Aryl Hydrocarbon Receptor Ligands of Widely Different Toxic Equivalency Factors Induce Similar Histone Marks in Target Gene Chromatin

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Posttranslational histone modifications are a critical regulatory mechanism of gene transcription. Previous studies from our laboratory have shown that contingent on binding to its cognate promoter motifs in the Cyp1a1 gene, activation of the aryl hydrocarbon receptor (AHR) by benzo[a]pyrene (BaP) treatment induces histone modifications in the Cyp1a1 promoter that are required for activation of gene transcription. Here, we have studied different AHR ligands, including polychlorinated biphenyls (PCBs) of different toxic equivalency factors (TEF), to determine whether changes in histone modifications are linked to different levels of Cyp1a1 expression or dependent on AHR-ligand affinity. We find that all ligands lead to the same pattern of histone modifications in a relationship that parallels the strength of their AHR-ligand affinity. Thus, whereas PCB126 (TEF 0.1), 3-methylcholanthrene, β-naphthoflavone, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) initiate a pattern of histone marks similar to those induced by BaP, PCB77 (TEF 0.0001) causes a lower level of change in the same marks and requires a longer activation time than PCB126, BaP, or TCDD. In contrast, the non–dioxin-like PCB153 recruits AHR to the Cyp1a1 enhancer causing a displacement of enhancer-associated histone H3 but does not cause the other observed histone mark changes nor does it induce transcription. These results indicate that AHR recruitment to the promoter is not sufficient to induce the histone modifications needed to activate gene expression and show that there is a good correlation between the regulatory chromatin changes associated with ligand-induced AHR target gene transcription and the resultant toxicity of the ligand.

Key Words: Ah receptor; dioxin-like compounds; PCBs; TEF; histone marks.
The promoter of the mouse Cyp1a1 gene has a 5′ transcriptional regulatory region, which we term hereafter the “proximal promoter,” located directly upstream of the transcription start site. The proximal promoter has no AHRE motifs but has binding motifs for several other transcription factors, including TATA-binding proteins (Jones and Whitlock, 1990). Several hundred base pairs further upstream of the proximal promoter, there is a distal “enhancer” region where the AHREs are clustered (Neuhold et al., 1989; Yanagida et al., 1990). Work in our laboratory established that, after AHR-mediated activation by a ligand such as benzo[a]pyrene (BaP), recruitment of RNA polymerase II to the promoter region of the Cyp1a1 gene requires the AHR-dependent displacement of an HDAC1/DNMT1 complex associated with the proximal promoter. Displacement of the complex allows for the initiation of posttranslational histone modifications in the nucleosomes associated with the proximal promoter region and the distal enhancer region of the gene (Schnekenburger et al., 2007a, 2007b). AHR recruitment in response to BaP therefore leads to a patterned change in histone modifications that requires displacement of the HDAC1/DNMT1 complex to take place and is associated with active transcription of the Cyp1a1 gene (Schnekenburger et al., 2007a, 2007b). The pattern of histone modifications that corresponds to active transcription after BaP treatment includes changes both at the enhancer domain where the AHR binds, and at the promoter, where the basal transcription machinery binds (Schnekenburger et al., 2007a, 2007b). These modifications include a large increase of Ser-10 phosphorylation in histone H3 and of Lys-16 acetylation in histone H4 in the Cyp1a1 enhancer domain and an increase of Lys-4 trimethylation in histone H3, Lys-14 acetylation in histone H3 and a decrease of Lys-4 dimethylation in histone H3 in the proximal promoter region (Schnekenburger et al., 2007a, 2007b). This pattern of histone modifications is in line with what is known as the “histone-code” for activation of transcription (Hon et al., 2009; Jenuwien and Allis, 2001; Zippo et al., 2009).

The work summarized above did not address the question of whether changes in histone modification occurring at the Cyp1a1 promoter are dependent on the affinity of AHR for BaP or whether other AHR ligands, including the very high affinity halogenated ligand TCDD, would cause the same pattern of histone modifications. Because PCBs are major environmental contaminants that differ in their relative toxicities, we wished to examine how the relative toxicities and AHR affinities of different coplanar PCBs affect the previously demonstrated pattern of epigenetic changes associated with gene activation. We set out to determine if the histone mark changes upstream of AHR target genes, which we had previously demonstrated to be associated with activation of transcription, are similarly affected by the AHR ligands affinity for the receptor. To do so, we compared three PCBs with different toxic equivalency factors (TEFs) and one non–dioxin-like PCB, to dioxin. TEFs are order of magnitude estimates of a DLC’s relative toxicity to TCDD; they were derived by the World Health Organization after scientific review of all the available in vivo and in vitro literature for each DLC AHR-mediated effects, to be used in risk characterization and assessment (Van den Berg et al., 1998, 2006). Here, we show that PCB77, with a TEF of 10⁻⁴, causes a different patterns of histone modifications than PCB 169, with a TEF of 10⁻², and PCB126, with a TEF of 10⁻¹. Likely to be responsible for this difference, PCB77 causes a low level of AHR recruitment to the gene enhancer that lasts for a longer period of time than activation by TCDD, PCB126, or PCB169.
NP-40, 1% deoxycholic acid). Immune complexes were eluted from the beads of protein A-agarose or protein G-agarose beads, depending on antibody/C176 H3 (Abcam; ab8580), or Histone 3 (Abcam; ab1791). Immune complexes were remaining 45% of the diluted lysate was similarly incubated with theappropriate nonspecific IgG. Antibodies used for chromatin immunoprecipitation/lysates was used for input, 45% of the diluted lysate was incubated overnight of protein-A agarose beads. Precleared lysates were diluted with 30-s intervals between bursts using a Bioruptor (Diagenode, Danville, NJ). kb by sonication in a crushed ice–water bath with six 30-s bursts of 200 W cell lysis buffer (5mM PIPES [pH 8.0], 85mM KCl, 0.5% NP-40, and the precleared sample was incubated on ice for 20 min. Nucleoli were isolated, and duplicate QRT-PCR was performed to determine the amount of Cyp1a1 mRNA relative to β-actin. Error bars represent SE. The * indicates statistical significance when compared with DMSO (p value < 0.05).

Chromatin immunoprecipitation. Hepa-1 cells were grown and treated as described for 90 min, unless otherwise noted. Following treatment, cells were incubated for 10 min at room temperature in 1% formaldehyde. The cross-linking reaction was quenched with 0.125% glycine for 10 min at room temperature, and the cells were rinsed three times with cold 1× PBS. Cells were scraped from the plate, pelleted by centrifugation, resuspended in cold cell lysis buffer (5mM PIPES [pH 8.0], 85mM KCl, 0.5% NP-40, and 1% protease inhibitor cocktail) (Roche; Indianapolis, IN) and incubated on ice for 10 min. Nucleoli were isolated, resuspended in nuclei lysis buffer (50mM Tris [pH 8.1], 10mM EDTA, 1% SDS, and 1% protease inhibitor cocktail) and incubated on ice for 10 min. Chromatin was sheared to a range of 0.3–0.6 kb by sonication in a crushed ice–water bath with six 30-s bursts of 200 W with 30-s intervals between bursts using a Bioruptor (Diagenode, Danville, NI). Cell debris was removed by centrifugation. Chromatin was precleared with a 50% slurry of protein-A agarose beads. Precleared lysates were diluted three times in dilution buffer (16.7mM Tris [pH 8.1], 167mM NaCl, 1.2mM EDTA, 1.1% Triton-x 100, 0.01% SDS). A 10% aliquot of the precleared lysates was used for input, 45% of the diluted lysate was incubated overnight at 4°C on a rotating platform with the specific antibody of interest, and the remaining 45% of the diluted lysate was similarly incubated with the appropriate nonspecific IgG. Antibodies used for chromatin immunoprecipitation (ChIP) were directed against AHR (Bio-Mol; Sa-210), AcK16-H4 (Upstate; 07-329), pS10-H3 (Millipore; Danvers, MA; 05-817), AcK14-H3 (Millipore; 07-353), 2MeK4-H3 (Abcam; Cambridge, MA; ab7766), 3MeK4-H3 (Abcam; ab65850), or Histone 3 (Abcam; ab1791). Immune complexes were recovered by 2-h incubation on a rotating platform at 4°C with a 50% slurry of protein A-agarose or protein G-agarose beads, depending on antibody specificity. Beads were pelleted by centrifugation and washed twice with dialysis buffer (50mM Tris-HCl [pH 8.0], 2mM EDTA, 0.2% Sarkosyl) and four times with IP wash buffer (100mM Tris-HCl [pH 9.0], 500mM LiCl, 1% NP-40, 1% deoxycholic acid). Immune complexes were eluted from the beads by incubation with elution buffer (50mM NaHCO3, 1% sodium dodecyl sulfate) while mildly vortexing. Elution was repeated and eluates combined. Cross-linking was reversed by increasing the NaCl concentration to 0.3M and incubating overnight at 65°C with RNase A. Proteins were digested with proteinase K at 45°C for 90 min. DNA was purified using QIAquick affinity chromatography columns (Qiagen) and eluted in distilled water. Primers specific for either the AHR-E cluster containing distal enhancer (forward: 5’-AGGCCTTCTTCCACTGCAACCTC-3’; reverse: 5’-TAAGGCGCTC- CATCCTTCTGC-3’) or proximal promoter (forward: 5’-TATCCCG- TATGCTTCTGC-3’; reverse: 5’-CACCCTAAGGTTAAGGGTA-3’) region of the mouse Cyp1a1 gene were used for QRT-PCR. Duplicate ChIPs were performed at least twice, and antibodies from the same lot were used for the replicates.

QRT-PCR analysis. QRT-PCR was performed at least in duplicate in a reaction mixture containing 1× SYBR green PCR master mix (Applied Biosystems; Carlsbad, CA) and 0.1μM of each primer. Samples were heated to 95°C followed by 35 cycles of a denaturing step at 95°C for 15 s and an annealing/elongation step of 60°C for 60 s using an ABI 7500 real-time PCR system (Applied Biosystems). Single product formation was confirmed by melting curve analysis after amplification. Analysis of results was performed using sequence detection software (SDS software version 1.3.1; Applied Biosystems).

Data analyses. The ΔCt for each sample was determined using the mean cycle threshold (Ct) of replicates from the input DNA, to normalize ChIP assay results, or the β-actin signal, to normalize gene expression assays. ΔΔCt values were determined by subtracting the ΔCt of control from the corresponding experimental ΔCt. The resulting values were converted to fold changes over control by raising 2 to the power of −ΔΔCt.

RESULTS

Different AHR Ligands Induce Similar Histone Modifications

To determine if AHR activation by other AHR ligands induce the same histone marks as those found for BaP, Hepa-1 cells were treated with concentrations of 3MC, BNF, TCDD, and BaP known to induce Cyp1a1 expression maximally. All ligands substantially induced Cyp1a1 mRNA expression, measured at maximal induction time of 8 h after treatment (Fig. 1). Histone marks were analyzed after a 90-min treatment, the maximal induction time previously determined for AHR (Schnekenburger et al., 2007a, 2007b). At this time point, concomitant with more than 10-times the increase in AHR recruitment to the enhancer region of Cyp1a1, there was a 3-fold increase of AcK16-H4 and greater than 6-fold increase of pS10-H3 at the enhancer in response to all ligands relative to DMSO (Fig. 2A). At the same time, all ligands increased the levels of AcK14-H3 associated with the Cyp1a1 proximal promoter by at least 4-fold over DMSO and each more than doubled the amount of 3MeK4-H3 and decreased by at least half the amount of 2MeK4-H3 associated with the Cyp1a1 promoter, compared with DMSO-treated controls (Fig. 2B). We conclude that all four ligands activated and completed AHR translocation and binding to its cognate sites in the Cyp1a1 enhancer equally well, initiating a common wave of changes in histone marks that resulted in the increase in Cyp1a1 transcription.
Ligand Toxicity Correlates with the Pattern of Histone Modifications

Next, we wished to determine whether the quality or quantity of the histone marks elicited by an AHR ligand would correlate to the toxicity of that particular ligand. For this purpose, we chose to use AHR ligands with different TEFs and determine whether they would cause patterns of histone modifications similar to those induced by TCDD or BaP. The ligands studied were the coplanar DLCs PCB126, PCB169, and PCB77 and the noncoplanar, non-DLC PCB153, with TEFs of $10^{-1}$, $10^{-2}$, $10^{-4}$ and 0, respectively (Van den Berg et al., 2006). Hepa-1 cells were treated with identical toxic equivalent (TEQ) levels of all three DLCs, TCDD, PCB126, and PCB77, and a concentration-response curve was determined using four concentrations of each compound with equivalent TEQ. The fold induction of Cyp1a1 expression induced by PCB126 did significantly differ from that of an equivalent TEQ of TCDD across all the tested concentrations (Fig. 3A), confirming the equivalency of the chosen concentrations. PCB77 induced greater than 10-fold higher Cyp1a1 expression than a similarly toxic level of TCDD at the lower concentration but at the highest concentration TCDD induced expression was 7-fold higher than PCB77 (Fig. 3A). PCB169 induces high levels of Cyp1a1 expression even at low concentrations (Fig. 3A). As expected from a noncoplanar PCB, the non-DLC PCB153 caused no change in Cyp1a1 mRNA expression across all tested concentrations (Fig. 3A). On the other hand, ChIP assays on Hepa-1 cells treated with PCB169, PCB126, and PCB77 showed significant differences in their effectiveness to promote AHR recruitment to the Cyp1a1 enhancer. PCB126 recruited nearly 20% more AHR than TCDD, whereas PCB77 recruited 60% less (Fig. 3B). PCB169-induced AHR recruitment was higher than TCDD when compared with a similar TEQ at any concentration (Fig. 3B).

In order to determine which histone modifications occurred in response to the dioxin-like PCBs, we treated Hepa-1 cells with two concentrations of each compound differing by one order of magnitude. We treated cells with TCDD at 0.5nM and 5nM or the corresponding toxic equivalent concentrations of PCB77 (500 and 5000nM), PCB126 (5 and 50nM), or PCB169 (50 and 500nM). We also treated cells with the non–dioxin-like PCB153 at concentrations equivalent to that of PCB77 (500 and 5000nM), the dioxin-like PCB with the lowest TEF that we used. All PCB treatments lowered the total amount of histone H3 associated with the distal enhancer to some extent, though a large decrease was only seen when cells were treated with the dioxin-like PCBs (Fig. 4A). When treated with TCDD or the PCB with the next highest TEF, PCB126, the amount of enhancer-associated phosphorylation of Ser10 in histone 3 increased more than 12-fold over control at the lowest tested concentrations and more than 20-fold when treated with the...
higher toxic equivalent concentrations (Fig. 4A). PCB77 and PCB169 caused double the amount of pSer10-H3 to be associated with enhancer after treatment with the lower tested concentration, and when cells were treated with higher concentrations of PCB77 and PCB126, the amount of pSer10-H3 was nearly an order of magnitude higher than control (Fig. 4A). As far as the acetylation of Lys16 in histone H4, another mark previously demonstrated to be associated with AHR-dependent Cyp1a1 induction, PCB126, PCB169, and TCDD caused similar increases at both tested toxic equivalent concentrations, whereas the lower concentration of PCB77 treatment led to more than double the amount of acetylation at this position in the Cyp1a1 enhancer than a similar treatment with TCDD (Fig. 4A). The higher dose of PCB77 lead to an increase of acetylated histone 4 that was also higher than that of cells treated with a similarly toxic concentration of TCDD (Fig. 4A). Interestingly, the non-DLC, noncoplanar PCB153 led to an increase in AHR binding and presumably activation of just greater than 50% that of TCDD, even greater than the level of binding induced by PCB77 (Fig. 4A). This PCB153-induced increase in AHR recruitment was dependent on concentration and caused the

FIG. 4. Different DLCs induce different levels of histone marks depending on their affinity for AHR. Hepa-1 cells were treated with 0.5 or 5nM TCDD, 5 or 50nM PCB126, 50 or 500nM PCB169, 500 or 5000nM PCB77, 500 or 5000nM PCB-153, or vehicle for 90 min. ChIP analyses were performed using antibodies against AHR, histone H3, or specific modified histones. QRT-PCR using specific primers for either the enhancer region (A) or the promoter region (B) of the Cyp1a1 gene was performed to determine the amount of chromatin target associated with each region. The ordinate represents the percent of the total input immunoprecipitated by each specific antibody. Error bars represent SE. An * indicates statistical significance when compared with control (p value < 0.05). A + symbol above a bar indicates statistical significance when compared with the equivalent TEQ of TCDD (p value < 0.05).
Cyp1a1 consistent with its inability to induce (Fig. 4A).

treatment (Fig. 4B). It should be noted that this decrease in 2MeK4-H3 associated with the promoter by an amount PCB126, PCB169, and PCB77 decreased the levels of histone 3 (Fig. 4B). The non–dioxin-like PCB153 also 2MeK4-H3 is concomitant with a loss of promoter-associated displacement of at least a small amount of the histone H3 associated with the enhancer region (Fig. 4A). Despite its apparent effect on AHR binding to its cognate sites and displacement of enhancer-associated monosomes, PCB153 treatment had a minimal effect on phosphorylation of S10-H3 or acetylation of K16-H4 in the enhancer region of Cyp1a1, consistent with its inability to induce Cyp1a1 gene expression (Fig. 4A).

In the proximal promoter domain of the Cyp1a1 gene, PCB126, PCB169, and PCB77 decreased the levels of 2MeK4-H3 associated with the promoter by an amount equivalent to the decrease caused in response to TCDD treatment (Fig. 4B). It should be noted that this decrease in 2MeK4-H3 is concomitant with a loss of promoter-associated histone 3 (Fig. 4B). The non–dioxin-like PCB153 also decreased the amount of dimethylated Lys-4 in H3 associated with the promoter proximal sequences but did so by an amount slightly less than that seen after the treatment with the dioxin-like PCBs, and this decrease was seen in the absence of a loss of promoter associated total histone H3 (Fig. 4B). A concomitant increase in trimethylation of lysine 4 of histone H3 was only detected after PCB77, PCB126, PCB169, and TCDD treatments (Fig. 4B). PCB77 caused an increase in trimethylation of Lys-4 in H3 that was nearly half that caused by TCDD, whereas PCB153 did not cause an increase in 3MeK4-H3 levels in comparison to DMSO at the lower tested concentration and increased 3MeK4-H3 only (Fig. 4B). Acetylation of Lys-14 in histone 3, another mark of modifications triggered by ligand-dependent AHR activation, was evident in the Cyp1a1 proximal promoter domain of TCDD-treated cells and to a lesser extent in PCB126- and PCB169-treated cells (Fig. 4B). PCB77 induced increase in the acetylation of Lys-14 in histone associated with the promoter was less than half that of either PCB126 or PCB169 and nearly one-third that of TCDD-treated cells (Fig. 4B). PCB153, in agreement with its inability to activate transcription showed no increase in AcK14 of histone 3 associated with the Cyp1a1 promoter (Fig. 4B).

**DISCUSSION**

Previous work from our laboratory has established that a pattern of histone modification changes occurs upstream of the Cyp1a1 when mouse cells are treated with the AHR agonist BaP (Schnekenburger et al., 2007a, 2007b). This pattern is dependent on AHR recruitment to the distal enhancer region of the gene and displacement of an HDAC1/DMNT1 complex at the promoter (Schnekenburger et al., 2007a, 2007b). The results of the present study show that the pattern of histone modifications triggered by ligand-dependent AHR activation in the promoter of the Cyp1a1 gene is common to all AHR ligands studied so far, including the various DLCs tested in this work, TCDD, BaP, 3-MC, and βNF. It is reasonable to conclude that this pattern is a conserved signature of gene expression induction by the AHR, as previously hypothesized.
Despite the fact that the different types of AHR agonists tested induce the same pattern of nucleosome modifications, treatment with PCB77, with a low TEF of $10^{-3}$, does not elicit these modifications to the same extent as the stronger AHR agonists, such as PCB126 with a TEF of $10^{-2}$ or TCDD (TEF 1), even after treatment with concentrations of equal TEQ, which induce gene transcription to similar levels. The main difference between high- and low-TEF ligands is the kinetics at which the modifications are established, with the low-TEF ligand being considerably slower than the high-TEF ligands. For example, the higher concentration PCB77 treatment induces a similar level of the transcription-associated histone H4 Lys-16 acetylation in the distal enhancer region as the other dioxin-like treatments, although PCB77 recruits much lower levels of AHR to that region (Figs. 3B and 4B). Also at the distal enhancer, PCB77 treatment leads to an increase of phosphorylation of S10 of histone 3 that is half that of either TCDD or PCB126 but similar in intensity as that of PCB169, which recruited the highest levels of AHR to the enhancer (Figs. 3B and 4A). Similar stark differences in proximal promoter region histone marks are also evident in response to PCB77 treatment. PCB77 showed a decrease in the 2MeK4-H3 mark accompanied by a decrease in total H3 associated with the proximal promoter in much the same way the other dioxin-like treatments did but caused an increase in 3MeK4-H3 and Ack14-H3 that was nearly half that induced by the high-TEF ligands (Fig. 4B). Our data suggest that part of this difference may be due to the kinetics of AHR-ligand binding and the related AHR activation, as PCB77 does not reach maximum AHR activation until after 120 min of treatment, compared with 90 min for both TCDD and BaP (Fig. 5). The histone mark modifications seen in response to PCB77 could be a snapshot of the early steps of chromatin modification that precede transcription, opening up new questions about the temporal–spatial kinetics of histone modification. It is likely that early AHR recruitment leads to histone modifications at the enhancer domain allowing for AHR to come into contact with the proximal promoter region, either by a sliding mechanism or by enabling chromatin looping (Steenland et al., 1997; Tian et al., 2003). This could facilitate later modification of histone marks at the proximal promoter ultimately leading to recruitment of the basal transcription machinery. Whether by a sliding or looping mechanism, this concept would agree with previous data showing that when the HDAC1/DMNT1 complex poised at the proximal promoter nucleosome was cross-linked to the promoter chromatin, AHR binding to the distal enhancer was retained, but its binding to the proximal promoter was blocked as were the changes in histone marks and the subsequent recruitment of RNA pol II associated with transcription (Schnekenburger et al., 2007a, 2007b). Similarly, suppression of the initial histone modifications at the distal enhancer would block the AHR from coming in contact with the proximal promoter and would inhibit the changes of histone marks that allow RNA pol II to be recruited. In this case, PCB77 and PCB126 (or TCDD) treatments would induce similar levels of Cyp1a1 expression albeit PCB77 would reach that level at a slower rate, which would explain the lower levels of histone mark modifications (Fig. 4) and RNA pol II recruitment (Supplementary fig. 1). If this hypothesis were correct, histone mark changes at the enhancer domain, such as acetylation of lysine 16 on histone 4, and loss of the 2MeK4-H3 mark might be early events occurring when levels of activated AHR are still low and function to facilitate subsequent changes. This would suggest that a cascade of changes must take place before Cyp1a1 transcription can proceed, requiring the recruitment of an appropriate amount of AHR to the distal enhancer and altering histone marks at the distal enhancer before changes can occur at the proximal promoter. For the FOSL gene enhancer, it has been shown that pS10-H3 is required to initiate acetylation of K16-H4, and that both are required for FOSL gene expression (Zippo et al., 2009). Therefore, a mechanism requiring first one set of histone mark changes to occur before a second set can be established would not be surprising. Further studies are needed to determine definitively the order in which these changes occur and which changes are necessary for the later changes to happen. It is possible that some of the changes that we observe are not necessary for later changes to take place.

The non-DLC PCB153 induced a low but significant increase of AHR enhancer recruitment without increasing Cyp1a1 expression. Previous work from other groups has shown that PCB153 can function as an antagonist of AHR-induced ethoxyresorufin-O-deethylase (Sanderson et al., 1996) and Cyp1a1 gene transcription (Suh et al., 2003). The mechanism for this antagonism was not well characterized, although it was shown that PCB153 antagonism of AHR activation was dependent on the agonist affinity and concentration, perhaps by direct competition with the agonist for binding (Suh et al., 2003). If PCB153 were to bind to the AHR ligand binding domain, it could induce nuclear translocation of the AHR complex and facilitate binding to its cognate sites in DNA, while simultaneously inhibiting association with other transcriptional cofactors required to complete transcription by assuming an improper tertiary structure or causing steric hindrance. Although PCB153 did recruit AHR to the enhancer of the Cyp1a1 gene, and this recruitment did cause a displacement of histone H3 from the enhancer region, it did not activate Cyp1a1 transcription at any tested concentration, and correspondingly, none of the activating histone marks that accompany transcription changed. However, PCB153 caused a decrease in the mark 2MeK4-H3 without further methylating this same site to the active transcription-associated mark 3MeK4-H3, which indicates that the methylation state of this site could have been changed to either the mono-methyl substitution or to a complete loss of methylation. It may be that recruitment of AHR to the enhancer could displace a histone lysine2...
methyltransferase that maintains the 2MeK4-H3 mark during inactive transcription, or alternatively, that PCB153 treatment activates a histone demethylase, such as a JARID1 family member, that removes the dimethylation mark. However, this loss is not reflective of a complete loss of the monosomes at the promoter region, as the amount of total histone H3 in PCB153 treated is similar to that of control (Fig. 4B). This suggest that the mechanism by which PCB153 removes the dimethylation of lysine 4 of histone 3 may be different than that which results in the loss of this mark by the DLCs, as each DLC significantly decreased the total H3 associated with the promoter region (Fig. 4B).

Our results support the view that AHR activation by ligand is a prerequisite for induction of a wave of chromatin modifications that are required for active transcription. Induction of this wave is not the exclusive effect of exposure to halogenated or polycyclic aromatic hydrocarbons but is the result of AHR activation by all ligands tested so far. The epigenetic regulatory changes associated with induction of AHR target genes by ligands of differing TEFs, particularly those located at the proximal promoter region, show a good correlation to their resulting toxicity and might be useful parameters in the assignment of toxic equivalency.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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The authors declare no conflicts of interest.

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