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Prostaglandin E_2 Induces *MUC8* Gene Expression via a Mechanism Involving ERK MAPK/RSK1/cAMP Response Element Binding Protein Activation in Human Airway Epithelial Cells*

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***MUC8* gene expression is overexpressed in nasal polyp epithelium and is also increased by treatment with inflammatory mediators in nasal epithelial cells. These data suggest that *MUC8* may be one of important mucin genes expressed in human airway. However, the mechanisms of various inflammatory mediator-induced *MUC8* gene expression in normal nasal epithelial cells remain unclear. We examined the mechanism by which prostaglandin E_2 (PGE2), an arachidonic acid metabolite, increases *MUC8* gene expression levels. Here, we show that ERK mitogen-activated protein kinase is essential for PGE2-induced *MUC8* gene expression in normal human nasal epithelial cells and that p90 ribosomal S 6 protein kinase 1 (RSK1) mediates the PGE2-induced phosphorylation of cAMP-response element binding protein. Our results also indicate that cAMP-response element at the −803 region of the *MUC8* promoter is an important site of PGE2-induced *MUC8* gene expression. In conclusion, this study gives insights into the molecular mechanism of PGE2-induced *MUC8* gene expression in human airway epithelial cells.**

Mechanicochemical proteins produced by airway epithelial cells are essential components of airway mucus, which plays an important role in the protection of the airways from bacterial and viral attack. Mucins are highly glycosylated, high molecular weight glycoproteins and major components of the mucus produced by respiratory tract epithelia. In the airway, virtually all forms of airway inflammation are associated with the overproduction of mucus, which can lead to airway obstruction (1).

Twenty mucin genes have been identified; however, it is not known which mucins are secreted in the various airway diseases. Thus, in terms of treatment for mucin hypersecretion, it is crucial that we identify the genes responsible for high viscosity mucus in airway diseases and develop means of reducing mucin production. The mucins are usually subdivided into two groups based on domain, namely, the membrane-bound and secreted mucins. Specifically, MUC2 (2), MUC5AC (3), MUC5B (4), MUC6 (5), MUC7 (6), MUC9 (7), and MUC19 (8) are secreted mucins, and MUC1 (9), MUC3 (10), MUC4 (11), MUC11 (12), MUC12 (12), MUC13 (13), MUC17 (14), MUC18 (15), and MUC20 (16) are membrane-bound mucins. However, the other mucin genes including *MUC8* (17) have not been fully characterized.

MUC8 may be an important airway mucin because its mRNA levels are up-regulated in chronic sinusitis with polyps (18) and by inflammatory mediators (19). In addition, because MUC8 protein is expressed in ciliated cells (20), it may be related to the differentiation or function of ciliated cells in airway epithelial cells. However, because only short partial sequences of the *MUC8* gene have been reported, molecular studies have been limited. In a previous study, we completely sequenced *MUC8* cDNA (GenBank™ accession number BK005559) and found that MUC8 protein contains secreted and membrane-bound mucin domains using the SMART program.[1]

Prostaglandins are arachidonic acid metabolites with a wide range of biological actions. Moreover, it is known that cyclooxygenase converts arachidonic acid to prostaglandin H_2, which is further metabolized to various prostaglandins and thromboxanes (22). These species are produced in a wide variety of tissues and function as lipid mediators. In particular, PGE2[2] mediates IL-1β-induced *MUC5AC* gene expression in human airway epithelium (23). Because mucin hypersecretion is a hallmark of airway inflammation, it is important that we determine how PGE2 regulates airway mucin gene expression.

In the present study, we examined the mechanism by which PGE2 increases *MUC8* gene expression levels. We found that extracellular signal-regulated kinase mitogen-activated protein kinase (ERK MAPK) is essential for PGE2-induced *MUC8* gene expression in normal human nasal epithelial (NHNE) cells. We also found that p90 ribosomal S 6 protein kinase 1 (RSK1) mediates the PGE2-induced phosphorylation of cAMP

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[2] The abbreviations used are: PGE2, prostaglandin E_2; IL, interleukin; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; NHNE, normal human nasal epithelial; RSK1, p90 ribosomal S 6 protein kinase 1; CRE, cAMP response element; CREB, CRE binding protein; MEK, MAPK/ERK kinase; DN, dominant negative.

response element binding protein (CREB). In addition, transcriptional activities of cloned *MUC8* promoter regions showed that CRE in *MUC8* promoter is an important site of PGE2-induced *MUC8* gene expression. Taken together, these studies provide insights into the mechanism of PGE2-induced *MUC8* gene expression and extend our understanding of mucin gene overexpression during airway mucosal inflammation.

EXPERIMENTAL PROCEDURES

Cell Cultures—Epithelia were isolated by scrapings from the inferior turbinate of healthy adult volunteers. None of volunteers had any history of allergic symptoms, nasal polyps, or asthma. They did not have a history of smoking and did not take any medicines for the past 6 months. The volunteers' permission and the approval from the Institutional Review Board at Yonsei University College of Medicine were obtained for the use of the specimens. The epithelial cells from the turbinates were treated with 1% Pronase (Type XIV protease, Sigma-Aldrich Chemical Co., St. Louis, MO) for 18 to 20 h at 4 °C. To remove fibroblasts, endothelial cells, and myoepithelial cells, isolated cells were placed in a plastic dish and cultured for 1 h at 37 °C. Isolated epithelial clusters were divided into single cells by incubating them with 0.25% trypsin/EDTA. Passage-2, NHNE cells were seeded in 0.5 ml of culture medium onto 24.5-mm, 0.45-μm pore size Transwell clear (Costar) culture inserts. Cells were cultured in a 1:1 mixture of bronchial epithelial cell growth medium (Clonetics):Dulbecco's modified Eagle's medium (Invitrogen) containing all supplements described previously (24). Cultures were grown submerged, and culture medium was changed on the first day and every other day thereafter. The human lung mucoepidermoid carcinoma cell line, NCI-H292, was purchased from the American Type Culture Collection (catalog no. CRL-1848, Manassas, VA) and cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) in the presence of penicillin-streptomycin at 37 °C in a 5% CO_2 humidified chamber. We obtained A549 cell from ATCC (catalog no. CCL-185, Manassas, VA).

Inhibitor, Antibodies, and Dominant Negative Vector—PD98059 and anti-α-tubulin antibody were purchased from Calbiochem, and anti-phospho p44/42 MAP kinase (Thr-202/Tyr-204) antibody, anti-phospho-p38 MAP kinase (Thr-180/Tyr-182) antibody, anti-phospho-SAPK/c-Jun NH_2-terminal kinase MAP kinase (Thr-183/Tyr-185) antibody, anti-phospho-RSK1 (Ser-380) antibody, and anti-phospho-CREB (Ser-133) antibody were purchased from Cell Signaling. Plasmid encoding kinase-deficient MEK1 mutant (*pcDNA5-MEK1DN*) was kindly provided by Dr. Jian-Dong Li (House Ear Institute, Los Angeles, CA).

Real-time Quantitative PCR—Primers and probes were designed using PerkinElmer Life Sciences Primer Express® software purchased from PE Biosystems. Commercial reagents (TaqMan PCR Universal PCR master mix, PE Biosystems) and conditions were applied according to the manufacturer's protocol. 1 μg of cDNA (reverse transcription mixture), oligonucleotides at a final concentration of 800 nM of primers, and 200 nM TaqMan hybridization probe were used in a 25-μl volume. The probe of real-time PCR was labeled with carboxylfluorescein at the 5′-end and with the quencher carboxytetramethylrhodamine at the 3′-end. The following primers and TaqMan probes were used: *MUC8*, forward (5′-TAACCCAATGCCACTCCTTC-3′) and reverse (5′-GGAGTGTAACCTGGCTGCTC-3′) and TaqMan probe (carboxylfluorescein-GGTTAGGGCTGACCACAGAA-carboxytetramethylrhodamine). β 2-Microglobulin, forward (5′-CGCTCCGTGGCCTTAGC-3′) and reverse (5′-GAGTACGCTGGATAGCCTCCA-3′) and TaqMan probe (carboxylfluorescein-TGCTCGCGCTACTCTCTCTTTCTGGC-carboxytetramethylrhodamine). Real-time reverse transcription-PCR was performed on a PE Biosystems ABI PRISM® 7700 sequence detection system (Foster City, CA). The thermocycler parameters were 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All reaction was performed in triplicate. Relative quantity of *MUC8* mRNA was obtained using a comparative threshold method, and results were normalized against β 2-microglobulin as an endogenous control. Data were analyzed using Student's *t* test for paired and unpaired values for statistical analysis.

Western Blot Analyses—NCI-H292 cells were grown to confluence in 6-well plates. After treating with 10 nM PGE2, cells were lysed with 2× Lysis buffer (250 nM Tris-Cl (pH 6.5), 2% SDS, 4% β-mercaptoethanol, 0.02% bromphenol blue, and 10% glycerol). Equal amounts of whole cell lysates were resolved by 10% SDS-PAGE and then transferred to a polyvinylidene difluoride membrane (Millipore). Membranes were blocked with 5% skimmed milk in Tris-buffered saline (TBS, 50 mM Tris-Cl (pH 7.5) and 150 mM NaCl) for 2 h at room temperature. Blots were then incubated overnight with primary antibodies in TTBS (0.5% Tween 20 in TBS). After washing with TTBS, the blots were further incubated for 1 h at room temperature with anti-rabbit or anti-mouse antibody (Cell Signaling) in TTBS and then visualized by ECL (Amersham Biosciences).

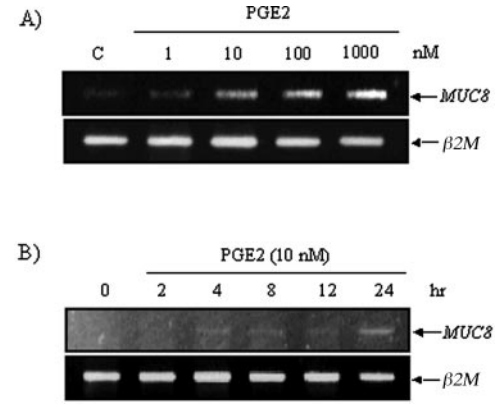

FIG. 1. **Effect of PGE2 on *MUC8* gene expression in NHNE cells.** Confluent cells were treated with PGE2 (1, 10, 100, and 1000 nM) for 24 h (*A*). Confluent cells were treated with PGE2 (10 nM) for 2, 4, 8, 12, and 24 h (*B*), and cell lysates were harvested for reverse transcription-PCR. *C*, control. β 2-Microglobulin (*β2M*) was employed as an internal control.

Electromobility Shift Analysis—PGE2-treated NCI-H292 cells were washed with ice-cold phosphate-buffered saline and pelleted. Pellets were resuspended in cell homogenization buffer (10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonylfluoride), incubated for 10 min on ice, and centrifuged. Cells were then resuspended in cell homogenization buffer containing 0.05% (V/V) Nonidet P-40 and then homogenized. Nuclei were the pelleted and resuspended in cell resuspension buffer (40 mM HEPES (pH 7.9), 0.4 M KCl, 1 mM dithiothreitol, 10% (V/V) glycerol, 0.1 mM phenylmethylsulfonylfluoride, 0.1% (W/V) aprotinin, and 0.3 M NaCl). This nuclear extract was then centrifuged at 24,000 rpm for 15 min at 4 °C, and the supernatant was aliquoted and stored at −70 °C. For electromobility shift assay, oligonucleotides corresponding to the consensus CRE sequence (5′-AGAGATTGCC*TGACG*TCAGAGAGCTAG-3′) and the MUC8 CRE sequence (5′-AACAGATAACAACTGACGCACTTCCGCCCG-3′) were synthesized, annealed, and end-labeled with [γ-^{32}P]ATP using T4-polynucleotidekinase (Promega). Nuclear extract was incubated at room temperature for 30 min with the ^{32}P-labeled CRE probes in binding buffer (Promega). Oligo-nuclear protein complexes were separated from the probes by electrophoresis through 6% nondenaturing polyacrylamide gel in 0.5× Tris borate-EDTA (TBE) buffer. Supershift experiment was conducted using 2 μl of anti-phospho-CREB antibody (Cell Signaling). The gel was dried and autoradiographed on a PhosphorImager.

Transient Transfection and Luciferase Analysis—The constructs of deleted promoter and point-mutated CRE site of *MUC8* gene were reported in our previous study.[1] NCI-H292 cells were transiently transfected with pGL3-basic, pGL3-MUC8 (−1644/+ 87), pGL3-MUC8 (−1190/+ 87), pGL3-MUC8 (−973/+ 87), pGL3-MUC8 (−549/+ 87), pGL3-MUC8 CREM1, and pGL3-MUC8 CREM2 constructs using a FuGENE6 Transfection Reagent (Roche Applied Science) according to the manufacturer's instructions, incubated for 48 h, treated with 10 nM of PGE2 for 24 h, harvested, and assayed for luciferase activity using a luciferase assay system (Promega) according to the manufacturer's instructions. β-Galactosidase activity was also assayed to standardize sample transfection efficiencies. To confirm that the luciferase activity of each construct was from PGE2, we assayed the activity of each construct in the absence of PGE2.

RESULTS

PGE2 Can Induce MUC8 Gene Expression—To determine whether PGE2 can induce *MUC8* gene expression within NHNE cells, we carried out reverse transcription-PCR after treating cells with various concentrations of PGE2. As the dose of PGE2 was increased from 1 to 1000 nM, *MUC8* gene expression was observed to gradually increase from 10 nM. As shown in Fig. 1*A*, 10 nM PGE2 significantly induced *MUC8* gene expression. However, no corresponding change was found in

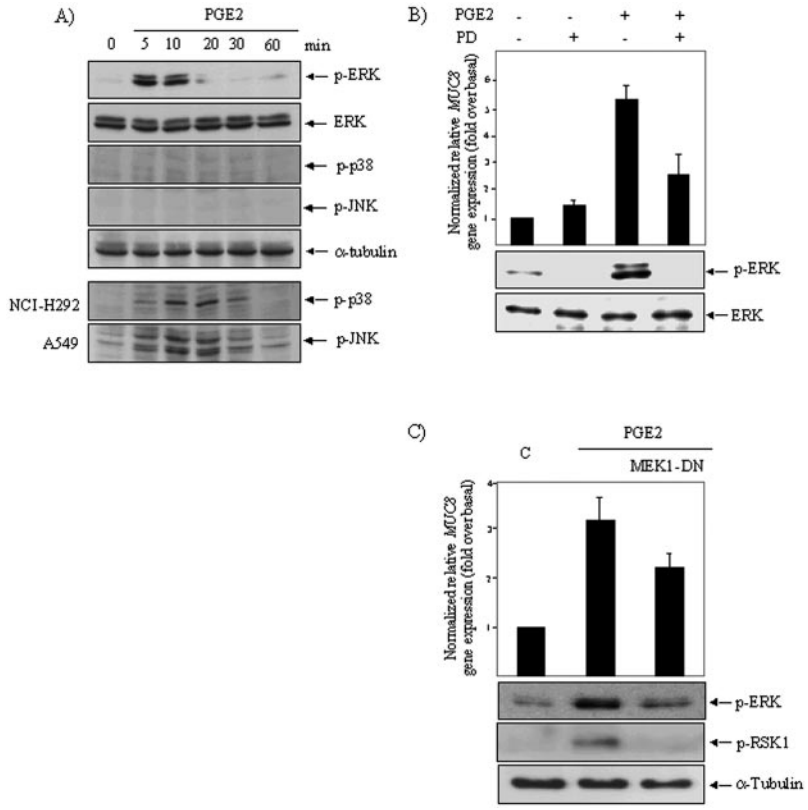

FIG. 2. **PGE2 induces *MUC8* gene expression via ERK MAP kinase signaling.** Confluent cells were treated with PGE2 (10 nM) for 5, 10, 20, 30, and 60 min, and cell lysates were harvested for Western blot analysis. Representative Western blots using phosphospecific antibodies showed transient activation of ERK but not p38 and c-Jun NH_2-terminal kinase, and the maximum effect is at 5 min (*A*). Confluent cells were pretreated for 1 h with 20 μM PD98059 and then stimulated for 24 h with PGE2 prior to collection of total RNA for real-time quantitative PCR. Pretreated cells were stimulated for 5 min with PGE2 prior to collection of cell lysates for Western blot analysis (*B*). The cells were transiently transfected with MEK1-dominant negative (MEK1-DN) construct and stimulated with PGE2 for 24 h prior to real-time quantitative PCR. The figures shown are representative of three independent experiments. Transfected cells were stimulated with PGE2 for 5 min prior to Western blot analysis (*C*).

the expression of the internal control, β 2-microglobulin. To determine whether PGE2 induced *MUC8* gene expression in a time-dependent manner, we examined *MUC8* expression after various exposures to 10 nM PGE2 (Fig. 1*B*). *MUC8* gene expression was found to be significantly increased after 24 h of exposure to PGE2. These results show that *MUC8* gene expression was significantly elevated by PGE2 treatment in NHNE cells. Ten nM PGE2 was used in subsequent experiments.

PGE2 Induces MUC8 Gene Expression via ERK MAP Kinase Signaling—To investigate which MAP kinase signal pathway is activated in NHNE cells stimulated by PGE2, we performed Western blot analysis using phosphospecific antibodies. ERK MAP kinase was maximally activated at 5 min, and this effect decreased after 20 min (Fig. 2*A*). However, no change was detected in the activations of phospho-p38 and c-Jun NH_2-terminal kinase. IL-1β-treated NCI-H292 cells (25) and A549 cells (26) were used as positive controls for p38 and c-Jun NH_2-terminal kinase activation, respectively. Thus, it appeared that stimulation by PGE2 activates the ERK MAP kinase pathway in NHNE cells. Because PGE2 significantly increased both *MUC8* gene expression and ERK MAP kinase activity, we investigated whether PGE2-induced *MUC8* gene expression involves the ERK MAP kinase pathway. As a next step, 20 μM PD98059, specific MAPK/ERK kinase 1/2 inhibitor, was applied before PGE2 treatment. The pretreatment of NHNE cells with PD98059 for 1 h clearly inhibited ERK MAP kinase and significantly suppressed PGE2-induced *MUC8* gene expression in NHNE cells (Fig. 2*B*). When the same experiments (Fig. 1 and Fig. 2, *A* and *B*) were performed using NCI-H292 cells (a human lung mucoepidermoid carcinoma cell line), the results obtained were the same as for NHNE cells (data not shown). To further confirm that ERK MAP kinase is involved in PGE2-induced *MUC8* gene expression, cells were transiently transfected with a DNA construct encoding MEK1 dominant negative (*pcDNA5-MEK1DN*), and the overexpression of MEK1 DN was found to suppress PGE2-induced ERK MAP kinase activity. Moreover, PGE2-induced *MUC8* gene expression was significantly and consistently suppressed by MEK1 DN (Fig. 2*C*). These results show that the activation of ERK MAP kinase via MEK1 is essential for PGE2-induced *MUC8* gene expression in NCI-H292 cells.

Effect of RSK1 on PGE2-induced MUC8 Gene Expression—To identify the molecules involved in the downstream signaling of ERK MAP kinase in PGE2-induced *MUC8* gene expression, we investigated RSK1 and MSK1. RSK1 has been reported to be activated by ERK MAP kinase in IL-1β-induced *MUC8* gene expression (27). In this study, the overexpression of MEK1 DN was found to suppress PGE2-induced RSK1 activity (Fig. 2*C*). This result shows that PGE2-induced RSK1 activation is via MEK1/ERK MAP kinase. And in NHNE cells, MSK1 was found to be activated by ERK MAP kinase in IL-1β-induced *MUC5AC* gene expression (28). However, MSK1 did not affect PGE2-induced *MUC8* gene expression (data not shown). The phosphorylation of RSK1 by PGE2 peaked at 5 min and then decreased at 20 min (Fig. 3*A*). To determine whether RSK1 plays an important role in PGE2-induced *MUC8* gene expression, we performed an RSK1 mutant study; RSK1 is a candidate mediator of cytokine-induced CREB phosphorylation at Ser-133. Overexpression of RSK1 DN (D205N, a dominant negative construct encoding RSK1 protein with an Asp-205 phosphorylation site mutated to Asn-205) suppressed PGE2-induced CREB and RSK1 phosphorylation (Fig. 3*B*), and PGE2-induced *MUC8* gene expression was significantly suppressed by RSK1 DN. These results show that RSK1 is required for PGE2-induced *MUC8* gene expression.

Effect of CREB on PGE2-induced MUC8 Gene Expression—To determine whether CREB plays a role in *MUC8* gene expression, we performed Western blot analysis using anti phospho-CREB antibody. The phosphorylation of CREB by PGE2 peaked at 10 min and then decreased at 60 min (Fig. 4*A*). Furthermore, PGE2-induced *MUC8* gene expression was significantly suppressed in cells transfected with plasmid encod-

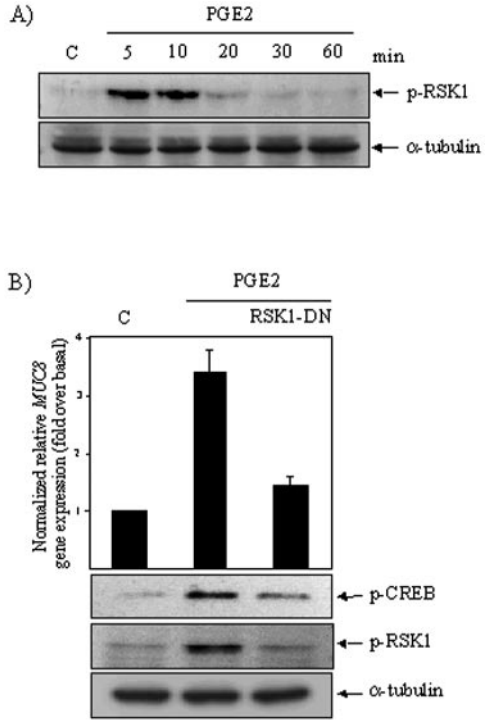

FIG. 3. **Effect of RSK1 on PGE2-induced *MUC8* gene expression.** Confluent cells were stimulated for the indicated times with PGE2, and then total proteins were collected for Western blot (*A*). The cells were transiently transfected with RSK1-dominant negative (RSK1-DN) construct and stimulated with PGE2 for 24 h prior to real-time quantitative PCR. The figures shown are representative of three independent experiments. Transfected cells were stimulated with PGE2 for 10 min prior to Western blot analysis (*B*).

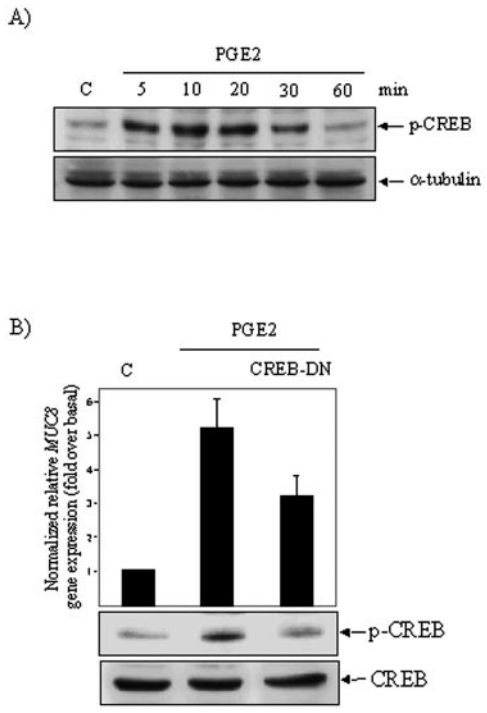

FIG. 4. **Effect of CREB on PGE2-induced *MUC8* gene expression.** Confluent cells were stimulated for the indicated times with PGE2, and then total proteins were collected for Western blot (*A*). The cells were transiently transfected with CREB-dominant negative (CREB S133A) construct and stimulated with PGE2 for 24 h prior to real-time quantitative PCR. The figures shown are representative of three independent experiments. Transfected cells were stimulated with PGE2 for 10 min prior to Western blot analysis (*B*).

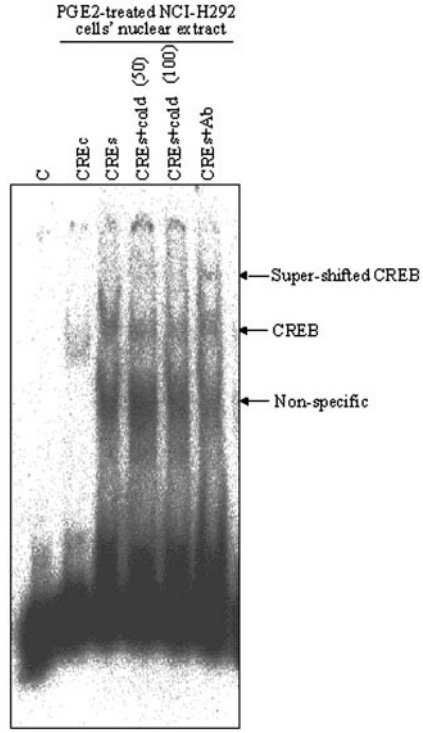

FIG. 5. **Identification of the CREB·CRE binding complex formed in response to PGE2.** Confluent cells were stimulated for 1 h with PGE2. Nuclear protein extract from PGE2-treated NCI-H292 cells was subjected to electromobility shift assay. Nuclear proteins were incubated with CREc, CREs, 50- and 100-fold excesses of cold probe, or anti-phospho-CREB antibody before electromobility shift assay. The labeled nuclear proteins were separated by electrophoresis on 6% polyacrylamide gels, and the gels were dried and exposed to autoradiography at −70 °C.

ing CREB DN (S133A, a dominant negative construct encoding CREB protein with its Ser-133 phosphorylation site mutated to Ala-133 (Fig. 4*B*). These findings suggest that the activation of CREB is essential for PGE2-induced *MUC8* gene expression via ERK MAP kinase and RSK1.

Identification of the CREB·CRE Binding Complex Formed in Response to PGE2—In a previous study, we cloned *MUC8* promoter (encompassing −1644 to + 87) to pGL3-basic,[1] a luciferase reporter vector. The CRE site is located at −803 of the *MUC8* promoter. To analyze the DNA binding activity of PGE2-activated CREB, we performed an electromobility shift assay using the nuclear extracts of PGE2 treated NCI-H292 cells. As shown in Fig. 5, the activities of consensus CRE (CREc) and *MUC8* specific CRE (CREs) oligonucleotides remarkably increased in response to PGE2. To identify specific CRE binding complexes, competition and supershift analysis were performed using 50-, and 100-fold excesses of non-radiolabeled (cold) CREc oligonucleotide and anti-phospho-CREB antibody, respectively. The specific band was found to be selectively inhibited by a consensus CRE competitor and to be supershifted by anti-phospho-CREB antibody (Fig. 5). These results indicate that activated CREB binds to a *cis*-acting element, CRE, in the *MUC8* promoter.

CRE Is Required for PGE2-induced MUC8 Transcription—Various deletion clones such as −1190 to +87, −973 to +87, and −549 to +87 were constructed based on the above −1644 to +87 clone.[1] NCI-H292 cells were then transiently transfected with the various deletion mutants and treated with PGE2 (10 nM) for 24 h, respectively. As shown in Fig. 6*A*, PGE2 selectively increased the luciferase activity of the −973 to −549 region of the *MUC8* promoter. However, its effect was reduced on fragments covering the −549 to +87 region, indicating that

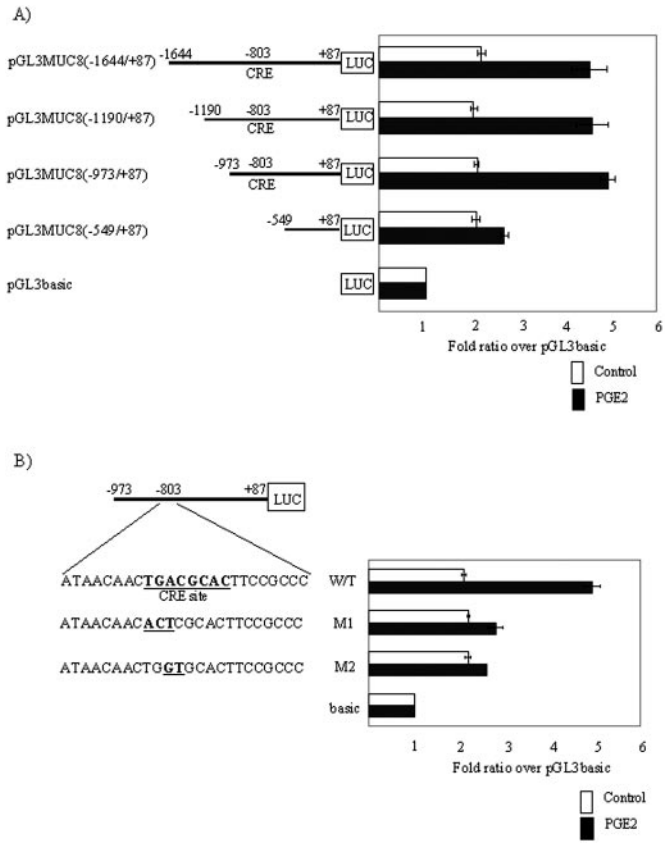

FIG. 6. **CRE is required for PGE2-induced *MUC8* transcription.** NCI-H292 cells were transiently transfected with various *MUC8* promoter luciferase reporter constructs and stimulated with PGE2 for 24 h. Luciferase activity was then assessed in PGE2-treated and -untreated cells (*A*). Cells were transfected with the *MUC8* promoter constructs containing mutated CRE sites as indicated (*B*). The luciferase activities were displayed after correction for transfection efficiency using the β-galactosidase activity of the cell lysates to standardize the values. The values shown are means ± standard deviations of experiments performed in triplicate.

the −1644 to −973 region of *MUC8* promoter may be necessary for response to PGE2. We examined whether CRE is required for PGE2-induced *MUC8* transcription by selectively mutating the CREB-binding site, CRE, at −803. As a shown in Fig. 6*B*, mutant constructs of CRE site in *MUC8* promoter, namely CREMI and CREM2, reduced responsiveness relative to the wild-type *MUC8* promoter construct. These results show that CRE in the regulatory region of the *MUC8* promoter is critical for the up-regulation of *MUC8* transcriptional activity by PGE2.

DISCUSSION

Mucus hypersecretion is a common feature of various inflammatory airway diseases. Accordingly, an understanding of the expression and regulation of airway mucin genes is of considerable importance. Previous studies have suggested that *MUC8* is a major mucin gene because inflammatory mediators up-regulate *MUC8* gene expression in *in vitro* culture (19), and *MUC8* gene expression is up-regulated in human nasal polyp epithelium stimulated by various inflammatory mediators (18).

However, the molecular mechanism of *MUC8* gene expression up-regulation by inflammatory mediators remains poorly understood. Recently, we described the signal transduction pathway by which IL-1β induces *MUC8* gene expression (27), and in the present study, we investigated the mechanism of *MUC8* gene expression up-regulation by PGE2 in normal human nasal epithelial cells. Our results show that only ERK MAP kinase activation is required for PGE2-induced *MUC8* gene expression (Fig. 2*A*), although several studies (29–31) have suggested that more than one MAP kinase is necessary for the signal transduction of various inflammatory mediators. Moreover, the activation of ERK MAP kinase by various stimulants mainly occurs through MEK1. Thus, in this study we investigated whether ERK MAP kinase activation by PGE2 is MEK1-dependent or -independent. The overexpression of MEK1 mutant significantly reduced PGE2-induced ERK MAP kinase phosphorylation and *MUC8* gene expression (Fig. 2*B*), showing that the ERK MAP kinase activation required to induce *MUC8* gene expression in human airway epithelial cells occurs via an MEK1-dependent pathway. However, the dominant negative effect of MEK1 did not completely block the *MUC8* gene expression as compared with control. This result can be explained by two possibilities. One is the low transfection rate and the other is that PGE2 stimulation may affect *MUC8* gene expression not only through the MAP kinase pathway but also through other pathways, such as G-protein-activated pathway (32) and phospholipase C pathway (33).

To date, the signal molecules involved in the downstream signaling of ERK MAP kinase for PGE2-induced *MUC8* gene expression have not been identified. However, recently it was reported that IL-1β-induced *MUC8* gene expression is mediated by sequential ERK MAPK/RSK1/CREB activation in airway epithelial cells (27). In the present study, we found that RSK1 and CREB are also important downstream molecules of ERK MAP kinase activation in PGE2-induced *MUC8* gene expression (Figs. 3 and 4). MSK1 and RSK1 are known substrates of CREB in NHNE cells (27, 28). However, MSK1 did not affect PGE2-induced *MUC8* gene expression (data not shown). On the other hand, RSK1 phosphorylates several transcription factors, *e.g.* CREB (34), c-Fos (35), CCAAT/enhancer binding protein (36), nuclear factor-κB (37), and the estrogen receptor (38), and interacts with transcriptional coactivator CREB-binding protein (also known as p300) (39). Moreover, PGE2 receptors, such as EP1–4, are coupled to cAMP up-regulation (40), which implies CREB activation. Also, CREB is a known potent regulator of the expression of mucin genes (*MUC2*, *MUC5AC*, *MUC5B*, and *MUC6*) in the p15 arm of chromosome 11 (11p15) (41). In a previous study, we reported the possible involvement of CREB in IL-1β-induced *MUC8* gene expression (27), but at that time because the *MUC8* promoter sequence was not known we could not determine whether CREB binds to a *MUC8* specific promoter. However, we already cloned the *MUC8* promoter region.[1] In the present study, our results show that the −973/−549 region of the *MUC8* promoter is involved in response to PGE2 and that CRE in the −803 region of the *MUC8* promoter is important for *MUC8* gene up-regulation by PGE2. Gerritsen *et al.* (42) reported that p300 and CREB-binding protein act as co-activators of p65 transactivation and may play an important role in the cytokine-induced expression of various immune and inflammation genes. These findings suggest that CREB may interact directly or indirectly with other transcription factor(s) and that non-DNA binding transcriptional co-activators, such as p300 and CREB-binding protein, which were believed to function as bridging proteins between DNA-binding transcription factors and basal transcription factors, play a role as integrators of diverse signaling pathways leading to *MUC8* gene expression.

Thus, to induce *MUC8* gene expression, IL-1β transduces through the IL-1β receptor/Ras/Raf/ERK/RSK1CREB cascade pathway (27), and PGE2 tranduces through the EP1–4/MEK1/ERK/RSK1/CREB cascade pathway. Although these two substances stimulate their own membrane receptors, they seem to

share common signaling molecules downstream of ERK MAP kinase.

Shimamoto *et al.* (21) reported that the Ca^{2+}-regulation of exocytic events and PGE2 release are activated in acetylcholine-stimulated antral mucous cells and that the PGE2 released induces cAMP accumulation, which enhances Ca^{2+}-regulated exocytosis. Gray *et al.* (23) reported that the induction of *MUC5AC* gene by IL-1β involves COX2-generated PGE2. From these reports, we cannot exclude the possibility that IL-1β stimulates PGE2 secretion and that secreted PGE2 induces *MUC8* gene expression in an autocrine manner.

In summary, our results demonstrate that ERK MAP kinase is essential for PGE2-induced *MUC8* gene expression and that the activations of RSK1 and CREB are required for the intracellular mechanisms that mediate *MUC8* gene expression. This study also demonstrated that CRE in the *MUC8* promoter may play a role in these processes by binding CREB. Further analysis of the signal pathways activated by various stimulators may yield deeper insights into the signaling mechanism of *MUC8* gene expression.

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Prostaglandin E_2 Induces *MUC8* Gene Expression via a Mechanism Involving ERK MAPK/RSK1/cAMP Response Element Binding Protein Activation in Human Airway Epithelial Cells

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