Mitogen-induced distinct epialleles are phosphorylated at either H3S10 or H3S28, depending on H3K27 acetylation

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ABSTRACT Stimulation of the MAPK pathway results in mitogen- and stress-activated protein kinase 1/2 (MSK1/2)-catalyzed phosphorylation of histone H3 at serine 10 or 28 and expression of immediate-early (IE) genes. In 10T1/2 mouse fibroblasts, phosphorylation of H3S10 and H3S28 occurs on different H3 molecules and in different nuclear regions. Similarly, we show that mitogen-induced H3S10 and H3S28 phosphorylation occurs in separate pools in human primary fibroblasts. High-resolution imaging studies on both cell types reveal that H3S10 and H3S28 phosphorylation events can be induced in a single cell but on different alleles, giving rise to H3S10ph and H3S28ph epialleles. Coimmunoprecipitation and inhibition studies demonstrate that CBP/p300-mediated H3K27 acetylation is required for MSK1/2 to phosphorylate S28. Although the K9ac and S10ph marks coexist on H3, S10 phosphorylation is not dependent on K9 acetylation by PCAF. We propose that random targeting of H3S10 or H3S28 results from the stochastic acetylation of H3 by CBP/p300 or PCAF, a process comparable to transcriptional bursting causing temporary allelic imbalance. In 10T1/2 cells expressing Jun, at least two of three alleles per cell were induced, a sign of high expression level. The redundant roles of H3S10ph and H3S28ph might enable rapid and efficient IE gene induction.

INTRODUCTION

In eukaryotes, DNA is wrapped around a histone octamer composed of two H2A/H2B dimers, two H3, and two H4 to form a nucleosome, the first level of chromatin organization. The nucleosome is not only a packaging unit; it is also a signaling module that enables a quick transcriptional response to external and internal stimuli. Each of the core histones is subject to an array of dynamic posttranslational modifications (PTMs; Huang et al., 2014), including acetylation or methylation of lysine and phosphorylation of serine. PTMs or marks are recognized by reader proteins via specific domains. In turn, readers recruit chromatin modifiers and remodelers, altering nucleosome structure and function (Bannister and Kouzarides, 2011; Turner, 2014).

On mitogenic or stress stimuli, the mitogen- and stress-activated protein kinases (MSKs) 1 and 2 (MSK1 and MSK2), activated by the ERK or p38 mitogen-activated protein kinase (MAPK) pathways, mediate the rapid and transient H3 phosphorylation at serine 10 (H3S10ph) and serine 28 (H3S28ph) in the regulatory regions of responsive genes (Soloaga et al., 2003). The 14-3-3 proteins bind to H3S10ph and H3S28ph (Macdonald et al., 2005; Winter et al., 2008b) and recruit BRG1, the ATPase subunit of the SWI/SNF remodeler (Drobic et al., 2010). After remodeling, transcription factors such as JUN are able to bind to regulatory regions of immediate-early (IE) genes, ensuring the onset of transcription (Drobic et al., 2010). Chromatin immunoprecipitation (ChIP) assays provide direct
Mitogen-induced H3 phosphorylation in human primary fibroblasts. Serum-starved CCD-1070Sk cells exposed to (A) 100 nM TPA or (C) 30 ng/ml EGF for the indicated times were fixed and subjected to immunofluorescence labeling with anti-H3S10ph and anti-H3S28ph antibodies. Cells were counterstained with DAPI. The distribution of H3S10ph and H3S28ph was visualized by fluorescence microscopy. (B, D) Overall signal intensities within the nucleus were determined using FIJI. The data were then exported as Excel files for statistical analysis and graphical representation.

Mitogen-induced H3S10ph and H3S28ph exist in separate pools in human primary fibroblasts

In mouse 10T1/2 fibroblast cells, mitogen-induced S10 and S28 phosphorylation events happen on distinct populations of H3 (Dunn and Davie, 2005; Dyson et al., 2005). To verify that this occurrence is physiologically relevant, we analyzed the distribution of these two modifications in human primary fibroblast CCD-1070Sk cells. First, we analyzed the temporal induction pattern of H3S10 and H3S28 phosphorylation upon treatment of serum-starved cells with 100 nM 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or 30 ng/ml epidermal growth factor (EGF). This was done by fluorescence microscopy after indirect immunofluorescence labeling of cells grown and fixed on coverslips. Figure 1 shows that phosphorylation of both S10 and S28 was induced in response to mitogenic stimulation of CCD-1070Sk cells. It is also noticeable that the TPA-induced phosphorylation of S10 and S28 was sustained for a longer period of time than the EGF-induced phosphorylation.

We quantified the overall changes of this stimulation by measuring the overall intensities within the nucleus using the open-source platform FIJI (Schindelin et al., 2012). Phosphorylation of H3S10ph and H3S28ph peaked 1 h after stimulation with both EGF and TPA. Stimulation with EGF resulted in a 2.5-fold increase of H3S10 phosphorylation and a 2.7-fold increase of H3S28ph phosphorylation. However, we observed a stronger response of TPA stimulation of serum-starved CCD-1070Sk cells, with a 3.8-fold increase in H3S10ph modification. Of interest, the TPA-induced phosphorylation of H3S28ph did not differ from that of EGF stimulated cells (2.7-fold increase).

Besides the changes in the phosphorylation of H3S10 and H3S28, we noticed an effect on the nuclear area, depending on the stimulant used (Supplemental Figure S1). Whereas the EGF-stimulation of serum-starved CCD-1070Sk cells did not result in a change of the nuclear area, TPA stimulation led to reduction by >20%.

Next we visualized the relative spatial nuclear distributions of H3S10ph and H3S28ph after 60 min of TPA stimulation of serum-starved CCD-1070Sk cells by fluorescence microscopy and image deconvolution. The merged images show that H3S10ph and H3S28ph have distinct subnuclear sites (Figure 2). The measurement of the H3S10ph and H3S28ph in a three-dimensional space showed that only a small percentile of signals was colocalizing. Of 267 H3S10ph and 426 H3S28ph signals, only 31 signals showed a
Transcribed H3S10ph and H3S28ph epialleles coexist in a single TPA-treated fibroblast cell

The observed absence of H3S10ph and H3S28ph colocalization in 10T1/2 and CCD-1070Sk nuclei can be explained in two different ways. MSK1/2 would phosphorylate H3S10 on one allele and H3S28 on another allele within a cell, or MSK1/2 would phosphorylate exclusively H3S10 or H3S28 on both alleles in a single cell. To distinguish between the two scenarios, we used DNA immuno-FISH. We first explored the nucleosomal response in serum-starved mouse fibroblast cell downstream of MAPK signaling. DNA immuno-FISH to detect Jun loci with H3S10ph/H3S28ph staining in 10T1/2 cells, which were serum starved and TPA treated for 30 min. Bar, 5 μm. Enlargements of each area with a Jun signal are shown.

To determine whether PTGS2 colocalizes with H3S10ph and/or H3S28ph, we examined 30 nuclei using TeloView. Similar to the colocalization study involving Jun, H3S10ph, and H3S28ph, we measured colocalization of PTGS2 with predominately H3S10ph (27%). Seven percent of the PTGS2 signals colocalized with H3S28ph, and 8% of the FISH signals showed simultaneous colocalization with the immunostained H3S10ph and H3S28ph. Analyzing the distribution of colocalization on nuclear level, we found that ~47% of all examined nuclei showed PTGS2/H3S10ph colocalization. Ten percent of the nuclei showed colocalization of PTGS2/H3S28ph. Approximately 30% of the nuclei showed simultaneous colocalization of H3S10ph with PTGS2 or H3S28ph with PTGS2, and 13% of the examined nuclei did not show any colocalization of PTGS2 and H3S10ph/H3S28ph.

To address the question of whether H3S10ph and H3S28ph epialleles were both transcribed, we performed sequential ChIP assays to determine whether each modification would occur simultaneously with the occupancy at the 5′ end of the gene by the elongation-competent RNA polymerase II, which has its CTD phosphorylated at serine 2 (RNAPII(S2ph)). To be able to communoprecipitate the elongating RNAPII with the promoter upstream regions marked by H3S10ph or H3S28ph, we sonicated the cells to a lesser extent

FIGURE 2: Relative intranuclear localization of H3S10ph and H3S28ph in TPA-treated human primary fibroblasts. Serum-starved CCD-1070Sk cells treated with 100 nM TPA for 60 min were subjected to immunofluorescence staining using anti-H3S10ph and anti-H3S28ph antibodies. Cells were digitally imaged. Boxed areas of merged image are shown enlarged in insets.

FIGURE 3: H3S10ph and H3S28ph epialleles coexist in a single mouse fibroblast cell downstream of MAPK signaling. DNA immuno-FISH to detect Jun loci with H3S10ph/H3S28ph staining in 10T1/2 cells, which were serum starved and TPA treated for 30 min. Bar, 5 μm. Enlargements of each area with a Jun signal are shown.
than in standard ChIP assays, generating an average fragment size of 800 base pairs. Figure 5 shows that RNAPII S2ph was associated with H3S10ph or H3S28ph at the 5’ end of the FOS and PTGS2 genes upon EGF treatment of the CCD-1070Sk cells.

In conclusion, mitogen-induced H3S10ph and H3S28ph epialleles were both actively transcribed in primary fibroblast cells.

Phosphorylation of S28 cooccurs with K27 acetylation on H3, whereas phosphorylation of S10 cooccurs with K9 acetylation

It was previously shown that CREB-binding protein (CBP)/p300 (also known as KAT3A/3B) and GCN5/p300/CBP-associated factor (PCAF; also known as KAT3A/3B) and GCN5/p300/CBP-associated factor (PCAF; also known as KAT3A/3B) were associated with MSK1 (Janknecht, 2003; Drobic et al., 2010; Sundar et al., 2012). Moreover, MSK1 and PCAF were corecruited to the regulatory regions of IE genes upon their induction by TPA (Drobic et al., 2010). PCAF mediates H3K9 acetylation, whereas CBP/p300 acetylate H3K27 (Jin et al., 2011). H3 acetylated at lysine 27 (H3K27ac) is the signature of active enhancers and promoters (Heintzman et al., 2009; Creighton et al., 2010; Rada-Iglesias et al., 2011), whereas H3K4me1 is implemented by MLL3/4 at poised or active enhancers (Heintzman et al., 2007; Herz et al., 2012; Hu et al., 2013). To explore the cross-talk between these marks deposited at regulatory regions by chromatin-modifier complexes, we performed coIP assays on SDS-treated nuclear lysates of serum-starved CCD-1070Sk cells treated with 30 ng/ml EGF. Equal amounts of input and immunoprecipitated DNA were quantified by real-time quantitative PCR. The enrichment values of the 5’ end of the FOS and PTGS2 genes are the mean of three independent experiments, and the error bars represent the SD.

Mitogen-induced H3S28 phosphorylation depends on K27 acetylation

Analysis of DNA immuno-FISH images (as shown in Figure 3) from serum-starved and TPA-induced 10T1/2 cells revealed a preferential phosphorylation of H3S10 over H3S28 at Jun alleles. To determine whether histone hyperacetylation could shift this distribution, we incubated serum-starved 10T1/2 cells with the HDAC inhibitor trichostatin A (TSA) for 30 min before TPA stimulation. Histone was acid-extracted and analyzed by immunoblotting (Figure 7A). After HDAC inhibition, a clear increase in H3S28ph, but not H3S10ph, was observed simultaneously with H3K9 and H3K27 hyperacetylation. Moreover, even when acetylation levels were increased, S10ph and S28ph did not cooccur on H3 tails, as H3S28ph did not communoprecipitate with H3S10ph (Supplemental Figure S8).

Results in Figure 7A suggested that H3K27ac might direct S28 phosphorylation by MSK1/2. To determine whether this was the case, we treated serum-starved 10T1/2 cells with C646 before TPA stimulation. C646 is a potent and selective inhibitor of p300/CBP (Bowers et al., 2010; Josefowicz et al., 2016), the KATs acetylating K27 (Jin et al., 2011). Figure 7B shows that K27ac level, and, even more, so S28ph level were decreased when cells were treated with C646, whereas S10ph and K9ac levels were not affected. Thus, K27ac directs MSK1/2 to phosphorylate H3 on S28.

We next determined whether H3K27ac was increased at the regulatory region of the IE Fosl1 gene when serum-starved 10T1/2 cells were stimulated with TPA for 30 min. ChIP assays showed that H3K27ac levels were increased at the upstream promoter (−1113, −187) and internal enhancer (+989) regions but not further downstream (Figure 8; Drobic et al., 2010). Inhibition of p300/CBP with C646 before addition of TPA to the 10T1/2 serum-starved cells prevented the increase in H3K27ac at the regulatory regions of the Fosl1 gene (Figure 8B).

We showed previously that inhibition of MSK1/2 activity with H89 prevented the recruitment of the MSK1/2 complex to the regulatory regions of IE genes (Drobic et al., 2010). Because MSK, which is associated with CBP/p300, stimulates the transactivation activity of CBP (Janknecht, 2003), we examined whether MSK1/2 inhibition prevented the TPA-responsive increases in
Figure 6: Cooccurrence of S28 phosphorylation with K27 acetylation or S10 phosphorylation with K9 acetylation on H3, but not vice versa. Nuclear extracts from serum-starved CCD-1070Sk cells stimulated with 30 ng/ml EGF were incubated with anti-H3S28ph, anti-H3K27ac, or control nonspecific IgG antibodies. Input and immunoprecipitated fractions (IP and IgG) were resolved by SDS–15%–PAGE and immunoblotted with the indicated antibodies.

Discussion

Analysis of the nucleosomal response at a single-cell level showed that mitogen induction of IE genes happened randomly on H3S10ph and/or H3S28ph epialleles in individual fibroblast cells from immortalized or finite cell lines. Our results showed that CBP/p300-mediated K27 acetylation was a signal for MSK1/2 to phosphorylate S28. On the other hand, H3 acetylated on K9 by PCAF would be phosphorylated on S10 because K9ac and S28ph did not coexist on a single H3 tail. However, S10 phosphorylation did not appear to depend on K9 acetylation because the increase in K9ac after HDAC inhibition was not paralleled by an increase in S10ph. In fact, a previous study using the MSK1/2 inhibitor H89 showed that H3K9ac at the regulatory regions of Fosl1. Figure 8C shows that MSK1/2 inhibition prevented the TPA-induced increase in H3K27ac. Together, these results provide evidence that MSK-CBP/p300 acetyltransferases H3K27ac and H3S28ph on the H3 tail of nucleosomes located in the regulatory regions of TPA-induced IE genes.

Figure 7: TPA-induced H3S28 phosphorylation depends on K27 acetylation. Histones acid extracted from serum-starved, TPA-stimulated 10T1/2 cells were resolved by 15% SDS–PAGE and immunoblotted with the indicated antibodies. Before blotting, membranes were stained with Ponceau-S. Before 30-min TPA stimulation, cells were treated or not with (A) TSA or (B) C646.
MATERIALS AND METHODS

Cell culture
Mouse fibroblast 10T1/2 cells (American Type Culture Collection [ATCC] CCL-226) and human primary fibroblast CCD-1070Sk cells (ATCC CRL-2091) were grown at 37°C in a humidified atmosphere containing 5% CO₂ in α-MEM and MEM (Life Technologies, Grand Island, NY), respectively, supplemented with 10% (vol/vol) fetal bovine serum (FBS) and 1% antibiotic-antimycotic (Life Technologies).

Indirect immunolocalization
Indirect immunolocalization was performed as described previously (He and Davie, 2006), using rabbit polyclonal antibodies against H3S10ph (1:250 dilution; Santa Cruz Biotechnology, Dallas, TX) and rat monoclonal antibodies against H3S28ph (1:250 dilution; Sigma-Aldrich). Peptide Competition assay was done by Standard Novus protocol (www.novusbio.com/support/support-by-application/peptide-competition/protocol.html) using H3 peptides from Abcam (human histone H3 (phospho S10) peptide [ab11477]; human histone H3 (phospho S28) peptide [ab14793; Supplemental Figure S9]). Alexa Fluor 488 donkey anti-rabbit immunoglobulin G (lgG; Molecular Probes, Eugene, OR) and Texas red- or AMCA-conjugated donkey anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) were used as secondary antibodies. DNA was counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Control experiments including epitope peptide blocking or primary antibody omission demonstrated the specificity of the antibodies used.

Quantification of fluorescence signals
For the analysis of the overall signal intensities within the nucleus, we used FIJI, an open source platform (Schindelin et al., 2012). For the filter, we used Gaussian blur at 10 for the counter stain (DAPI) throughout the experiments. The thresholds were adjusted to match the counterstained area (DAPI). The overall intensities, as well as the area, were measured for each channel. The data were then exported as Excel files for statistical analysis.

DNA immuno-FISH
DNA FISH assays were performed as described (Lomvardas et al., 2006). BAC clones RP24-28281B and RP11-1122M4, carrying the mouse Jun and human PTGS2 loci, respectively (BACPAC Resources, CHORI, Oakland, CA), were labeled with Digoxigenin (DIG) by nick translation (Roche Diagnostics, Indianapolis, IN) to generate probes for FISH. 10T1/2 cells were grown on coverslips, serum starved for 24 h, and treated with 100 nM TPA for 30 min; CCD-1070Sk cells were serum starved for 48 h and treated with 100 nM TPA for 60 min. After indirect immunolocalization of H3S10ph and H3S28ph, the antigen–antibody complexes were cross-linked with 1 mM dithiobis(succinimidyl propionate) (Thermo Fisher Scientific, Waltham, MA) for 30 min at room temperature. The genomic DNA was denatured using 0.1 N NaOH for 2 min and hybridized to the denatured probes (72°C for 10 min) overnight at 37°C. After extensive washing steps, the probe was detected using primary anti-DIG antibody (Roche Diagnostics) and DyLight 594-conjugated secondary antibody (Jackson ImmunoResearch Laboratories).
Measurement of colocalizing signals
For the colocalization studies, we used Teloview (Vermolen et al., 2005). This program allows the three-dimensional measurement of the signal coordinates \((x, y, z)\) from their center of gravity. The measurements were performed for H3S10ph and H3S28ph, as well as for H3S10ph, H3S28ph, and the FISH-signals Jun and PTGS2. Thirty nuclei were analyzed for each immuno-FISH experiment. Matching coordinates were classified as colocalizing signals.

ChIP and sequential ChIP assays
High-resolution ChIP using micrococcal nuclease digestion and sequential ChIP assays were performed as previously described (Drobic et al., 2010), except that cross-linked chromatin was sheared to ~800–base pair fragments by sonication. Antibodies to H3S10ph (Santa Cruz Biotechnology), H3S28ph (Sigma-Aldrich), RNAPII S2ph (Abcam, Cambridge, MA), or an isotype-matched nonrelated IgG as negative control (EMD Millipore) were used. Primers used to amplify UPRs were as follows: FOS forward, 5′-CCCCCTT-ACACAGGATGTC3′- and reverse, 5′-CCCCCAAGTTAGGGGTTTC-3′; and PTGS2 forward, 5′-AGGAAGGAGGGGATCAGAC-3′, and reverse, 5′-AAAAATCGGAAACCCAGGAAG-3′. Mouse Fos/F primers are described in Drobic et al. (2010). Enrichment values are the mean of three independent experiments. Error bars indicate SD.

Histone extraction and immunoblot analysis
Histones were acid extracted from 10T1/2 and CCD-1070K cells, resolved by 15% SDS-PAGE, and stained immunologically with anti-H3 (Abcam), anti-H3S10ph (Santa Cruz Biotechnology), anti-H3S28ph (Sigma-Aldrich), anti-H3K27ac (Millipore), anti-H3K9ac (Abcam), or an isotype-matched nonrelated IgG as negative control (EMD Millipore) were used. Primers used to amplify UPRs were as follows: FOS forward, 5′-CCCCCTT-ACACAGGATGTC3′- and reverse, 5′-CCCCCAAGTTAGGGGTTTC-3′; and PTGS2 forward, 5′-AGGAAGGAGGGGATCAGAC-3′, and reverse, 5′-AAAAATCGGAAACCCAGGAAG-3′. Mouse Fos/F primers are described in Drobic et al. (2010). Enrichment values are the mean of three independent experiments. Error bars indicate SD.

Histone communoprecipitation
Cells were resuspended in cell lysis buffer (5 mM 1,4-piperazinediethanesulfonic acid, pH 8.0, 85 mM KCl, 0.5% NP-40) containing phosphatase and protease inhibitor cocktails, and a 10% SDS–PAGE and subjected to immunoblot analysis. Three CCD-1070K cells) biological repeats were performed.

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REFERENCES
Bannister AJ, Kouzarides T (2011). Regulation of chromatin by histone modifications. Cell Res 21, 381–395.
Borel C, Ferreira PG, Santer F, Delaneau O, Fort A, Popadyn KY, Gariei M, Falcionnet E, Ribaux P, Guipponi M, et al. (2015). Biased allelic expression in human primary fibroblast single cells. Am J Hum Genet 96, 70–80.
Bowers EM, Yan G, Mukherjee C, Orly A, Wang L, Holbert MA, Crump NT, Hazzalin CA, Liszczak G, Yuan H, et al. (2010). Virtual ligand screening of the p300/CBP histone acetyltransferase: identification of a selective small molecule inhibitor. Chem Biol 17, 471–482.
Chadee DN, Hendzel MJ, Tyulipski CP, Alls CD, Bazett-Jones DP, Wright JA, Davie JR (1999). Increased Ser-10 phosphorylation of histone H3 in mitogen-stimulated and oncogene-transformed mouse fibroblasts. J Biol Chem 274, 24914–24920.
Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Alls CD (2000). Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. Mol Cell 5, 905–915.
Clayton AL, Rose S, Barratt MJ, Mahadevan LC (2000). Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation. EMBO J 19, 3714–3726.
Creighton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, Hanna J, Lodato MA, Frampton GM, Sharp PA, et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci USA 107, 21931–21936.
Deng Q, Ramskold D, Reinius B, Sandberg R (2011). Single-cell RNA-seq reveals dynamic, random monoallelic gene expression in mammalian cells. Science 343, 193–196.
Drobic B, Perez-Cadahia B, Yu J, Kung SK, Davie JR (2010). Promoter chromatin remodeling of immediate-early genes is mediated through H3 phosphorylation at either serine 28 or 10 by the MSK1 multi-protein complex. Nucleic Acids Res 38, 3196–3208.
Dunn KL, Davie JR (2005). Stimulation of the Ras-MAPK pathway leads to independent phosphorylation of histone H3 on serine 10 and 28. Oncogene 24, 3492–3502.
Dunn KL, He S, Wark L, Delceupe GP, Sun JM, Yu Chen H, Mai S, Davie JR (2009). Increased genomic instability and altered chromosomal protein phosphorylation timing in HRAS-transformed mouse fibroblasts. Genes Chromosomes Cancer 48, 397–409.
Dyson MH, Thomson S, Inagaki M, Goto H, Arthur SJ, Nightengale K, Ilora FJ, Mahadevan LC (2005). MAP kinase-mediated phosphorylation of distinct pools of histone H3 at S10 or S28 via mitogen- and stress-activated kinase 1/2. J Cell Sci 118, 2247–2259.
