Genistein restores functional interactions between ΔF508-CFTR and ENaC in *Xenopus* oocytes

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Running Title: Genistein restores interactions between ΔF508-CFTR and ENaC
Summary

The cystic fibrosis transmembrane conductance regulator (CFTR), in addition to its Cl⁻ channel properties, has regulatory interactions with other epithelial ion channels including the epithelial Na⁺ channel (ENaC). Both the open probability and surface expression of wild type CFTR Cl⁻ channels are increased significantly when CFTR is co-expressed in *Xenopus* oocytes with αβγENaC and conversely, the activity of ENaC is inhibited following wild type CFTR activation. Using the *Xenopus* oocyte expression system, a lack of functional regulatory interactions between ΔF508-CFTR and ENaC was observed following activation of ΔF508-CFTR by forskolin and IBMX. Whole cell currents in oocytes expressing ENaC alone decreased in response to genistein, but increased in response to a combination of forskolin and IBMX, followed by genistein. In contrast, ENaC currents in oocytes co-expressing ENaC and ΔF508-CFTR remained stable following stimulation with forskolin/IBMX/genistein. Furthermore co-expression of ΔF508-CFTR with ENaC enhanced the forskolin/IBMX/genistein-mediated activation of ΔF508-CFTR. Our data suggest that genistein restores regulatory interactions between ΔF508-CFTR and ENaC, and that combinations of protein repair agents, such as 4-phenylbutyrate and genistein, may be necessary to restore ΔF508-CFTR function *in vivo*. 
The cystic fibrosis phenotype is a result of mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR)\(^1\) (1). In addition to functioning as a cAMP-activated, ATP-dependent Cl\(^-\) channel, CFTR influences the transepithelial transport of other solutes, including Na\(^+\) via the epithelial sodium channel (ENaC), Cl\(^-\) via an outwardly rectifying Cl\(^-\) channel, HCO\(_3^\)-, glutathione and ATP (2-20).

Functional interactions between CFTR and ENaC have been observed in both epithelial and non-epithelial cells (2-8). The activation of CFTR is generally associated with an inhibition of ENaC, although activation of CFTR leads to activation of ENaC in the sweat duct (9) suggesting that the regulatory interactions between these 2 transporters are complex. The co-expression of CFTR and ENaC in *Xenopus* oocytes results in a decrease in ENaC-mediated Na\(^+\) transport in the presence of activated CFTR (4-7), a regulatory interaction that mimics CFTR/ENaC interactions in the airway. Furthermore, CFTR Cl\(^-\) conductance is increased in presence of ENaC (5-7).

The ΔF508-CFTR mutation is the most prevalent mutation in patients with cystic fibrosis (21). The mutant protein has impaired exit from the endoplasmic reticulum and is targeted for rapid proteasomal degradation (22-24). Selected chemical or pharmacological agents, such as glycerol or 4-phenylbutyrate, improve the functional expression of ΔF508-CFTR and may

\(^1\) Abbreviations used in this paper: CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial sodium channel; cRNA, complementary RNA; IBMX, 3-isobutyl-1-methylxanthine; NMDG, N-methyl-D-glucamine; WT, wild type; ns, not significant
affect the interaction of ΔF508-CFTR with specific molecular chaperones (25-28). Genistein, an isoflavone with tyrosine kinase and topoisomerase inhibitor activities, enhances functional ΔF508-CFTR Cl⁻ channel expression in epithelial and non-epithelial cells via increases in channel open probability (29). Previous studies have concentrated on assessing changes in mutant CFTR-mediated Cl⁻ transport in response to chemical or pharmacological agents, such as 4-phenylbutyrate, glycerol, and genistein. However, assessment of mutant CFTR-mediated regulation of Na⁺ transport in response to these pharmacological agents has received limited attention. We utilized the Xenopus oocyte expression system to examine whether genistein restores ΔF508-CFTR regulatory interactions with ENaC, as previous studies have shown that ΔF508-CFTR is expressed at the oocyte cell surface (30). We observed that ΔF508-CFTR does not inhibit the functional expression of ENaC, in agreement with previous data (31). Furthermore, ENaC did not enhance the functional expression of ΔF508-CFTR. However, we observed that genistein restores functional interactions between ΔF508-CFTR and ENaC, suggesting that combinations of pharmacologic agents may prove beneficial for the repair of mutant CFTR function.
Experimental Procedures

Materials

Forskolin, IBMX and genistein were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were purchased from Fisher Chemical Co.

Expression of human CFTR (wild type (WT) and ΔF508) and mouse ENaC in Xenopus oocytes

Human CFTR (WT and ΔF508) and mouse ENaC were expressed in Xenopus oocytes essentially as previously described (7). Briefly, human WT-CFTR, human ΔF508-CFTR, and mouse α-, β-, and γENaC cRNAs were prepared using a cRNA synthesis kit (mMESSAGE mMACHINE, Ambion Inc, Austin, TX) according to the manufacturer’s protocol. cRNA concentrations were determined spectrophotometrically. Oocytes obtained from adult female Xenopus laevis (NASCO, Fort Atkinson, WI) were enzymatically defolliculated and maintained at 18º C in modified Barth’s saline (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 0.3 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 0.82 mM MgSO₄, 15 mM Hepes, pH 7.6 supplemented with 10 µg/ml sodium penicillin, 10 µg/ml streptomycin sulfate and 100 µg/ml gentamicin sulfate). Each batch of oocytes obtained from an individual frog were injected with either α-, β-, and γ-subunits of ENaC (0.33 ng/subunit), WT-CFTR (10 ng), ΔF508-CFTR (10 ng), or a combination of ENaC and CFTR (WT or ΔF508) cRNAs dissolved in RNase-free water using a Nanoject II microinjector (Drummond Scientific, Broomall, PA).

Electrophysiological analyses

Whole-cell current measurements were performed 24 to 48 hours after injection using
the two-electrode voltage clamp method as previously described (7). Oocytes were placed in a 1 ml chamber containing modified ND96 (96 mM NaCl, 1 mM KCl, 0.2 mM CaCl$_2$, 5.8 mM MgCl$_2$, 10 mM Hepes, pH 7.4), and impaled with micropipettes of 0.5-5 MΩ resistance filled with 3M KCl. The whole-cell currents were measured by voltage clamping the oocytes in 20 mV steps between −140 mV to +60 mV adjusted for baseline transmembrane potential. Whole cell currents (I) were digitized at 200 Hz during the voltage steps, recorded directly onto a hard disk and analyzed using pClamp 8 software (Axon Instruments, Foster City, CA). Ion replacement studies were performed in an identical manner except that equimolar N-methyl-D-glucamine (NMDG) replaced Na$^+$ in the ND96 solution. To reduce error due to series resistance, the voltage clamp (Axon Geneclamp 500B) was configured to clamp the bath potential to 0 mV. In this configuration, we independently monitored the oocyte membrane potential during our clamp protocol. We routinely observed membrane potentials that were <5% depolarized from our target holding potentials, despite high conductances observed in oocytes expressing ENaC and WT-CFTR.

The difference in whole-cell currents measured in the absence and presence of 10 µM amiloride was used to define the amiloridesensitive Na$^+$ current that was carried by ENaC. Activation of WT- or ΔF508-CFTR was accomplished by perfusion of the oocyte with buffer supplemented with 10 µM forskolin and 100 µM IBMX for 25 minutes (7). In some experiments this first step was followed by an incubation with 10 µM forskolin, 100 µM IBMX and 50 µM genistein for 20 minutes. In all experiments, ΔF508-CFTR Cl$^-$ current was defined as the difference between current measured 20 min after perfusion with forskolin/IBMX (or 15 min
after perfusion with forskolin/IBMX/genistein), and the current measured prior to forskolin/IBMX (+/-genistein) stimulation. Whole-cell currents were recorded at –100 mV for comparisons. All measurements were performed at room temperature.

Statistical Analyses

Statistical comparisons were performed using the Student’s t test. A pair wise t test was used for pre/post treatment in experiments using an individual oocyte. A 2-tailed t test was used when comparing currents obtained from oocytes injected with a cRNA for a single transporter (i.e., ENaC or CFTR (WT or ΔF508)) versus oocytes co-injected with a cRNAs for both ENaC and CFTR (WT or ΔF508). p values < 0.05 were accepted to indicate statistical significance.
Results

Expression of ΔF508-CFTR and ENaC in Xenopus oocytes

The Xenopus oocyte expression system was used to examine the functional expression of ΔF508-CFTR and its functional interaction with ENaC. Although ΔF508-CFTR is a trafficking defective mutant, ΔF508-CFTR is delivered to the oocyte plasma membrane (30) as oocytes are maintained at 18°C. Temperatures ≤ 27°C are permissive for ΔF508-CFTR trafficking (32,33). The two-electrode voltage clamp technique was used to measure whole-cell currents at varying clamping potentials in oocytes expressing ΔF508-CFTR (Figure 1A). I/V curves were obtained prior to and after 20 min of incubation with 10 µM forskolin/100 µM IBMX to activate endogenous protein kinase A. Whole cell currents, measured at a clamp potential of 100 mV, increased 7.5-fold from -0.2 ± 0.0 to -1.5 ± 0.3 µA (n=19) in response to forskolin/IBMX, in agreement with previous observations (30). Oocytes expressing αβγENaC exhibited amiloride-sensitive whole cell currents (Fig. 1B), as previously reported (7). The amiloride-sensitive whole cell current, measured at a clamp potential of 100 mV, was 5.1 ± 1.4 µA (n=9). In agreement with previous observations, amiloride-sensitive currents were unchanged in response to 10 µM forskolin/100 µM IBMX (Fig 2, $I_{Na} = -4.3 \pm 1.0 \mu A$ (-forskolin/IBMX) versus $-4.6 \pm 1.1 \mu A$ (+forskolin/IBMX), p=ns; Fig 3, $I_{Na} = -5.1 \pm 1.4 \mu A$ (-forskolin/IBMX) versus $-5.6 \pm 1.5 \mu A$ (+forskolin/IBMX), p=ns) (31,34).

Co-expression of WT-CFTR and αβγENaC in Xenopus oocytes

Several groups have previously reported that when WT-CFTR and ENaC were co-
expressed in *Xenopus* oocytes, ENaC-mediated Na⁺ currents were inhibited in response to CFTR activation (4-7). Furthermore, ENaC enhances forskolin/IBMX-stimulated CFTR Cl⁻ currents (5-7). Figure 2 shows whole cell currents measured at a holding potential of −100mV in oocytes injected with cRNAs for WT-CFTR (10 ng) or αβγENaC (0.33 ng/subunit), or co-injected with cRNAs for both WT-CFTR and αβγENaC. Oocytes co-injected with both CFTR and ENaC expressed an amiloride-sensitive whole cell current (-1.3 ± 0.3 µA at 100 mV) in the absence of forskolin/IBMX. Following CFTR activation with forskolin/IBMX, the amiloride-sensitive whole cell current was reduced to -0.7 ± 0.2 µA (Fig. 2, n=15, p<0.01).

The forskolin/IBMX-stimulated amiloride-insensitive whole cell current measured at a clamp potential of -100 mV in oocytes co-expressing WT-CFTR and ENaC (-7.6 ± 2.1 µA; n=15) was 3.5-fold greater than that obtained in oocytes expressing CFTR alone (-2.2 ± 0.4 µA; n=18; p=0.01; Fig 2). The larger forskolin/IBMX-stimulated amiloride-insensitive current in oocytes expressing both CFTR and ENaC, compared with oocytes expressing CFTR alone, is in agreement with previous reports that ENaC enhances functional CFTR expression (5-7).

*Co-expression of ΔF508-CFTR and αβγENaC*

Whole cell currents measured at a clamp potential of 100 mV in oocytes injected with cRNAs for either ΔF508-CFTR (10 ng), αβγENaC (0.33 ng/subunit), or both ΔF508-CFTR and αβγENaC are shown in Fig. 3. Oocytes co-injected with both ΔF508-CFTR and ENaC expressed amiloride-sensitive whole cell currents (-5.1 ± 0.7 µA) in the absence of forskolin/IBMX. Following ΔF508-CFTR activation with forskolin/IBMX, the amiloride-sensitive whole cell current was essentially unchanged (-4.9 ± 0.6 µA, n=19, p=ns), in agreement with previous
studies (31). Furthermore, forskolin/IBMX-stimulated whole cell currents in oocytes expressing ΔF508-CFTR alone (-1.3 ± 0.3 µA) and amiloride-insensitive forskolin/IBMX-stimulated whole cell currents in oocytes expressing ΔF508-CFTR and ENaC (-1.6 ± 0.3 µA) were similar in magnitude (n=19; p=ns). These data are consistent with a lack of functional regulatory interactions between ΔF508-CFTR and ENaC expressed in *Xenopus* oocytes.

*Genistein restores functional interactions between ΔF508-CFTR and αβγENaC*

Previous studies reported that the open probability of ΔF508-CFTR is lower than the open probability of WT-CFTR (35). Furthermore, the open probability of both ΔF508-CFTR and WT-CFTR have been reported to increase in response to the isoflavone genistein (29). We therefore examined whether genistein restored functional interactions between ΔF508-CFTR and ENaC. Linear whole cell I/V relationships were observed in oocytes expressing ΔF508-CFTR (Fig. 4A) or WT-CFTR (Fig. 4B) after 15 min of incubation with 10 µM forskolin/100 µM IBMX/50 µM genistein. Similarly, oocytes expressing αβγENaC and stimulated with forskolin/IBMX/genistein exhibited amiloride-sensitive whole cell currents with a linear I/V relationship (Fig. 4C).

Previous studies suggested that chronic treatment with genistein inhibits ENaC by reducing surface expression of Na⁺ channels in A6 renal epithelial cells (36,37). In contrast, protein kinase A activates ENaC in part via an increase in surface expression of channels (38,39). Amiloride-sensitive whole cell currents were measured in oocytes expressing ENaC prior to and after 15 min incubation with 10 µM forskolin/100 µM IBMX/50 µM genistein (Fig. 5). Whole cell amiloride-sensitive currents modestly, but significantly increased in response to
forskolin/IBMX/genistein (-7.1 ± 1.0 µA (- forskolin/IBMX/genistein) versus -9.0 ± 0.9 µA (+ forskolin/IBMX/genistein); n=23; p = 0.004; Fig 5). In contrast, amiloride-sensitive currents decreased in response genistein alone (-7.3 ± 1.5 (ENaC -genistein); -4.0 ± 0.6 (ENaC +genistein); n=8, p=0.015).

Forskolin/IBMX-stimulated whole cell currents in oocytes expressing ΔF508-CFTR alone increased 5.7-fold following the addition of 50 µM genistein (-0.3 ± 0.1 µA (-genistein) versus -1.7 ± 0.4 µA (+genistein); n=23; p<0.001; Fig. 5). Furthermore, the amiloride-insensitive component of the forskolin/IBMX/genistein-stimulated current in oocytes expressing ΔF508-CFTR and αβγENaC was 3.6-fold greater than the forskolin/IBMX/genistein-stimulated current measured in oocytes expressing ΔF508-CFTR alone (-1.7 ± 0.4 µA (ΔF508-CFTR, n=23) versus -6.2 ± 0.9 µA (ΔF508-CFTR/ENaC, n=24); p<0.001; Figs 5 and 6). A 2.4 ± 0.5-fold (p<0.05) increase in forskolin/IBMX/genistein-stimulated amiloride-insensitive current in oocytes co-expressing ΔF508-CFTR/ENaC (n=12), when compared to oocytes expressing ΔF508-CFTR alone (n=13) was also observed when bath Na⁺ was replaced with the impermeant cation NMDG⁺ (Fig 6). These data suggest that ENaC enhances the forskolin/IBMX/genistein-mediated activation of ΔF508-CFTR. Furthermore, this enhanced activation ΔF508-CFTR is not dependent on ENaC-mediated Na⁺ transport.

Whole cell amiloride-sensitive currents observed after stimulation by forskolin/IBMX/genistein in oocytes expressing ENaC and ΔF508-CFTR (-5.6 ± 0.6 µA, n=24) were significantly lower than the amiloride-sensitive currents obtained after stimulation by forskolin/IBMX/genistein in oocytes expressing ENaC alone (-9.0 ± 0.9 µA, n=23, p=0.003).
Although amiloride-sensitive whole cell currents significantly increased in oocyte expressing ENaC alone following stimulation by forskolin/IBMX/genistein (Fig. 5), the amiloride-sensitive currents recorded in oocytes co-expressing ENaC and ΔF508-CFTR remained stable following stimulation with forskolin/IBMX/genistein (-5.2 ± 0.7 (-forskolin/IBMX/genistein) versus -5.6 ± 0.6 μA (+forskolin/IBMX/genistein); n=24; p=ns). These data suggest that activation of ΔF508-CFTR by forskolin/IBMX in the presence of genistein partially restores CFTR-mediated inhibition of ENaC activity.

Functional interactions between WT-CFTR and ENaC were examined in oocytes treated with genistein (Fig. 7). Whole cell currents measured in oocytes co-expressing WT-CFTR and ENaC in response to forskolin/IBMX/genistein were -13.0 ± 1.9 μA (n=12; Fig. 7), 5.2-fold greater than forskolin/IBMX/genistein-stimulated currents in oocytes expressing CFTR alone (-2.5 ± 0.5 μA, n=13 p<0.001). Furthermore, oocytes co-expressing WT-CFTR and ENaC responded to forskolin/IBMX/genistein with a modest, but statistically insignificant reduction in amiloride-sensitive whole cell currents (Fig. 7; -3.2 ± 0.6 μA, (-forskolin/IBMX/genistein); -2.6 ± 0.5 μA, (+forskolin/IBMX/genistein); n=12; p=ns).
Discussion

CFTR is a Cl⁻-selective channel as well as a regulator of other epithelial channels and transport proteins. In cystic fibrosis, the absence of CFTR in the airway epithelia leads to hyperactive ENaC, which is hypothesized to lead to enhanced reabsorption of airway fluids and impaired mucociliary clearance (40). ENaC hyperactivity results in hyperpolarization of airway epithelia, as is evidenced by increases in the change in transepithelial potential of the nasal airway in response to amiloride (41). In contrast, activation of CFTR in sweat ducts leads to an enhancement in ENaC activity, suggesting that regulatory interactions between CFTR and ENaC may be tissue specific (9).

Previous studies have demonstrated that regulatory interactions between CFTR and ENaC in airway epithelia are replicated in Xenopus oocytes. Functional expression of WT-CFTR, but not ΔF508-CFTR is associated with an inhibition of functional ENaC expression in oocytes (31). Such CFTR-mediated inhibition of ENaC is associated with a decrease in Na⁺ channel open probability, not with changes in levels of ENaC mRNA or protein expression (8,42,43). The forskolin/IBMX-regulated inhibition of ENaC by CFTR does not require expression of the full length CFTR protein. Inhibition of ENaC was observed when ENaC is co-expressed either with CFTR truncation mutants containing an intact first nucleotide binding domain (NBD-1) (44) or with a CFTR peptide fragment containing NBD-1 and the regulatory (R) domain (45). A similar NBD-1/R peptide fragment containing the G551D mutation did not demonstrate forskolin/IBMX-regulated inhibition of ENaC (45). Furthermore, Ji and co-workers demonstrated that regulatory interactions between CFTR and ENaC were dependent on the presence of the carboxyl terminus of the β-subunit and the amino terminus of the and γ-subunit
of ENaC (6).

It has been suggested that the ability of CFTR mutants to transport Cl⁻ correlates with their ability to inhibit ENaC (4,46). Recent data have correlated levels of Cl⁻ transport in oocytes expressing WT-CFTR with the degree of ENaC inhibition; higher levels of Cl⁻ transport due to increased WT-CFTR expression are associated with increases in the extent of inhibition of ENaC (46). In contrast, our data with both WT-CFTR (Figs. 2 and 7) and ΔF508-CFTR (Figs. 3 and 5) suggest that levels of Cl⁻ transport do not correlate with the degree of inhibit ENaC inhibition. For example, although we observed high levels of CFTR-mediated Cl⁻ currents in oocytes co-injected with CFTR and ENaC following treatment with forskolin/IBMX/genistein, we did not observe significant inhibition of ENaC in oocytes expressing ENaC and CFTR following activation of CFTR (Fig. 5). Furthermore, the enhanced activation ΔF508-CFTR by ENaC is not dependent on ENaC-mediated Na⁺ transport (Fig. 6). These data argue that Cl⁻ and Na⁺ conductances are not the sole determinants of CFTR/ENaC interactions.

Nagel and co-workers suggested that the apparent CFTR-mediated inhibition of ENaC reflected a series resistor error (47). Our voltage clamp was configured to clamp the bath potential to 0 mV to diminish the likelihood of series resistor errors. Furthermore, in this configuration we independently monitored oocyte membrane potential during our clamp protocol and, even at high conductances, routinely observed membrane potentials that were <5 % depolarized from our target holding potentials. In addition, the cAMP-regulated conductance in oocytes co-expressing CFTR and ENaC was significantly greater than that observed in oocytes expressing CFTR alone (Figure 5), in agreement with a recent observations (5-7). This apparent
stimulation of CFTR by the presence of ENaC (Figures 2, 7) and the restoration of stimulation of
ΔF508-CFTR by ENaC in the presence of genistein, cannot be explained by series resistance
errors (47). These data therefore provide further support for the presence of regulatory
interactions between these proteins.

Our data are potentially important in devising strategies for effecting chemical or
pharmacological repair of mutant CFTR function. ΔF508-CFTR retains chloride transport
properties but is mistrafficked and targeted for rapid intracellular degradation (22-24). It was
hypothesized that repair of trafficking would sufficiently restore CFTR function to ameliorate the
CFTR deficiency in cystic fibrosis (48). In fact, ΔF508-CFTR-mediated chloride transport can
be restored by a number of physical or pharmacological maneuvers in vitro (49), and to a small
extent in vivo using the protein-repair agent sodium 4-phenylbutyrate (50). However, in this
clinical trial there was no change in nasal epithelial amiloride-sensitive potential in vivo with 4-
phenylbutyrate (50). This observation and the present data suggest that combinations of protein
repair agents, such as 4-phenylbutyrate and genistein, may be necessary to restore ΔF508-CFTR
function in vivo.
Acknowledgments

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References


Figure Legends

Figure 1. Expression of ΔF508-CFTR and αβγENaC in Xenopus oocytes

(A) ΔF508-CFTR was expressed in oocytes and TEV performed as described under Experimental Procedures. Whole-cell currents were measured by voltage clamping oocytes in 20mV steps between 140 mV to +60 mV adjusted for baseline transmembrane potential. Shown are I/V relationships in the absence (closed circles) or presence (open circles) of 10 µM forskolin and 100 µM IBMX. (B) αβγENaC was expressed in oocytes and TEV performed. I/V relationships in the absence (open diamonds) or presence (closed diamonds) of 10 µM amiloride are shown. Means ± SEM are illustrated. Error bars contained within the symbols are not apparent.

Figure 2. Expression of WT-CFTR and ENaC in Xenopus oocytes

WT-CFTR and ENaC were expressed separately or together in oocytes and TEV performed as described under Experimental Procedures. (A) Changes in whole cell currents (-100 mV holding potential) after stimulation with 10 µM forskolin/100 µM IBMX that were not inhibited by 10 µM amiloride are illustrated (closed bars). (B) Amiloride-sensitive whole cell currents (-100 mV holding potential) were determined in oocytes expressing ENaC or co-expressing ENaC and WT-CFTR prior to (dark gray bars) and following (open bars) stimulation with 10 µM forskolin/100 µM IBMX. Data obtained from the same WT-CFTR/ENaC co-injected oocytes are presented in panels (A) and (B). Means ± SEM are illustrated.

Figure 3. Co-expression of ΔF508-CFTR and αβγENaC in Xenopus oocytes
ΔF508-CFTR and ENaC were expressed separately or together in oocytes and TEV performed as described under Experimental Procedures. (A) Changes in whole cell currents (-100 mV holding potential) after stimulation with 10 µM forskolin/100 µM IBMX that were not inhibited by 10 µM amiloride are illustrated (closed bars). (B) Amiloride-sensitive whole cell currents (-100 mV holding potential) were determined in oocytes expressing ENaC or co-expressing ENaC and ΔF508-CFTR prior to (dark gray bars) and following (open bars) stimulation with 10 µM forskolin/100 µM IBMX. Data obtained from the same ΔF508-CFTR/ENaC co-injected oocytes are presented in panels (A) and (B). Means ± SEM are illustrated.

**Figure 4.** Effect of genistein on ΔF508-CFTR, CFTR, and αβγENaC

(A) ΔF508-CFTR, (B) WT-CFTR, or (C) αβγENaC were expressed in oocytes and whole-cell currents were measured in response to voltage clamping oocytes between -140 mV to +60 mV in 20mV steps. In (A) and (B), the I/V relationships for ΔF508-CFTR and WT-CFTR, respectively, are shown before (closed symbols) and after (open symbols) treatment with 10 µM forskolin/100 µM IBMX/50 µM genistein. Panel (C) illustrates whole cell currents in ENaC-injected oocytes after treatment with 10 µM forskolin/100 µM IBMX/50 µM genistein before (open squares) and after (closed squares) addition of 10 µM amiloride. Means ± SEM are illustrated. Error bars contained within the symbols are not readily apparent.

**Figure 5.** Genistein restores regulation interactions between ΔF508-CFTR and αβγENaC

ΔF508-CFTR and ENaC were expressed separately or together in oocytes and TEV performed
as described under Experimental Procedures. (A) Changes in whole cell currents (-100 mV holding potential) after stimulation with 10 µM forskolin/100 µM IBMX (closed bar) or 10 µM forskolin/100 µM IBMX/50 µM genistein that were not inhibited by 10 µM amiloride (light gray bars) are illustrated. (B) Amiloride-sensitive whole cell currents (-100 mV holding potential) were determined in oocytes expressing ENaC or co-expressing ENaC and ΔF508-CFTR prior to (dark gray bars) and following (open bars) stimulation with 10 µM forskolin/100 µM IBMX/50 µM genistein. Data obtained from the same ΔF508-CFTR/ENaC co-injected oocytes are presented in panels (A) and (B). Means ± SEM are illustrated.

**Figure 6.** ΔF508-CFTR/ENaC regulatory interactions restored by genistein do not require Na+ transport

Whole cell currents (100 mV holding potential) were determined in oocytes expressing ΔF508-CFTR alone (open bars), or co-expressing ΔF508-CFTR and αβγENaC (closed bars). *ND96:* Changes in whole cell currents that were not inhibited by 10 µM amiloride (presumed to be ΔF508-CFTR-mediated) were determined following stimulation with 10µM forskolin/100 µM IBMX/50 µM genistein in standard ND96 (NaCl) buffer. *NMDG-Cl96:* Changes in whole cell currents were determined following stimulation with 10µM forskolin/100 µM IBMX/50 µM genistein in oocytes bathed in a Na+-free solution (Na+ was replaced with N-methyl-D-glucamine (NMDG)). Data (means ± SEM) are expressed relative to the mean change in whole cell current of oocytes injected with ΔF508-CFTR alone.

**Figure 7.** Regulatory interactions between WT-CFTR and αβγENaC in the presence of genistein
WT-CFTR and ENaC were expressed separately or together in oocytes and TEV performed as described under Experimental Procedures. (A) Changes in whole cell currents (-100 mV holding potential) after stimulation with 10 μM forskolin/100 μM IBMX (closed bar) or 10 μM forskolin/100 μM IBMX/50 μM genistein that were not inhibited by 10 μM amiloride (light gray bars) are illustrated. (B) Amiloride-sensitive whole cell currents (-100 mV holding potential) were determined in oocytes co-expressing ENaC and WT-CFTR prior to (dark gray bars) and following (open bars) stimulation with 10 μM forskolin/100 μM IBMX/50 μM genistein. Data obtained from the same WT-CFTR/ENaC co-injected oocytes are presented in panels (A) and (B). Means ± SEM are illustrated.
Figure 1

A.

B.
Figure 2

A.

Stimulated CFTR current (μA)

WT-CFTR (n=18)

WT-CFTR (n=15)

p=0.01

B.

Amiloride-sensitive current (μA)

ENaC (n=15)

WT-CFTR ENaC (n=15)

p=0.007

p=ns

p=0.001

p=0.02
Figure 3

A.

Stimulated CFTR current (μA)

ΔF508-CFTR (n=19)

ΔF508-CFTR

ENaC (n=19)

p=ns

B.

Amiloride-sensitive current (μA)

ENaC (n=9)

ΔF508-CFTR

ENaC (n=19)

p=ns

p=ns

p=ns

p=ns
Figure 6

**Relative Stimulation Amiloride-Insensitive Current**

- **ENaC**
  - **ND 96**
    - $-\quad +$
    - (n=24)
    - p<0.001
  - **NMDG-Cl 96**
    - $-\quad +$
    - (n=13)
    - (n=12)
    - p=0.021

- **p=ns**
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