Requirement for High Mobility Group Protein HMGI-C Interaction with STAT3 Inhibitor PIAS3 in Repression of α-Subunit of Epithelial Na\(^+\) Channel (α-ENaC) Transcription by Ras Activation in Salivary Epithelial Cells*

Previously, we have demonstrated that oxidative stress or Ras/ERK activation leads to the transcriptional repression of α-subunit of epithelial Na\(^+\) channel (ENaC) in lung and salivary epithelial cells. Here, we further investigated the coordinated molecular mechanisms by which α-ENaC expression is regulated. Using both stable and transient transfection assays, we demonstrate that the overexpression of high mobility group protein I-C (HMGI-C), a Ras/ERK-inducible HMG-I family member, represses glucocorticoid receptor (GR)/dexamethasone (Dex)-stimulated α-ENaC-reporter activity in salivary epithelial cells. Northern analyses further confirm that the expression of endogenous α-ENaC gene in salivary Pa-4 cells is suppressed by an ectopic HMGI-C overexpression. Through yeast two-hybrid screening and co-immunoprecipitation assays from eu-

Mark D. Zentner‡, H. Helen Lin§, Hong-Tao Deng‡, Kwang-Jin Kim¶¶, Hsiu-Ming Shih**, and David K. Ann‡ ‡‡ §§

From the Departments of ‡Molecular Pharmacology and Toxicology, §Medicine, ¶¶Physiology and Biophysics, and ‡‡Biomedical Engineering, Will Rogers Institute, Pulmonary Research Center, and §§Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, California 90033, and the **Division of Molecular and Genomic Medicine, National Health Research Institutes, Taipei 11529, Taiwan, Republic of China

The glucocorticoid receptor (GR)\(^1\) belongs to the Type I nuclear receptor superfamily. Upon activation, GRs bind to the glucocorticoid response elements (GRE) and activate target gene transcription by either remodeling the chromatin structure or recruiting transcription initiation complex(es) containing RNA polymerase II to the promoter. Inhibition of GRE-mediated transcription can occur through the activation of signaling pathway(s), such as MAPK/ERK. Through the studies identifying genes targeted by Ras/ERK pathway activation, our laboratory previously demonstrated that ERK activation led to the transcriptional repression of the α-subunit of the amiloride-sensitive epithelial Na\(^+\) channel (α-ENaC) gene (1). We further reported that Ras/ERK-mediated signaling pathways down-regulated α-ENaC steady-state mRNA level by antagonizing GR/dexamethasone (Dex)-dependent transactivation of α-ENaC expression (1–3). Moreover, we showed that both protein synthesis-dependent and protein synthesis-independent pathways elicited by Ras/ERK activation are involved in attenuating GR/Dex-stimulated α-ENaC transcription in salivary and lung epithelial cells (1–3). A bona fide GRE, identified by us within the −1334 to −1306 base pair of rat α-ENaC promoter/enhancer region, which is conserved in human α-ENaC 5′-flanking region (4), is critical for glucocorticoid-mediated induction and ERK-dependent repression of α-ENaC expression in both types of epithelial cells (2, 3). However, the exact molecular pathways involved in the antagonistic cross-talk between Ras- and GR-mediated pathways that govern the overall transcriptional regulation of the α-ENaC gene remain largely unknown to date.

In addition to repressing α-ENaC transcription, Ras/ERK pathway activation induces the expression of high mobility group I (HMGI-I) protein, HMGI-C (5). HMGI-C, also known as a chromosomal architectural transcription factor, is a small and highly charged nuclear phosphoprotein that modulates DNA conformation-dependent, rather than DNA sequence specific, transcriptional activities (reviewed in Refs. 6 and 7). Inactivation of the HMGI-C gene results in a pymgy phenotype and a loss of fat tissue in mice, suggesting that HMGI-C plays an important role in cell proliferation and differentiation (8, 9). The exact molecular mechanisms by which HMGI-C impinges

Received for publication, April 10, 2001, and in revised form, June 1, 2001
Published, JBC Papers in Press, June 4, 2001, DOI 10.1074/jbc.M103153200

1 The abbreviations used are: GR, glucocorticoid receptor; GRE, glucocorticoid response element; MAPK, mitogen-activated protein kinase; α-ENaC, α-subunit of epithelial Na\(^+\) channel; Dex, dexamethasone; HMGI, high mobility group; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis.

This paper is available online at http://www.jbc.org

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
on transcription remain to be elucidated. In general, high mobility group HMG-I proteins are proposed to exert their effects through facilitating the assembly of functional nucleoprotein complexes (10). It is likely that nucleoprotein assembly facilitated by HMG-I members is promoted by either modifying DNA conformation or by recruiting nuclear proteins to an enhancer via binding of HMG-I family members to its interacting DNA element.

A number of in vitro and in vivo studies have established a direct correlation between the high level expression of the HMG-I family members and increased degree of neoplastic transformation (11, 12). This is particularly evident for HMGI-C, which is mainly expressed in fully transformed cells, in addition to being involved in early embryonic development (11, 13). Indeed, the cell transformation induced by retrovirus (14) or ectopic Raf-1 overexpression (39) is reversed when the synthesis of HMGI-C protein is inhibited with a HMGI-C antisense approach. Although the HMGI-C protein or mRNA level is low or undetectable in differentiated or nonproliferating cells, the expression of HMGI-C can be transiently induced by Ras/ERK pathway activation and growth-stimulating factors (5, 15). However, little is known about the biological consequence of transient HMGI-C expression in non-transformed or non-malignant cells.

In the past few years, emerging biochemical and genetic evidence have indicated that changes in the expression levels of HMG-I family elicit either stimulatory or inhibitory effects on HMG-I family members and increased degree of neoplastic transformation (16). In addition to being involved in early embryonic development (11, 13). Indeed, the cell transformation induced by retrovirus (14) or ectopic Raf-1 overexpression (39) is reversed when the synthesis of HMGI-C protein is inhibited with a HMGI-C antisense approach. Although the HMGI-C protein or mRNA level is low or undetectable in differentiated or nonproliferating cells, the expression of HMGI-C can be transiently induced by Ras/ERK pathway activation and growth-stimulating factors (5, 15). However, little is known about the biological consequence of transient HMGI-C expression in non-transformed or non-malignant cells.

In the past few years, emerging biochemical and genetic evidence have indicated that changes in the expression levels of HMG-I family elicit either stimulatory or inhibitory effects on target gene expression (16). Here, we report an additional and heretofore unexpected role for HMGI-C in the down-regulation of α3-ENaC gene expression. These transcriptional regulatory effects of HMGI-C appear to be mediated by its association with the protein inhibitor of activated STAT3 (PIAS3), which has been previously shown to bind to activated STAT3 and to block STAT3 DNA binding activity (17). PIAS3, a protein of 583 amino acids, has a molecular mass of 68 kDa and binds specifically to STAT3, but not to other STATs. Furthermore, PIAS3 belongs to a family of proteins, some of which inhibit the transactivation of other STAT-responsive genes (reviewed in Ref. 18). We demonstrate herein that HMGI-C interacts with PIAS3 in a cooperative way to inhibit GR/Dex-stimulated transcription of the α3-ENaC gene and enhance PIAS3-mediated repression on STAT3-dependent transactivation. To our knowledge, this is the first report to provide a molecular mechanism by which the Ras/ERK signaling utilizes specific components of the chromosomal architecture protein, i.e. HMGI-C, and cytokine signaling pathway, i.e. PIAS3, collectively to repress GR-stimulated α3-ENaC transcription and STAT3-dependent transactivation, respectively. Furthermore, these results provide a molecular explanation for the inhibitory effects on glucocorticoid activated gene expression by the Ras/ERK pathway in general, and for the Ras/ERK-mediated suppression of STAT3-responsive gene(s) as well.

MATERIALS AND METHODS

Pa-4 and Pa-4/HMGI-C Cells—The rat parotid epithelial cell line Pa-4, also known as parotid C5 cells (19), was plated on Primary culture dishes (Falcon) in Dulbecco’s modified Eagle’s/F-12 (1:1) medium (supplemented with 2.5% fetal calf serum, insulin (5 μg/ml), transferrin (5 μg/ml), epidermal growth factor (25 ng/ml), hydrocortisone (1.1 μM), glutamate (5 mM), and kanamycin monosulfate (60 μg/ml)), and maintained in a humidified atmosphere of 5% CO2 and 95% air at 37 °C using an end over end rotator.

Northern Analysis—Total RNA was extracted from Pa-4 or Pa-4/HMGI-C cells with TRizol™ Reagents (Molecular Research Center, Inc.) per the manufacturer’s instructions. The quality and quantity of RNA were analyzed by fractionating an equal amount of RNA from each sample on a 1.5% agarose gel in the presence of 2.2 M formaldehyde, stained with ethidium bromide, and compared among different samples. For Northern analyses, equal amounts of RNA (18 μg/sample) from parental, transfected, or treated cells were electrophoresed through a denaturing agarose gel, transferred to nylon membranes (ICN Biomedicals, Inc.), and UV cross-linked. All blots were pre-hybridized for 1 h with QuickHyb™ (Stratagene) to establish hybridization conditions for the respective probe. To screen various blots, [32P]dCTP-labeled probes were synthesized using either rat α3-ENaC (bases 1–909 (1)) or mouse PIAS3 (bases 576–1176 (17)) cDNA fragments as a template. A rabbit β-actin probe was also included to ensure that the quality and quantity of RNA between lanes were comparable. All blots were washed in a 0.1 × SSC, 0.5% SDS solution at 60 °C. To improve autoradiographic detection, exposure was carried out overnight at −80 °C using intensifying screens.

Transient Transfection—HA-tagged HMGI-C was cloned into pCNA3.1 expression vector as follows. An EcoRI site encompassing 5′-GAGGATCCGCGCCCGGTTAGG-3′ and a XbaI encompassing 3′-primer, 5′-GATCCTAAGTTGGCCGGCCCTTAAT-3′ were employed in a PCR amplification using HMGI-C cDNA as a template. The resulting PCR product was cloned into HA-expression vector, pBluescript SK (a kind gift from Dr. Michael Stallcup, University of Southern California). By digesting the resulting plasmid with BamHI/XbaI, the amplified PCR DNA fragment with a HA-tag was excised from pBluescript SK vector and recloned into pCNA3.1 that had been linearized with the same restriction enzymes. After confirming with DNA sequence analyses, the expression of the resultant HA-HMGI-C pCNA3.1 expression plasmid was verified by the in vitro transcription/translation system (Promega) with T7 RNA polymerase, obtaining a translation product of ~14 kDa. The antisense HMGI-C construct was prepared as described previously (15). The PCR fragment of HMGI-C was amplified from HMGI-C cDNA using a BamHI site encompassing 5′-primer, 5′-GAGGATCCGCGCCCGGTTAGG-3′ and EcoRI site encompassing 3′-primer, 5′-CGAATTCGCTAATCTTCCCTGAGG-3′. Subsequently, the PCR fragment was cloned into pSg5 (Stratagene) by ligating the desired insert and vector DNA following the digestion of both PCR fragment and vector with BamHI and EcoRI.

Plasmids were transiently transfected into Pa-4 or Pa-4/HMGI-C cells by the Lipofectamine™ mediated method as described previously (20). The transfection experiments, 0.1 μg of pRenilla luciferase plasmid, pRL-TK (Promega), was included as an indicator plasmid to normalize for transfection efficiency. Plasmid encoding for FLAG-PIAS3 was generously provided by Dr. Kei Shuai (University of California at Los Angeles). The plasmid molar ratio and total amount of transfected DNA (2 μg) were kept constant by supplementing with appropriate amounts of the pCMV, and/or pRK52A (21). Twenty-four hours after the start of transfection, media was changed and cells were treated as indicated and harvested 24 h thereafter.

Immunoprecipitation—For immunoprecipitation, the lysate volume equivalent to 100 μg of nuclear protein was suspended in 1 ml of Dignum D buffer (20 mM HEPEs, pH 7.9, 20% glycerol, 0.1% Nonidet P-40, 75 mM NaCl, 100 mM KCl, 0.2 mM EDTA, 3% bovine serum albumin) and pre-cleared with 40 μl of a pre-equilibrated protein A-Sepharose slurry for 30 min. The supernatant was then incubated with 0.2 μg of anti-FLAG antibody for 30 min, and followed by 90 min incubation with 30 μl of pre-equilibrated 50% (w/v) protein A-Sepharose slurry. The Sepharose beads were sedimented, washed three times with an incubation buffer (20 mM HEPEs, pH 7.9, 75 mM KCl, 2.5 mM MgCl2, 1 mM dithiothreitol, 0.1% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 μg/ml leupeptin, 1 mM NaVO4), and resuspended in 4 × SDS gel loading buffer (62 mM Tris, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 0.005% (w/v) bromophenol blue). All the incubations above were carried out at 4 °C using an end over end rotator.

Western Analysis—Parental and HMGI-C/Lex A transfected L-40 yeast cells were pelleted and washed in ice water. Cell pellets were resuspended in 1 ml of pre-warmed (60 °C) cracking buffer containing glass beads, lysed by vortexing for 1 min, and boiled for 4 min. Fifty μl of supernatant were loaded on a gradient (4–20%) SDS-PAGE, electroblotted on Immobilon-P membrane (Millipore) and probed with an anti-Lex A antibody (Upstate Biotechnology). HA-HMGI-C was detected using murine anti-HA monoclonal antibody (Covance). PIAS3 was detected using murine anti-FLAG M5 monoclonal antibody
Hormasridish peroxidase-conjugated secondary antibody (Amersham Pharmacia Biotech) and the enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech) were used to visualize proteins of interest.

Yeast Two-Hybrid System Screening—L-40 cells with a genotype of *MATa ade2 his3 rpl1,2 lys2,2 leu1-A- lacZ, and URA3-Lex A-HIS3 (22) were cultured in either complete (Yc), enriched (YPAD), or synthetically depleted (–WUK, –WUKHL media). The Ye, YPAD, –WUK, and –WUKHL media were prepared as follows. Ye contained per liter basis, 1.2 g of yeast nitrogen base (without amino acids), 5 g of ammonium sulfate, 10 g of succinic acid, 6 g of NaOH, 0.1 g each of adenine, arginine, cysteine, leucine, lysine, threonine, tryptophan, and uracil, and 0.05 g each of aspartic acid, histidine, isoleucine, methionine, phenylalanine, proline, serine, tyrosine, and valine. YPAD contained per liter basis, 10 g of yeast extract, 20 g of peptone, and 0.1 g of adenine. Synthetically depleted –WUK medium contained the same components as Ye with the omission of tryptophan, uracil, and lysine, whereas –WUKHL medium lacks histidine and leucine as well. All yeast cells were grown at 30 °C.

DNA construct used for yeast two-hybrid screening was performed as follows. The rat full-length HMGI-C coding sequence was PCR amplified using forward and reverse primers of 5’-GAGAATTCTAGGCG-CACCCGCTGAGG-3’ and 5’-GAGGATCCTGTTAATGCAGCCGC-CCCTAAT-3’. The desired PCR product was cloned into pCR®-TOPO (Invitrogen), excised with EcoRI, and subcloned into pGAD10, with the LexA coding sequence of the DNA-binding domain expression vector, BMT116 (23). To identify proteins that interact with HMGI-C, a mouse 17-day-old embryo two-hybrid cDNA library, harboring cDNA inserts from a mouse 17-day embryo and in-frame with the GAL4 coding sequence of the activation domain expression vector pGAD10, was obtained from CLONTECH Laboratories, Inc. Yeast strain L-40 was used and yeast two-hybrid screening was performed as described in detail elsewhere (24). Briefly, L-40 cells, co-transformed with HMGI-C/BMT116 and cDNA/pGAD10, were grown for 16 h in –WUK medium prior to being plated onto –WUKHL plates. Candidates were selected and assayed for β-galactosidase activity as follows: L-40 cells were lifted from the plates with sterile nitrocellulose membrane filters and assayed for β-galactosidase by ortho-thiazolylblue for 30 min and incubated with Z buffer (10 μM β-mercaptoethanol, 0.8 mM 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 60 mM NaHPO4, 40 mM NaH2PO4 10 mM KCl, 1 mM MgSO4, pH 7.0) at 37 °C for 24 h. The interaction between HMGI-C and proteins encoded by the cDNA library activates His3 and LacZ reporter genes. His3 confers upon yeast the ability to grow on histidine-free selective medium, whereas LacZ produces β-galactosidase that can be detected colorimetrically by filter assays (24).

Analysis of cDNAs Encoding HMGI-C Interacting Proteins—Total plasmid was rescued from positive candidates using Zymoprep™ Yeast Plasmid Rescue Kit (Zymo Research). To isolate plasmids encoding library cDNA, transfomers that grew on ampicillin (100 μg/ml) containing medium were selected and further characterized by restriction enzyme digestions and asymmetric PCR-based sequencing (Applied Biosystems). Sequences were further analyzed by comparing them with those stored at GenBank™ and EMBL data bases at the National Center for Biotechnology Information (NCBI).

RESULTS

A Distal GRE Site Is Pivotal for HMGI-C-mediated Repression of α-ENaC Expression in Salivary Epithelial Cells—Previously, our laboratory utilized a mRNA differential display approach to identify downstream targets of Ras/ERK pathway activation that may be involved in modulating salivary epithelial cell differentiation and proliferation program(s). The small nuclear protein HMGI-C and the α-subunit of the amiloride-sensitive sodium channel, α-ENaC, were identified as the Ras/ERK positive and negative responsive genes in salivary Pa-4 cells, respectively (5). Because the kinetics of ERK-mediated induction of HMGI-C expression closely correlated well with that of ERK-mediated repression of the α-ENaC transactivation, we considered whether these two proteins were a downstream effector of the ERK pathway that repressed GR-mediated expression of tissue specific genes, such as α-ENaC.

To examine the possibility that HMGI-C expression alone was sufficient to repress α-ENaC transcription in cultured salivary epithelial cells, Pa-4 cells were transiently transfected with an ERK/GR-responsive –1.4α-ENaC/Luc reporter gene in the absence or presence of GR/Dex and increasing concentrations of a HMGI-C expression plasmid (Fig. 1A). The choice of utilizing –1.4α-ENaC/Luc reporter in our assay was based on our previous observation that the GRE, located between nucleotides −1334 to −1306 in the rat α-ENaC 5′-flanking region, within −1.4α-ENaC/Luc reporter construct plays an essential role in dictating the overall α-ENaC expression level in salivary and lung epithelial cells (2, 3). Transfected Pa-4 cells were allowed to recover overnight, serum-deprived for 8 h, and Dex treated for 16 h prior to the reporter assays. As shown in Fig. 1A, co-transfected HMGI-C inhibited the GR/Dex-stimulated −1.4α-ENaC/Luc reporter activities in a concentration-dependent manner (lane 6 versus lanes 7 through 10).

Furthermore, low concentrations of co-transfected HMGI-C exerted a very modest inhibitory effect on −1.4α-ENaC/Luc expression in the absence of GR/Dex (Fig. 1A, lanes 2 and 3 versus lane 1). This modest inhibition of basal −1.4α-ENaC/Luc activity by HMGI-C probably reflects the residual glucocorticoid hormone in the culture system after serum starvation. While the GR/Dex treatment reproducibly induced the reporter activity by at least 5-fold, consistent with our previous report (3), the Dex-stimulated reporter activity was substantially inhibited by more than 30–50% with low concentrations of ectopic HMGI-C expression (Fig. 1A, lanes 7 and 8 versus lane 6) and as much as by 80–90% with substantially abundant HMGI-C expression (Fig. 1A, lanes 9 and 10 versus lane 6), respectively. Western analysis was performed to assure that there was no correlation between the protein expression levels of transfected HMGI-C and the amounts of HMGI-C plasmids utilized in each assay (data not shown). Together, these data suggest that the ectopic expression of HMGI-C alone, especially at low concentration, is sufficient to substantially repress GR-mediated α-ENaC expression without affecting its basal levels (Fig. 1A, lanes 2, 3, 7, and 8 versus lanes 1 and 6) in Pa-4 cells.

Steroid hormone receptors, such as the GR, enhance basal gene transcription by binding to their cognate DNA response sequences upstream or downstream of the core promoter. Since we had previously shown that the repression of α-ENaC transcription by ERK activation was dependent upon the presence of an intact GRE, we investigated whether HMGI-C also utilizes the same cis-element to elicit its inhibitory effect. To do this, we evaluated α-ENaC reporter expression in Pa-4 cells by comparing GR/Dex-stimulated transcription of the wild type and GRE-mutated reporter constructs in the presence or absence of ectopic HMGI-C expression. Expression constructs, −1.4α-ENaC/Luc and p(−1334/−1306)GL2-P that harbors one copy of α-ENaC GRE upstream of a heterologous SV40 promoter (3), and their corresponding GRE-mutated constructs were individually and transiently transfected in Pa-4 cells along with GR expression plasmid in combination with 0.15 μg of HA-HMGI-C expression construct (Fig. 1B). Consistent with our previous report (3), Dex-stimulated reporter activities from both wild type constructs were reproducibly repressed by HMGI-C expression (Fig. 1B, lanes 4 and 12 versus lanes 2 and 10). Moreover, site-specific mutations on the GREs within both reporter constructs completely abolished this HMGI-C-mediated repression on GR/Dex-dependent transactivation of reporter constructs (Fig. 1B, lanes 5–8 and 13–16 versus lanes 1–4 and 9–12). As illustrated in Fig. 1, A and B, low levels of HMGI-C expression had almost no effect on the activities of these reporters in the absence of GR/Dex, supporting the notion that HMGI-C does not function as a general transcription repressor. Together, we conclude that α-ENaC GRE is indispensable for HMGI-C-mediated suppression of α-ENaC transcription.

HMGI-C Attenuates the Dex Stimulation of Steady-state Lev-
presses endogenous α-ENaC expression within its own native, higher order chromatin structure. To do this, a stably transfected clone, Pa-4/HMGI-C, which exhibited constitutive levels of HA-HMGI-C expression was established. Both Pa-4 and Pa-4/HMGI-C cells were cultured under serum-containing, dehydrated, or dehydrated/Dex-treated conditions, and then total RNAs from these cells were extracted for Northern blot analyses of endogenous α-ENaC expression. Moreover, the establishment of stably integrated Pa-4/HMGI-C cells as host cells for our assays also allowed us to exclude the possibility that the observed repression of GR/Dex-stimulated α-ENaC reporter is a result of promoter dilution due to the sequestering of common transcription factor(s), a potential artifact from transient cotransfection experiments.

As shown in Fig. 2A, there was a >90% decrease in the basal steady-state α-ENaC mRNA level in Pa-4/HMGI-C cells when compared with that in the parental cells cultured in serum-containing conditions (lane 1 versus lane 4). Moreover, the α-ENaC expression in serum-deprived Pa-4/HMGI-C cells could not be induced by 16 h of 100 nM Dex treatment (Fig. 2A, lane 5 versus lane 6), in contrast to the robust induction by the same treatment observed in parental Pa-4 cells (Fig. 2A, lane 2 versus lane 3) as reported previously (3). This latter observation reinforces the results of HMGI-C-mediated inhibition of the stimulated −1.4α-ENaC/Luc reporter activities by transient transfection assays as illustrated in Fig. 1, A and B.

Notably, the Dex-inducible elevation of α-ENaC mRNA levels was almost completely abolished in Pa-4/HMGI-C cells, confirming that HMGI-C is capable of repressing Dex-stimulated endogenous α-ENaC expression. The steady-state β-actin mRNA level was used to normalize the α-ENaC mRNA level in both parental and Pa-4/HMGI-C cells under different culture conditions and to assure the quality of RNA preparation. The lack of repression of β-actin mRNA level by HMGI-C expression also supports that HMGI-C-mediated repression is GRE dependent. Furthermore, GR/Dex treatment failed to stimulate the reporter activities from transfected −1.4α-ENaC/Luc and p−1334/−1306GL2-P reporter constructs in Pa-4/HMGI-C cells to the same extent as those observed in Pa-4 cells (Fig. 2B, lanes 2 and 6 versus lanes 1 and 5). Although HMGI-C-mediated inhibition was GRE-dependent (Fig. 2B, lanes 4 and 8 versus lanes 3 and 7), the demonstrated inhibitory effect by HMGI-C in stably transfected Pa-4/HMGI-C cells was less pronounced than that obtained from transient transfection assays carried out in Pa-4 cells (Fig. 2B versus Fig. 1B). This discrepancy is probably due to the relatively lower HMGI-C expression level in Pa-4/HMGI-C cells, compared with that in transiently transfected cells (data not shown). This is also consistent with the concentration dependence of HMGI-C-mediated repression shown in Fig. 1A. Taken together, our data confirm that HMGI-C expression dose dependently attenuates α-ENaC transcription stimulated by Dex through the previously identified GRE, located between nucleotides −1334 and −1306, in the α-ENaC 5′-flanking region.

HMGI-C Interacts with PIAS3 in Yeast Two-hybrid Screening and in Pa-4 Cells—To gain more insight into the repressor role of HMGI-C in the GR signal transduction pathway, we set out to identify proteins that interact with HMGI-C. To this end, the entire coding region of HMGI-C fused to the LexA DNA-binding domain was constructed as a bait for yeast two-hybrid screening (Fig. 3A). We used a cDNA expression library that had been generated from day 17 post conception murine embryos. The screen was performed by plating a total of 2 million yeast cells that had been co-transformed with the bait and library plasmids in a histidine-deficient medium. The emerging colonies were picked and tested for β-galactosidase activities.

![Fig. 1. The effect of HMGI-C overexpression on GR/Dex transactivation of α-ENaC expression.](http://www.jbc.org/)
The library plasmids of α-ENaC positive and histidine auxotrophic clones were isolated for further characterization. The cDNA insert of isolated yeast clone 12, showing a strong interaction upon retransformation with HMGI-C/LexA judged by the relative β-galactosidase activity, encoded C-terminal amino acid residues 317 to 550 of the protein inhibitors of activated STAT3, PIAS3.

To test whether PIAS3 and HMGI-C interact in mammalian cells, we prepared total cellular extracts from Pa-4/HMGI-C cells transiently transfected with either FLAG-PIAS3 or FLAG-PIAS3 and GR, respectively. Protein extracts from these transfected cells were immunoprecipitated utilizing an anti-FLAG antibody, which recognizes the FLAG-tagged PIAS3 in the transfected Pa-4/HMGI-C cells. Immunoprecipitates were then analyzed by SDS-PAGE and Western blotting using an anti-HA antibody to detect coprecipitated HMGI-C. In all sets of experiments, HMGI-C could be reproducibly co-precipitated with an antibody against tagged PIAS3 in the transfected Pa-4/HMGI-C cells (Fig. 3B). Similar experiments were carried out in Pa-4 cells, where extracts from cells without tagged HMGI-C expression did not produce any detectable signals after immunoprecipitation with an anti-FLAG antibody followed by Western blot with anti-HA antibody (data not shown), indicating the specificity of interaction between HMGI-C and PIAS3. Moreover, co-transfection with GR allowed HMGI-C and PIAS3 to be co-precipitated with a higher efficiency, compared with that recovered from Pa-4/HMGI-C cells transfected with PIAS3 alone (Fig. 3B). However, we failed to detect GR from the complex immunoprecipitated with the anti-HA antibody, suggesting an uncertainty whether HMGI-C/PIAS3 is capable of forming a stable complex with GR directly.

PIAS3 Represses GR/Dex-mediated α-ENaC Transcription—Since PIAS3 is a known transcription regulator for STAT3-dependent pathway, we further tested whether PIAS3 was also involved in modulating GR/Dex-stimulated transcription. A transient transfection reporter assay utilizing −1.4α-ENaC/Luc with increasing amounts of PIAS3 expression construct in the presence or absence of GR was performed. Results shown in Fig. 4A indicated that co-transfection with increasing amounts of PIAS3 expression constructs led to a reduction of GR/Dex-stimulated −1.4α-ENaC/Luc expression in a concentration-dependent manner, confirming that PIAS3 could function as a repressor for GR/Dex-dependent α-ENaC transactivation. Moreover, only small amounts of exogenous PIAS3 expression construct were needed to repress α-ENaC transactivation. This is probably due to the fact that endogenous PIAS3 is constitutively expressed in Pa-4 cells (data not shown). Therefore, a...
Co-repression of Both GR and STAT3 Signaling Pathways by HMGI-C/PIAS3—To determine whether functional expression of HMGI-C and PIAS3 together modulates signal transduction-coupled transcription(s) in addition to GR pathway, we investigated if the HMGI-C expression could enhance or relieve PIAS3-mediated STAT3 inhibition. A pLucTKS3, a reporter gene for the STAT3-binding site (25), can be efficiently activated in salivary Pa-4 cells by the activation of the non-receptor cytoplasmic tyrosine kinase, Etk (26). Therefore, Etk-mediated pLucTKS3 activation in Pa-4 cells was employed as a model to examine the role of HMGI-C/PIAS3 in modulating the transcription stimulated by the activated STAT3. Co-transfection of PIAS3 expression constructs led to a reduction of Etk/STAT3-stimulated pLucTKS3 transcriptional activation in Pa-4 cells was employed as a model to examine the role of HMGI-C/PIAS3 in modulating the transcription stimulated by the activated STAT3 (Fig. 5A and Ref. 17). While activation of the pLucTKS3 reporter was only modestly reduced by co-transfection of the HMGI-C expression construct alone (Fig. 5A, lanes 5 and 6 versus lane 2), PIAS3-mediated repression of the pLucTKS3 reporter was robustly enhanced by co-transfection of a low amount of the HMGI-C expression construct (Fig. 5A, lane 7 versus lane 3). This observation, together with those results shown in Fig. 4B, led us to propose that the HMGI-C/PIAS3 complex functions cooperatively as a repressor for both GR and STAT3 signaling pathways.

Furthermore, GR/Dex treatment failed to relieve the HMGI-C/PIAS3-mediated STAT3 repression (Fig. 5B, lane 5 versus lane 4), implicating that the HMGI-C/PIAS3 complex is likely to be present in excess and there is no antagonism between GR and STAT3 pathways. In addition, GR/Dex treatment had no effect on Etk-stimulated pLucTKS3 activation (Fig. 5B, lane 3 versus lane 2). Together, albeit direct protein-protein interaction between STAT3 and GR is unlikely as indicated by our negative results on coimmunoprecipitation and by others (27), the HMGI-C/PIAS3 complex is capable of suppressing the transcription stimulated by either signaling pathway. Whole cell
of PIAS3, HMGI-C, and Etk activation. Neither the STAT3 expression level nor activation of endogenous STAT3 was suppressed by co-expression of HMGI-C/PIAS3 (Fig. 5C). Intriguingly, Etk was capable of stimulating STAT3 activation, as visualized by Tyr-705 phosphorylation, in cell extracts prepared from HMGI-C/PIAS3 transfected and Etk activated Pa-4 cells (Fig. 5C, lane 6 versus lanes 2 and 4). Hence, it excludes the possibility that inhibition of STAT3-dependent transcription by the HMGI-C/PIAS3 complex is mediated by blocking of Etk-stimulated Tyr-705 phosphorylation of STAT3. Moreover, since HMGI-C augments the repression caused by PIAS3 of Etk-stimulated pLucTKS3 activities without dampening STAT3 tyrosine phosphorylation, it suggests that HMGI-C represses STAT3-dependent transcription through its interaction with PIAS3.

Previously, we have shown that Ras activation represses Dex-stimulated α-ENaC expression in salivary and lung epithelial cells (2, 3). Since Ras pathway activation has also been shown to induce HMGI-C expression (5), we investigated whether Ras activation alone was sufficient to attenuate STAT3-dependent gene activation. To do this, we assessed the effect of Ras activation on STAT3-responsive reporter activity using transient transfection assays. As illustrated in Fig. 6A, the constitutively active Ras V12 elicited a modest inhibitory effect on pLucTKS3-reporter activities when co-transfected into Pa-4 cells (lane 3 versus lane 2). However, when co-transfected with PIAS3, Ras V12 exhibited a marked repression on reporter activity (Fig. 6A, lane 6 versus lanes 2 and 4). This latter piece of data was also reminiscent of the repression by the PIAS3/HMGI-C complex shown in Fig. 6A (lane 8 versus lane 6).

To further explore the role of Ras-mediated HMGI-C induction in repressing GR- and STAT3-dependent transcription, co-transfection experiments with antisense HMGI-C construct were performed to modulate the amount of HMGI-C protein induced by Ras V12. Pilot studies have indicated that the expression level of HA-HMGI-C in Pa-4/HMGI-C cells can be substantially reduced by the introduction of an antisense HMGI-C construct. Similarly, antisense approach against HMGI(Y) expression has been reported to inhibit the transcription of genes regulated by HMGI(Y) proteins (28, 29). Thus, antisense HMGI-C construct was employed to evaluate the mechanistic role of HMGI-C in Ras-mediated repression of GR- and STAT3-dependent transcription, respectively (Fig. 6, B and C). As is evident from Fig. 6B, expression of antisense HMGI-C substantially relieved the repression of GR/Dex-stimulated −1.4α-ENaC reporter activities by Ras V12. This observation provided strong evidence that HMGI-C is the protein synthesis-dependent downstream mediator induced by Ras/ERK activation to repress α-ENaC expression, as we proposed herein. By contrast, the abrogation of Ras V12/PIAS3-mediated repression on STAT3-stimulated pLucTKS3 reporter activities by antisense HMGI-C was less robust (Fig. 6C). Since parallel experiments were performed using the same host cells for transfection, it is unlikely that the difference in restoring GR- and STAT3-signaling pathways in Ras V12 transfected cells was due to transfection or inhibition efficiency of antisense HMGI-C. Hence, it is conceivable that the induction of HMGI-C by Ras activation is sufficient, but not absolutely essential, for PIAS3 to exert its repressive effect on STAT3-dependent transcription. Nonetheless, this observation reinforces the results, shown in Figs. 3, 4, and 5A, of the repressive effects of the PIAS3/HMGI-C complex on activation of GR- and STAT3-dependent transcription in epithelial cells.

D. Li and D. K. Ann, unpublished observation.
Cross-talk Among GR, STAT3, and Ras/ERK Signaling Pathways

Fig. 6. HMGI-C is involved in Ras-mediated repression of both STAT3 and GR signaling pathways. A. Ras-activation represses Etk-stimulated pLucTKS3 reporter activity. Salivary Pa-4 cells were transfected with 0.9 μg of pLucTKS3, a STAT3-dependent reporter construct, or a combination of vector control, a constitutively active Ras V12, HMGI-C, and increasing amounts of PIAS3 in the presence (+) or absence (−) of Etk activation as indicated. The transfection, luciferase assays, and data analyses were carried out as described in the legend to Fig. 1A. The extent of repression of Etk-stimulated pLucTKS3 reporter activities by the co-transfection of Ras V12 and low concentration of PIAS3 (lane 6) is comparable to those of extracts from cells transfected with a high concentration of PIAS3 (lane 7) or cells co-transfected with HMGI-C and PIAS3 (lane 8). Error bars were calculated from three independent experiments performed in duplicate. B, antisense HMGI-C relieves −1.4α ENaC/Luc transcriptional repression by Ras V12. Salivary Pa-4 cells were transfected with 0.6 μg of −1.4α ENaC/Luc reporter and 0.3 μg of vector control (−) or GR expression construct in the presence or absence of 0.3 μg of Ras V12 without (−) or with increasing amounts of antisense HMGI-C. The transfection, cell treatment, luciferase assays, and data analyses were carried out as described in the legend to Fig. 1A. Co-transfection of antisense HMGI-C relieves the Ras-mediated inhibition of GR/Dex-stimulated −1.4α ENaC/Luc activity in a concentration-dependent manner. C, Ras V12/PIAS3-mediated pLucTKS3 repression involves HMGI-C. Pa-4 cells were transfected with pLucTKS3, Ras V12, PIAS3, or Etk constructs in the presence or absence (−) of antisense HMGI-C. The transfection, luciferase assays, and data analyses were carried out as described in A. Etk-mediated STAT3 activation is repressed by Ras V12/PIAS3 and this repression can be partially reversed by antisense HMGI-C.

DISCUSSION

In this study, we present for the first time experimental evidence supporting the hypothesis that biochemical and functional interaction between the non-histone chromosomal protein, HMGI-C, and STAT3 inhibitory protein, PIAS3, represses GR/Dex-stimulated α-ENaC transcription and STAT3-dependent transcription in epithelial Pa-4 cells (Figs. 4 and 5). The physical interaction between HMGI-C and PIAS3 was demonstrated by two independent approaches, including the yeast two-hybrid system and transient transfections followed by co-immunoprecipitation, suggesting that the formation of such a complex leads to physiologically relevant regulation of cell function. We also demonstrated that both GR/Dex and Etk/STAT3 signaling pathways are antagonized by the Ras pathway activation, a known inducer of HMGI-C expression, adding further support to this hypothesis (Fig. 6). These findings are consistent with the notion that overexpression of HMGI-C is causally associated with the modulation of expression from a complex set of genes. The observation that HMGI-C is capable of repressing the Dex-stimulated endogenous α-ENaC expression (Fig. 2A) also serves to establish the possible physiological and/or pathophysiological importance of this study.

The inhibitory effect of HMGI-C on GR/Dex-stimulated α-ENaC transcription was demonstrated in both transiently and stably HMGI-C transfected salivary Pa-4 epithelial cells (Figs. 1 and 2). The exact mechanism by which the interaction between HMGI-C and PIAS3 leads to the repression of GR/Dex-mediated enhancement on α-ENaC transcription remains to be established. HMGI-C binds to a stretch of 4 to 13 AT-rich nucleotides in the minor groove of DNA (30). While the low expression level of HMGI-C alone had virtually no inhibitory effect on GR/Dex-stimulated α-ENaC promoter activity, the same expression level of HMGI-C together with PIAS3 elicited a marked repression on reporter activities (Fig. 4B, lane 8 versus lane 6). Furthermore, an intact GRE was required for HMGI-C to exert its repressive effect on GR/Dex-dependent α-ENaC transcription (Figs. 1B and 2B). Together, these data suggest that HMGI-C works in concert with PIAS3 to repress the Dex-stimulated transcription of α-ENaC via its GRE.

It has been postulated that the assembly and disassembly of higher order nucleoprotein complexes, comprised of families of transcription activators and repressors, could be a means of bridging together divergent signaling pathways to activate or repress specific genes. This complex assembly/disassembly process may depend upon architectural transcription factors(s), such as HMGI-C. Alternatively, HMGI-C could have interacted with DNA near the α-ENaC GRE, rendering a lower affinity of α-ENaC GRE to the liganded GR. In this scenario, PIAS3 acts to stabilize HMGI-C/DNA interaction through its protein-protein interaction with HMGI-C, thereby antagonizing GR-stimulated α-ENaC transcription. This would implicate that an interaction between HMGI-C and PIAS3 alone is sufficient to abrogate GR/Dex stimulatory activity. One appealing hypothesis is that the HMGI-C-PIAS3 complex might repress only those GR-target genes that harbor HMGI-C contact sites close to the GRE. We attempted to detect an interaction between GR and HMGI-C/PIAS3 by coimmunoprecipitation without success. Nonetheless, this does not rule out the possible interaction between GR and HMGI-C/PIAS3, since this complex may be too unstable to be detected under the standard assay conditions. Alternatively, additional factors may also be required for the formation of a more stable complex.

There is an increasing body of evidence attesting the roles of STAT3 in cell growth, anti-apoptosis, and malignant transformation. PIAS3 has been shown to bind the activated and tyrosine-phosphorylated STAT3 and prevents DNA binding of the STAT3 dimer (17), albeit how this is achieved by PIAS3 is still elusive. Based on our results, we postulate that HMGI-C attenuates binding of the activated STAT3 to DNA and thereby antagonizing STAT3-mediated gene activation. GRs and STAT transcription factors represent two different sets of signaling molecules, activating gene transcription by binding to their
mutated in the channel subunits, increasing the rate of Na\(^+\) transport by inhibiting Dex-dependent signals in the modulation of epithelial gene expression, such as that of α-ENaC and STAT3-response genes, at least in part, through modulating the potency of GR and/or STAT3 signaling pathways. Conclusive evidence for this model will require targeted mutation of the endogenous HMGI-C gene of an experimental animal or in a cell culture model, and the subsequent evaluation of the strength of GR- and STAT3-dependent signals in the modulation of the expression of target genes, such as α-ENaC and STAT3-response genes.

In summary, we have shown that the interaction between HMGI-C and PIAS3 is central to the repression of stimulated α-ENaC transcription via GRE- and STAT3-dependent trans-activation, respectively. These findings reveal a novel signaling network comprised of Ras, STAT3, and glucocorticoid hormone, and suggest that Ras/EKR pathway activation may modulate other glucocorticoid receptor- and STAT3-targeted gene expression in various epithelial cells. Furthermore, although PIAS3 is constitutively expressed in many cells, expression of HMGI-C is induced by the activation of Ras/EKR pathway in differentiated cells. Therefore, we further envision that this induction may serve both to recruit and stabilize PIAS3 within a transcription repressor complex to effectively antagonize GR- and possibly STAT3-stimulated gene transcription. Since both STAT3 and glucocorticoid hormone signaling pathways have essential roles in multiple cellular processes including cell growth, differentiation, cell cycle control, apoptosis, and development, our findings bear particular importance by underscoring a novel mechanism underlying the possible cross-talk occurring among the GR, STAT3, and Ras/EKR signaling pathways.

Acknowledgments—We sincerely thank Drs. Malcolm Sneed and Michael Stallcup for helpful discussions and advice during the course of this work. We extend our gratitude to Dr. Kei Shuai for providing the PIAS3 expression construct and Dr. Michael Stallcup for generously providing reagents used in yeast two-hybrid screening.

REFERENCES
1. Zentner, M. D., Lin, H. H., Wen, X., Kim, K. J., and Ann, D. K. (1998) J. Biol. Chem. 273, 30770–30776
2. Wang, H. C., Zentner, M. D., Deng, H. T., Kim, K. J., Wu, R., Yang, P. C., and Ann, D. K. (2000) J. Biol. Chem. 275, 8600–8609
3. Lin, H. H., Zentner, M. D., Ho, H. L., Kim, K. J., and Ann, D. K. (1999) J. Biol. Chem. 274, 21544–21554
4. Sayegh, R., Auerbach, S. D., Li, X., Lofts, R. W., Husted, R. F., Stokes, J. B., and Thomas, C. P. (1999) J. Biol. Chem. 274, 12431–12437
5. Li, D., Lin, H. H., McMahon, M., Ma, H., and Ann, D. K. (1997) J. Biol. Chem. 272, 25682–25670
6. Bustin, M., and Reeves, R. (1996) Prog. Nucleic Acids Res. Mol. Biol. 54, 35–100
7. Bustin, M. (1999) Mol. Cell. Biol. 19, 5237–5246
8. Zhou, X., Benson, K. F., Ashar, H. R., and Chada, K. (1995) Nature 376, 771–774
9. Anand, A., and Chada, K. (2000) Nat. Genet. 24, 377–380
10. Falvo, J. V., Thanos, D., and Maniatis, T. (1995) Cell 83, 1101–1111
11. Giansotti, V., Punzi, B., D’Andrea, P., Berlingieri, M. T., Di Fiore, P. P., Fuso, A., Vecchio, G., Philip, R., Crane-Robinson, C., Nicolas, R. H., Wright, C. A., and Goodwin, G. H. (1987) EMBO J. 6, 3310–3321
12. Vallone, D., Battista, S., Fierantoni, G. M., Fedele, M., Casalino, L., Santoro, M., Viglietto, G., Fusco, A., and Verde, P. (1997) EMBO J. 16, 5310–5321
13. Tallini, G., and Dal Cin, P. (1999) Adv. Anat. Pathol. 6, 237–246
14. Berlingieri, M. T., Manfioletti, G., Santoro, M., Bandiera, A., Visconti, R., Giansotti, V., and Fuso, A. (1995) Mol. Cell. Biol. 15, 1545–1553

FIG. 7. A putative model for GR- and STAT3-dependent transcription regulated by HMGI-C in salivary epithelial cells. This diagram depicts an integrated molecular model in which the expression of α-ENaC and STAT3 response genes is modulated through dynamic interactions among activated Ras, glucocorticoid hormone- and Etk/STAT3-response genes, at least in part, through modulating the potency of GR and/or STAT3 signaling pathways. Conclusive evidence for this model will require targeted mutation of the endogenous HMGI-C gene of an experimental animal or in a cell culture model, and the subsequent evaluation of the strength of GR- and STAT3-dependent signals in the modulation of the expression of target genes, such as α-ENaC and STAT3-response genes.

One central question that this study poses is the relevance between the inhibition of GR signaling mediated by PIAS3/HMGI-C and its biological effects. Previously, we have demonstrated that the increased oxidative stress impairs Na\(^+\) transport by inhibiting Dex-dependent α-ENaC GRE activation via both ERK-dependent and thioredoxin-sensitive pathways (2). Based on results from our earlier studies and current report, we envision that the relative level of PIAS3/HMGI-C, at least in part, determines potency of glucocorticoid action on various cellular functions, such as ENaC modulation. Collectively, we propose that the dynamic equilibrium between the induction (i.e. GR) and inhibitory (i.e. Ras/ERK activation) signals likely dictate physiological or pathological outcomes. Hence, during embryonic development, when cells are proliferating, the expression of tissue-specific genes, such as α-ENaC, would be undesirable for the proliferation stage of specific organs and would therefore be silenced by the programmed embryonic expression of HMGI-C. During the fetal stage of lung development, Na\(^+\) transport is tapered. In contrast, once cells have differentiated, the appropriately controlled expression of these genes would be critical for its biological function. In the case of α-ENaC, constitutive expression levels of PIAS3 in tight epithelial tissues, such as airways of the lung, collecting duct, and salivary glands, would allow regulation of α-ENaC expression by way of small variations in Ras/ERK-inducible HMGI-C expression. Based on our results, we envision that Ras/ERK-inducible HMGI-C is the potential modulator to integrate PIAS function in multiple cellular regulatory mechanisms, including the transactivation by STAT3, GR, and possibly other nuclear receptors, mediating the cross-talk between Ras/ERK and STAT3/nuclear receptor signaling pathways. As depicted in Fig. 7, our results support a putative model in which HMGI-C plays an integral role in modulating epithelial gene expression, such as that of α-ENaC and STAT3-response genes, at least in part, through modulating the potency of GR and/or STAT3 signaling pathways. Conclusive evidence for this model will require targeted mutation of the endogenous HMGI-C gene of an experimental animal or in a cell culture model, and the subsequent evaluation of the strength of GR- and STAT3-dependent signals in the modulation of the expression of target genes, such as α-ENaC and STAT3-response genes.

In summary, we have shown that the interaction between HMGI-C and PIAS3 is central to the repression of stimulated α-ENaC transcription via GRE- and STAT3-dependent trans-activation, respectively. These findings reveal a novel signaling network comprised of Ras, STAT3, and glucocorticoid hormone, and suggest that Ras/EKR pathway activation may modulate other glucocorticoid receptor- and STAT3-targeted gene expression in various epithelial cells. Furthermore, although PIAS3 is constitutively expressed in many cells, expression of HMGI-C is induced by the activation of Ras/EKR pathway in differentiated cells. Therefore, we further envision that this induction may serve both to recruit and stabilize PIAS3 within a transcription repressor complex to effectively antagonize GR- and possibly STAT3-stimulated gene transcription. Since both STAT3 and glucocorticoid hormone signaling pathways have essential roles in multiple cellular processes including cell growth, differentiation, cell cycle control, apoptosis, and development, our findings bear particular importance by underscoring a novel mechanism underlying the possible cross-talk occurring among the GR, STAT3, and Ras/EKR signaling pathways.

Acknowledgments—We sincerely thank Drs. Malcolm Sneed and Michael Stallcup for helpful discussions and advice during the course of this work. We extend our gratitude to Dr. Kei Shuai for providing the PIAS3 expression construct and Dr. Michael Stallcup for generously providing reagents used in yeast two-hybrid screening.
Cross-talk Among GR, STAT3, and Ras/ERK Signaling Pathways

21. Lanahan, A., Williams, J. B., Sanders, L. K., and Nathans, D. (1992) Mol. Cell. Biol. 12, 3919–3929
22. Reeves, R., Edberg, D. D., and Li, Y. (2001) Mol. Cell. Biol. 21, 575–594
23. Chung, C. D., Liao, J., Liu, B., Rao, X., Jay, P., Berta, P., and Shuai, K. (1997) Science 278, 1803–1805
24. Shuai, K. (1999) Prog. Biophys. Mol. Biol. 71, 405–422
25. Quissell, D. O., Barzen, K. A., Redman, R. S., Camden, J. M., and Turner, J. T. (1998) In Vitro Cell Dev. Biol. Anim 34, 58–67
26. Lin, H. H., Tu, Z. J., and Ann, D. K. (1996) J. Biol. Chem. 271, 27637–27644
27. Bruder, J. T., Heidecker, G., and Rapp, U. R. (1992) Genes Dev. 6, 545–556
28. Vojtek, A. B., Hollenberg, S. M., and Cooper, J. A. (1993) Cell 74, 205–214
29. Bartel, P., and Fields, S. (1997) The Yeast Two-hybrid System, Oxford University Press, New York
30. Vojtek, A. B., and Hollenberg, S. M. (1995) Methods Enzymol. 255, 331–342
31. Turkson, J., Bowman, T., Garcia, R., Caldenhoven, E., De Groot, R. P., and Jove, R. (1998) Mol. Cell. Biol. 18, 2545–2552
32. Wen, X., Lin, H. H., Shih, H. M., Kung, H. J., and Ann, D. K. (1999) J. Biol. Chem. 274, 38204–38210
33. Cella, N., Groner, B., and Hynes, N. E. (1998) Mol. Cell. Biol. 18, 1783–1792
34. Himes, S. R., Reeves, R., Attema, J., Nissen, M., Li, Y., and Shannon, M. F. (2000) J. Immunol. 164, 3157–3168
35. Thanos, D., and Maniatis, T. (1992) Cell 71, 777–789
36. Reeves, R., and Nissen, M. S. (1990) J. Biol. Chem. 265, 8573–8582
37. Zhong, Z., Wen, Z., and Darnell, J. E., Jr. (1994) Science 264, 95–98
38. Beato, M., Herrlich, P., and Schutz, G. (1995) Cell 83, 851–857
39. Ktaja, N., Aittomaki, S., Silvennoinen, O., Palvimo, J. J., and Janne, O. A. (2000) Mol. Endocrinol. 14, 1986–2000
40. Stewart, P. M. (1999) Lancet 353, 1341–1347
41. Champigny, G., Voilley, N., Linguigia, E., Friend, V., Barbry, P., and Lazdunski, M. (1994) EMBO J. 13, 2177–2181
42. Volk, K. A., Sigmund, R. D., Snyder, P. M., McDonald, P. J., Welsh, M. J., and Stokes, J. B. (1995) J. Clin. Invest. 96, 2748–2757
43. Stokes, J. B., and Sigmund, R. D. (1998) Am. J. Physiol. 274, C1699–1707
44. Jui, H. Y., Tseng, R. J., Wen, X., Fang, H. I., Huang, L. M., Chen, K. Y., Kung, H. J., Ann, D. K., and Shih, H. M. (2000) J. Biol. Chem. 275, 41124–41132
45. Li, D. (1997) Ph.D. thesis, University of Southern California
Requirement for High Mobility Group Protein HMGIC Interaction with STAT3 Inhibitor PIAS3 in Repression of $\alpha$-Subunit of Epithelial $\text{Na}^+$ Channel ($\alpha$-ENaC) Transcription by Ras Activation in Salivary Epithelial Cells

Mark D. Zentner, H. Helen Lin, Hong-Tao Deng, Kwang-Jin Kim, Hsiu-Ming Shih and David K. Ann

J. Biol. Chem. 2001, 276:29805-29814.
doi: 10.1074/jbc.M103153200 originally published online June 4, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M103153200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 20 of which can be accessed free at http://www.jbc.org/content/276/32/29805.full.html#ref-list-1