Biochimica et Biophysica Acta 1840 (2014) 693–700

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Histone code of genes induced by co-treatment with a glucocorticoid hormone agonist and a p44/42 MAPK inhibitor in human small intestinal Caco-2 cells

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ARTICLE INFO

Article history:
Received 16 June 2013
Received in revised form 26 September 2013
Accepted 16 October 2013
Available online 24 October 2013

Keywords:
Histone code
Differentiation
Caco-2 cells
Glucocorticoid hormone
p44/42 MAPK

ABSTRACT

Background: Inactivation of glucocorticoid hormones and p44/42 mitogen-activated protein kinase (MAPK) is thought to be important in small intestinal maturation and expression of genes related to intestinal differentiation and functions.
Methods: We investigated target genes induced by co-treatment for 48 h with a glucocorticoid hormone agonist, dexamethasone (Dex), and a p44/42 MAPK inhibitor, PD98059 (PD), in a small intestine-like cell line (Caco-2) using microarray analysis. We also investigated whether expression changes of the target genes induced by the co-treatment are associated with histone modifications around these genes.
Results: Co-treatment of Caco-2 cells with Dex and PD enhanced several genes related to intestinal differentiation and functions such as *SCNN1A*, *FXYD3*, *LCT* and *LOX*. Induction of the *SCNN1A* gene was associated with increased presence of acetylated histone H3 and H4 and di-methylated histone H3 at lysine (K) 4 around the transcribed region of the gene, and induction of the *FXYD3* gene was associated with increased presence of acetylated histones H3 and H4 from the promoter/enhancer to the transcribed region of the gene. Induction of *LCT* and *LOX* genes was associated with increased presence of acetylated histone H4 on the promoter/enhancer region of the genes.
Conclusions: Histone acetylation and/or histone H3 K4 methylation around the promoter/enhancer or/and transcribed regions of target genes are associated with induction of the genes by co-treatment with Dex and PD in Caco-2 cells.
General significance: The histone code is specific to each gene with respect to induction by glucocorticoid hormone and inhibition of p44/42 MAPK in Caco-2 cells.

1. Introduction

Small intestinal absorptive cells rapidly mature during their transition from the crypt to the villus, and start to express many small intestine-specific genes involved in digestion and absorption of nutrients. A previous study of ours has demonstrated that expression of the gene encoding the hexose transporter protein SLC2A5 (GLUT5), which is a fructose transporter and participates in fructose absorption from the lumen to the enterocytes, is enhanced in the proximal small intestine (jejunum) by injection of the glucocorticoid hormone agonist dexamethasone (Dex) into suckling rats [1]. It has also been reported that expression of the gene encoding the sucrase–isomaltase (SI) protein, which participates in digestion of starch hydrolysates and sucrose, was enhanced by glucocorticoid hormone injection in the rat jejunum during the suckling period [2]. In addition, our previous study has demonstrated that co-treatment with a glucocorticoid agonist (Dex) and PD98059 (PD), an inhibitor for p44/42 MAPK (mitogen-activated protein kinase), induces *SLC2A5* gene expression in a small intestine-like cell line (Caco-2), and this induction is associated with enhanced binding of the glucocorticoid hormone receptor (GR) to the promoter/enhancer region of the gene [3]. These results indicate that glucocorticoid hormone and inhibition of p44/42 MAPK are concerned with the induction of gene expression during the differentiation of enterocytes in the small intestine. However, it is not yet known whether other genes related to small intestinal differentiation and function are up-regulated by co-treatment with Dex and PD in Caco-2 cells, and the molecular regulations underlying the transcription of such genes are unclear.

Recent studies have shown that abrupt changes in gene expression, which occur frequently in differentiating cells, are accompanied by a major chromatin structural change that is triggered by modifications of the histone tail, such as acetylation, methylation and phosphorylation [4,5]. Among several histone modifications that have been identified, acetylation of histone H3 at lysine (K) 9 and 14, of histone H4 at K5/8/

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http://dx.doi.org/10.1016/j.bbagen.2013.10.026

12/16 and methylation of histone H3 at K4 are the most extensively studied, because regulation of these histone modifications is related to transactivation [6–8]. Di-methylation of histone H3 at K4 is the most extensively studied modification at this position, because the lysine residue of histone H3 is highly di-methylated around many genes and is induced prior to the acetylation of histones and transactivation of genes; di-methylation of histone H3 at K4 induces acetylation of histones [9,10]. Acetylation of histones leads to the recruitment of transcription initiation complexes onto the promoter/enhancer, and transcription elongation complexes onto the transcribed region close to the transcription initiation site, through binding of bromodomain-containing proteins to acetylated histones [11–13]. Indeed, we have demonstrated that acetylated histone H3 and H4 around the promoter/enhancer and transcribed regions of the *SLC2A5* gene are involved in the induction of this gene in Caco-2 cells when they are co-treated with Dex and PD [14]. However, it is still not known whether expression of other genes related to intestinal differentiation and function in Caco-2 cells are enhanced by co-treatment with Dex and PD, and whether the induction of these genes is associated with modifications of histones around their genes.

In the present study, we explored the target intestinal genes induced by co-treatment with Dex and PD in Caco-2 cells, and examined whether histone modifications such as acetylation of histone H3 at K9/14 and H4 at K5/8/12/16 and di-methylation of histone H3 at K4 are involved in the induction of these target genes in Caco-2 cells.

2. Materials and methods

2.1. Cell culture

Caco-2 cells from the American Type Culture Collection (Rockville, MD, USA) were seeded at a density of 0.6×10^4 cells/cm^2 in culture

Table 1
Up- and down-regulated genes in response to co-treatment with Dex and PD in Caco-2 cells.

Description	Symbol	$\log_2$ Ratio	UniGene ID	P-value
Up-regulated genes induced by PD alone				
Beta-galactoside-binding lectin	*LGALS16*	1.88465	Hs.667238	0.002
ATP-binding cassette, sub-family C, member 3	*ABCC3*	1.31825	Hs.463421	<0.001
HERV-FRD provirus ancestral Env polyprotein	*HERV-FRD*	1.30735	Hs.631996	<0.001
MAS-related GPR, member X1	*MRGPRX1*	1.24077	Hs.711459	0.001
Lactase	*LCT*	1.18945	Hs.551506	<0.001
Lysyl oxidase	*LOX*	1.15227	Hs.102267	0.000
Stanniocalcin 1	*STC1*	1.12259	Hs.25590	0.002
Hydroxyacid oxidase 2 (long chain)	*HAO2*	1.04792	Hs.659767	<0.001
T-box 4	*TBX4*	1.03606	Hs.143907	<0.001
Aquaporin 1 (Colton blood group)	*AQP1*	1.01407	Hs.76152	<0.001
Up-regulated genes induced by co-treatment with Dex and PD				
Solute carrier family 2	*SLC2A5*	3.14425	Hs.530003	<0.001
Leucine-rich repeat LGI family, member 4	*LGI4*	1.91098	Hs.65256	<0.001
Phosphodiesterase 6A, cGMP-specific, rod, alpha	*PDE6A*	1.84461	Hs.567314	<0.001
Zymogen granule protein 16 homolog	*ZG16*	1.67227	Hs.632195	<0.001
Alpha-fetoprotein	*AFP*	1.66484	Hs.518808	<0.001
ATPase, class V, type 10B	*ATP10B*	1.64131	Hs.109358	<0.001
Hypoxia inducible factor 3, alpha subunit	*HIF3A*	1.50744	Hs.420830	<0.001
FXYD domain containing ion transport regulator 3	*FXYD3*	1.49654	Hs.301350	<0.001
Glutathione S-transferase alpha 1	*GSTA1*	1.48267	Hs.446309	<0.001
Sodium channel, nonvoltage-gated 1 alpha	*SCNN1A*	1.46336	Hs.591047	<0.001
Sarcolipin	*SLN*	1.15365	Hs.334629	<0.001
Dual oxidase 2	*DUOX2*	1.14846	Hs.71377	<0.001
Cytochrome P450, family 1, subfamily A, polypeptide 1	*CYP1A1*	1.12699	Hs.72912	0.001
Apolipoprotein C-III	*APOC3*	1.09424	Hs.73849	<0.001
ATP-binding cassette, sub-family A (ABC1), member 1	*ABCA1*	1.06869	Hs.719214	<0.001
Secreted phosphoprotein 1	*SPP1*	1.04683	Hs.313	<0.001
ATPase type 13A4	*ATP13A4*	1.04643	Hs.674423	<0.001
Tumor protein p53 inducible nuclear protein 1	*TP53INP1*	1.03818	Hs.492261	<0.001
Zinc finger and BTB domain containing 16	*ZBTB16*	1.00268	Hs.591945	<0.001
Progastricsin (pepsinogen C)	*PGC*	1.00011	Hs.1867	<0.001
Down-regulated genes induced by Dex alone				
Glycerol-3-phosphate acyltransferase, mitochondrial	*GPAM*	−1.2691	Hs.42586	<0.001
Down-regulated genes induced by PD alone				
Early growth response 1	*EGR1*	−2.05348	Hs.326035	<0.001
Ets variant 5	*ETV5*	−1.57444	Hs.43697	<0.001
NADPH oxidase 1	*NOX1*	−1.4321	Hs.592227	<0.001
N-deacetylase/N-sulfotransferase 4	*NDST4*	−1.27185	Hs.591700	0.001
Gamma-aminobutyric acid (GABA) A receptor, alpha 3	*GABRA3*	−1.23791	Hs.123024	<0.001
FBJ murine osteosarcoma viral oncogene homolog	*FOS*	−1.18213	Hs.707896	<0.001
SRY (sex determining region Y)-box 2	*SOX2*	−1.15055	Hs.518438	<0.001
Four jointed box 1	*FJX1*	−1.12964	Hs.39384	0.005
Sprouty homolog 4	*SPRY4*	−1.11383	Hs.323308	<0.001
CD44 molecule	*CD44*	−1.06947	Hs.502328	<0.001
Regulator of G-protein signaling 5	*RGS5*	−1.05572	Hs.24950	<0.001
Solute carrier family 37	*SLC37A2*	−1.04332	Hs.352661	<0.001
Small nucleolar RNA, C/D box 14E	*SNORD14E*	−1.0066	Hs.180414	0.001
Down−regulated genes induced by co-treatment with Dex and PD				
Inter-alpha (globulin) inhibitor	*ITIH2*	−1.31585	Hs.75285	<0.001
ATPase, H+ transporting	*ATP6V1G3*	−1.13711	Hs.127743	<0.001

collagen plates (Iwaki, Tokyo, Japan) in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), 1% non-essential amino acids (Invitrogen, Tokyo, Japan), 20 mM HEPES (pH 7.4), 1× antibiotic–antimycotic mixed stock solution (Nacalai Tesque, Kyoto, Japan) and 2 mM L-glutamate (Invitrogen) at 37 °C in a humidified atmosphere of 5% CO_2. Five days after seeding, the Caco-2 cells were cultured in DMEM containing 10% FCS stripped of glucocorticoid hormone by treatment with charcoal/dextran, and treated with 1 μM of the glucocorticoid hormone agonist Dex and 50 μM of the mitogen-activated protein kinase kinase (MEK) inhibitor PD, or vehicle (dimethyl sulfoxide (DMSO)) alone, for 48 h. The IC50 of PD for MEK1 and MEK2 are 4 μM and 50 μM, respectively [15], and it does not inhibit activation of other highly related dual-specificity protein kinases, or influence the activity of over 18 other Ser/Thr protein kinases [16]. PD can therefore be considered a specific inhibitor for MEK.

2.2. Microarray analyses

For microarray analyses, we selected total RNA extracts from three samples in each group, i.e., Dex treatment, PD treatment, both treatments, or DMSO, to assess biological reproducibility. Aliquots containing 500 ng of total RNA were individually converted to cRNA and labeled with biotin using a two-cycle labeling kit (Affymetrix, Tokyo, Japan) according to the manufacturer's instructions. Next, aliquots containing 10 μg of biotin-labeled cRNA were separately hybridized to Human Gene 1.0 ST Arrays (Affymetrix) according to the manufacturer's protocol. After completing the washing steps, the microarray plates were analyzed using a GeneChip Scanner 3000 (Affymetrix). Data analysis was performed using the GeneChip operating system (GCOS; Affymetrix), Partec (Ryoka Systems, Tokyo, Japan) and Excel (Microsoft). Variable spots detected by an algorithm in Partec software in each plate were defined as non-expressed genes and removed accordingly. The biotin-labeled signals were normalized by the global median normalization method based on the Partec algorithm. Scatter plots of the mean between DMSO and Dex alone, DP alone or Dex and PD, are shown in Supplemental Fig. 1. Because a previous report has shown that differences between groups of greater than or equal to ±1 after log2 transformation by Affymetrix microarray correlate with real-time RT-PCR data at 98% probability [17], we selected genes with signals greater than or equal to ±1 after log2 transformation. When the differences in the microarray signals between the group treated with drug, i.e., Dex alone, PD alone, or Dex and PD, and that treated with DMSO were greater than or equal to 1 after log2 transformation, and exhibited significance using Bonferroni's test based on analysis of variance (ANOVA), the gene signals were subjected to further analyses. Among the 28,870 gene signals detected in the microarray, 20 were significantly up-regulated (0.069%) by co-treatment with Dex and PD compared with treatment with DMSO. These genes up-regulated by co-treatment with Dex and PD are listed in Table 1.

2.3. Real-time RT-PCR

Total RNA was subjected to reverse transcription using Superscript III reverse transcriptase (Invitrogen). Test genes (*SCNN1A*, *FXYD3*, *LCT*, *LOX*, *ZG16*, *HIF3A*, *PDE6A* and *LGALS16*) and *ACTB* cDNAs were amplified by real-time PCR using a Light Cycler System (Roche Diagnostics, Tokyo, Japan) and SYBR Green I (Takara, Shiga, Japan). The cycle threshold (CT) values of test genes and *ACTB* detected by real-time RT-PCR were converted into signal intensities by the delta–delta method [18]. The sequences of the primers used for real-time RT-PCR are indicated in Supplemental Table 1.

2.4. Chromatin immunoprecipitation (ChIP) assay

Cells were incubated in a fixation solution (1% formaldehyde, 4.5 mM HEPES pH 8.0, 9 mM NaCl, 0.09 mM EDTA and 0.04 mM EGTA) in 10% fetal bovine serum DMEM for 30 min at 37 °C. The reactions were terminated by the addition of glycine to a final concentration of 150 mM. After being washed in FACS solution (1× PBS, 2% bovine serum and 0.05% NaN_3), the samples were sonicated in SDS lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1% SDS and 0.5 mM PMSF) to generate DNA fragments of size 200–500 bp. The ChIP assay used 1 μg anti-acetyl histone H3 (K9/14) antibody (Millipore, Tokyo, Japan), anti-acetyl histone H4 (K5/8/12/16) antibody (Millipore), anti-di-methyl-histone H3 (K4) antibody (Millipore) or normal rabbit IgG, and was performed as described previously [19]. The precipitated DNA was subjected to real-time PCR using primers that corresponded to the indicated sites in the promoter/enhancer, transcription start and transcribed regions. The CT values of the ChIP signals detected by real-time PCR were converted to the percentage of the ChIP signal for the input DNA, which was calculated by the delta–delta method [18], using the formula $100 \times [2^{(\text{CT IP input} - \text{CT sample})}]$. The sequences of the primers used in ChIP assays are indicated in Supplemental Table 2.

2.5. Statistical analysis

For the real-time RT-PCR and ChIP assays, results are expressed as mean ± standard error of the mean (SEM). The significance of differences between groups was determined by Tukey's multiple range test based on one-way ANOVA. A *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Genes up-regulated by co-treatment with Dex and PD in Caco-2 cells

The number of genes induced greater than 2-fold by PD alone and by co-treatment with Dex and PD was 10 and 20, respectively (Table 1). Microarray analysis did not identify any up-regulated genes in response to Dex alone. We tested mRNA levels of several of the up-regulated genes using real-time RT-PCR. As shown in Fig. 1, the mRNA levels of *SCNN1A*, *FXYD3*, *LCT*, *LOX*, *HIF3A*, *ZG16*, *PDE6A* and *LGALS16* genes were enhanced by the Dex/PD co-treatment in Caco-2 cells.

3.2. Changes of histone modifications around targeted genes induced by co-treatment with Dex and PD in Caco-2 cells

The ChIP signals for normal rabbit IgG around all genes tested were <0.2% per input.

With respect to the *SCNN1A* gene, the ChIP signals of acetylated histone H3 at K 9/14 were markedly enhanced by co-treatment with Dex and PD from the promoter (−600 bp) to the transcribed (+4850 bp) region of the gene (Fig. 2B). The ChIP signals of acetylated histone H4 at K5/8/12/16 and di-methylated histone H3 at K4 were markedly enhanced by co-treatment with Dex and PD at 1900 bp in the transcribed region (Fig. 2C, D).

Around the *FXYD3* gene, the ChIP signals of acetylated histone H3 at K 9/14 and acetylated histone H4 at K5/8/12/16 were markedly enhanced by co-treatment with Dex and PD throughout the upstream/transcribed regions of the gene (acetylated histone H3 from −5100 bp to over 4900 bp, acetylated histone H4 from −5100 bp to 4850 bp) (Fig. 2F, G).

Around the *LCT* gene, the ChIP signals of acetylated histone H4 at K5/8/12/16 were markedly enhanced by co-treatment with Dex and PD on the promoter/enhancer region of the *LCT* gene (from −3100 bp to −150 bp) (Fig. 3C).

For the *LOX* gene, the ChIP signals of acetylated histone H4 at K5/8/12/16 were markedly enhanced by co-treatment with Dex and PD on the promoter/enhancer region of the gene (from −3100 bp to −2100 bp and from −600 bp to −150 bp) (Fig. 3G). Di-metylated histone H3K4 arround all genes tested was not remarkably changed by the treatments (Figs. 2–3D, H).

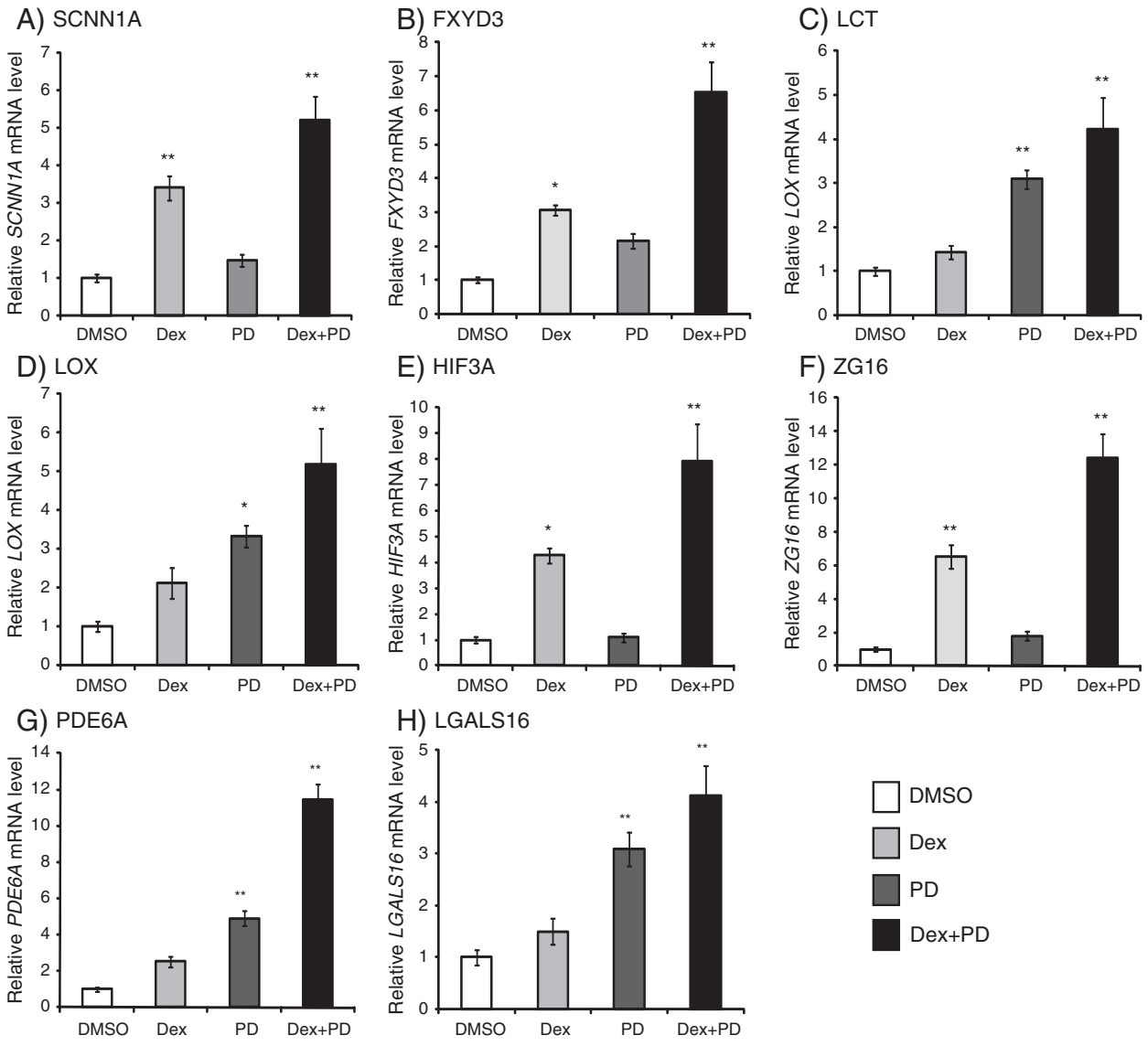

Fig. 1. Real-time RT-PCR analysis of up-regulated genes identified by microarray analysis of Caco-2 cells co-treated with Dex and/or PD. A) *SCNN1A*. B) *FXYD3*. C) *LCT*. D) *LOX*. E) *HIF3A*. F) *ZG16*. G) *PDE6A*. H) *LGALS16*. Caco-2 cells were co-treated with Dex (1 μM) with/without PD (50 μM). Results of real-time RT-PCR for each mRNA were normalized to *ACTB* mRNA and are expressed as arbitrary units. *, ** indicate significant differences compared with control cells (DMSO) at $P < 0.05$ and $P < 0.01$, respectively.

4. Discussion

In this study, we have demonstrated in Caco-2 cells that expression of several genes related to intestinal differentiation and function, such as *SCNN1A*, *FXYD3*, *LCT*, *LOX*, *HIF3A*, *ZG16*, *PDE6A* and *LGALS16*, was induced by co-treatment with Dex and PD. The sodium channel, nonvoltage-gated 1 alpha (*SCNN1A*) gene encodes a protein that mediates the rate-limiting step in conductive sodium absorption across epithelial-lined organs including the small intestine and kidney [20,21]. The FXYD domain-containing ion transport regulator 3 (*FXYD3*) gene encodes a known chloride channel or chloride channel regulator [22]. Lactase (LCT) is an enzyme that hydrolyzes lactose to glucose and galactose [23]. The lysyl oxidase (LOX), which oxidizes peptidyl lysine residues in collagen and elastin, is essential for extracellular matrix maturation and related differentiation [24,25]. The hypoxia inducible factor 3 alpha (*HIF3A*) subunit gene encodes one of the alpha subunits of the hypoxia-inducible factors (HIFs). HIFs are heterodimeric transcription factors that reportedly regulate intestinal absorption of nutrients such as iron by adapting to low oxygen conditions in the small intestine [26]. Zymogen granule protein 16 homolog (ZG16) is known to regulate apical secretion of digestive enzymes in the small intestine [27]. Photoreceptor phosphodiesterase 6α (PDE6A) regulates the intracellular levels of the second messenger, cGMP, which is known to induce intestinal maturation in Caco-2 cells [28]. Lectin, galactoside-binding soluble 16 (LGALS16) is a modulator of cell adhesion and associated differentiation via acting on certain carbohydrate and protein ligands [29]. A previous study of ours has demonstrated that the small intestinal fructose transporter *SLC2A5* is induced by co-treatment with Dex and PD in Caco-2 cells [3]. The genes up-regulated by co-treatment with Dex and PD are putatively related to intestinal differentiation and function. In addition, it has been reported that an injection of glucocorticoid hormone agonist into rat pups during the suckling–weaning period, when the concentration of epidermal growth factor, a p44/42 MAPK activator, in milk is known to decrease, enhanced *SLC2A5* gene expression [30]. Thus, our results and insights from the previous studies mentioned above indicate that glucocorticoid hormone action and p44/42 MAPK inhibition coordinately regulate small intestinal differentiation and induce expression of genes related to intestinal function and differentiation.

In this study, we investigated whether histone modifications are associated with induction of genes co-treated with Dex and PD in Caco-2 cells. We found that induction of the *SCNN1A* gene was associated with

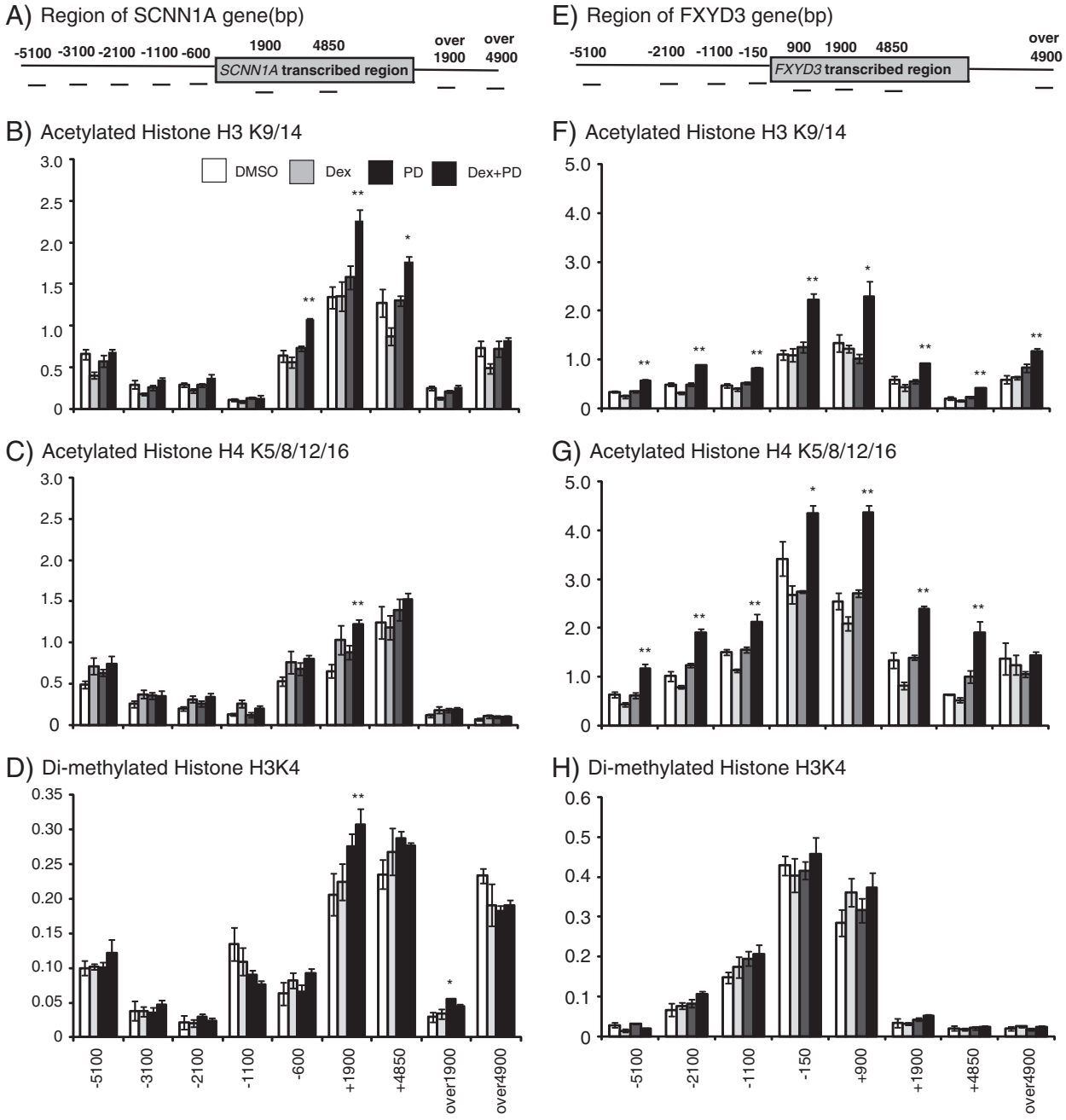

Fig. 2. Histone modifications around the *SCNN1A* and *FXYD3* genes in response to treatment with Dex and/or PD in Caco-2 cells. A–D) *SCNN1A*. E–H) *FXYD3*. The samples were collected from Caco-2 cells treated with Dex and/or PD for ChIP assays. ChIP signals were detected by real-time PCR following immunoprecipitation of the indicated nuclear proteins bound to the *SCNN1A* and *FXYD3* genes, and were normalized to input signals. The abscissa denotes the region on the *SCNN1A* and *FXYD3* genes relative to the transcription initiation site. *, ** indicate significant differences compared with control cells (DMSO) at $P < 0.05$ and $P < 0.01$, respectively.

an increased presence of acetylated histones H3 and H4 around transcribed regions of the gene, whereas induction of *FXYD3* and *SLC2A5* genes was associated with an increased presence of histones H3 and H4 from the promoter/enhancer to the transcribed regions of the gene [31]. In contrast, changes of a histone modification (histone H4 acetylation) around *LCT* and *LOX* genes co-treated with Dex and PD were observed in the promoter/enhancer regions. In addition, we found that there are three types of genes that are up-regulated by co-treatment of Dex and PD, i.e., those with enhanced binding of acetylated histone H3 (*SCNN1A*), those with enhanced binding of acetylated histone H4 (*LCT* and *LOX*), and those with enhanced binding of both acetylated histone H3 and H4 (*FXYD3* and *SLC2A5*). One of our previous studies has demonstrated that inhibition of p44/42 MAPK in Caco-2 cells enhances glucocorticoid hormone-mediated GR translocation from the cytoplasm to the nucleus, and the associated GR binding to the promoter/enhancer region of *SLC2A5* gene, by inhibiting the phosphorylation of the GR at Serine 203 [3]. Thus, enhanced histone acetylation in response to the co-treatment may be regulated by histone acetyltransferases (HATs) associated with the GR in the nucleus. However, the HATs that potentially regulate histone acetylation around the genes up-regulated by the co-treatment have not been identified. It has been reported that histone H4 is acetylated by CBP/p300 and PCAF, coactivators that are known to bind to nuclear receptors including the GR and nuclear transcriptional factors following activation by hormone and nutrient signals [32]. In addition, GCN5, a component of transcription initiation and elongation complexes, acetylates histone

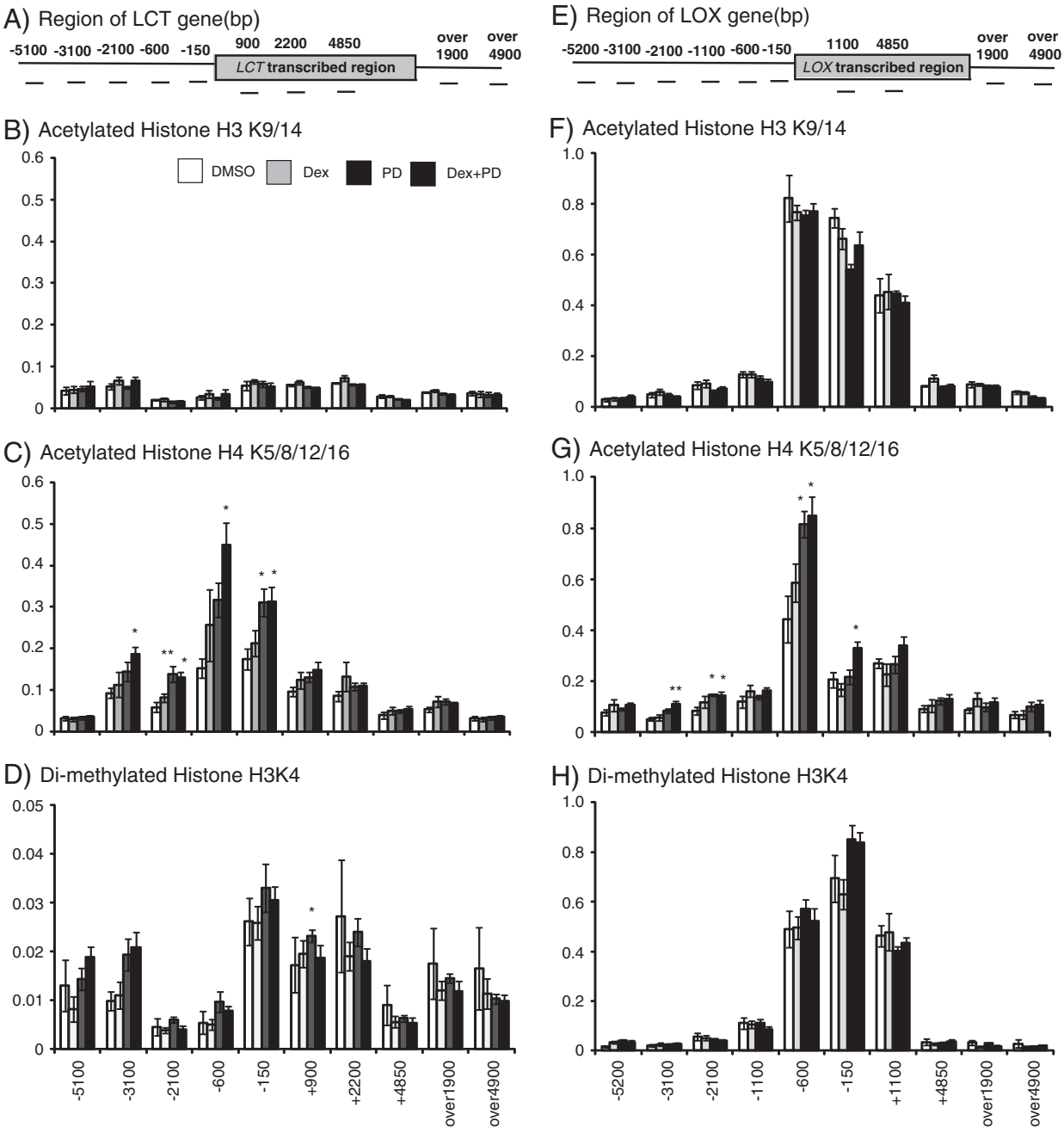

Fig. 3. Histone modifications around the *LCT* and *LOX* genes in response to treatment with Dex and/or PD in Caco-2 cells. A–D) *LCT*. E–H) *LOX*. Samples were collected from Caco-2 cells treated with Dex and/or PD for ChIP assays. ChIP signals were detected by real-time PCR following immunoprecipitation of the indicated nuclear proteins bound to the *LCT* and *LOX* genes, and were normalized to input signals. The abscissa denotes the region on the *LCT* and *LOX* genes relative to the transcription initiation site. *, ** indicate significant differences compared with control cells (DMSO) at $P < 0.05$ and $P < 0.01$, respectively.

H3 at K9 and K14 [33]. GCN5 enhances the mRNA transcriptional elongation step by acetylating histones around the transcribed region [33,34]. The acetylated histone on the transcribed region recruits an acetylated histone binding protein, bromodomain protein (brd) 4, and the Brd4-associated complex positive-transcription elongation factor b (P-TEFb) to the transcribed region [12]. Indeed, our previous study has demonstrated that GCN5 is recruited around the transcription initiation site and the transcribed region of intestinal genes such as *Si* and *Slc5a1* in response to a high-carbohydrate diet in rats [35]. This study also found that acetylation of histone H3 at K9/K14 was higher in regions near the transcription initiation site, or in the transcribed region, than in the promoter and enhancer regions, whereas histone H4 tends to be higher in the promoter and enhancer regions. Thus, histone H4 in the promoter and enhancer regions may be acetylated by GR-associated HATs such as CBP, p300 and PCAF, whereas histone H3 regions near the transcription initiation site, and the transcribed region, may be acetylated by GCN5. We therefore speculate that acetylated histone H4 in the promoter/enhancer region of the up-regulated genes recruits the GR-inducible transcription initiation complex, whereas histone H3 acetylated at K9/K14 in the transcribed region of the up-regulated genes recruits transcription elongation complexes including P-TEFb. This should be the focus of further studies.

It should be noted that expression of the *SI* gene was not enhanced by Dex in Caco-2 cells, although it has been reported that p44/42 MAPK inhibition enhanced *SI* gene expression in sub-confluent Caco-2 cells [36]. It was demonstrated that jejunal *Si* gene expression were

enhanced by injection of a glucocorticoid hormone agonist hydrocortisone in suckling rats [2]. It has been reported that the GR is bound to the promoter/enhancer region of the *Slc2a5* gene in the jejunum of rats during the suckling–weaning transient period [1] and also to the promoter/enhancer region of the *SLC2A5* gene in Caco-2 cells treated with Dex and PD [3]. However, GR binding around the gene encoding *SI* in Caco-2 cells and in suckling–weaning rats has not yet been reported. Thus, it is still unclear whether jejunal induction of the *Si* gene by glucocorticoid hormone during the suckling–weaning period in rats is directly regulated by the GR. Whether the GR binds to the promoter/enhancer region of *Si* in the jejunum of suckling–weaning rats needs to be examined. Whether non-responsiveness of *SI* gene expression to co-treatment with Dex and PD in Caco-2 cells is due to the fact that the GR is not bound to the promoter/enhancer region of the gene also needs to be examined.

It should be noted that we used a colon cancer cell line Caco-2 as a small intestinal absorptive cell model. Many previous studies have demonstrated that several small intestinal genes such as *SI*, *LCT* and *SLC2A5* are expressed in the Caco-2 cells [3,37,38]. This is partially because the Caco-2 cells express a highly caudal type homeobox (Cdx)-2, which is an important transcriptional factor for differentiation of absorptive cells in the small intestine [38]. These insights in previous reports indicate that Caco-2 cells have partial characteristics of small intestinal absorptive cells. In this study, we tried to treat Caco-2 cells with glucocorticoid hormone and p44/42 MAPK inhibition signals, which are important for small intestinal differentiation. We found that co-treatment with Dex and PD induced expression of the *SLC2A5* and *LCT* genes in the Caco-2 cells. Thus, co-treatment with Dex and PD may induce partial differentiation of small intestinal absorptive cells in Caco-2 cells. In our studies, it was shown that acetylation of histone H3 and H4 around *SLC2A5* [31] and *LCT* genes in this study was enhanced by co-treatment with Dex and PD in Caco-2 cells. In addition, a previous study has demonstrated that *Slc2a5* mRNA and histone H3 K9/14 acetylation in rats was induced during the suckling–weaning transient period, which is a period when the serum concentration of a glucocorticoid hormone corticosterone increased and the provision of epidermal growth factor, activator of p44/42 MAPK, from their mother, decreased [1]. These results indicate that induction of *SLC2A5* in Caco-2 cells induced by Dex and PD seems to be correlated with a phenomenon in the jejunum during the suckling–weaning transient period in rats. It should be examined whether other genes up-regulated by Dex and/or PD in Caco-2 cells are expressed in the small intestinal absorptive cells, and the expression and histone acetylation around the genes are induced by glucocorticoid hormone injection during the suckling–weaning transient period in the small intestine of rats.

In conclusion, we have demonstrated in Caco-2 cells that the histone code of each gene induced by co-treatment with a glucocorticoid hormone agonist and a p44/42 MAPK inhibitor is specific and could be related to different transcriptional regulations.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagen.2013.10.026.

Acknowledgements

This work was supported by fellowships from a Grant-in-Aid for JSPS Researcher Fellowship for Young Scientists (23-9920), a Grant-in-Aid for Young Scientists (22680054) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT), the Global COE Program, the Center of Excellence for Innovation in Human Health Sciences of MEXT, and a grant from the Hoansha Foundation.

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