Oxidative Stress Disrupts Glucocorticoid Hormone-dependent Transcription of the Amiloride-sensitive Epithelial Sodium Channel α-Subunit in Lung Epithelial Cells through ERK-dependent and Thioredoxin-sensitive Pathways*

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The amiloride-sensitive epithelial Na⁺ channel (ENaC) plays a critical role in the maintenance of alveolar fluid balance. It is generally accepted that reactive oxygen and nitrogen species can inhibit ENaC activity and aggravate acute lung injury; however, the molecular mechanism for free radical-mediated ENaC inhibition is unclear. Previously, we showed that the expression of the α-subunit of ENaC, α-ENaC, which is indispensable for ENaC activity, is repressed in salivary epithelial cells. Here, we investigated whether exogenous H₂O₂ modulates α-ENaC gene expression in lung epithelial cells through a similar molecular mechanism. Utilizing transient transfection reporter assays and site-directed mutagenesis analyses, we found that the glucocorticoid response element (GRE), located at -1334 to -1306 base pairs of the α-ENaC 5'-flanking region, is the major enhancer for the stimulated α-ENaC expression in A549 lung epithelial cells. We further demonstrate that the presence of an intact GRE is necessary and sufficient for oxidants to repress α-ENaC expression. Consistent with our hypothesis, exogenous H₂O₂-mediated repression of α-ENaC GRE activity is partially blocked by either a specific inhibitor for extracellular signal-regulated kinase (ERK) pathway activation, U0126, or dominant negative ERK, suggesting that, in part, activated ERK may mediate the repressive effects of H₂O₂ on α-ENaC expression. In addition, overexpression of thioredoxin restored glucocorticoid receptor action on the α-ENaC GRE in the presence of exogenous H₂O₂. Taken together, we hypothesize that oxidative stress impairs Na⁺ transport activity by inhibiting dexamethasone-dependent α-ENaC GRE activation via both ERK-dependent and thioredoxin-sensitive pathways. These results suggest a putative mechanism whereby cellular redox potentials modulate the glucocorticoid receptor/dexamethasone effect on α-ENaC expression in lung and other tight epithelia.

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1 The abbreviations used are: AP-1, activator protein-1; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; ENaC, epithelial sodium channel; ARDS, acute respiratory distress syndrome; Dex, dexamethasone; GR, glucocorticoid receptor; GRE, glucocorticoid response element; bp, base pairs; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; MEKK, MEK kinase; TRX, thioredoxin; wt, wild type.
MAP kinase activation is cell type- and/or stimulus-specific. It is therefore important to define, for a given cell type, the specific pathway(s) that links external stimuli to the target genes that promote changes in cell phenotype.

The amiloride-sensitive epithelial sodium channel, ENaC, is localized to the apical surface of salt transporting epithelia, notably the collecting duct of the kidney, salivary glands, colon, and respiratory tract. In the lung, ENaC activity is essential in controlling the amount of liquid within the air space. Both pharmacological and genetic studies have shown that at birth ENaC function is critical for clearing lung fluid (15, 16). However, there are certain conditions where pulmonary fluid clearance appears impaired. For example, acute respiratory distress syndrome (ARDS) is characterized by the presence of refractory hypoxemia, reduced lung compliance, increased permeability pulmonary edema, and diffuse alveolar infiltration on chest radiographs. Pathologically, ARDS develops as a result of alveolar-capillary membrane disruption with leakage of protein-rich exudate and migration of inflammatory cells (neutrophils and macrophages) into the air space (17). Neutrophils within the pulmonary circulation are activated, causing the release of free radicals and reactive oxygen species, which are increasingly regarded as the key mediators of pulmonary tissue damage (18). The specific effect(s) elicited by reactive oxygen and/or nitrogen species on lung epithelial cells during injury is currently under intense investigation.

Recently, it has been demonstrated that nitric oxide inhibits ENaC activity in distal lung epithelial cells (19). Yet, to date, the molecular mechanism underlying oxidative stress-induced ENaC inhibition in lung epithelia is unclear. Because ENaC activity is dependent upon its α-subunit expression (see Ref. 20 for a review), we decided to use exogenous H₂O₂ treatment in A549 cells as an experimental paradigm to investigate whether or not oxidative stress represses the sodium channel α-subunit gene, α-ENaC expression. Previously, our laboratory has utilized salivary epithelial cells as a model system to elucidate the molecular mechanism responsible for dexamethasone (Dex)-dependent and Ras-repressible α-ENaC expression (21, 22). We reported that Ras activation leads to the stimulation of the ERK effector pathway and consequently to the inhibition of glucocorticoid receptor (GR)/Dex-stimulated α-ENaC transcription in parotid epithelial cells. A bonafide glucocorticoid response element (GRE) has been identified by us within the −1334 to −1306 bp of rat α-ENaC promoter/enhancer region and has been shown to be critical for glucocorticoid induction in salivary epithelial cells (21). This Dex-dependent regulatory motif is also conserved in the human α-ENaC 5′-flanking region (23). However, whether or not the cross-talk between Ras- and GR-mediated pathways, governing overall transcriptional regulation of the α-ENaC gene, also exists in nonsalivary cells, as lung epithelial cells, has yet to be demonstrated. In addition, elucidating the specific pathways involved in the mediation of α-ENaC gene repression by oxidative stress may lead to the development of strategies that prevent and/or treat acute lung or oxidative injury in general.

The present study was undertaken to unravel the role of oxidative stress- and Ras-mediated signaling pathways in modulating GRE-dependent α-ENaC expression in lung epithelial cells. Data reported herein demonstrate for the first time that both exogenous H₂O₂-mediated oxidative stress and Ras activation suppress Dex-dependent α-ENaC transcriptional activation in lung A549 cells. This antagonistic cross-talk between H₂O₂- and Ras-mediated pathways and the glucocorticoid hormone pathway occurs via the previously identified α-ENaC GRE in its homologous promoter or a heterologous promoter context via overlapping as well as nonoverlapping mechanisms. We further establish that ERK, but not MEKK1/JNK, is the major MAP kinase-signaling pathway utilized by exogenous H₂O₂ to repress Dex-mediated α-ENaC induction. Moreover, we also report that an anti-oxidant system, thioredoxin (TRX), is involved in regulating GR/Dex-dependent α-ENaC transcription. Together, these data suggest that both ERK-dependent and TRX-sensitive pathways play a critical role in allowing exogenous and endogenous oxidative stress to attenuate the GR/Dex effect on α-ENaC expression in lung epithelial cells and possibly in other tight epithelia.

MATERIALS AND METHODS

Reagents—Dex and H₂O₂ were obtained from Sigma. Dex was resuspended to 1 mM in ethanol and stored at −80 °C. U0126 (Promega), prepared as a 1 mM stock solution in dimethyl sulfoxide (Me₂SO) and stored at −20 °C.

Cell Culture and DNA Constructs—The human lung carcinoma A549 cells (24) were purchased from American Type Culture Collection. These cells were maintained in Ham’s F12 (Kaighn’s modification, pH 7.4, Sigma) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Life Technologies, Inc.), 100 units/ml penicillin G and 100 µg/ml streptomycin.

Reporter constructs—1.4α-ENaC/Luc and GREmt1.4α-ENaC/Luc were engineered from α-ENaC/CATs (wt) and its GRE mutant (GREmt) (21), respectively. Individual 1.3-kilobase Sau3AI (vector)-DNA fragments (−1413 to −154 bp) were excised from wt and GREmt α-ENaC/CATs and ligated into SacI/NheI-digested promoterless pGL2-Basic reporter plasmid to generate 1.4α-ENaC/Luc and GREmt1.4α-ENaC/Luc, respectively. A reporter construct, p1334−1306GL2-P, containing one copy of α-ENaC GRE was generated as described previously (21). In brief, a pair of complementary and 5′-phosphorylated oligomers, which correspond to −1334 to −1306 bp of α-ENaC, were annealed by incubating 200 pmol of each oligomer in a buffer containing 50 mM Tris-HCl (pH 7.6) and 10 mM MgCl₂, followed by heating to 85 °C for 2 min and stepwise cooling at 65 °C (15 min), 37 °C (15 min), 25 °C (15 min), and 4 °C (15 min). These annealed oligos, which have a built-in 5′-phosphorylated XhoI overhang, were ligated to XhoI-linearized pGL2-Reporter plasmid DNA (Promega). The pcDNA-TRX expression construct was engineered by inserting an EcoRI-digested MTS87 insert into the EcoRI cloning site of pcDNA3.1 (Invitrogen). MTS87 was cloned from a full-length (550 bp) monkey TRX cDNA (25). The sequence of all plasmid constructs was confirmed by DNA sequence analyses. A constitutive gene expression construct was a generous gift from Dr. D. Johnson (University of Southern California), whereas MEKK1, JNK, β-actin/Luc and −73 Collagenase/Luc expression constructs were kindly provided by Dr. E. Zendi (University of Southern California).

Transient and Stable Transfection—Plasmid DNAs were transiently transfected into A549 or TRX-A549 cells (see below) using SuperFect™ (Qiagen) per the manufacturer’s instructions. In all experiments, 0.1 µg of luciferase reporter plasmid, pGL-TK (Promega), was co-transfected with pCMVβ-gal to ensure transfection efficiency. The total amount of DNA in each transfection of cells grown in a 35-mm dish was kept constant at 2 µg by supplementing with pCMV vector. Cells were serum-deprived for 8 h by replacing growth medium with a medium containing 0.05% stripped serum at 24 h after the start of transfection. For H₂O₂ treatment, the culture medium was removed, and cells were washed with 3 ml of prewarmed phosphate-buffered Kreb’s Ringer solution, which contains 1.0 mM MgSO₄, 1.3 mM CaCl₂, 10 mM HEPES, 5 mM glucose, 144 mM NaCl, 5 mM KCl, 8.5 mM NaH₂PO₄, and 1.4 mM NaHCO₃, pH 7.4, prior to incubating cells with various concentrations of H₂O₂ in phosphate-buffered Kreb’s Ringer solution for 20 min. These cells were washed once with regular culture medium and incubated with a medium containing 0.05% stripped serum followed by cell harvesting 16 h thereafter. The firefly- and Renilla-luciferase activities were measured using the Dual Luciferase™ Reporter Assay Kit (Promega) following the manufacturer’s instruction. All transient transfection assays were carried out in duplicate at least three times using two separate preparations of plasmid DNA.

The stably transfected TRX-overexpressing A549 cells were established by transfecting A549 cells with pcDNA-TRX expression construct using the DEAE-dextran-mediated method (Promega) according to the manufacturer’s instruction. After selection with Geneticin (G418, 1200 µg/ml), ten resistant clones were isolated, expanded, and screened for their TRX expression level by Western blot analyses. Among 10 clones, clone 2 exhibited the highest TRX expression level and was used in this study; it is hereafter referred to as TRXs-A549.

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Northern Blot Analysis—Total RNA was isolated from A549 cells using TRizol® Reagent (Life Technologies, Inc.) as instructed by the manufacturer. The quality and quantity of RNA were determined by both spectrophotometric analysis and fractionation of RNA on a 1.3% agarose gel in the presence of 2.2% formaldehyde followed by staining with ethidium bromide to compare the ratio of 28 S to 18 S ribosomal RNAs. For Northern blot analyses, equal amounts of RNA (18 µg each) were loaded onto an agarose/formaldehyde gel, fractionated by size, transferred to a 0.2-µm nylon membrane (ICN Biomedicals, Inc.), and UV cross-linked. All blots were prehybridized for 1 h in QuickHyb® (Stratagene®). The hybridization was carried out according to the manufacturer’s instruction. [32P]-labeled α-ENaC probes were prepared from an isolated 5′-ENaC cDNA fragment (bases 1–905) using a Random Primed DNA Labeling Kit (Boehringer Mannheim). After hybridization, blots were washed three times at 60 °C for 2 h each in a 0.1× SSC (0.15 M NaCl and 0.015 M sodium citrate) solution containing 0.5% SDS. All blots were reprobed with a human β-actin probe to ensure that the quality and quantity of RNA among different samples were comparable. The radioactive signal was visualized by autoradiography where blots were exposed to BioMax™MS films at −80 °C overnight, using BioMax™ TranScreen-HE (Eastman Kodak Company) to improve sensitivity. Blots were also quantitated through the use of electronic autoradiography with an Instantimager 228 (Packard Instrument Co). The amount of α-ENaC message was quantitatively compared between lanes normalizing against the corresponding β-actin level.

Western Blot Analysis—Equal amounts of soluble proteins from A549 or TRXaA549 whole cell lysates were subjected to a 10 or 15% SDS-polyacrylamide gel electrophoresis. After electrophoresis, proteins were electroblotted onto Immobilon™-P membrane (Millipore) and incubated with the respective primary antibody including anti-ERK1 (C-20, Santa Cruz Biotechnology, Inc.), anti-Active™ mitogen-activated protein kinase (Promega), anti-phospho-stress-activated protein kinase/JNK (New England BioLabs), anti-phospho-p38 MAPK (New England Biolabs) or anti-TRX. The polyclonal anti-TRX antibody was generated by immunizing rabbits with a synthetic N-terminal 19-mer peptide (K’QIESKAQFELADDAGDK)® from monkey TRX® (25). Horseradish peroxidase-conjugated goat anti-rabbit IgG (Amersham Pharmacia Biotech) was used to detect the bound primary antibody. Immune complexes were visualized using an enhanced chemiluminescence (ECL) detection kit (Amersham Pharmacia Biotech) following the manufacturer’s instruction.

RESULTS

Endogenous α-ENaC Expression Is Up-Regulated by Dexamethasone Treatment in Lung Epithelial A549 Cells—The glucocorticoid hormone, Dexamethasone, has been demonstrated to increase fetal and postnatal lung α-ENaC mRNA levels in rat (26). Moreover, Dexamethasone treatment enhances short circuit current, an index for active Na⁺ absorption, across primary cultured rat alveolar epithelial cell monolayers. To determine whether or not glucocorticoids increase α-ENaC expression in A549 cells and could therefore be used as a model to explore the molecular mechanism by which glucocorticoids modulate ENaC activity in lung epithelial cells, Northern blot analyses were performed on total RNA taken from A549 cells treated with 100 nM Dex for either 4 or 24 h (Fig. 1). A single band corresponding to the reported size of the α-ENaC message (~3700 bases) (27) was detected. Dex treatment induced the expression of α-ENaC within 4 h, suggesting that DEX-enhanced α-ENaC expression, in part, accounts for the increase in amiloride-sensitive Na⁺ transport. This suggests that glucocorticoid hormone may not only induce α-ENaC expression during lung development but may also modulate α-ENaC expression in adult human lung epithelial cells as well (26).

To determine whether glucocorticoids up-regulate α-ENaC at the transcriptional level in A549 cells, we subcloned the 1.3-kilobase DNA 5′-flanking region, −1413 to −154 bp (relative to the translation initiation ATG (21)), of rat α-ENaC into a promoterless reporter plasmid, pGL2-basic, to create −1.4α-ENaC/Luc (Fig. 2A). These constructs were used based on our previous observation that the GRE located between nucleotide −1334 to −1306 of the rat α-ENaC 5′-flanking region plays an essential role in dictating the overall α-ENaC expression level in salivary cells (21). To ascertain the contribution of the identified α-ENaC GRE in modulating −1.4α-ENaC/Luc activity in A549 cells, we mutated the GRE within the context of the full-length −α-ENaC/reporter to create GREmt−1.4α-ENaC/Luc (Fig. 2A). The wild type −1.4α-ENaC/Luc and GREmt−1.4α-ENaC/Luc reporter constructs were transiently transfected in A549 cells and the associated luciferase activities were assayed. As shown in Fig. 2B, 100 nM Dexamethasone treatment alone was able to stimulate luciferase activities of the wild type −1.4α-ENaC/Luc by 10-fold (lane 1 versus 2), suggesting that A549 cells harbor an endogenously functional GR that up-regulates α-ENaC expression at the transcriptional level. Point mutations within the α-ENaC GRE abolished the enhancement observed in the presence of DEX (Fig. 2B, lane 6 versus 5), confirming that the GRE, located between −1334 and −1306 bp of α-ENaC 5′-flanking region, is critical for the DEX-stimulated α-ENaC gene expression in A549 cells. Taken together, these data indicate that the previously identified GRE is the major transcriptional enhancer for modulating α-ENaC expression by glucocorticoid hormone in human lung epithelial cells.

GR/Dex-mediated Stimulation of α-ENaC GRE Is Attenuated by Either Ras Pathway Activation or Exogenous H₂O₂ Treatment—To ascertain that the cross-talk between Ras- and GR/Dex-mediated signaling pathways that we recently reported for salivary epithelial Pa-4 cells (21, 22) also dictate overall α-ENaC expression levels in nonsalivary cells, we assessed the effect of Ras on α-ENaC promoter/enhancer activity using transient transfection and reporter assays in lung A549 cells. As illustrated in Fig. 2B, the constitutively active Ras V12 markedly repressed −1.4α-ENaC/Luc activity when co-transfected into DEX-treated A549 cells (lane 2 versus 4). However, Ras V12 alone elicited a modest inhibitory effect on the transfected reporter activities (lane 3 versus 1). Although the point mutations within the identified GRE did not reduce the basal reporter expression (Fig. 2B, lane 5 versus 1), they substantially abrogated Dex-mediated induction (Fig. 2B, lane 6 versus 2). The Ras-mediated suppression of Dex-enhanced −1.4α-ENaC/Luc reporter activities (Fig. 2B, lanes 2 and 4) was also abolished by the α-ENaC GRE mutation in A549 cells (Fig. 2B, lanes 6 and 8). This repression is reminiscent of our
previous identification of Ras activation as a negative mediator of Dex-stimulated α-ENaC gene transcription in salivary cells (21), which appears to be also retained in lung epithelial cells (Fig. 2B). We next examined the effect on α-ENaC expression of exogenous H$_2$O$_2$, an agent that has been reported to interfere with sodium homeostasis in the alveolar epithelium (28) and implicated in acute lung injury (28). The addition of exogenous H$_2$O$_2$, up to 1 mM, to culture medium of A549 cells did not cause obvious cytotoxicity; nor did it appear to have an adverse effect on transcription in general, β-actin (Fig. 2C, lane 17 versus lanes 18–21) or p65 expression (data not shown). H$_2$O$_2$ evoked no repression of the unstimulated −1.4α-ENaC/Luc activity (Fig. 2C, lanes 1–5), whereas the Dex-stimulated luciferase activity from the −1.4α-ENaC/Luc reporter was considerably lower in 100 μM H$_2$O$_2$-treated cells than that of control A549 cells (Fig. 2C, lane 7 versus 6). H$_2$O$_2$ inhibited the Dex-stimulated −1.4α-ENaC/Luc activity with an apparent IC$_{50}$ of approximately 100 μM (Fig. 2C, lanes 6–10). The residual reporter activity from GREmt−1.4α-ENaC/Luc was not affected by exogenous H$_2$O$_2$ treatment (Fig. 2C, lanes 12–16), reinforcing that α-ENaC repression was not a result of nonspecific cell damage by H$_2$O$_2$ treatment. Given that the mutation of the α-ENaC GRE abolished HO$_2$-mediated repression (Fig. 2C, lanes 12–16) and that exogenous H$_2$O$_2$ had no inhibitory effect on basal activity (Fig. 2C, lanes 1–5), we conclude that exogenous H$_2$O$_2$ preferentially suppresses stimulated α-ENaC expression via the identified α-ENaC GRE. Together, these data indicate that the presence of an intact GRE in the α-ENaC 5′-flanking region is necessary for both treatment with exogenous H$_2$O$_2$ and Ras activation to evoke their inhibitory effect on α-ENaC expression in lung epithelial cells.

**Redox-sensitive Mechanism Inhibits GR/Dex Effect on α-ENaC GRE**—Based on our data in Fig. 2C, we hypothesized that oxidative stress by H$_2$O$_2$ attenuates α-ENaC expression via repressing the GR/Dex effect on the activity of the identified α-ENaC GRE. To further test this hypothesis, we transiently transfected A549 cells with a reporter construct harboring the identified α-ENaC GRE coupled to a SV40 minimal promoter-driven luciferase reporter, p(-1334/-1306)GL2-P (Fig. 2D). After transfection, these cells were maintained in the absence or presence of various concentrations of H$_2$O$_2$ for 20 min and then incubated with 100 nM Dex for 16 h prior to harvesting. This reporter system provides a sensitive and direct measurement of the effect of exogenous H$_2$O$_2$ on α-ENaC GRE function.
As shown in Fig. 2D, treatment with 100 nM Dex, in the absence of exogenous H₂O₂, enhanced the reporter activity in transfected A549 cells by greater than 20-fold (lane 6 versus 1). This Dex-mediated enhancement was consistent with, but higher than, that observed in -1.4a-ENaC/Luc-transfected cells (Fig. 2, B and C). As shown in Fig. 2C, the GR/Dex-independent gene expression is insensitive to exogenous H₂O₂ treatment. In contrast, treatment with H₂O₂ for 20 min inhibited the Dex-induced response by 30–80% in a concentration-dependent manner with 100 μM to 1 mM H₂O₂, respectively, in A549 cells (Fig. 2D, lanes 6–10), confirming that the diminished Dex stimulation of -1.4a-ENaC/Luc expression results from the abrogation of the GR/Dex effect on the α-ENaC GRE. The extent of inhibition by 100 μM H₂O₂ was greater in -1.4α-ENaC/Luc-transfected cells than that in p(-1334/-1306)/GL2-P transfected cells. One possible explanation for this phenomenon is that the identified GRE located at -1334 to -1306 bp of α-ENaC 5'-flanking region may not be the only target affected by H₂O₂ treatment. Activated Ras significantly antagonized Dex-stimulated luciferase activity in A549 cells (Fig. 2D, lane 12 versus 6), implying that the repression of GR/Dex-mediated transactivation of the α-ENaC GRE by Ras pathway activation may be common in many types of epithelial cells. In support of this, Ras has been proposed as a potential target of reactive oxygen species (29), suggesting that the H₂O₂/Ras downstream effector(s) cascade may also be involved in modulating α-ENaC expression in A549 cells.

In the absence of Dex, luciferase activity from the minimal SV40 promoter was not influenced by H₂O₂ treatment (Fig. 2D, lanes 1–5). Furthermore, the combination of Dex and H₂O₂ treatment had no effect on the activity from the cotransfected indicator plasmid, pRL-TK, whereas mutation on the GRE in p(-1334/-1306)/GL2-P almost abolished the attenuation of Dex-stimulated p(-1334/-1306)/GL2-P activities by H₂O₂ and Ras (data not shown). Conceivably, the oxidative stress caused by exogenous H₂O₂ selectively repressed GR/Dex-inducible gene expression by inhibiting GRE action. Results from transfection assays using reporter constructs harboring either a homologous α-ENaC promoter or a heterologous SV40 promoter (Fig. 2, B and C) demonstrate that the presence of an intact GRE is necessary and sufficient for exogenous H₂O₂ and Ras activation to repress α-ENaC expression in lung epithelial cells. Because treatment of A549 cells with H₂O₂ elicited no effect on the level of total cellular GR protein by Western analysis (data not shown), depletion of total GR content is not likely to be the cause of the observed H₂O₂-induced reduction in Dex responsiveness. In line with this reasoning, we further examined the molecular mechanism underlying H₂O₂-mediated repression of GR/Dex transactivation activity.

**Differential Activation of ERK and JNK Pathways by Exogenous H₂O₂ Treatment in A549 Cells**—Because H₂O₂ treatment induces MAP kinase activity in several cell lines, such as NIH 3T3 and PC12 cells (30), we investigated whether exogenous H₂O₂ could activate ERK, JNK, or a p38 kinase in A549 cells. Activation of these MAP kinases requires phosphorylation of specific tyrosine and threonine residues on ERK1/ERK2, JNK, and p38. Because sublethal 200 μM exogenous H₂O₂ is able to elicit a substantial inhibitory effect on α-ENaC GRE function without obvious cytotoxic damage (Fig. 2, C and D), we assessed the tyrosine/threonine phosphorylation of these MAP kinases in A549 cells exposed to 200 μM exogenous H₂O₂ over time, performing Western blots of whole cell soluble lysates with either phospho-p44/p42, phospho-JNK, or phospho-p38 antibodies (Fig. 3). We found that exogenous H₂O₂ treatment activated both JNK and ERK MAP kinases with different kinetics of ERK and JNK activation. As shown in Fig. 3, the induction of ERK activation was transient with an increase in ERK phosphorylation within 15 min followed by a decline to the basal level after 2 h. The activation of JNK was delayed, starting between 30 min and 1 h after H₂O₂ treatment, which further increased up to 2 h post-treatment.

In contrast to the results obtained with ERK and JNK, we did not detect any H₂O₂-induced activation of p38 kinase, by immunoblotting with the anti-active p38 antibody, for up to 2 h after treatment (Fig. 3). Uniform loading of the cellular proteins onto gels was demonstrated by reprobing the blots with an anti-ERK antibody that recognized both ERK1 and ERK2. There was no change in the amount of either ERK1 or ERK2 over the time period examined, indicating that the observed changes in the signals of activated ERK and JNK resulted from a stimulation of ERK or JNK upstream kinases, respectively. These observations suggest that 200 μM H₂O₂ potently activates both ERK and JNK MAP kinases but not the p38-signaling pathway in A549 cells. Clearly, the increase in ERK activation preceded the JNK activation, although the relationship between these two events is not clear. Taken together, both exogenous H₂O₂ treatment and activation of Ras are capable of repressing the Dex-stimulated luciferase activities of transduced -1.4α-ENaC/Luc (Fig. 2, B and C) and p(-1334/-1306)/GL2-P (Fig. 2D), respectively. Because Ras downstream effectors, ERK and JNK, were stimulated by H₂O₂ treatment in A549 cells (Fig. 3), it is conceivable that the activated ERK and JNK pathways could account for, at least in part, the repression of Dex-dependent α-ENaC expression by exogenous H₂O₂.

**ERK Pathway Inhibitors Attenuate the H₂O₂-mediated Repression of Dex-mediated Transcriptional α-ENaC GRE Activation**—We have recently shown that the activated Ras/Raf/ERK pathway represses the stimulated α-ENaC promoter/enhancer activity and the steady-state α-ENaC message level (21, 22). It is tempting to speculate that exogenous H₂O₂-induced activation of ERK kinase mediates repression of α-ENaC expression in A549 cells. Hence, we determined if U0126, a widely used specific inhibitor of ERK pathway activation, could inhibit H₂O₂-mediated repression through the α-ENaC GRE. U0126 binds to MEK1/MEK2 and blocks its ability to activate ERK. This inhibitor acts as a highly specific noncompetitive inhibitor of MEKs in the ERK pathway (31). It has been reported that U0126 inhibits the MEK1 and MEK2 activities at μM concentration and elicits little effect on other kinases, such as protein kinase C, Abl, Raf, MEKK, ERK, JNK, MKK-3, MKK-4/SEK, MKK-6, cdk2, or cdk4 (31, 32). We pretreated p(-1334/
GL2-P transfected A549 cells were cultured in 0.05% stripped- serum containing medium for 8 h and subsequently treated with 50 μM U0126 (+) (lanes 3–6) or vehicle (−) (lanes 1, 2, and 7–10) for 20 min and then incubated with various concentrations of H2O2 for 20 min prior to treatment with vehicle (−) (lane 1) or 10−7 M Dex (−) (lanes 2–10) overnight. Luciferase assays, normalization, and data analyses were performed as described in Fig. 2B.

−1306/1306GL2-P transfected cells with 50 μM U0126 for 20 min prior to exogenous H2O2 exposure, which led to a reversal of H2O2-mediated repression (Fig. 4, lanes 3–6 versus 7–10). This reversal was most dramatic with either 100 or 200 μM H2O2 (Fig. 4, lanes 7 and 8 versus 3 and 4). A partial (approximately 50%) blocking effect was observed even at 500 and 1000 μM H2O2 concentrations (Fig. 4, lanes 9 and 10 versus 3 and 5 and 6). Consistent with the pharmacological blockade of ERK, A549 cells cotransfected with p−1334/−1306GL2-P and a dominant negative ERK (33) attenuated H2O2-induced repression at concentrations up to 1000 μM (data not shown). Together with our previous report (22), these data provide a direct link between ERK activation and exogenous H2O2-mediated repression, reinforcing that ERK activation is required for oxidative stress to attenuate Dex-inducible α-ENaC expression.

The MEKK1/JNK-signaling Module Has No Inhibitory Effect on GR/Dex-Mediated Activation of the α-ENaC GRE—Because enhanced JNK activity was also detected in A549 cells following H2O2 treatment, we also investigated the role of the JNK pathway in exogenous H2O2-mediated repression of the α-ENaC GRE. To do this, A549 cells were cotransfected with a p−1334/−1306GL2-P reporter construct and a constitutively active form of the JNK activator, MEKK1 (34), JNK alone, or a combination of the two. A MEKK1 dose-dependent activation of AP-1-dependent luciferase activity (Fig. 5, inset) was established to assure the validity of our transfection assay. With two different amounts of MEKK1 and JNK expression constructs, cotransfection of constitutively active MEKK1 and/or JNK exhibited little, if any, inhibitory effect on GR/Dex enhancement over the α-ENaC GRE (Fig. 5). Overexpression of MEKK1 alone appeared to have a very modest stimulatory effect on the Dex-induced luciferase activity. Although this observation was highly reproducible, the nature of this enhancement is still unclear. Together, we conclude that the Raf/ERK but not the MEKK1/JNK-signaling module is the major MAP kinase signaling pathway utilized by exogenous H2O2 to down-regulate the GR/Dex effect on the α-ENaC GRE (Figs. 4 and 5).

Overexpression of TRX Rescues the H2O2-mediated Suppression of Gene Expression Stimulated by Dex—As shown in Fig. 4, the pretreatment of 50 μM U0126 or cotransfection of dominant negative ERK (data not shown) failed to completely restore the stimulatory effect of Dex on the α-ENaC GRE in the presence of higher concentrations of H2O2, suggesting that oxidative stress by H2O2 may also repress the Dex effect via an alternative pathway involving cellular redox mechanisms. Reactive oxygen species, such as H2O2, can be scavenged by TRX, thus protecting cells against injury by H2O2 (35). TRX is also reported to activate various transcription factors, such as nuclear factor-κB and AP-1 (36, 37). Thus, we next studied whether TRX can protect Dex-mediated up-regulation of α-ENaC gene activation against H2O2-induced inhibition, using a stably transfected clone TRXs−A549, which exhibited the highest level of TRX expression (Fig. 6A, lane 3). In contrast to the results shown in Fig. 2, Dex-induced luciferase activity was sustained at levels >20-fold higher than that of the basal level in TRXs−A549 cells in the presence of increasing concentrations of exogenous H2O2 (Fig. 6B, lanes 3, 5, 7, and 9). Notably, the fold induction by Dex in the presence of either 500 or 1000 μM H2O2 in TRXs−A549 cells was at least at a comparable level with that observed in control A549 cells in the absence of H2O2 (Fig. 6B, lanes 7 and 9 versus lane 1). Thus, these data indicate that TRX restored, even in the presence of excess H2O2, GR/Dex-dependent up-regulation of α-ENaC expression. It is of interest to note that TRX was more efficient than an ERK blockade in preventing the negative effect of H2O2 on GRE action, especially when higher concentrations of exogenous H2O2 were administered (Fig. 6B versus Fig. 4). However, in the absence of exogenous H2O2, overexpression of TRX also enhanced Dex-stimulated luciferase activity, suggesting that TRX is capable of protecting GR function perhaps against endogenously generated oxidative stress. This was further confirmed by the observation that the stimulated luciferase activity of −1.4α-ENaC/Luc-transfected TRXs−A549 cells was further enhanced 2-fold over that observed in the parental A549 cells (Fig. 6C, lane 2 versus 3). To test whether TRX augments GR transactivation activity by attenuating the Ras/ERK pathway, we cotransfected −1.4α-
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**Fig. 6.** The effect of TRX overexpression on H$_2$O$_2$- and Ras-mediated α-ENaC gene expression. A, Western blot analysis of cellular lysate taken from TRX stably transfected A549 clones using an anti-TRX antibody. A549 cells were transfected with a pcDNA-TRX expression construct by the DEAE-dextran-mediated method. After selection with Geneticin (G418, 1200 μg/ml), 10 individual clones were randomly selected and expanded. Cellular lysates of parental and clones were prepared. Twenty micrograms of total protein from each sample were fractionated by size using 15% polyacrylamide gels, transferred to Immobilon™-P membranes, and immunoblotted with anti-TRX antibody. A representative Western blot analysis indicating the TRX expression level of the parental A549 cells (P) and 5 TRX-transfected cell lines (S1–S5) is shown in A. Clonal cells (S2) that exhibited the highest TRX expression level were referred to as TRXs-A549 clones and were used in the studies presented in B and C. B, repression of Dex-mediated gene activation by treatment with H$_2$O$_2$ is restored by overexpression of TRX. A549 and TRXs-A549 cells were transiently transfected with 0.9 μg of the pc(-1334/-1306)GL2.P reporter construct. Transient transfections were carried out as described in Fig. 2B. Cells were serum-starved for 8 h and treated with various concentrations of H$_2$O$_2$ or vehicle for 20 min followed by the addition of 10$^{-7}$ M Dex to the culture medium and incubated overnight. The level of induction was calculated as described in Fig. 2D. C, TRX overexpression enhances Dex-stimulated GR transactivation activity without attenuating the Ras/ERK pathway. A549 and TRXs-A549 cells were transfected with 1.2 μg of pc(−144α-ENaC/Luc) in the presence (+) or absence (−) of 0.7 μg of Ras V12 plasmid. The transient transfection was carried out as described in Fig. 2B. Sixteen hours after the start of transfection, cells were serum-starved for 8 h, followed by 10$^{-7}$ M Dex (+) or vehicle (−) to the culture medium and incubated overnight. The level of induction was calculated as described in Fig. 2D.

ENaC/Luc, constitutively activated Ras V12 in TRXs-A549 cells, and measured the luciferase activity in the absence or presence of Dex. As seen in Fig. 6C (lane 3 versus 5), activated Ras V12 efficiently repressed the Dex-stimulated luciferase activity to the same extent in TRXs-A549 cells. Similar results were also observed in different clones of TRXs-A549 cells. It thus appears that overexpression of TRX could potentiate GR function but elicits no modulating effect on Ras-mediated pathways. Moreover, because Ras activation represses Dex-stimulated α-ENaC promoter/enhancer activity in a comparable manner in both parental and TRXs-A549 cells, Ras-mediated repression most likely takes place downstream of the proposed TRX enhancement.

**TRX Enhances Endogenous Expression of α-ENaC mRNA—** To investigate whether TRX stimulates α-ENaC GRE function in its native configuration, endogenous α-ENaC expression was examined in cells with enhanced expression of TRX under different experimental conditions of oxidative stress. To do this, A549 cells and TRXs-A549 cells were treated with 100 nM Dex or vehicle for 6 h and then cultured in the presence or absence of exogenous 200 μM H$_2$O$_2$ for 10 h. As shown in Fig. 7, A549 cells stimulated with 100 nM Dex alone exhibited an induction in α-ENaC steady-state mRNA level, whereas treatment of A549 cells with 200 μM H$_2$O$_2$ led to a down-regulation of the α-ENaC message level. This latter observation is consistent with the results of our reporter construct assays shown in Fig. 2. Notably, the fold induction of Dex-stimulated mRNA levels in TRXs-A549 cells was higher than the fold induction observed in parental A549 cells (Fig. 7, lane 2 versus 5), confirming that TRX may be capable of protecting GR function against endogenously generated oxidative stress (Fig. 6C, lane 2 versus 5). Furthermore, exogenous H$_2$O$_2$-induced repression of α-ENaC was not detected to any significant degree in TRXs-A549 cells (Fig. 7, lanes 5 and 6), demonstrating the protective effects of TRX on GR/Dex transactivation ability. β-actin mRNA expression was used to normalize the α-ENaC mRNA level in parental and TRXs-A549 cells and to assure the viability of the treated cells. Consistent with the results shown in Fig. 2C, oxidative stress had little effect on β-actin transcription.

**Fig. 7.** TRX enhances GR/Dex transactivation and protects against H$_2$O$_2$-mediated suppression of α-ENaC mRNA. Northern blot analyses were performed to analyze the changes in α-ENaC mRNA expression in control A549 and TRXs-A549 cells. Cells were cultured in 0.05% serum-starved medium for 8 h prior to treatment with vehicle (−/−) (control), 10$^{-7}$ M Dex (−/+), or 10$^{-7}$ M Dex and 200 μM H$_2$O$_2$ (+/+). In control and Dex-treated groups, cells were incubated with vehicle or 10$^{-7}$ M Dex for 16 h. In Dex and H$_2$O$_2$-treated group, cells were first treated with 10$^{-7}$ M Dex for 6 h, subsequently treated with 200 μM H$_2$O$_2$ for 20 min as described in Fig. 2C, followed by adding 10$^{-7}$ M Dex to the culture medium, and incubated for 10 h thereafter. Eighteen micrograms of total RNA from each sample were electrophoresed, blotted, and probed with $^{32}$P-labeled α-ENaC and β-actin DNA, as indicated. The experiment was repeated three times with one representative Northern blot analysis shown. Dex-induced α-ENaC mRNA levels in A549 and TRXs-A549 cells were also quantitated by electronic autoradiograph and normalized against β-actin mRNA levels as shown in the lower panel (mean ± S.E.).
tion and its message level. These data reinforce the results shown above on the regulatory effects on α-ENaC gene reporter activity by exogenous H$_2$O$_2$. Dex treatment, and TRX overexpression and serve to establish the physiological and/or pathophysiological relevance of this study. Together, our data confirm that the overexpression of TRX enhances the Dex-stimulated endogenous α-ENaC expression through the identified GRE.

**DISCUSSION**

This study investigated exogenous H$_2$O$_2$-induced signal transduction pathways and their role in impairing the GR/Dex-mediated up-regulation of α-ENaC expression at the transcriptional level in lung epithelial A549 cells. First, GR/Dex-induced α-ENaC expression in A549 cells was suppressed by exogenous H$_2$O$_2$. H$_2$O$_2$-induced suppression was completely abrogated by mutation of the α-ENaC GRE in both homologous and heterologous promoter constructs (Fig. 2). The observed down-regulation of α-ENaC expression was not because of nonspecific cell damage by exogenous H$_2$O$_2$, but rather H$_2$O$_2$ appeared to specifically inhibit GR-mediated α-ENaC induction. Second, whereas 200 μM H$_2$O$_2$ treatment activated both ERK and JNK kinases (Fig. 3) in A549 cells, only ERK activation appears to attenuate α-ENaC GRE function (Fig. 5). The diminishing effect of H$_2$O$_2$ on cells pretreated with either a pharmacological or biological ERK pathway inhibitor indicates that ERK activation is involved in oxidative stress-mediated suppression of the α-ENaC GRE action. Finally, overexpression of TRX blocked H$_2$O$_2$-elicited inhibition at the α-ENaC GRE (Figs. 6 and 7), indicating that both ERK activation and TRX-sensitive pathways are involved in H$_2$O$_2$-mediated suppression of α-ENaC expression in A549 cells. Our data suggest a dual pathway by which GR/Dex-stimulated α-ENaC gene expression is repressed in oxidatively stressed lung epithelium.

Increased oxidative stress has been suggested to modulate the activities of transcription factors, calcium regulatory proteins, and other molecules expressed in cell and organelle membranes in many different cell types by various mechanisms (38–42). Despite these investigations, the potential pathophysiological contribution of oxidative stress in attenuating (or augmenting) any ENaC subunit gene expression has not been conclusively established in epithelial cells. Oxidation-reduction mechanisms are in fact a potential physiological means for reversible regulation of protein function and provide a likely target through which exogenous and endogenous oxidants can interfere with the function of signal transduction pathways. For example, it is now widely accepted that oxidants are able to induce transient activation of many signal transduction pathways, such as ERK and JNK, which we also demonstrate in A549 cells in this study (Fig. 3). However, the exact mechanism as to how reactive oxygen species modulate each signal transduction pathway is still unclear and may involve: the activation of protein kinase C (43), the promotion of guanine nucleotide exchange on Ras (29), or the covalent dimerization and activation of receptor tyrosine kinases (44). There is some controversy in the literature regarding the activation of MAP kinases by H$_2$O$_2$ treatment. For example, Torres and Forman (45) reported that an increase in the endogenous H$_2$O$_2$ production by respiratory burst induces ERK but not p38 kinase activity, whereas exogenous H$_2$O$_2$ activates p38 kinase but not ERK in rat alveolar macrophages. By contrast, the increase in endogenous H$_2$O$_2$ generated by respiratory burst was shown to induce p38 kinase activation in human polymorphonuclear neutrophils. The p38 kinase activation by exogenous H$_2$O$_2$ was also reported in several other cell lines, such as NIH 3T3 cells (30) and HEK 293 cells (46). We do not envision that these studies are necessarily in conflict because there may be an alternative pathway or a different requirement for activating each MAP kinase in different cells. Our data on the induction of ERK and JNK, but not p38 kinase, activities in A549 cells (Fig. 3) further support the notion that the activation of each signaling pathway(s) by oxidants is cell type- and stimulus-specific.

Although our data do not exclude the involvement of other putative cellular molecule(s) or signaling module(s) in oxidant-induced lung injury, they do suggest that the GR would be a direct target of oxidative stress caused by H$_2$O$_2$ to downregulate α-ENaC expression in lung epithelial cells. This conclusion is supported by two complementary observations: (i) the iden-

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**Fig. 8.** A putative model for regulation of α-ENaC expression in lung epithelial cells by oxidants. This diagram depicts an integrated molecular model of α-ENaC gene regulation, illustrating the dynamic interactions among oxidative stress-, activated Ras, TRX, and glucocorticoid hormone-mediated signaling pathways. For details, see “Discussion.” PKC, protein kinase C.
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tified GRE is necessary and indispensable for exogenous H$_2$O$_2$-mediated repression of α-ENaC enhancer/promoter activity (Fig. 2C) and (ii) H$_2$O$_2$-elicited modification (phosphorylation and/or oxidation (47)) of the GR is able to suppress Dex-mediated transactivational potency on the α-ENaC GRE (Fig. 2D). This conclusion is entirely consistent with the demonstrated reversal of exogenous H$_2$O$_2$-mediated inhibition on the Dex-stimulated α-ENaC p-133α/1306GL2-P reporter activity by U0126 pretreatment and TRX overexpression, respectively (Figs. 4 and 6).

We are aware that cellular damages, other than inhibiting ENaC activity, may also be evoked from a relatively high dose of exogenous H$_2$O$_2$. Notably, however, overexpression of TRX was capable of restoring Dex-stimulated α-ENaC expression in the presence of high concentrations, such as 1 mM, of exogenous H$_2$O$_2$ to the level observed in the absence of exogenous H$_2$O$_2$ (Fig. 6B). As an important part of the intracellular antioxidant buffering system, TRX is a small (13 kDa) disulfide reductase (48). Our data on the reversal of H$_2$O$_2$-mediated inhibition of GRE activation in TRX-overexpressing cells strongly suggest that redox-dependent modification of the transactivation potential of the GR, based on analogous paradigms, contributes to the H$_2$O$_2$-mediated repression of GR-dependent gene expression. Perhaps the most intriguing part of our present findings is that TRX overexpression is also capable of potentiating GR function in the absence of exogenous H$_2$O$_2$. Considering that reactive oxygen species are known to be generated as a consequence of stimulation via cytokines (49), growth factors (4), and phagocytosis (50), and that TRX reverses the oxidative stress-mediated repression on cellular GR/Dex action, TRX may be important in normal cell physiology as well as inflammatory processes.

Based on the data presented herein, we envision two potential pathways by which the endogenous and exogenous reactive oxygen species, such as H$_2$O$_2$ and/or nitric oxide, suppress α-ENaC expression in lung epithelial cells (Fig. 8). In the first pathway (doubled-lined arrow), exogenous H$_2$O$_2$ diffuses across cell membrane rapidly and oxidizes the GR directly, thereby inhibiting GR/Dex-mediated signal transduction. Similar results with the oxidation of the GR have been observed by Hutchison et al. (47) who found that inhibition of the GR DNA binding domain activity via H$_2$O$_2$ can be accounted for entirely by the formation of disulfide bonds between cysteine residues within the DNA binding domain. Our data showing that overexpression of TRX restores the effect of Dex on α-ENaC GRE function in the presence of relatively high concentrations of H$_2$O$_2$ supports their findings. In the second pathway (→), oxidants may indirectly mediate the inhibition of GR transactivation activity via ERK activation. This resembles what we have previously proposed, that the Ras- and GR-mediated pathways act antagonistically to modulate α-ENaC expression (21). Based on the data in Fig. 6C, it is likely that the TRX-sensitive pathway is upstream of the Ras/ERK-dependent pathway or these two pathways are independent of each other, because overexpression of TRX failed to reverse the Ras-mediated repression of α-ENaC promoter/enhancer activity. These two pathways may also work synergistically to confer a cell type-specific responsiveness to oxidative stress.

One potential relevance of our findings presented herein may lie in the understanding of cellular mechanisms leading to the impairment of sodium reabsorption under various experimental and disease conditions. Results from several investigations have demonstrated that pharmacologically induced inhibition in epithelial sodium channel activities causes respiratory distress syndrome in otherwise normal animals (15, 18). Moreover, an inverse correlation between the impairment of ENaC activity and the resolution of alveolar pulmonary edema in ARDS has been suggested (51, 52). The resolution of alveolar pulmonary edema is dependent upon ameliorating the fluid leakage into the distal lung air space coupled with efficient restoration of fluid reabsorption from the alveolar air space. Interestingly, a beneficial effect has been described when corticosteroids were given during the late stage of ARDS (53–56), whereas a short term high dose treatment with corticosteroids at the onset of ARDS was ineffective to ameliorate ARDS (57, 58). We speculate that at the onset of ARDS, the burden of oxidative stress may be overwhelming, resulting in the disruption of corticosteroid-mediated transcriptional activation of α-ENaC. However, oxidative stress may have returned to the basal level during the late stage of ARDS. This then allows corticosteroids to exert their beneficial effect not only through altering the inflammatory response of the host but also through up-regulation of α-ENaC expression, thus increasing ENaC activity and ultimately enabling a decrease in edema associated with ARDS.

In summary, together with our previous observations (21, 22), we conclude that GR-mediating signals communicate with both the redox signaling and Ras pathway(s) at multiple regulatory levels to dictate the overall transcriptional control of α-ENaC expression in lung epithelial A549 cells. Our findings suggest that the dynamic balance among the oxidative stress-, Ras activation-, TRX-, and GR-mediated pathways could play an important role in maintaining lung alveolar sodium (and thus fluid) homeostasis in health and disease. Further studies are necessary to clarify not only the underlying molecular mechanisms of TRX action but also their pathophysiological significance in the lung and other organs.

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REFERENCES

1. Suzuki, Y. J., Forman, H. J., and Sevanian, A. (1997) Free Radic Biol. Med. 22, 269–285
2. Papa, S., and Skulachev, V. P. (1997) Mol. Cell. Biochem. 174, 305–319
3. Sen, C. K., and Packer, L. (1996) FEBS Lett. 10, 709–720
4. Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K., and Finkel, T. (1995) Science 270, 296–299
5. Kaul, N., and Forman, H. J. (1996) Free Radic Biol. Med. 21, 401–405
6. Kaul, N., Gopalakrishnana, R., Gundimeda, U., Choi, J., and Forman, H. J. (1998) Arch. Biochem. Biophys. 350, 79–86
7. Dass, K. C., Lewis-Molock, Y., and White, C. W. (1995) Am. J. Physiol. 269, L588–L602
8. Xanthoudakis, S., and Curran, T. (1992) EMBO J. 11, 653–665
9. Schreck, R., Rieber, P., and Baeuerle, P. A. (1991) EMBO J. 10, 2247–2258
10. Nise, K., Shibanuma, M., Ono, S., and Kuraki, T. (1991) Eur. J. Biochem. 201, 99–106
11. Ashery, S. R., and Fitzgerald, D. J. (1999) J. Biol. Chem. 274, 5038–5046
12. Hartfiel, C. L., Alam, J., Cook, J. L., and Choi, A. M. (1997) Am. J. Physiol. 273, L180–L198
13. Scheaffer, H. J., and Weber, M. J. (1999) Mol. Cell. Biol. 19, 2435–2444
14. Davis, R. J. (1994) Trends Biochem. Sci. 19, 470–473
15. O’Broovich, H., Hannam, V., Seear, M., and Mullen, J. B. (1990) J. Appl. Physiol. 68, 1758–1762
16. Hummler, E., Barker, P., Gatzy, J., Beermann, F., Verduino, C., Schmidt, A., Boch-C, and Rosier, B. C. (1995) Nat. Genet. 12, 325–328
17. Spragg, R. G., and Smith, R. M. (1991) in The Lung: Scientific Foundations (Crystal, R. G., and West, J. B., eds) pp. 2003–2017, Raven, New York
18. Chabot, F., Mitchell, J. A., Gutteridge, J. M., and Evans, T. W. (1998) Eur. Respir. J. 11, 745–757
19. Ding, J. W., Dickie, J., O’Broovich, H., Shintani, Y., Raffi, B., Hackam, D., Muranaka, Y., and Rotstein, O. D. (1998) Am. J. Physiol. 274, L378–L387
20. Garby, H., and Palmer, L. G. (1997) Physiol. Rev. 77, 559–596
21. Lin, H. H., Zentner, M. D., Ho, H.-L. L., Kim, K.-J., and Lin, D. K. (1999) Arch. Biochem. Biophys. 254, 2154–2155
22. Zentner, M. D., Lin, H. H., Wen, X., Kim, K. J., and Ann, D. K. (1999) J. Biol. Chem. 274, 1758–1754
23. Lieber, M., Smith, B., Szakal, A., Nelson-Rees, W., and Todor, G. (1976) Int. J. Cancer 7, 62–70
24. An, G., and Wu, R. (1992) Biochem. Biophys. Res. Commun. 183, 170–175
25. Tepic, S., Ueda, J., Canessa, C., Rosseff, B. C., and O’Broovich, H. (1995) Am. J. Physiol. 269, C805–C812
26. Canessa, C. M., Horisberger, J. D., and Rosseff, B. C. (1993) Nature 361, 467–470
Mechanism of Oxidant-mediated α-ENaC Transcriptional Repression

28. Kim, K. J., and Suh, D. J. (1993) *Am. J. Physiol.* 264, L308–L315
29. Lander, H. M., Ogiste, J. S., Teng, K. K., and Novogrodsky, A. (1995) *J. Biol. Chem.* 270, 21195–21198
30. Guyton, K. Z., Liu, Y., Gorospe, M., Xu, Q., and Holbrook, N. J. (1996) *J. Biol. Chem.* 271, 4138–4142
31. Favata, M. F., Horiuchi, K. Y., Manos, E. J., Daulerio, A. J., Stradley, D. A., Feerer, W. S., Van Dyk, D. E., Pitts, W. J., Earl, R. A., Hobbs, F., Copeland, R. A., Magolda, R. L., Scherle, P. A., and Trzaskos, J. M. (1998) *J. Biol. Chem.* 273, 18623–18632
32. Tolwinski, N. S., Shapiro, P. S., Goueli, S., and Ahn, N. G. (1999) *J. Biol. Chem.* 274, 6168–6174
33. Robbins, D. J., Zhen, E., Owaki, H., Vanderblit, C. A., Ebert, D., Geppert, T. D., and Cobb, M. H. (1993) *J. Biol. Chem.* 268, 5097–5106
34. Xia, Y., Wu, Z., Su, B., Murray, B., and Karin, M. (1998) *Genes Dev.* 12, 3369–3381
35. Nakamura, H., Matsuda, M., Furuke, K., Kitazoka, Y., Iwata, S., Toda, K., Inamoto, T., Yamaoka, Y., Ozawa, K., and Yodoi, J. (1994) *Immunol. Lett.* 42, 3369–3381
36. Matthews, J. R., Wakasugi, N., Virelizier, J. L., Yodoi, J., and Hay, R. T. (1992) *Nucleic Acids Res.* 20, 3821–3830
37. Hirota, K., Matsui, M., Iwata, S., Nishiyama, A., Mori, K., and Yodoi, J. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 3633–3638
38. Guyton, K. Z., and Kensler, T. W. (1993) *Br. Med. Bull.* 49, 523–544
39. Pahl, H. L., and Baehner, P. A. (1984) *Bioessays* 16, 497–502
40. Rao, G. N., Katki, K. A., Madamanchi, N. R., Wu, Y., and Birrer, M. J. (1999) *J. Biol. Chem.* 274, 6003–6010
41. Tirosh, A., Patashnik, R., Bashan, N., and Rudich, A. (1999) *J. Biol. Chem.* 274, 10595–10602
42. Grant, C. M., Quinn, K. A., and Dawes, I. W. (1999) *Mol. Cell. Biol.* 19, 2650–2656
43. Gopalkrishna, R., and Anderson, W. B. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 6758–6762
44. van der Vliet, A., Hristova, M., Cross, C. E., Eisierich, J. P., and Goldkorn, T. (1998) *J. Biol. Chem.* 273, 31860–31866
45. Torres, M., and Forman, H. J. (1999) *Arch. Biochem. Biophys.* 366, 231–239
46. Wesselborg, S., Bauer, M. K. A., Vogt, M., Schmitz, M. L., and Schulze-Osthoff, K. (1997) *J. Biol. Chem.* 272, 12422–12429
47. Hutchinson, K. A., Matic, G., Moshenehi, S., Bresnick, E. H., and Pratt, W. B. (1991) *J. Biol. Chem.* 266, 10505–10509
48. Holmgren, A. (1985) *Annu. Rev. Biochem.* 54, 337–371
49. Klebanoff, S. J., Vadas, M. A., Harlan, J. M., Sparks, L. H., Gamble, J. R., Agosti, J. M., and Waltersdorph, A. M. (1986) *J. Immunol.* 136, 4220–4225
50. Shepherd, V. L. (1986) *Semin. Respir. Infect.* 1, 49–106
51. Matthay, M. A., and Wiener-Kronish, J. P. (1990) *Am. Rev. Respir. Dis.* 142, 1250–1257
52. Sakuma, T., Okaniwa, G., Nakada, T., Nishimura, T., Fujimura, S., and Matthay, M. A. (1994) *Am. J. Respir. Crit. Care Med.* 150, 305–310
53. Keel, J. B., Hauser, M., Stocker, R., Baumann, P. C., and Speich, R. (1998) *Respiration* 65, 256–264
54. Olivier, D. (1998) *Respiration* 65, 256–257
55. Brun-Buisson, C., and Brochard, L. (1998) *J. Am. Med. Assoc.* 280, 182–183
56. Meurhi, G. U., Headley, A. S., Golden, E., Carson, S. J., Umberger, R. A., Kelso, T., and Tolley, E. A. (1998) *J. Am. Med. Assoc.* 280, 159–165
57. Bernard, G. R., Luce, J. M., Sprung, C. L., Rinaldo, J. E., Tate, R. M., Sibbald, W. J., Kariman, K., Higgins, S., Bradley, R., Metz, C. A., Harris, T. R., and Brigham, K. L. (1987) *N. Engl. J. Med.* 317, 1566–1579
58. Luce, J. M., Montgomery, A. B., Marks, J. D., Turner, J., Metz, C. A., and Murray, J. F. (1988) *Am. Rev. Respir. Dis.* 138, 62–68
Oxidative Stress Disrupts Glucocorticoid Hormone-dependent Transcription of the Amiloride-sensitive Epithelial Sodium Channel α-Subunit in Lung Epithelial Cells through ERK-dependent and Thioredoxin-sensitive Pathways

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