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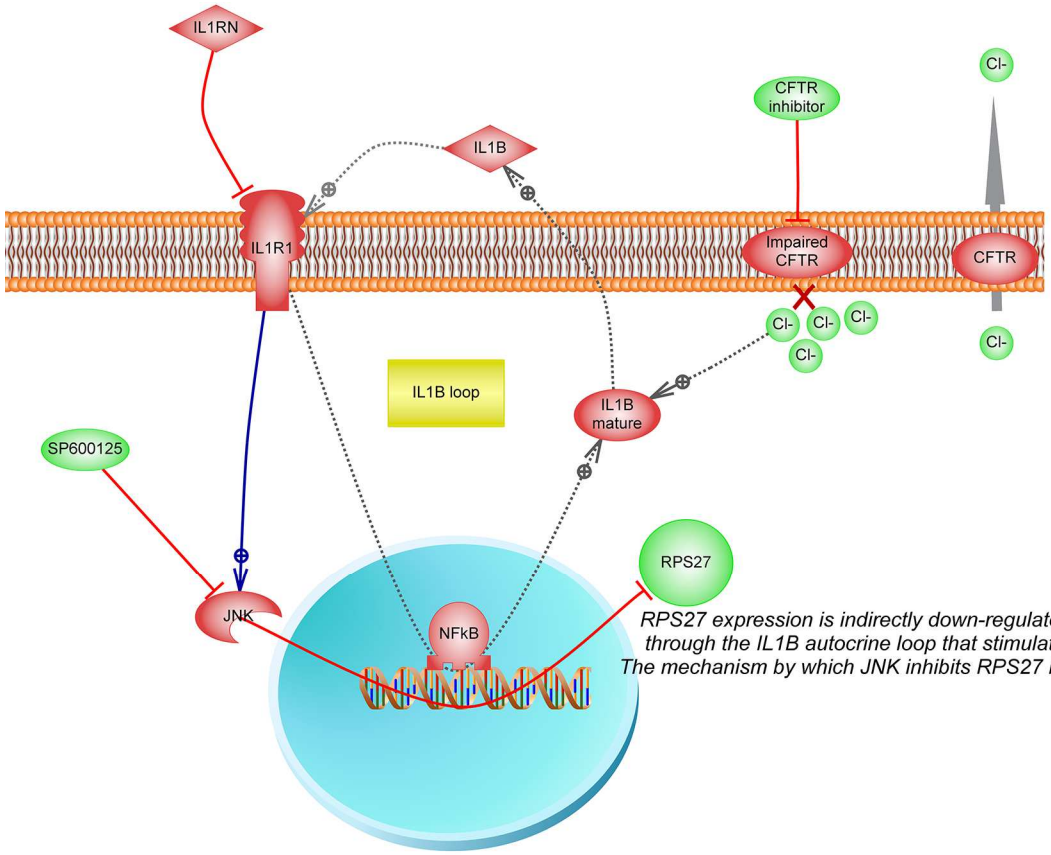

RPS27 expression is indirectly down-regulated by Cl- through the IL1B autocrine loop that stimulates JNK. The mechanism by which JNK inhibits RPS27 is unknonw

CFTR Modulates *RPS27* Gene Expression Using Chloride Anion as Signaling Effector

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Short Tittle: CFTR and Cl^- signaling

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Abstract

In Cystic Fibrosis (CF), the impairment of the CFTR channel activity leads to a variety of alterations, including differential gene expression. However, the CFTR signaling mechanisms remain unclear. Recently, culturing IB3-1 CF cells under different intracellular Cl^- concentrations ($[Cl^-]_i$), we observed several Cl^--dependent genes and further characterized one of them as *RPS27*. Thus, we hypothesized that Cl^- might act as a signaling effector for CFTR signaling. Here, to test this idea, we study *RPS27* expression in T84 cells modulating the CFTR activity by using CFTR inhibitors. First, we observed that incubation of T84 cells with increasing concentrations of the CFTR inhibitors CFTR(inh)-172 or GlyH-101 determined a progressive increase in the relative $[Cl^-]_i$ (using the Cl^- fluorescent probe SPQ). The $[Cl^-]_i$ rise was concomitant with a dose-dependent down-regulation of *RPS27*. These results imply that CFTR inhibition produce Cl^- accumulation and that *RPS27* expression can be modulated by CFTR inhibition. Therefore, Cl^- behaves as a signaling effector for CFTR in the modulation of *RPS27* expression. In addition, the IL-1β receptor antagonist IL1RN or the JNK inhibitor SP600125, both restored the down-regulation of *RPS27* induced by CFTRinh-172, implying a role of autocrine IL-1β and JNK signaling downstream of Cl^- in *RPS27* modulation.

1. Introduction

Alterations in the CFTR channel expression or activity (Cystic Fibrosis Transmembrane Conductance Regulator), produced by mutations in its gene, cause the disease cystic fibrosis (CF) [1]. Previously, by using differential display (DD), we reported the existence of several CFTR-dependent genes [2, 3]; among them *SRC* [4, 5], *CISD1* [6], and *MTND4* [3, 7, 8]. In particular, the expression of *SRC* was found increased in CF cells and linked to *MUC1*

overexpression [4]. In this way, the proto-oncogene SRC was the first intermediate found for the CFTR signaling pathway. Contrary to *SRC*, *CISD1* (nuclear genome) and *MTND4* (mitochondrial genome), both encoding mitochondrial proteins, showed a decreased expression in CF cells [6, 7]; this was then correlated with a reduced mitochondrial Complex-I (mCx-I) activity [8, 9]. Other authors, by using microarrays analysis, also reported the presence of differentially regulated genes in CF cells [10-25]. Thus, the existence of CFTR-dependent genes has been well demonstrated.

However, the CFTR signaling mechanisms involved in regulation of the CFTR-dependent genes are unclear and could implicate several different mechanisms related to the CFTR expression, localization and activity. We have observed that the expression of *SRC*, *MTND4* and *CISD1* was modulated in cells treated with different CFTR inhibitors (NPPB, glibenclamide, CFTR(inh)-172), suggesting that the CFTR chloride transport activity was involved in the signaling mechanism affecting the expression of these genes [4, 6, 7, 26]. The CFTR activity might lead to changes in the intracellular chloride concentration ($[Cl^-]_i$), as it was reported by other authors [27-30]. Thus, we hypothesized that $[Cl^-]_i$ could be involved in the modulation of certain CFTR-dependent genes and act as a signaling effector for CFTR. Recently, applying differential display (DD) to human IB3-1 cells incubated with increased $[Cl^-]_i$ (using tributyltin and nigericin), we found that several genes were under $[Cl^-]_i$ modulation. One of them, identified as *RPS27* [31], also known as metallopanstimulin-1 (MPS-1) or ribosomal protein S27 [32, 33], showed a biphasic response against Cl^- [31]. Thus, the CFTR signaling involved in the regulation of CFTR-dependent gene expression could be initiated by Cl^- acting as a signaling effector. The aim of the present work was to demonstrate, by using the *RPS27* expression as marker, that Cl^- constitutes the first element in the CFTR-signaling pathway. To test this hypothesis, *RPS27* expression was measured in T84 cells treated with the CFTR inhibitors CFTR(inh)-172 and GlyH-101 to modulate the CFTR

activity and induce Cl^- accumulation. The results showed that *RPS27* was regulated by the CFTR activity, in a dose-dependent manner, in the presence of increasing concentrations of CFTR inhibitors. In addition, Cl^- was accumulated in agreement with the increased concentrations of both inhibitors, thus modulating the expression of the Cl^--dependent gene *RPS27*. These results suggest that the Cl^- anion behaves as a signaling effector for CFTR in the regulation of *RPS27* gene expression.

2. Materials and Methods

2.1 Materials

CFTR(inh)-172 (5-[(4-Carboxyphenyl) methylene]-2-thioxo-3-[(3-trifluoromethyl)phenyl-4-thiazolidinone) and GlyH-101 (N-(2-naphthalenyl)-[(3,5-dibromo-2,4-ihydroxyphenyl)methylene] glycine hydrazide) were purchased from Calbiochem (San Diego, CA). The fluorescent Cl^- probe SPQ (6-methoxy-N-[3-sulfopropyl]quinolinium) was from Invitrogen (Carlsbad, CA). Interleukin-1 receptor antagonist (ILR1N, Cat. No. SRP3327), dimethyl sulfoxide (DMSO, culture grade) and valinomycin were from Sigma-Aldrich (St. Louis, MO) and the JNK inhibitor SP600125 from Alomone Labs (Jerusalem, Israel). Stock solutions for each inhibitor were prepared at 1000 X in DMSO and control cultures were treated with equal amounts of DMSO (final concentration 0.1–0.3%). All other reagents were analytical or molecular biology grade.

2.2 Cells and culture conditions

T84 (CCL-248) cells, which are human colon carcinoma epithelial cells that express wt-CFTR [8, 34, 35], were cultured in DMEM/F12 (Life Technologies, GIBCO BRL, Rockville, MD) supplemented with 5% FBS (Internegocios S.A., Buenos Aires, Argentina), 100 U/ml penicillin and 100 µg/ml streptomycin (Life Technologies, GIBCO BRL, Rockville, MD).

Cultures were grown at 37 ºC in a humidified air atmosphere containing 5% CO_2. Cells were plated at a density of 15 x 10^3 cells/cm^2 and cultured by using 0.08 ml of media/cm^2. Before the assays, the cells were cultured 24 h in serum-free DMEM/F12. The incubations in the presence of CFTR(inh)-172 and GlyH-101 were performed for 4 h in serum-free medium. For treatments with the JNK inhibitor SP600125 (10 μM) or the interleukin-1 receptor type I (IL1R1) inhibitor IL1RN (100 ng/ml), the cells were pre-incubated for 30 min with these inhibitors and then treated with CFTRinh-172.

2.3 Measurement of the intracellular chloride concentration ([Cl^-]i)

The [Cl^-]i of T84 cells incubated in the presence of increased concentration of CFTR inhibitors was measured by using the chloride sensitive probe SPQ [35-37], as previously described [38]. Briefly, cells were seeded in 96-well plates, black walls, clear bottom (Greiner Bio-One, Germany; Cat. # 655090) at a density of 10,000 cells/cm^2 and grown for 4 days, using 200 μl of DMEM/F12 plus 5% FBS. After reaching confluence, the cells were incubated for 24 h in serum-free DMEM/F12 and loaded overnight with 5 mM SPQ in the same medium. The SPQ-loaded monolayers were washed four times with 0.2 ml/well of Hank´s buffer (136.9 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 3.7 mM NaH_2PO_4, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.7 mM $MgSO_4$, 5.5 mM D-glucose and 10 mM HEPES). The cells were then incubated in serum-free DMEM/F12 with different concentrations of CFTR(inh)-172 or GlyH-101 (0-7 μM). Appropriate vehicle (DMSO) was added at each concentration point. Medium was replaced by Hank´s buffer to avoid phenol red interference. To estimate the $[Cl^-]_i$, calibration curves were made using two high K^+ buffers (High KCl buffer: 1.3 mM Ca-gluconate, 100 mM KCl, 40 mM K-gluconate, 3.7 mM NaH_2PO_4, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.7 mM $MgSO_4$, 5.5 mM D-glucose; and High KNO_3 buffer: 1.3 mM Ca-gluconate, 100 mM KNO_3, 40 mM K-gluconate, 3.7 mM NaH_2PO_4, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.7 mM $MgSO_4$, 5.5 mM D-glucose), containing the ionophores

nigericin (5 µM) and tributyltin (10 µM). The fluorescence intensity (F) was measured in a microplate reader (NOVOstar BMG LABTECH GmbH, Ortenberg, Germany) at 37 °C, after 4 h of equilibration with CFTR inhibitors. Six wells were incubated in presence of potassium thiocyanate 140 mM plus valinomycin 5 µM to obtain the background fluorescence (Fb), which was subtracted to each fluorescence value (F) of the curve, and the F-Fb value was normalized to 1 for the F-Fb value of 0 µM inhibitor [38, 39].The Stern-Volmer constant (Ksv) was calculated from the calibration curves and the [Cl^-] was estimated by using the equation Fo/F - 1= *Ksv* [Cl^-]. The values were expressed in mM, plotted, fitted to a dose-response curve, and the EC_{50}s corresponding to each CFTR inhibitor were calculated.

2.4 *RPS27* expression measurement by reverse transcription-qPCR

Reverse transcription-qPCR (RT-qPCR) was used to analyze the *RPS27* mRNA expression, as we previously described [31]. Briefly, total RNA samples (1 µg) from T84 cells incubated at different concentrations of CFTR inhibitors (CFTR(inh)-172 and GlyH-101) (0, 0.1, 0.5, 1, 2.5, 5 and 7 µM) for 4 h in serum-free DMEM/F12. The RNA was reverse transcribed by using M-MLV reverse transcriptase (Promega) (100 U) and 8 µM Oligo-dT, according to the manufacturer's instructions. The *RPS27* expression was referred to *18S* expression. Primers for *RPS27* were: Fw-(*RPS27*) 5´-GGCGGTGACGACCTACGCAC-3´, Rv-(*RPS27*) 5´-TAGCATCCTGGGCATTTCACATCCA-3´. Primers for 18S rRNA were: Fw-(*18S*) 5´-CCGATAACGAACGAGACTCTGG-3´ and Rv-(*18S*) 5´-TGAACGCCACTTGT CCCTCTAAG-3´. RT-qPCR were performed by using an ABI 7500real-time PCR system (Applied Biosystems Inc., Foster City, CA), and the ΔΔCt method was used to obtain the expression levels relative to internal standards (IS) expression by using software from Applied. Previously reported RT-qPCR conditions were used [31].

2.5 Statistics

Unless otherwise indicated, all the assays were performed at least by duplicates. The results corresponded to three independent experiments (n=3) and were expressed as mean ± SEM (n) with n showing the number of independent experiments. RT-qPCR reactions were carried-out by using intra-assay quadruplicates. The final RT-qPCR quantification values were obtained as the means of the relative quantification (RQ) values for each independent experiment (n=3). The different curves and regressions were fitted and the R^2 value were used to obtain the corresponding t values: $t = \sqrt{R^2(n-2)/(1-R^2)}$ [40]. One-way ANOVA and the Turkey's test were applied to determine significant differences among samples (* indicates $p< 0.05$).

3. Results

3.1 The inhibition of the CFTR activity increases the intracellular Cl^- concentration.

To test the hypothesis that the Cl^- anion might have a role as signaling effector for CFTR, we first modulated the CFTR activity by using pharmacological CFTR inhibitors. We expected that the CFTR inhibition with increasing concentrations of inhibitors would cause a progressive increase in the $[Cl^-]_i$. The CFTR inhibitors used for this purpose were CFTR(inh)-172 and GlyH-101, which are highly potent and specific [8, 41-43], possessing different binding sites at the channel (intracellular and extracellular, respectively [41, 43]). T84 cells were used as a model system, since these cells express abundant wt-CFTR. T84 cells were incubated in the presence of increased concentration of inhibitors for 4 h. The consequent changes in the $[Cl^-]_i$ were measured by using SPQ fluorescence, which is quenched by Cl^-. As shown in Fig. 1a and 1b, the $[Cl^-]_i$ increased in a dose-dependent manner in the presence of

increased concentration of the CFTR inhibitors: CFTR(inh)-172 (EC_{50} = 2.1 ± 0.5 (4) μM; sigmoidal fit, R^2 = 0.97, p< 0.001) and GlyH-101 (EC_{50} = 2.0 ± 0.1 (5) μM; sigmoidal fit, R^2 = 0.99, p< 0.001). These results show that the progressive inhibition of the CFTR activity, as expected, also resulted in a progressive accumulation of intracellular Cl^-. Performing an ANOVA analysis, a significant [Cl^-] increase (p<0.01, indicated as **) was seen at concentrations over 5 μM of both inhibitors compared to control cells.

3.2 Increased $[Cl^-]_i$ induced a progressive down-regulation of RPS27

We had previously reported that increased $[Cl^-]_i$ induced a down-regulation of *RPS27*, in a dose-dependent manner [31]. In that case, the $[Cl^-]_i$ was modulated by using a double-ionophore strategy (tributyltin and nigericin) that allows the equilibration between the intracellular $[Cl^-]_i$ and the extracellular $[Cl^-]_e$, independently of the activity of Cl^- channels. Now, to test the hypothesis that the Cl^- anion may behave as a signaling effector for the CFTR channel activity, we measured if the changes in the $[Cl^-]_i$ caused by the CFTR inhibition above described could also result in *RPS27* down-regulation. T84 cells were preincubated for 24 h in serum-free DMEM/F12, and then incubated with different concentrations of CFTR(inh)-172 and GlyH-101 for 4 h. After incubation, the *RPS27* mRNA levels were measured by real time-qPCR. As shown in Fig. 2a and 2b, the *RPS27* expression decreased in a dose-dependent manner after incubation with increasing doses of the CFTR inhibitors CFTRinh-172 (EC_{50} = 1.6 ± 0.3 (3) μM; sigmoidal fit, R^2 = 0.99, p< 0.001,) and GlyH-101 (EC_{50} = 2.0 ± 0.5 (3) μM; sigmoidal fit, R^2 = 0.98, p< 0.001). The *RPS27* mRNA expression was significative decreased in cells exposed to 5 μM and 7 μM of both inhibitors compared to control cells (*, p < 0.05). In addition, a significant sigmoidal correlation was observed between *RPS27* expression and the $[Cl^-]_i$ calculated from the SPQ values obtained using each CFTR inhibitor. The EC_{50}s for *RPS27* levels vs $[Cl^-]_i$ where similar with both inhibitors,

although the value obtained with GlyH-101 (EC_{50} = 47 ± 3 mM) was closer to the value obtained previously by using the two ionophores to change the $[Cl^-]_i$ (EC_{50} = 47 ± 7 mM) [31]. Altogether, these results strongly suggest that Cl^- may act as a signaling effector for CFTR, in this case modulating the *RPS27* expression.

3.3 CFTR activity regulates RPS27 gene expression through IL-1β and JNK signaling

We have previously reported that lung epithelial IB3-1 CF cells and colon Caco-2/pRS26 cells, both with impaired CFTR activity, have increased IL-1β expression (mRNA and secreted protein) [44]. Interestingly, in IB3-1 epithelial CF cells, the IL-1β secretion was modulated by changes in $[Cl^-]_i$, in a biphasic way, with maximal IL-1β secretion at $[Cl^-]_i$ 75 mM [38]. The secreted IL-1β in turn activates an autocrine positive feed-back loop, which resulted in the increased expression of its own mRNA [38]. The disruption of this autocrine loop by using IL-1β blocking antibodies or the IL-1 receptor type I inhibitor (IL1RN or anakinra) normalized the mitochondrial Complex I+III activity (NADH cytochrome c reductase activity) and total ROS levels, and improve the mitochondrial ROS levels [44]. In addition, IL1RN normalized the IL-1β expression in IB3-1 cells stimulated with Cl^- 75 mM (in the presence of tributyltin and nigericin) [38], and reduced the c-Src activity and mitochondrial ROS levels in Caco-2/pRS26 [5, 38]. The JNK inhibitor SP600125 had a similar inhibitory effect on the IL-1β mRNA responses to Cl^- changes in IB3-1 cells [38]. On the other hand, *RPS27* also had a biphasic response to $[Cl^-]_i$ in IB3-1 cells, although in the opposite way, with minimal *RPS27* expression at $[Cl^-]_i$ 75 mM [31]. Therefore, we hypothesized that the increased accumulation of $[Cl^-]_i$ observed in T84 cells treated with CFTR(inh)-172 might also stimulate the secretion of IL-1β and its positive autocrine feed-back loop, and that IL-1β could in turn be responsible for the *RPS27* down-regulation. In that case, the reduction in the *RPS27* expression in the presence of CFTRinh-172 should be

reverted by using a blocker of the IL-1β loop. On the other hand, it was also known that T84 cells express IL-1β [39]. Thus, T84 cells were pre-incubated in the presence of IL1RN (100 ng/ml) or SP600125 (10 μM), and after 30' CFTR(inh)-172 (5 μM) was added to the cells. After incubation for 4 h the expression of *RPS27* was measured. In agreement with our hypothesis, as shown in Fig. 3, treatments with SP600125 or IL1RN inhibited the down-regulation of *RPS27* induced by CFTR(inh)-172. These data suggest that IL-1β and its autocrine positive feed-back loop are involved in *RPS27* gene expression, downstream of Cl^-.

4. Discussion

In a previous work, using lung epithelial IB3-1 CF cells, we observed several differentially expressed genes under Cl^- dependency [31]. One of these differentially expressed genes was further characterized and corresponded to *RPS27* [31], which codify a protein involved in DNA repair, transcription, growth regulation and carcinogenesis [32, 33, 45]. It was therefore hypothesized, although not probed in that work, that the Cl^- anion might act as a signaling effector for channels and transporters able to modulate the $[Cl^-]_i$, in particular CFTR [31]. The aim of the present work was to demonstrate this hypothesis for CFTR. The strategy implemented in that work to modulate $[Cl^-]_i$ was to equilibrate the $[Cl^-]_i$ to the $[Cl^-]_e$ by using nigericin and tributyltin [31]. Here the strategy was different, since we want to modify the $[Cl^-]_i$, changing the CFTR activity, in order to demonstrate that Cl^- may act as a signaling effector for CFTR. Since we already knew that *RPS27* was a Cl^--dependent gene [31], its expression was used to verify the functional effects of the CFTR inhibition and the consequent Cl^- accumulation.

The results first demonstrated that an increased CFTR inhibition determined a progressive Cl^- accumulation inside T84 cells, as it was previously observed in different model systems [27-30]. Both CFTR inhibitors increased the $[Cl^-]_i$ in a dose-dependent manner. Then, *RPS27*

expression was measured in the presence of increasing concentrations of these CFTR inhibitors. As shown, increased concentrations of the CFTR inhibitors, that progressively increase $[Cl^-]_i$, also induced a negative dose-response in *RPS27* expression. A significant correlation was observed between the *RPS27* expression levels and the $[Cl^-]_i$ measured for each inhibitor. Altogether, these and the previous results [31] suggest a role of Cl^- as signaling effector for CFTR, which was the aim of this work.

Following the Cl^- accumulation, multiple mechanisms could be involved in *RPS27* modulation. We have found previously an increased secretion of interleukin-1β (IL-1β) in CF cells [44], and demonstrated that an IL-1β autocrine loop was responsible for the inhibition of the mitochondrial Complex I activity and ROS generation in these cells [44]. Interestingly, Cl^- modulated the IL-1β loop affecting the IL-1β maturation and secretion, which in turn started the IL-1β loop [44]. Therefore, we hypothesized that here the Cl^--dependency of *RPS27* might also be the consequence of an active IL-1β loop, as a downstream signaling mechanism for Cl^-. Confirming this hypothesis, the IL-1β loop inhibitor IL1RN [44] was able to revert the *RPS27* down-regulation induced by CFTR inhibition, suggesting that the IL-1β loop is downstream of Cl^- in the CFTR signaling that results in *RPS27* down-regulation. The JNK inhibitor SP600125 also reverted the RPS27 levels, although it does not inhibit the IL-1β loop [44]; therefore, the JNK effects should be outside and downstream of the IL-1β loop as illustrated in Fig. 4. In this regard, the role of IL-1β in stimulating JNK has been well documented in the past [46, 47].

Previously, other studies have shown the effect of extracellular and intracellular chloride in gene regulation, incubating the cells in media with low or high Cl^- concentrations [48-52]. On the other hand, Succol et al., using a genetic and pharmacological approach to produce changes in the $[Cl^-]_i$, showed that $[Cl^-]_i$ regulates the expression of alpha3-1 and delta-containing GABA(A) receptors in mice primary cerebellar neurons, suggesting that Cl^- was

acting as an intracellular signal [53]. Other studies, discussed previously [31], have also shown similar results regarding the possible role of Cl^- as a signaling effector.

5. Conclusions

In conclusion, the results obtained, summarized in Fig. 4, suggest that inhibition of CFTR determines Cl^- accumulation in T84 cells, which in turn modulates *RPS27* gene expression, through an autocrine effect of IL-1β. More importantly, the results suggest that Cl^- is the first element in the CFTR signaling pathway, acting as a signaling effector for CFTR. The exact mechanism by which Cl^- modulates *RPS27* expression is not clear yet, although it involves the IL-1β loop and JNK signaling. These results might contribute to better understand different pathological conditions in which the CFTR activity or the chloride homeostasis are affected.

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Figure Legends

Fig. 1. The inhibition of the CFTR activity results in the accumulation of intracellular chloride. T84 cells were incubated with different concentration of CFTR(inh)-172 (a) or GlyH-101 (b) for 4 h and the SPQ fluoresce was used to measure the $[Cl^-]_i$ by spectrofluorometry [35]. The results were expressed in mM. Measurements corresponded to 4 independent experiments for CFTR(inh)-172 and 5 independent experiments for GlyH-101.

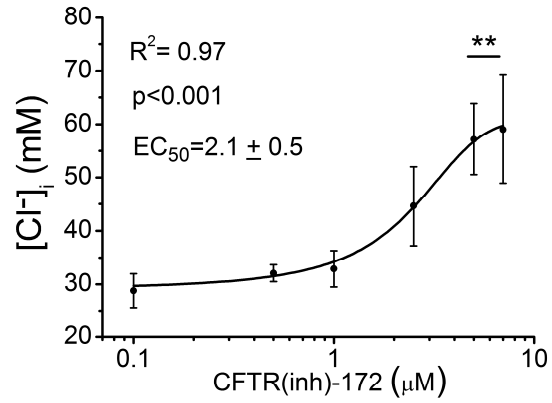
Fig. 2. CFTR inhibition down-regulates *RPS27* gene expression. The *RPS27* mRNA expression was measured by applying RT-qPCR to total RNA obtained from T84 cells incubated with different concentration of CFTR(inh)-172 (a) or GlyH-101 (b) for 4 h. The relative expression values were adjusted to a sigmoidal dose-response curve and the t-values were calculated from the R^2. The values were expressed as means ± SEM (n) from three independent experiments (n=3). Correlation curves between *RPS27* and $[Cl^-]$i for CFTR(inh)-172 (c) and GlyH101 (d) treatments were calculated using a sigmoidal fit.

Fig. 3. CFTR activity regulates *RPS27* gene expression through IL-1β and JNK signaling. *RPS27* mRNA expression was measured by RT-qPCR from total RNA obtained from T84 cells incubated with CFTR(inh)-172 (5 μM) for 4 h, with or without SP600125 10 μM (JNK inhibitor) or IL1RN 100 ng/ml (interleukin 1 receptor antagonist). The expression of control cells was taken as 100%. Measurements correspond to four independent experiments (n=4); data were expressed as mean ± SE. * indicates $p<0.05$ (ANOVA one-way analysis and Tukey post-hoc test).

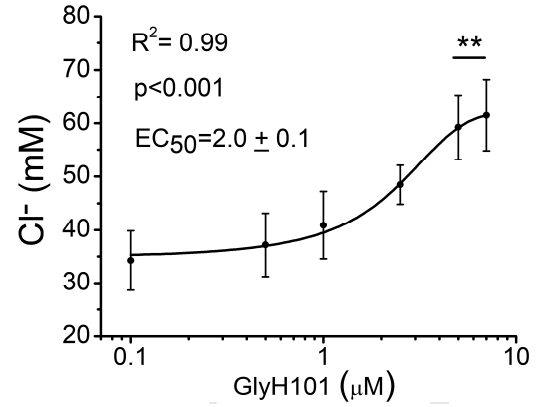
Fig. 4. Graphic summary. The illustration shows the results obtained here and the interaction between the different effectors. Results from previous works are shown by dotted lines. The

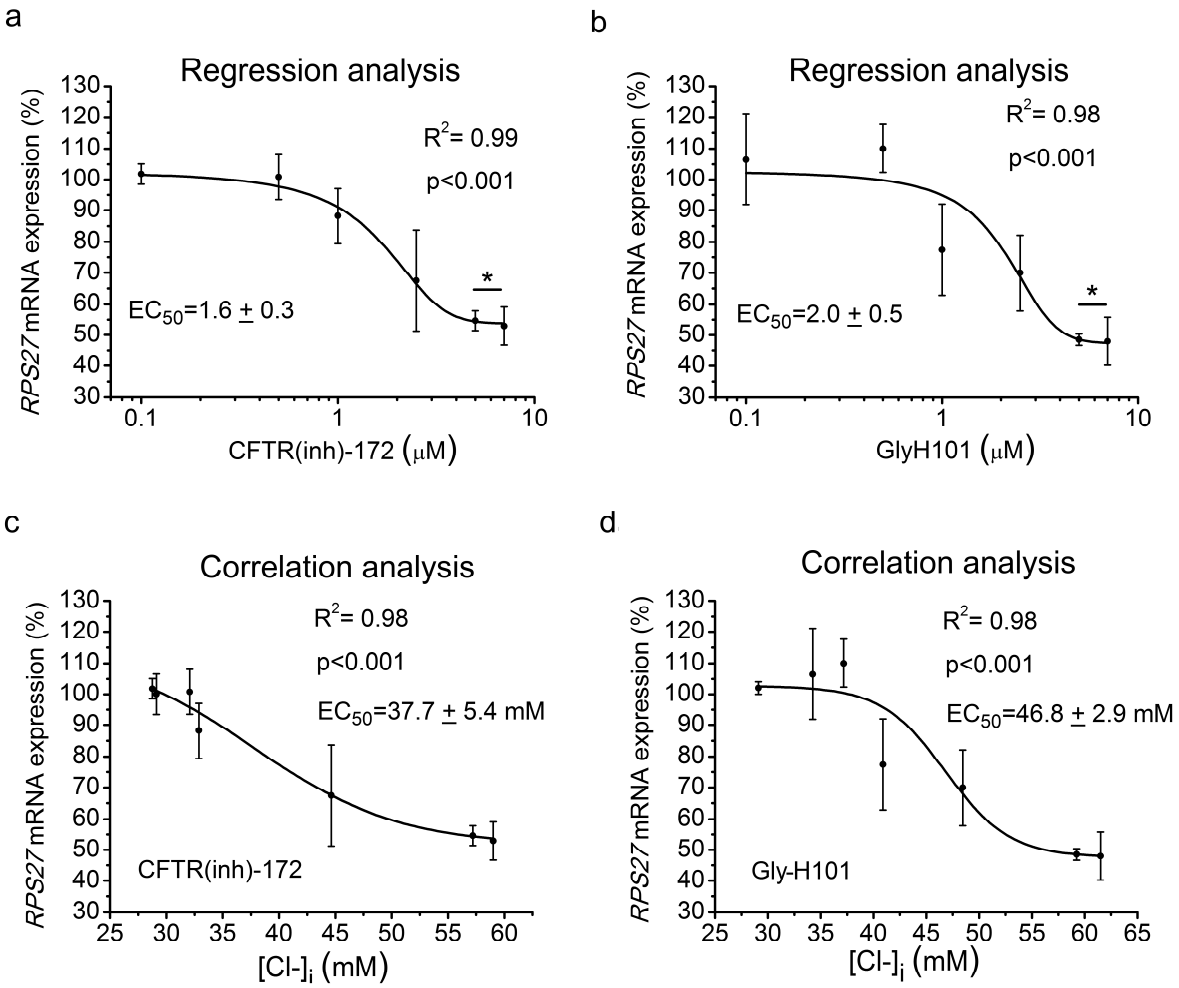
figure was drawn by using Pathway Studio v.10 (Elsevier). The CFTR inhibition results in the accumulation of Cl^-, which in turn stimulates IL-1β secretion [38]. The secreted IL-1β starts an autocrine positive feed-back loop that inhibits *RPS27* expression. Thus, incubation with the interleukin-1 receptor type I antagonist IL1RN blocks the loop and restore *RPS27* mRNA levels. Its expression was also restored by using the JNK inhibitor SP600125, suggesting that the *RPS27* inhibition is mediated through IL-1β→JNK signaling. Thus, Cl^- behaves as a signaling effector for CFTR, in this case stimulating IL-1β autocrine signaling, which in turn down-regulates the *RPS27* gene expression.

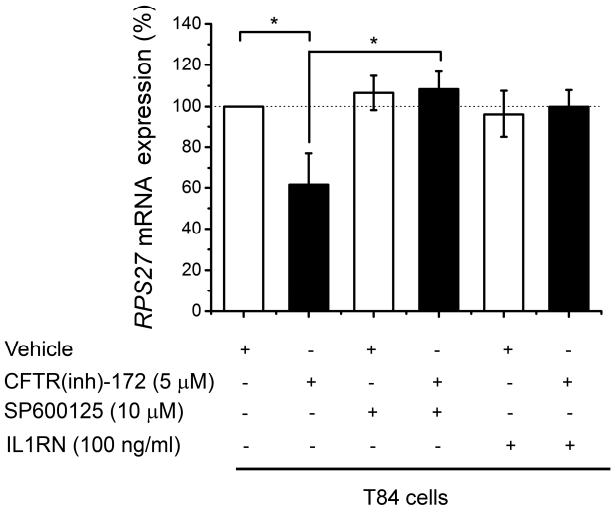
a

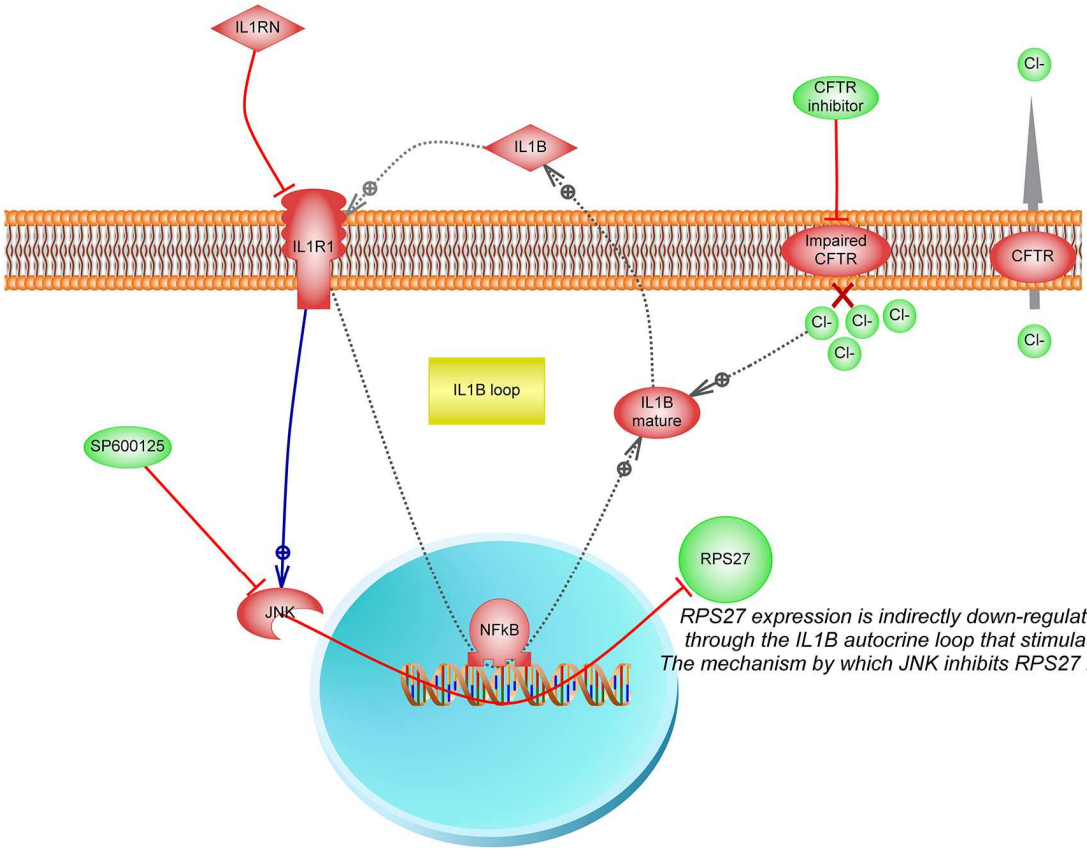


b




a
Regression analysis
R^2= 0.99
p<0.001
EC_{50}=1.6 ± 0.3
*
RPS27 mRNA expression (%)
CFTR(inh)-172 (μM)
b
Regression analysis
R^2= 0.98
p<0.001
EC_{50}=2.0 ± 0.5
*
RPS27 mRNA expression (%)
GlyH101 (μM)
c
Correlation analysis
R^2= 0.98
p<0.001
EC_{50}=37.7 ± 5.4 mM
CFTR(inh)-172
RPS27 mRNA expression (%)
$[Cl-]_i$ (mM)
d
Correlation analysis
R^2= 0.98
p<0.001
EC_{50}=46.8 ± 2.9 mM
Gly-H101
RPS27 mRNA expression (%)
$[Cl-]_i$ (mM)





RPS27 expression is indirectly down-regulated by Cl- through the IL1B autocrine loop that stimulates JNK. The mechanism by which JNK inhibits RPS27 is unknonw