTR channels most likely as an effect of destabilization and/or saturation of the ER quality control system by truncated CFTR chains. These ΔF508-CFTR-dependent currents are strongly impaired when the truncated constructs possess either ΔF508 or arginine-to-lysine mutations of RXR motifs in NBD1, suggesting that the ΔF508 mutation may interfere with recognition of CFTR RXRs by the ER quality control system.

2. Materials and methods

2.1. DNA constructs and site-directed mutagenesis of RXR motifs

The bicistronic pCINeo/IRES-green fluorescent protein (GFP) vectors expressing either CFTR, ΔF508-CFTR or MDR1 have been described in detail elsewhere [21–24].

All the CFTR and ΔF508-CFTR truncation constructs are schematically represented in Fig. 1. The WF1 construct expressing 177 amino acids (aa) from the N-terminal part of CFTR was amplified by polymerase chain reaction (PCR), using the oligonucleotides 5′-CTAGCTAGCGACCCCAGCGCCCGAGAGACC (*Nhe*I site underlined) and 5′-CCGCTCGAG**CTA**ACTTATTTTATCTAGAACACG (*Xho*I site underlined; in-frame stop codon in bold) and the pCINeo/IRES-GFP/CFTR vector as a DNA template. The obtained PCR fragment was digested with *Nhe*I and *Xho*I and cloned back into the *Nhe*I- and *Xho*I-cut pCINeo/IRES-GFP/CFTR vector.

The N-terminal half-size versions of CFTR and ΔF508-CFTR that comprise the complete NBD1 (for determination of the N- and C-terminal boundaries of NBD1 see [25]), WF2 and MF2 were constructed as follows. The 1097 bp fragment corresponding to the region between V^{272} and L^{636} was PCR-amplified, using the oligonucleotides 5′-CTGTTAAGGCATACTGCTGGGAAGAAGC and 5′-CGGAATTC**TA**CTCGAGTAGATTTTGGAGTTCTGAAAATGTCCC (*Eco*RI site underlined; in-frame stop codon in bold) and the pCINeo/IRES-GFP/CFTR (for WF2) or pCINeo/IRES-GFP/ΔF508-CFTR

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Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; NBD, nucleotide binding domain; GFP, green fluorescent protein; PCR, polymerase chain reaction; aa, amino acid

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(for MF2) vector as a DNA template. After digestion with *Cel*II and *Eco*RI, the obtained PCR fragments were cloned into *Cel*II- and *Eco*RI-cut pCINeo/IRES-GFP/CFTR.

The RR1 and RR2 fragments expressing 129 aa of NBD1 were amplified by PCR, using the oligonucleotides 5′-CTAGCTAGC CACC**ATG**GTGATTATGGGAGAACTGGAG (*Nhe*I site underlined; in-frame ATG codon in bold) and 5′-GCGAATTC**CTA**ATC-ATCGAGGTTAGCCATCAGTTTACAGAC (*Eco*RI site underlined; in-frame stop codon in bold) and pCINeo/IRES-GFP/CFTR (for RR1) or pCINeo/IRES-GFP/MR2-7 (for RR2) as a DNA template. *Nhe*I- and *Eco*RI-digested PCR products were cloned into the respective restriction sites in the pCINeo/IRES-GFP vector.

The site-directed mutagenesis of arginine residues in RXR motifs of truncated CFTR constructs was performed using the standard PCR overlap extension technique as previously described [26]. The base pair substitutions (underlined) were introduced using following oligonucleotides: for R29K mutation: 5′-GAAAGGATACAAACAGC-GCCTGGA (sense) and 5′-TCCAGGCGCTGTTTGTATCCTTTC (antisense); for R516K mutation: 5′-CTATGATGAATATAAATA-CAGAAGCGTC (sense) and 5′-GACGCTTCTGTATTTATATTC-ATCATAG (antisense); for R555K mutation: 5′-GGTCAACGAG-CAAAAATTTCTTTAGC (sense) and 5′-GCTAAAGAAATTTTT-TGCTCGTTGACC (antisense). To amplify 5′ and 3′ PCR products that overlap with a mutated residue, two separate PCRs were performed, using the respective sense and antisense mutagenic oligonucleotides in pair with the outermost oligonucleotides used for amplification of WF1 and WF2 constructs (see oligonucleotide sequences above) and pCINeo/IRES-GFP/CFTR as a DNA template. The obtained PCR fragments were mixed together and used in the second step PCR, yielding full-length mutant products. In the last step, these mutated fragments from the second step PCR were cloned into the pCINeo/IRES-GFP vector by the strategies used for generation of WF1- and WF2-expressing vectors. The double and triple mutant constructs were generated consequently in vectors with single mutations of RXRs in the truncated CFTR.

The absence of additional mutations in all PCR-amplified DNA fragments used in this study was always verified by sequence analysis.

2.2. Cell culture and DNA transfection

Cultured African green monkey kidney fibroblast cells (cell line COS-1), obtained from the American Type Culture Collection, were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 2 mmol/ml L-glutamine, 2 U/ml penicillin and 2 mg/ml streptomycin at 37°C in a fully humidified atmosphere of 10% CO_2 in air. The cells were detached by exposure to 0.05% trypsin in a Ca^{2+}- and Mg^{2+}-free solution, reseeded on gelatin-coated coverslips, and kept in culture for 2–4 days before use. Only the non-confluent cells were used for the patch-clamp method.

COS-1 cells were transiently co-transfected with pCINeo/IRES-GFP vectors containing the respective DNA constructs, using Trans-IT®-COS Transfection Reagent (Mirus, Madison, WI, USA). For each co-transfection experiment, equal amounts of both vector DNAs were mixed together at the same transfection cocktail. Twenty-four hours after transfection, the cells were trypsinized and seeded on gelatin-coated coverslips and used for electrophysiological measurement within 2–4 days. The transfected cells were visually identified in the patch-clamp setup by the presence of the GFP expression that was coupled with expression of the respective constructs in the bicistronic pCINeo/IRES-GFP vector.

2.3. Electrophysiology

The COS-1 cell optimized protocols for solutions and the whole-cell mode of the patch-clamp technique were used [27]. The extracellular solution contained (in mM): 150 NaCl, 6 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 10 glucose, 10 HEPES; titrated with NaOH to pH 7.4; osmolarity was 320 ± 5 mOsm (vapor pressure osmometer, Wescor 5500, Schlag, Gladbach, Germany). In all experiments, before the experimental protocol was started, KCl was replaced by CsCl to block completely the inwardly rectifying K^+ channel. The CFTR channel was activated by a cAMP-increasing cocktail containing 100 μM IBMX (3-isobutyl-1-methylxanthine) and 10 μM forskolin (both from Sigma) dissolved in isotonic solution. The pipette solution contained (in mM): 20 CsCl, 20 tetraethylammonium chloride, 100 aspartate, 4 EGTA, 4 Mg-ATP, 5 HEPES; titrated to pH 7.2 with CsOH. This solution is slightly hypotonic (290 ± 5 mOsm) in comparison with the standard extracellular solution to prevent spontaneous activation of volume-sensitive chloride currents.

Coverslips carrying the seeded cells were placed in a recording chamber mounted on the stage of an Axiovert 10 inverted microscope (Zeiss). Rapid solution exchange and extracellular application of drugs were achieved using a multi-barreled pipette connected to solution reservoirs, and was controlled by a set of magnetic valves. Patch electrodes were pulled from Vitrex capillary tubes (Modulohm, Herlev, Denmark) on a DMZ-Universal puller (Zeitz Instruments, Augsburg, Germany). When filled with pipette solution they had a DC resistance between 2 and 5 MΩ. An Ag–AgCl wire was used as reference electrode. Ag–AgCl electrodes of sintered pellets (IVM Systems, Healdsburg, CA, USA) were used to avoid contamination of the bath and pipette solutions. Membrane currents were recorded using an EPC-7 patch-clamp amplifier (List Electronic, Lambrecht/Pfalz, Germany) and filtered with an eight-pole Bessel filter (Kemo, Beckenham, UK). For control of voltage-clamp protocols and data acquisition, we used the pCLAMP 6 software (Axon Instruments, Foster City, CA, USA) run on an IBM-compatible PC, which was connected to the amplifier via a TL-1 DMA interface (Axon Instruments). Membrane currents were measured in the whole-cell mode of the patch-clamp technique [28]. The cell capacitance and series resistance were assessed using the analog compensation circuit of the EPC-7 amplifier. Generally, between 50 and 80% of the series resistance was electronically compensated to minimize voltage errors. A ramp protocol, consisting of one step to −100 mV and a 400 ms linear voltage ramp to +100 mV, was applied every 10 or 5 s from a holding potential of −20 mV. In all experiments, time zero corresponds to the rupture of the membrane. Time courses of the whole-cell currents were measured from voltage ramps at −80 and +80 mV. Current–voltage relations were obtained from the currents measured during the linear voltage ramp. Experiments were performed at room temperature (22–25°C). Electrophysiological measurement data were analyzed using the WinASCD software package (Guy Droogmans, ftp://ftp.cc.kuleuven.ac.be/pub/droogmans/winascd.zip). Statistical analysis and graphical presentations were performed using Origin version 7.0 (OriginLab, Northampton, MA, USA). Pooled data are given as the mean ± S.E.M. Significance was calculated using Student's unpaired *t*-test and is presented as asterisks ($*P < 0.05$).

3. Results

3.1. ΔF508-CFTR function is restored upon co-expression with truncated CFTR

Transient expression of ΔF508-CFTR from the CMV-EI promoter in COS-1 cells results in the appearance of a significant cAMP-activated Cl^- current (Fig. 2A,E), indicating that a fraction of the mutant channels escapes the ER quality control and reaches the cell surface. To evaluate this cellular model in the context of functional ΔF508-CFTR rescue, COS-1 cells transfected with ΔF508-CFTR were incubated for 24 or 48 h at 26°C and then used for the patch-clamp method. Forty-eight hours incubation of the cells at 26°C resulted in an almost three-fold increase of Cl^- currents through ΔF508-CFTR channels when compared to cells cultured at 37°C (231.2 ± 11.4 versus 87.5 ± 6.8 pA/pF). The shorter incubation (24 h) had a less pronounced effect on the ΔF508-CFTR chloride channel function (current densities of 117.5 ± 15.8 pA/pF), demonstrating a correlation between the duration of the low temperature treatment and levels of the ΔF508-CFTR channel rescue.

We tested whether co-expression of ΔF508-CFTR with truncated wild type CFTR fragments, which include RXR motifs, might result in plasma membrane localization and function of ΔF508-CFTR channels. We generated GFP vectors with truncated CFTR constructs, WF1 and WF2, which express the N-terminal 177 and 636 aa fragments of wild type CFTR, containing one and three RXR motifs, respectively (Fig. 1B). We did not generate a construct that comprised

all four RXR motifs (WF2 extended over the R domain) because this truncated CFTR variant was shown to function as a cAMP-activated Cl^- channel [29–31].

Co-expression of ΔF508-CFTR with WF2 in COS-1 cells results in an almost six-fold increase of cAMP-activated Cl^- current when compared to cells co-transfected with ΔF508-CFTR and empty vectors (510.9 ± 79.2 versus 87.5 ± 6.8 pA/pF; Fig. 2I), indicating the presence of functional channels in the plasma membrane. The density of the current through ΔF508-CFTR was similar to current observed in cells that expressed only wild type CFTR (645.8 ± 52.1 pA/pF; Fig. 2I). No significant differences in time courses, current–voltage relationships or reversal potentials of these two currents were observed (Fig. 2C,G and Fig. 2B,F). The presence of WF1 in ΔF508-CFTR-expressing cells resulted in a moderate 2.5-fold increase of cAMP-activated Cl^- current (Table 1). No current was observed when COS-1 cells were transfected only with WF2 vector (Fig. 2D,H), ruling out the possibility of reconstitution of functional channels by oligomerization of truncated CFTR fragments in the plasma membrane.

3.2. *ΔF508-CFTR function is not efficiently rescued when co-expressed with the N-terminal half-size ΔF508-CFTR construct*

Since two RXR motifs are localized in NBD1 of CFTR, we examined whether ΔF508 mutation might affect rescue properties of WF2. The MF2 construct is identical to WF2 except for the presence of the ΔF508 mutation (Fig. 1B). cAMP-activated Cl^- current was almost 2.5-fold reduced in COS-1 cells co-transfected with ΔF508-CFTR and MF2 vectors when compared to cells co-expressing ΔF508-CFTR and WF2 (167.9 ± 29.1 versus 510.9 ± 79.2 pA/pF; Fig. 3). This current was similar to that observed in cells with ΔF508-CFTR and WF1 co-expression (Table 1), suggesting that ΔF508 interferes with the accessibility of RXR motifs in NBD1.

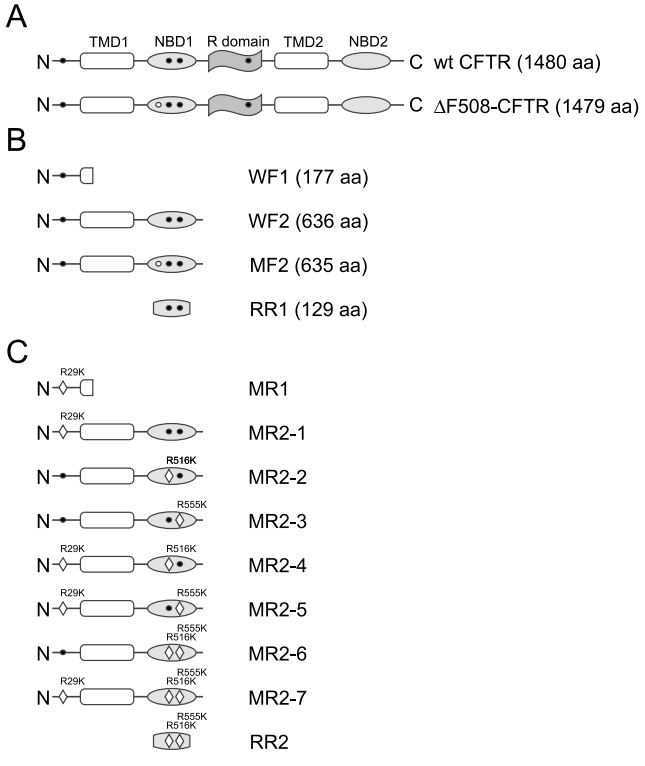

Fig. 1. Schematic representation of full-length (A), truncated (B) and RXR-mutated (C) CFTR and ΔF508-CFTR constructs. The black circles indicate the localization of the RXR motifs. An empty circle corresponds to the ΔF508 mutation. Arginine-to-lysine mutations in RXRs are indicated by diamonds. TMD, transmembrane domain; NBD, nucleotide binding domain; R domain, regulatory domain.

Table 1
Mutations of RXRs in truncated CFTR constructs impair their properties to rescue ΔF508-CFTR function

Truncated CFTR construct	Current density (pA/pF)	*n*	*P*
WF1	226.5 ± 41.4	8	–
MR1	155.5 ± 24.4	6	NS
WF2	510.9 ± 79.2	6	–
MR2-1	495.8 ± 105.7	5	NS
MR2-2	267.3 ± 44.5	7	0.018
MR2-3	288.7 ± 43.6	9	0.019
MR2-4	295.4 ± 60.8	8	0.048
MR2-5	266.5 ± 48.6	6	0.025
MR2-6	270.6 ± 14.2	5	0.025
MR2-7	213.6 ± 39.7	8	0.003

ΔF508-CFTR-dependent current densities were measured at +80 mV in cells co-transfected with ΔF508-CFTR and truncated CFTR-expressing vectors. Values are means ± S.E.M.; *n* is number of experiments; significance ($P < 0.05$) was calculated, using Student's unpaired *t*-test. NS, not significant.

3.3. *RXRs in NBD1 of truncated CFTR constructs are essential for efficient rescue of ΔF508-CFTR function*

To determine the role of particular RXRs in the ΔF508-CFTR rescue, we created a series of arginine-to-lysine mutations in RXRs of WF1 and WF2 constructs (Fig. 1C). Co-expression of ΔF508-CFTR with mutated constructs, possessing R516K and R555K mutations either alone or in combination, led to an about two-fold decrease of ΔF508-CFTR-dependent current when compared to cells that co-expressed ΔF508-CFTR and WF2 (Table 1). This suggests that both RXRs in NBD1 contribute equally to interaction with proteins of the ER system. Surprisingly, the R29K mutation did not significantly affect the rescuing properties of WF1 as well as WF2 (Table 1), suggesting the presence of another retention/retrieval motif in the N-terminal tail of CFTR.

To further evaluate the role of NBD1 in the ΔF508-CFTR rescue process, we constructed RR1 and RR2 vectors, which expressed 129 aa of the CFTR NBD1 with both RXRs (from M^{469} to N^{597}; Fig. 1B). The RR2 construct is identical to RR1 except for the presence of R516K and R555K mutations in RXRs. Co-expression of RR1 with ΔF508-CFTR resulted in a significant 60% increase of cAMP-activated Cl^- current when compared to cells co-transfected with ΔF508-CFTR and empty vectors (146.8 ± 21.3 versus 87.5 ± 6.8 pA/pF; Fig. 4). This increase of current was not observed when ΔF508-CFTR was co-expressed with RR2, demonstrating that RXRs in the NBD1 fragment are sole determinants of the ΔF508-CFTR rescue.

4. Discussion

Although CFTR was identified almost 13 years ago, no efficient pharmaceutical treatments or a gene therapy for CF have been identified so far [32]. Much effort is focused on the correction and improvement of the deficient maturation of the ΔF508-CFTR channel at the ER level. Transient expression of

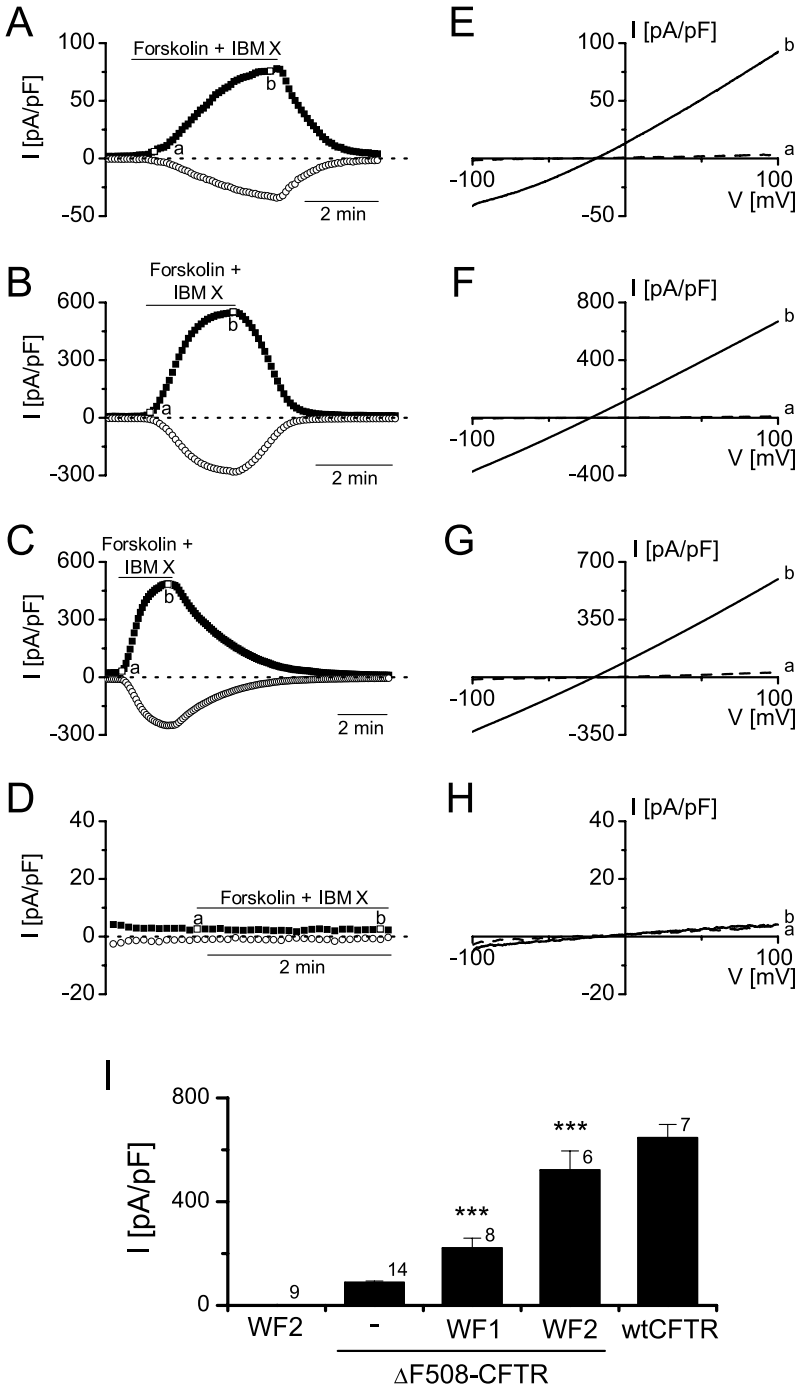

Fig. 2. Co-expression of WF1 or WF2 with ΔF508-CFTR results in rescue of mutant channel functions. A–D: Time course of CFTR currents obtained from measurements at +80 mV (filled squares) and −80 mV (empty circles) in cells co-transfected with ΔF508-CFTR and empty vectors (A), with wild type CFTR and empty vectors (B), with ΔF508-CFTR and WF2 vectors (C) and transfected only with the WF2 vector (D). E–H: Current–voltage relationships before (a) and after (b) ΔF508-CFTR activation with the forskolin (10 μM)/IBMX (100 μM) cocktail at the time indicated by the empty squares in A–D). I: Comparison of ΔF508-CFTR chloride current densities in cells co-expressing indicated CFTR and ΔF508-CFTR constructs. Bars denote mean ± S.E.M. Numbers of analyzed cells are indicated. Significance was calculated using Student's unpaired *t*-test (***$P < 0.001$).

ΔF508-CFTR from either a vector (Fig. 2A,E) or using recombinant vaccinia virus in Vero cells [4] results in the appearance of cAMP-activated Cl^- current, indicating that a certain pool of the mutant channels can reach the cell surface. As proposed previously [7], such inefficiency of the ER quality control may result from saturation of its components by an increased number of nascent ΔF508-CFTR chains. Here we show that co-expression of ΔF508-CFTR with truncated wild type CFTR constructs results in functional rescue of ΔF508-CFTR channels. This rescue mechanism seems to be exclusive to truncated CFTR versions since co-expression of ΔF508-CFTR with truncated MDR1 did not result in the ΔF508-CFTR rescue (data not shown). Most likely, the truncated CFTR chains compete with full-length ΔF508-CFTR to inter-

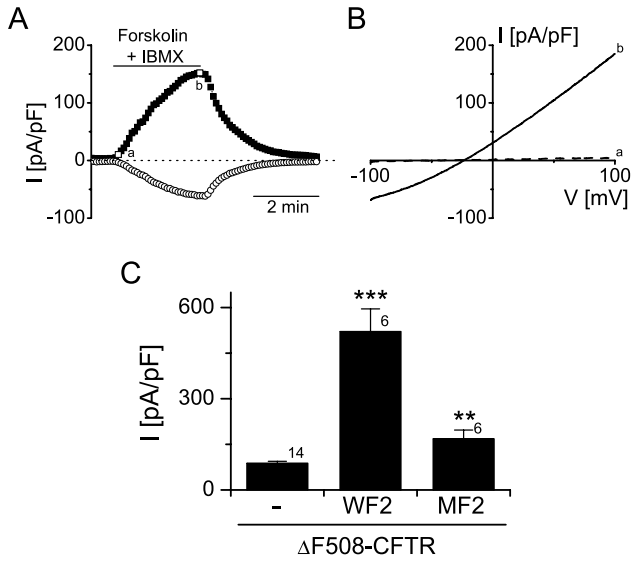

Fig. 3. ΔF508 mutation in the MF2 construct strongly impairs rescue of the ΔF508-CFTR channel. A: Time course of CFTR currents obtained from measurements at +80 mV (filled squares) and −80 mV (empty circles) in cells co-transfected with ΔF508-CFTR and MF2 vectors. B: The current–voltage relationship before (a) and after (b) ΔF508-CFTR activation with the forskolin (10 μM)/IBMX (100 μM) cocktail at the time indicated by the empty squares in A. C: ΔF508-CFTR-dependent current densities from cells co-transfected with ΔF508-CFTR and either empty (−), WF2 or MF2 vectors. Bars denote mean ± S.E.M. Numbers of analyzed cells are indicated. Significance was calculated using Student's unpaired *t*-test (***$P < 0.001$).

act with components of the ER quality control system. Therefore, they might be recognized as premature or not fully translated proteins and subsequently degraded by the ER-associated protein degradation pathway [33–36]. Concomitantly, full-length ΔF508-CFTR chains that lack the interaction in the ER may bypass quality control and enter the secretory pathway to the plasma membrane.

Expression of truncated CFTR constructs, WF1, WF2 and MF2, in the absence of the ΔF508-CFTR expression vector does not result in cAMP-activated Cl^- currents (Fig. 2D,H and data not shown). This is at variance with previously published reports, which showed that similar CFTR truncates can function as cAMP-activated chloride channels in *Xenopus* oocytes [29,37,38] and IB3-1 cells [29], a human bronchial epithelial cell line derived from a CF patient compound heterozygous for the ΔF508 mutation (ΔF508/W1282X) [39]. We have no explanation for the functional expression of truncated CFTR versions in *Xenopus* oocytes. However, it is well described that expression of small proteins in *Xenopus* oocytes very often induces up-regulation of endogenous Cl^- channels [40,41]. IB3-1 cells are CF cells. We propose from our results that the observed cAMP-activated current in IB3-1 cells expressing truncated CFTR is an effect of functional rescue of endogenous ΔF508-CFTR channels. Further experiments will be focused on the functional rescue of endogenous ΔF508-CFTR, using expression of truncated CFTR or artificially engineered polypeptides.

Each of the truncated CFTR fragments used for ΔF508-CFTR rescue contains bi-arginine RXR motifs. Arginine-to-lysine mutation in NBD1's RXRs of truncated CFTR constructs (R516K and R555K mutations) strongly impairs their ΔF508-CFTR rescue properties. Similar results were obtained when the truncated construct contains ΔF508 mutation (MF2), suggesting that this mutation interferes with RXR accessibility. It has recently been proposed that RXR accessibility could be lost by the assembly of subunits in the ER [42]. The assembled complexes can then be exported from the ER via the coated protein complex II vesicles. In the ER–Golgi intermediate compartment (ERGIC) and in the *cis*-Golgi, correctly oligomerized proteins are transported towards the plasma membrane while unassembled or partially assembled subunits bind to the coated protein complex I (COPI) via RXRs and are recycled to the ER in COPI vesicles for a next round of quality control. It should be noted that by default 'mistakes' of the ER quality control, some unassembled or partially assembled subunits could also escape from the ER. Based on this mechanism, we propose that the ΔF508 mutation would create a conformational change in NBD1 of

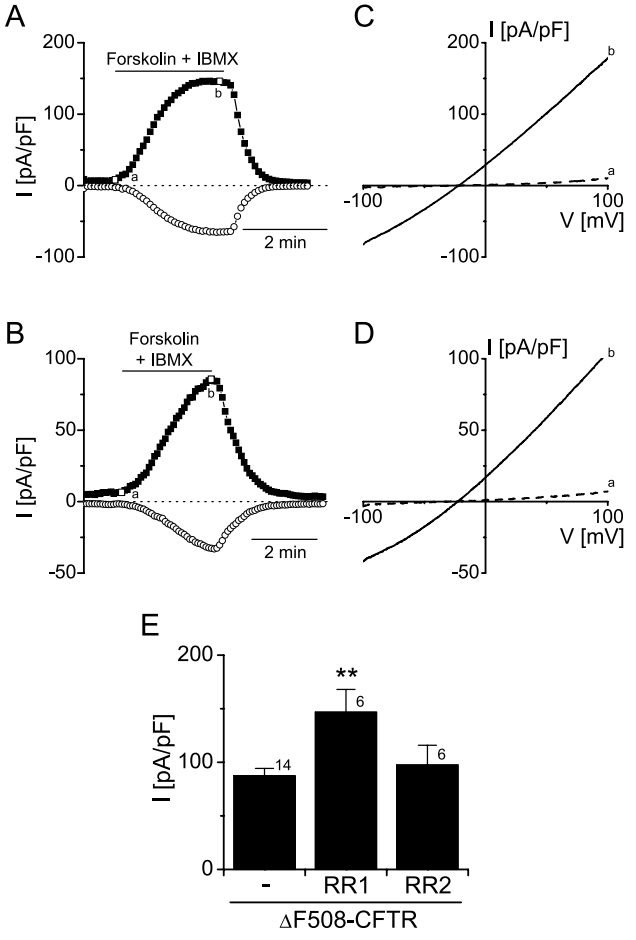

Fig. 4. Co-expression of ΔF508-CFTR with the wild type CFTR NBD1 rescues ΔF508-CFTR channel function. A,B: Time course of CFTR currents obtained from measurements at +80 mV (filled squares) and −80 mV (empty circles) in cells co-transfected with ΔF508-CFTR and either RR1 (A) or RR2 vectors (B). C,D: Current–voltage relationships before (a) and after (b) ΔF508-CFTR activation with the forskolin (10 μM)/IBMX (100 μM) cocktail at the time indicated by the empty squares in A,B. E: Comparison of ΔF508-CFTR-dependent chloride current densities in cells co-transfected with ΔF508-CFTR and either empty (−), RR1- or RR2-expressing vectors. Bars denote mean ± S.E.M. and numbers of analyzed cells are indicated. Significance was calculated using Student's unpaired *t*-test (**$P < 0.01$).

CFTR, resulting in constitutive accessibility of RXRs to ER and/or COPI proteins. Therefore, the majority of ΔF508-CFTR is retained and degraded in the ER or, if exported to the ERGIC, retrotransported to the ER via COPI vesicles and then degraded. The fact that second-site mutations in NBD1 of ΔF508-CFTR partially correct processing and functional defects of this mutant channel strongly supports this latter hypothesis (note that R553Q and R553M mutations correspond to the arginine in the RAR motif) [16–18].

In conclusion, we show here that expression of short CFTR polypeptides could be a promising approach to rescue ΔF508-CFTR function. Identification of ER proteins that interact with RXR motifs of CFTR as well as elucidation of cellular fates of CFTR truncates will be indispensable to understand the critical steps of the ER quality control and trafficking of CFTR channels.

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