MAST205 Competes with Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)-associated Ligand for Binding to CFTR to Regulate CFTR-mediated Fluid Transport

Aixia Ren1, Weiqiang Zhang2,†, Sunitha Yarlagadda3, Chandrima Sinha3, Kavisha Arora4, Chang-Suk Moon4, and Anjaparavanda P. Naren1,†

From the 1Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee 38163 and the 2University of Tennessee Cystic Fibrosis Care and Research Center at Le Bonheur Children’s Hospital, Memphis, Tennessee 38103

Background: CFTR is an important cAMP-regulated chloride channel.

Results: MAST205 and CAL compete for binding to CFTR to regulate the expression level and function of CFTR.

Conclusion: MAST205 is a regulator for CFTR.

Significance: Targeting the MAST205-CFTR complex has potential clinical implications for treating CFTR-related diseases such as cystic fibrosis and secretory diarrheas.

The PDZ (postsynaptic density-95/discs large/zona occludens-1) domain-based interactions play important roles in regulating the expression and function of the cystic fibrosis transmembrane conductance regulator (CFTR). Several PDZ domain-containing proteins (PDZ proteins for short) have been identified as directly or indirectly interacting with the C terminus of CFTR. To better understand the regulation of CFTR processing, we conducted a genetic screen and identified MAST205 (a microtubule-associated serine/threonine kinase with a molecular mass of 205 kDa) as a new CFTR regulator. We found that overexpression of MAST205 increased the expression of CFTR and augmented CFTR-mediated fluid transport in a dose-dependent manner. Conversely, knockdown of MAST205 inhibited CFTR function. The PDZ motif of CFTR is required for the regulatory role of MAST205 in CFTR expression and function. We further demonstrated that MAST205 and the CFTR-associated ligand competed for binding to CFTR, which facilitated the processing of CFTR and consequently upregulated the expression and function of CFTR at the plasma membrane. More importantly, we found that MAST205 could facilitate the processing of F508del-CFTR mutant and augment its quantity and channel function at the plasma membrane. Taken together, our data suggest that MAST205 plays an important role in regulating CFTR expression and function. Our findings have important clinical implications for treating CFTR-associated diseases such as cystic fibrosis and secretory diarrheas.

The cystic fibrosis transmembrane conductance regulator (CFTR)2 belongs to the superfamily of ATP binding cassette transporters. It functions as a cAMP-activated anion channel responsible for transepithelial chloride (Cl−) and bicarbonate (HCO3−) transport in the lung, pancreas, liver, intestine, sweat ducts, and reproductive system (1–4). CFTR has been implicated in two major diseases: cystic fibrosis (CF) and enterotoxin-induced secretory diarrheas such as cholera. CF is the most common lethal inherited disease among Caucasians, affecting ~1 in 2,500 Caucasians in the United States (3, 4). F508del is the most common CFTR mutation with >90% of CF patients carrying it on at least one allele (5, 6). The ideal therapy for CF associated with F508del mutation would be to promote its folding efficiency to increase the quantity of protein at the cell surface and restore its defective gating properties. Study of CFTR interacting partners and identification of its major regulators will help to find ways to improve the folding efficiency and/or to slow down the degradation of mutant CFTR.

Accumulating evidence has demonstrated that CFTR interacts with a variety of partners (e.g. channels/transporters, receptors, and scaffolding proteins) in a complex network (CFTR interactome) that regulates the expression and/or function of CFTR at the plasma membrane (7, 8). The C terminus of CFTR possesses a type I PDZ-binding motif, TRL, which binds PDZ proteins, including Na+/H+ exchanger regulatory factor 1 (NHERF1), Na+/H+ exchanger regulatory factor 2 (NHERF2), Cap70 (CFTR-associated protein 70), and PDZK1 (PDZ domain-containing protein in the kidney 1) (9–12). A recent study has demonstrated that the C terminus of CFTR was required for reinsertion of CFTR from the recycling endosomes to the plasma membrane and for prolonging the half-life of CFTR at the plasma membrane (13). The PDZ-domain-containing protein interactions have also been demonstrated to play important roles in CFTR biosynthesis, processing, and trafficking (14, 15). The CFTR-associated ligand (CAL) is a Golgi-associated PDZ protein that localizes mainly in the trans-Golgi network and, to a smaller extent, at the plasma membrane of...
MAST205 Regulates CFTR Expression and Function

lysozones. CAL has been shown to interact with syntaxin 6, a Q-SNARE protein that is involved in trafficking between endosomes and the trans-Golgi network, to assist in the proper sorting of membrane proteins (15). CAL was also reported to bind CFTR through its PDZ motif and down-regulate CFTR expression (8, 14). Overexpression of CAL has been shown to reduce the half-life of CFTR at the plasma membrane and promote CFTR degradation in lysosomes. In contrast, silencing of CAL has been shown to restore function to CFTR and rescue the mutant F508del-CFTR (14, 16, 17). The CAL-induced reduction in CFTR expression and distribution in cells can be restored by the overexpression of NHERF-1, acting through a competition mechanism for binding to the PDZ motif of CFTR (18). These findings suggest that CAL negatively regulates CFTR intracellular processing and trafficking.

In this study, we identified a novel CFTR binding partner, MAST205 (the microtubule-associated serine/threonine kinase with a molecular mass of 205 kDa), which is highly expressed in spermatids and is also found ubiquitously at lower levels (19, 20). MAST205 has a serine/threonine protein kinase domain and a PDZ domain. MAST205 has been shown to interact with several proteins, including β2-syntrophin, protocadherin LKC, and the Na+/H+ exchanger NHE3 (21–25). It has been reported that MAST205 forms a complex with TNF receptor-associated factor 6, an E3 ubiquitin ligase, resulting in the inhibition of TNF receptor-associated factor 6 activation. Valiente et al. showed that the binding of MAST205 to PTEN (phosphatase and tensin homolog) via its PDZ domain contributes to PTEN protein stability (22). In this study, we show that MAST205 is part of the CFTR-containing macromolecular complex and that MAST205 competes with CAL for binding to CFTR and therefore increases the level of CFTR expression and channel function.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—A rabbit anti-MAST205 serum was generated by immunization with a fusion protein containing amino acids 719–970 of MAST205. A mouse anti-MAST205 antibody (Abnova; Walnut, CA) was purchased from Abcam (Cambridge, MA). A rabbit anti-CFTR antibody (CF3) was purchased from Bethyl Laboratories, Inc. (Montgomery, TX). An anti-MAST205 serum was generated by immunization with a fusion protein containing amino acids 862–1430 of MAST205. A mouse anti-MAST205 antibody was purchased from ABNOVA (Walnut, CA). A rabbit anti-CAL antibody (Abnova; Walnut, CA; 1:100) and rabbit anti-MAST205 antibody R3194 (27) (1:100) for 60 min at 37 °C. After washing, the tissues were incubated for 60 min at 37 °C with PLA probes, consisting of secondary antibodies (anti-mouse and anti-rabbit) conjugated to oligonucleotides. Following washes, circularization and ligation of oligonucleotides were performed in ligase-containing solution for 30 min at 37 °C. Cells were then rinsed briefly and incubated for 90 min at 37 °C with the amplification solution prior to hybridizing the amplified product with complementary probe labeled with Alexa Fluor 568. Fluorescence images were taken using an LSM 700 confocal microscope (Carl Zeiss Microscopy, LLC; Thornwood, NY).

**In Vitro Protein Competition Assays**—To test the competition of MAST205 and CAL for binding to CFTR, individual truncated proteins GST-His<sub>6</sub>-S-CFTR (amino acids 1430–1480), His<sub>6</sub>-S-CAL (amino acids 0–454) and His<sub>6</sub>-S-MAST205 PDZ domain protein (amino acids 1104–1192) were expressed in Escherichia coli and purified. Equal amounts of His<sub>6</sub>-S-CAL (1 μg) and GST-His<sub>6</sub>-S-CFTR (1 μg) were incubated with the increasing concentrations of His<sub>6</sub>-S-MAST205 PDZ domain protein (0–25 μg) for competition. Glutathione beads were added to precipitate the proteins. The beads were washed, and the proteins were eluted from beads using 1 × SDS-PAGE sample buffer. The proteins were separated via SDS-PAGE, transferred to PVDF membrane, and subjected to blotting for CAL, CFTR, and MAST205.

**Immunoprecipitation and Immunoblotting**—Plasmid of HA-tagged MAST205 was transfected into HEK293-FLAG-wt-CFTR cells. Forty-eight h post-transfection, the cells were harvested, lysed, and centrifuged. Anti-FLAG beads were added to the supernatant and incubated overnight at 4 °C. The beads were washed three times with the lysis buffer and subjected to
SDS-PAGE. The proteins were transferred to PVDF membrane and immunoblotted for CFTR and MAST205.

Measurement of CFTR-mediated I

Influx using a YFP-based Halide Sensor—HEK293-FLAG-wt-CFTR cells or HEK293 parental cells were cotransfected with pME18s-HA-hMAST205 (or shuttle vector) and pCDNA3-TM-Cl– sensor (28). After 24 h, the transfected cells were seeded in 24-well plates and cultured for another 24 h. The cells were washed twice with 500 μl of washing buffer per well (washing buffer: 136 mM NaNO₃, 20 mM HEPES, 3 mM KNO₃, 2 mM Ca(NO₃)₂, 11 mM glucose, pH 7.2), and then 100 μl of washing buffer was added to each well. Fluorescence recordings were recorded at 37 °C on a FLUOstar-Omega (BMG Labtech; Ortenberg, Germany) microplate reader equipped with HQ500/20X (500 ± 10 nm) excitation and HQ535/30 M (535 ± 15 nm) emission filters and two syringe pumps. Intracellular YFP fluorescence was monitored continuously for 3 min for each well. At 5 s after the start of fluorescence recording, 100 μl of an influx buffer (the composition is the same as the washing buffer except that NaNO₃ was replaced with 272 mM NaI) containing 20 μM forskolin (FSK) to activate the CFTR-mediated I

Real-time RT-PCR Analysis of CFTR mRNA Level upon Transfection with MAST205—Total RNA was isolated from HEK293-FLAG-wt-CFTR cells transfected with different concentrations of HA-MAST205 (0–4 μg plasmid) at 48 h post-transfection. To control for RNA degradation during DNase treatment and reverse transcription, HEK293-FLAG-wt-CFTR cDNA products were amplified for the housekeeping gene β-actin before amplification for CFTR. For amplification of β-actin, 0.2 μg of cDNA were mixed with 1 unit of Taq polymerase, 200 μM dNTPs, and 20 pmol of each of the PCR primers: 5’-TGACGGGGTCTACCCACACTGTGCCCATCTA-3’ and 5’-CTAGAAGACATTCGGTGGACGATGGAGGG-3’. CFTR were amplified using the PCR primers: 5’-GAGACACTGTGCCTCACAC-3’ and 5’-CAGATTGCACCCCATGGAGG-3’ (spanning the region between nucleotides 531 and 778). Real-time PCR was performed using SYBR Green-based detection with each 20 μl reaction containing ~30 ng of cDNA, 200 μM primer, and 1×SYBR Green PCR master mix (Kapa Biosystems, Woburn, MA). Samples were cycled under the following conditions: 95 °C for 2 min, 50 cycles of 60 °C for 14 s, and 95 °C for 7 s. Each reaction was performed in triplicate; mRNA levels for CFTR in CFTR cells transfected with HA-MAST205 (or vector) to determine whether the interaction occurs in cells. We found that CFTR protein could be co-immunoprecipitated with MAST205 but not in the vector-transfected cells (Fig. 1C). We tested the expression of MAST205 in different mouse tissues using Western blotting. Our results indicated that MAST205 was expressed in the lung, testis, kidney, small intestine, brain, and liver and expressed poorly in the colon (Fig. 2A). The two bands of MAST205 most likely represent the splice variants of MAST205. The colocalization of MAST205 and CFTR in polarized human gut epithelial cells (HT29-CL19A) was examined using immunostaining. We found that MAST205 was localized to both the apical and basolateral membrane of HT29-CL19A cells, with a higher abundance on the apical plasma membrane where it is colocalized with CFTR (Fig. 2B). We also tested the expression pattern of MAST205 and CFTR in the mouse jejunum epithelium using immunostaining. The H&E-stained sec-

MAST205 Regulates CFTR Expression and Function

CFTR Interacts with MAST205—The PDZ motif of CFTR has been reported to play an important role in regulating the function, localization, and stability of CFTR (13). We performed a PDZ protein array screening to identify new CFTR interacting partners. The purified GST-His₅-S-CFTR protein was used as the bait in this assay. As shown in Fig. 1A, this C terminus of CFTR interacted with several PDZ domain proteins in the array, including NHERF1 (C1 and C2) and NHERF2 (B13–16), which have previously been reported to interact with CFTR (31). Interestingly, we observed a robust interaction of CFTR with MAST205 (A3–4, Fig. 1A). To further confirm the direct association of MAST205 and CFTR, we performed a pair-wise binding assay between purified GST-his-S-CFTR and His₅-S-MAST205 PDZ-domain proteins. We found that the purified PDZ domain of MAST205 bound to the C terminus of CFTR, but not to purified GST protein (Fig. 1B). We also performed co-immunoprecipitation experiments in HEK293-FLAG-wt-CFTR cells transfected with HA-MAST205 (or vector) to determine whether the interaction occurs in cells. We found that CFTR-mediated HT29-CL19A cells transfected with the vehicle construct were used as controls.

Knockdown of MAST205 Expression by using Lentiviral Transduction—HT29-CL19A cells were seeded in 12-well plates. The lentiviral particles containing MAST205 shRNA (purchased from Santa Cruz Biotechnology, Santa Cruz, CA) were used to infect HT29-CL19A cells. After transduction, a stable cell line expressing the shRNA was generated via selection using 2 μg/ml puromycin. The expression level of MAST205 after knockdown was detected by Western blotting.

Surface Labeling Assay—The expression of CFTR at the plasma membrane was determined by using surface labeling assay (30). Briefly, the transfected cells were fixed with 3.7% formaldehyde for 10 min, blocked with 1% BSA for 30 min, and treated with α-FLAG-HRP for 90 min. HRP substrate 1-step Ultra TMB (Pierce; Rockford, IL) was added to each well for 10–20 min, and the reaction was stopped by adding an equal amount of 2 M H₂SO₄. The absorbance was read at 450 nm on the FLUOstar Omega microplate reader.

Statistical Analysis—The data are presented as mean ± S.E. Statistical analysis was determined by Student’s t test (two-tailed), and p < 0.05 was considered significant.

RESULTS

CFTR Interacts with MAST205—The PDZ motif of CFTR has been reported to play an important role in regulating the function, localization, and stability of CFTR (13). We performed a PDZ protein array screening to identify new CFTR interacting partners. The purified GST-His₅-S-CFTR protein was used as the bait in this assay. As shown in Fig. 1A, this C terminus of CFTR interacted with several PDZ domain proteins in the array, including NHERF1 (C1 and C2) and NHERF2 (B13–16), which have previously been reported to interact with CFTR (31). Interestingly, we observed a robust interaction of CFTR with MAST205 (A3–4, Fig. 1A). To further confirm the direct association of MAST205 and CFTR, we performed a pair-wise binding assay between purified GST-his-S-CFTR and His₅-S-MAST205 PDZ-domain proteins. We found that the purified PDZ domain of MAST205 bound to the C terminus of CFTR, but not to purified GST protein (Fig. 1B). We also performed co-immunoprecipitation experiments in HEK293-FLAG-wt-CFTR cells transfected with HA-MAST205 (or vector) to determine whether the interaction occurs in cells. We found that CFTR protein could be co-immunoprecipitated with MAST205 but not in the vector-transfected cells (Fig. 1C). We tested the expression of MAST205 in different mouse tissues using Western blotting. Our results indicated that MAST205 was expressed in the lung, testis, kidney, small intestine, brain, and liver and expressed poorly in the colon (Fig. 2A). The two bands of MAST205 most likely represent the splice variants of MAST205. The colocalization of MAST205 and CFTR in polarized human gut epithelial cells (HT29-CL19A) was examined using immunostaining. We found that MAST205 was localized to both the apical and basolateral membrane of HT29-CL19A cells, with a higher abundance on the apical plasma membrane where it is colocalized with CFTR (Fig. 2B). We also tested the expression pattern of MAST205 and CFTR in the mouse jejunum epithelium using immunostaining. The H&E-stained sec-
MAST205 Regulates CFTR Expression and Function

**A**

| Bin | PDZ domain |
|-----|-------------|
| A1-2 | Whirlin PDZ3 |
| A3-4 | MAST205 PDZ3 |
| A5-6 | K15491 |
| A7-8 | hINADLPDZ8 |
| A9-10 | hINADLPDZ9 |
| A11-12 | hINADLPDZ1 |
| A13-14 | hINADLPDZ2 |
| A15-16 | hINADLPDZ5 |

**B**

GST-His-S-CFTR — His-S-MAST205 — GST

**C**

Input IP: HA-MAST205

MAST205

GST-His-S-MAST205

GST

**D**

Positive control

**E**

Blank

**FIGURE 1. CFTR interacts with MAST205.** A, identification of CFTR binding partners by using a PDZ protein array. A proteomic array containing several PDZ domain proteins was overlaid with 100 mM GST-His-S-CFTR-C-terminal tail protein (last 50 amino acids of CFTR). B, the purified His-S-MAST205 PDZ domain protein binds to GST-His-S-CFTR protein in *in vitro* binding assay. C, CFTR was co-immunoprecipitated (IP) with MAST205 in transiently transfected HEK293-FLAG-wt-CFTR cells. Data are representative of three independent experiments.

MAST205 Regulates CFTR Expression and Function—Given that overexpression of MAST205 potentiates CFTR channel function and that knockdown of MAST205 attenuates CFTR channel function (Fig. 3), we sought to elucidate the molecular mechanisms underlying these phenomena. To this end, pME18S-HA-hMAST205 plasmid in various quantities was transfected in HEK293-FLAG-wt-CFTR cells. Our data demonstrated that overexpression of MAST205 increased the expression level of CFTR at 48 h post-transfection in a dose-dependent manner (Fig. 4A). The expression of another CFTR-interacting protein, MRP4 (multi-drug resistant protein 4), was not affected by overexpression of MAST205 (Fig. 4A). We further investigated whether overexpression of MAST205 affects the endogenous CFTR expression in HT29-CL19A cells. CFTR was immunoprecipitated using 24–1 anti-mouse CFTR antibody from cells transiently transfected with various amounts of MAST205 and then immunoblotted with anti-rabbit CFTR antibody. Consistent with the observations in HEK293 cells, overexpression of MAST205 increased CFTR expression in a dose-dependent manner (Fig. 4B).

We used real-time RT-PCR to test the CFTR mRNA levels in HEK-FLAG-wt-CFTR cells upon transfection with different quantities of MAST205 for 24 h. We found that CFTR mRNA quantities of MAST205 for 24 h. We found that CFTR mRNA...
levels were not affected by overexpression of MAST205 (Fig. 4C), suggesting that MAST205 did not affect the transcriptional regulation of CFTR. Given that the C-terminal PDZ motif of CFTR interacts with MAST205 (Fig. 1A and B), we continued to test whether deletion of the PDZ motif (the last three amino acids TRL) had any effect on the CFTR (resulting protein is referred to as /H9004TRL-CFTR) expression level after MAST205 transfection. We cotransfected /H9004TRL-CFTR and MAST205 into HEK293 cells and used Western blotting to test CFTR expression levels. We observed that overexpression of MAST205 did not affect the expression level of /H9004TRL-CFTR (Fig. 4D), suggesting that the observed regulatory effect of MAST205 on CFTR expression requires the C-terminal PDZ motif of CFTR. Taken together, these results clearly demonstrate that MAST205 regulates CFTR expression level, with overexpression of MAST205 up-regulates total CFTR expression, and that such regulation requires the C-terminal PDZ motif of CFTR.

MAST205 Competes with CAL for Binding to CFTR—Our data showed that overexpression of MAST205 increased the expression level of CFTR without affecting the transcription of CFTR. Therefore, we reasoned that MAST205 may be involved in biosynthesis or intracellular trafficking of CFTR. To test whether MAST205 participates in CFTR trafficking, we performed co-immunoprecipitation experiments for CFTR and CAL. We found that overexpression of MAST205 reduced the interaction between CAL and CFTR in a dose-dependent manner, whereas the expression level of CAL was unaffected (Fig. 5B), suggesting that MAST205 competes with CAL for binding to CFTR in cells. To test whether the competition occurs in vitro, we purified the following recombinant proteins: GST-His6-S-CFTR, His6-S-CAL, and His6-S-MAST205 PDZ-domain using the bacterial expression system and performed an in vitro competition binding assay. The purified GST-His6-S-CFTR protein was incubated with purified His6-S-CAL protein, to which increasing quantities of His6-S-MAST205 PDZ-domain protein (0–25 μg) were added. Glutathione beads were used to precipitate the protein complex. The Western blotting data demonstrated that MAST205 inhibited the interaction between CAL and CFTR in a dose-dependent manner (Fig. 5C).

MAST205 Increases the Expression of F508del-CFTR and Its Function—Approximately 70% of CF patients are homozygous for the F508del-CFTR mutation. Finding methods to increase the F508del-CFTR expression level at the cell surface and
restore its gating properties are of paramount importance in the development of CF therapy. We explored the effect of MAST205 overexpression on F508del-CFTR expression and channel function. We observed that overexpression of MAST205 increased the expression level of both mature and immature forms of F508del-CFTR (Fig. 6A). A similar increase in Cl⁻ channel function was also observed using I⁻ influx assay (Fig. 6B). These results suggest that increasing the expression of MAST205 may be an attractive approach for rescuing F508del-CFTR.

**DISCUSSION**

In this study, we identified MAST205 as a novel regulator for CFTR expression and function. We found that overexpression of MAST205 increases the expression level and function for both wild type and F508del-CFTR.
Our results showed that the effect of MAST205 on CFTR requires the C-terminal PDZ motif of CFTR. PDZ-mediated protein-protein interactions have been demonstrated to play an important role in the biosynthesis, processing, trafficking, and degradation of CFTR (13, 34, 35). Our data demonstrated that the binding of MAST205 and CFTR inhibited the PDZ-based binding between CAL and CFTR. It has been reported that overexpression of CAL leads to a dramatic decrease at the plasma membrane pool of both wild type CFTR and F508del-CFTR (14, 17). Recent studies suggested that TC10, a small Rho-GTPase interacting with CAL and inhibiting CFTR-CAL binding, increased CFTR expression at the plasma membrane (36). When NHERF1 was coexpressed with CAL, the inhibitory effect of CAL on CFTR surface expression was overcome (18).

Our results showed that the effect of MAST205 on CFTR through promoting lysosomal degradation of CFTR (14, 17). Endogenous CAL limits F508del-CFTR half-life, and knockdown of CAL expression level increases the surface pool of functional F508del-CFTR in human airway epithelial cells (17). Recent studies suggested that TC10, a small Rho-GTPase interacting with CAL and inhibiting CFTR-CAL binding, increased CFTR expression at the plasma membrane (36). When NHERF1 was coexpressed with CAL, the inhibitory effect of CAL on CFTR surface expression was overcome (18).
Here, we observed that overexpression of MAST205 increased the expression level of F508del-CFTR at the plasma membrane and potentiated its channel function. Our results further showed that MAST205 acts by inhibiting the binding of CAL to CFTR, consequently abrogating the negative regulatory effect of CAL and leading to the increased expression level of wt and F508del-CFTR. Therefore, it is likely that the CFTR-MAST205 association inhibits the CAL-mediated CFTR trafficking to the lysosomes and up-regulates CFTR (wt and F508del) trafficking through trans-Golgi network to plasma membrane (Fig. 7). All of these findings suggest that inhibitors of CFTR-CAL binding will enhance the plasma membrane pool of CFTR. Structural studies on the PDZ domains of MAST205 and CAL may provide a framework for developing novel drugs to enhance the membrane expression and activity of mutant CFTR such as F508del.

MAST205 contains a Ser/Thr kinase domain. A recent study has shown that CFTR levels were increased by cAMP/PKA stimulation through phosphorylation-dependent binding with 14-3-3, suggesting that posttranslational regulatory events also increase CFTR biogenesis (38). It has been reported that MAST205 can phosphorylate NHE3 and that the functional kinase activity of MAST205 is required for inhibition of NHE3 activity (23). It is possible that CFTR is also a substrate for phosphorylation by MAST205. In addition, MAST205 is a microtubule-associated protein. It has been found that membrane trafficking of CFTR is microtubule-dependent and can be disrupted by using nocodazole (39, 40). Therefore, it is also possible that MAST205 may directly phosphorylate CFTR and increase its trafficking through the microtubule cytoskeleton to increase the cell surface CFTR expression level. Further studies are needed to address these hypotheses.

As mentioned above, coexpression of MAST205 inhibits the activity of NHE3, a key protein that mediates the transcellular reabsorption of Na+/H+ and HCO3-/H+. NHE3 is the dominant Na+/H+ exchanger in the small intestine and plays important roles in maintaining normal gastrointestinal physiology. Impaired absorption due to malfunction or reduced expression of NHE3 can increase the fluidity of diarrhea (41). However, hyperstimulation of CFTR is another major pathway for secretory diarrheas (42). Because MAST205 interacts with and regulates both CFTR and NHE3, it is possible that MAST205 can cross-regulate NHE3 and CFTR by assembling NHE3, CFTR, and other signaling components into a macromolecular complex. Such a complex can also be a target for therapeutic interventions in diarrheal diseases because down-regulation of MAST205 activates NHE3 and inhibits CFTR channel function. Xiong et al. (24) identified a peptide from the N terminus of MAST205 that inhibits the lipopolysaccharide-stimulated activation of NF-κB. It is reasonable to speculate that MAST205 peptides or small molecules that inhibit the binding of MAST205 to CFTR have the potential to combat diarrheal diseases.

In summary, our findings add a novel binding partner to the CFTR interacting protein family. Overexpression of MAST205 increases the expression level of CFTR and therefore increases CFTR-dependent fluid secretion. Such an effect is, at least in
part, due to the competition between MAST205 and CAL for binding to CFTR, thus inhibiting the CAL-mediated degradation process (Fig. 7). Our findings not only expand our knowledge of the CFTR interactome but may discover new ways for the therapy of CF. Furthermore, our study provides a rationale for studying the formation and regulation of the CFTR-MAST205-NHE3 macromolecular complex, which has clinical implications for therapy of CFTR-related diseases, including secretory diarrheas.

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MAST205 Regulates CFTR Expression and Function
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