Tumor Promoter Arsenite Stimulates Histone H3 Phosphoacetylation of Proto-oncogenes c-fos and c-jun Chromatin in Human Diploid Fibroblasts*

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Although epidemiological studies have long established that inorganic arsenic is a potent human carcinogen, the underlying mechanisms are still poorly understood. Recent studies suggest that inorganic arsenic may act as a tumor promoter by perturbing key signaling transduction pathways. We have shown previously that arsenite can potently activate the mitogen-activated protein kinase cascades and induce the expression of proliferation-associated genes, including proto-oncogenes c-jun and c-fos. In order to elucidate further the molecular mechanisms underlying its tumor-promoting properties, we investigated the signaling events involved in arsenite-mediated induction of c-fos and c-jun. We found that induction of both c-fos and c-jun by arsenite can be substantially inhibited by the MEK-selective inhibitor U0126, suggesting that the ERK pathway is critically involved in their up-regulation. Interestingly, arsenite dramatically induced the phosphorylation and acetylation of histone H3 preceding the induction of mRNAs encoding c-fos and c-jun. Finally, chromatin immunoprecipitation assays revealed that arsenite treatment markedly induced the phosphorylation/acetylation of histone H3 associated with the c-fos and c-jun genes through an ERK-dependent pathway. Our results strongly suggest that arsenic-triggered alterations in chromatin structure perturb specific gene transcription, including that of proto-oncogenes c-jun and c-fos, and may thereby contribute to the carcinogenic process.

Arsenite, the inorganic trivalent arsenic compound, is a potent human carcinogen with significant worldwide exposure through natural contamination of food and drinking water. Epidemiological studies have shown that chronic arsenic exposure is associated with an increased incidence of skin, lung, and bladder cancers. At the present arsenic regulatory standard of 50 ppb in drinking water, issued by the United States Environmental Protection Agency in 1982, the cancer risk due to arsenic contamination is estimated to be comparable with that of environmental tobacco smoke or radon in homes. A recent analysis conducted by the National Academy of Sciences indicated that the risk of lung and bladder cancer from arsenic in drinking water is several times higher than that estimated by the Environmental Protection Agency when it developed its 10-ppb standard (projected to go into effect in 2006). According to the new analysis, at the arsenic standard of 10 ppb in drinking water, the combined lifetime risk of lung and bladder cancer is between 30 and 37 per 10,000 people. In fact, ~350,000 individuals in the United States drink water containing arsenic levels higher than 50 ppb. In many developing countries, arsenic contamination in drinking water can be as high as 1,800 ppb and represents a very serious threat to public health.

Although epidemiological studies have long established that arsenite is potently carcinogenic, the underlying mechanisms remain poorly understood. Two plausible models have been proposed to explain the carcinogenic actions of arsenic. According to the first and more controversial model, arsenite would cause genetic abnormalities affecting chromosome structure without directly interacting with DNA. In the second model, arsenite is proposed to act as a tumor promoter. The latter hypothesis is supported by the findings that arsenite can induce anchorage independence in human diploid fibroblasts, enhance cell proliferation in bladder epithelium in mice, and potentiate the mutagenic effects of short wavelength ultraviolet (UV) radiation in cultured cells. Consistent with the notion that arsenite acts on signaling pathways that regulate cell proliferation, a number of proliferation-associated genes, notably the proto-oncogenes c-fos and c-jun, have been found to be induced rapidly in response to arsenite. Both proto-oncogenes encode components of the mitogen-inducible immediate-early transcription factor AP-1 and have been implicated in promoting cell proliferation by affecting G1 to S-phase progression. Moreover, it is well established that the abnormal expression of both c-fos and c-jun can induce cell transformation. Recently, c-jun induction in response to proliferative stimuli was shown further to be critical for the down-regulation of both the tumor suppressor p53 and the cyclin-dependent kinase inhibitor p21/WAF-1/CIP1. Therefore, perturbation of the expression of c-fos and c-jun by arsenic compounds is likely to play an important role in carcinogenesis.

The signal transduction pathways that regulate c-fos and c-jun transcription have been thoroughly investigated. The mitogen-activated protein (MAP) kinase pathway plays a crucial role in the induction of both c-fos and c-jun in response to many...
extracellular stimuli, such as growth factors and cellular stress (18, 19). Recent studies (20, 21) have indicated that growth factor stimulation may lead to phosphorylation and acetylation of histone H3 and its associated proteins. These modifications can be detected by western blotting using antibodies specific for phospho-histone H3 (Ser(P)-10) or phospho-acetylhistone H3 (Ser(P)-10/Ac-lysine-14) (22).}

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Treatment**—Early passage WI-38 human diploid fibroblasts were cultured in minimum essential medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone, Logan, UT). Subconfluent cultures were rendered quiescent by incubating the cells in minimum essential medium containing 0.5% fetal bovine serum for 36 h prior to treatment. Sodium arsenite (Sigma) and triostatin A (RTS, Sigma) were directly added to the medium. U0126 (Promega, Madison, WI), SB203580 (Sigma), SB202190 (Calbiochem), PD169316 (TSA, Sigma) were directly added to the medium. U0126 (Promega, Madison, WI), SB203580 (Sigma), SB202190 (Calbiochem), PD169316 (Calbiochem) and p38 MAP kinase inhibitor (p38 INH, Calbiochem) were dissolved in dimethyl sulfoxide and added to the medium to a final concentration of 10 μM 15 min prior to treatment.

**Northern Blot Analysis**—Total RNA was isolated with STAT-60 (Tel-Test B, Friendswood, TX) and Northern blot analysis performed as described previously (23). Messenger RNAs encoding c-fos and c-jun were detected using probes derived from either the human EST clone AL133966 or the mouse genomic clone pD-46 (gift from W. Woodgett, see Ref. 24), respectively. To monitor differences in loading and transfer among samples, blots were stripped and rehybridized with an end-labeled oligonucleotide complementary to the 18 S rRNA.

**Antibodies, Western Blotting, and Immunofluorescence**—To analyze the phosphorylation status of various MAP kinases, Western blotting was performed as described previously (23) using 20 μg of total cell lysate. Rabbit polyclonal antibodies recognizing phospho-ph38, total p38, and phospho-p44/p42 ERK MAP kinases were from Cell Signaling (Beverly, MA). A mouse monoclonal antibody against p44/p42 ERK was from Transduction Laboratories (Lexington, KY). To test the modification of histone H3, histone H4, and phospho-histone H3 antibodies were extracted using sulfuric acid (23) and then analyzed by Western blotting using rabbit polyclonal antibodies recognizing either phospho-histone H3 (Ser(P)-10) (Cell Signaling), phosphoacetyl-histone H3 (Ser(P)-10/Ac-Lys-14), Upstate Biotechnology, Inc., Lake Placid, NY), phospho-histone H2A.X (p-Ser-139, Upstate Biotechnology, Inc.), acetyl-histone H3 (Ac-Lys-14, Upstate Biotechnology, Inc.), acetyl-histone H3 (Ac-Lys-9,14, Upstate Biotechnology, Inc.), or a rabbit antibody recognizing total histone H3 (Cell Signaling).

For the immunofluorescence studies, Alexa 488-conjugated goat anti-rabbit and Alexa 568-conjugated goat anti-mouse secondary antibodies (Molecular Probes, Eugene, OR) were used according to the manufacturer’s specifications. Phospho-histone H3 was detected using a rabbit polyclonal antibody specifically recognizing human phospho-histone H3 (Ser-10) histone H3 (Cell Signaling). Total histone H3 was detected using a mouse monoclonal antibody (25). DAPI staining was performed as described previously (26). Samples were visualized by either fluorescence microscopy (Carl Zeiss, New York) or confocal microscopy (Zeiss LSM-410 inverted confocal microscope equipped with a 63× NA 1.4 oil immersion objective). The confocal pinhole was set to obtain a spatial resolution of 0.4 μm in the horizontal plane and 1 μm in the axial dimension. Image processing and presentation were done using MetaMorph 6.3.6 software (Universal Imaging, Inc., West Chester, PA).

**ERK, MAPKAPK23, and MSK1 Activity Assays**—MSK1 was immunoprecipitated from cell lysates using a polyclonal antibody (Upstate Biotechnology, Inc.), and its activity was determined by assessing the phosphorylation of a synthetic peptide (Crosstide) in the presence of γ-32P/ATP (23). ERK activity was determined by immunocomplex kinase assays using myelin basic protein (MBP, Sigma) as a substrate as described previously (26). MAPKAPK23 activity was determined by immunocomplex kinase assays using recombinant His25 (25-kDa heat-shock protein, Stressgen, Vancouver, Canada) as a substrate as described previously (27).

**Chromatin Immunoprecipitation (ChIP) and Real Time PCR**—Chromatin was cross-linked using formaldehyde and broken down to fragments with an average size of ~2 kbp through brief sonication according to Clayton et al. (20). The resulting chromatin solution was divided equally into four fractions that were subsequently used for isolating input DNA (fraction 1) or performing ChIP assays (fractions 2–4). Chromatin solutions were first incubated for 2 h at 4 °C with 10 μl of rabbit pre-immune serum or with 10 μl of purified antibody specifically recognizing phospho-histone H3 (Ser(P)-10) or 40 μl of purified antibody specifically recognizing phospho-acetylhistone H3 (Ser(P)-10/Ac-Lys-14). Protein synthesis was blocked by addition of 1 M ammonium acetate (final concentration 200 μg/ml), sonicated λ phase DNA (5 μg), and protein A-Sepharose beads (Amersham Biosciences) were added to the solutions and incubated overnight with gentle rotation at 4 °C. The beads were washed extensively as described previously (23). The resulting immunoprecipitates or input chromatin solutions were then incubated with RNase A (50 μg/ml) for 1 h at 37 °C and then digested for 16 h using proteinase K (100 μg/ml). The input and immunoprecipitated chromatin were incubated at 65 °C for ≥6 h to reverse the formaldehyde cross-links. The DNA was extracted with phenol/chloroform, precipitated with ethanol, and dissolved in 30 μl of water.

Real time PCR was performed using SYBR® Green PCR Master Mix and a LightCycler Detection System (Roche Diagnostics, Mannheim, Germany). For the DNA extraction, cells were washed with an average size of 2 kbp through brief sonication according to Hypis definition. The vRas gene was amplified with an average size of 2 kbp through brief sonication according to Clayton et al. (20). The resulting chromatin solution was divided equally into four parts that were subsequently used for isolating input DNA (fraction 1) or performing ChIP assays (fractions 2–4). Chromatin solutions were first incubated for 2 h at 4 °C with 10 μl of rabbit pre-immune serum or with 10 μl of purified antibody specifically recognizing phospho-histone H3 (Ser(P)-10) or 40 μl of purified antibody specifically recognizing phospho-acetylhistone H3 (Ser(P)-10/Ac-Lys-14). Protein synthesis was blocked by addition of 1 M ammonium acetate (final concentration 200 μg/ml), sonicated λ phase DNA (5 μg), and protein A-Sepharose beads (Amersham Biosciences) were added to the solutions and incubated overnight with gentle rotation at 4 °C. The beads were washed extensively as described previously (23). The resulting immunoprecipitates or input chromatin solutions were then incubated with RNase A (50 μg/ml) for 1 h at 37 °C and then digested for 16 h using proteinase K (100 μg/ml). The input and immunoprecipitated chromatin were incubated at 65 °C for ≥6 h to reverse the formaldehyde cross-links. The DNA was extracted with phenol/chloroform, precipitated with ethanol, and dissolved in 30 μl of water.

**RESULTS**

**Arsenite Stimulates Histone H3 Phosphorylation/Acetylation and Activates MSK1**—It has been shown that in mouse fibroblasts, expression of several oncogenes, including Ha-Ras, v-mos, and v-fes, leads to enhanced phosphorylation of histone H3 (28). Likewise, stimulation of cells with three well established tumor promoters, TPA, okadaic acid, and UVC, also increased histone H3 phosphorylation (28–30). Recent studies (20, 28, 31, 32) have indicated that phosphorylation of histone H3 is coupled to its acetylation, and such modifications are critical for the induction of immediate-early genes in response to extracellular stimuli. To address whether arsenite triggers histone H3 modifications, histone H3 phosphorylation in normal human lung fibroblast WI-38 cells was examined by immunofluorescence, using an antibody that recognizes phospho-histone H3 (Ser(P)-10). Compared with control cells, where
the level of phospho-histone H3 was low, arsenite triggered a rapid increase of phospho-histone H3 in the nucleus. As shown in Fig. 1A, the levels of phospho-histone H3 after a 60-min treatment with arsenite were comparable with those seen after treatment with a combination of EGF and anisomycin, shown previously (20) to enhance potently histone H3 phosphorylation.

EGF-stimulated histone H3 phosphorylation is reportedly restricted to a small subset of nucleosomess that are associated with active gene transcription (20, 21, 31, 33, 34). Likewise, in Drosophila salivary glands, heat shock significantly stimulates histone H3 phosphorylation at a few loci where the heat shock protein genes are located (35). To investigate whether arsenite stimulates phosphorylation of histone H3 throughout the entire nucleus or only at a few loci, confocal microscopy was performed. A mouse monoclonal antibody recognizing both phosphorylated and unphosphorylated histone H3 was used to visualize all histone H3 proteins (Fig. 1B). In unstimulated cells, only a few loci were intensely stained by the phospho-histone H3 antibody (Fig. 1B). By contrast, in cells stimulated with EGF + anisomycin, phospho-histone H3 was markedly elevated and displayed a punctate staining pattern in the nucleus (Fig. 1B). As seen in the EGF + anisomycin treatment group, arsenite stimulation also resulted in a dramatic increase in phospho-histone H3, revealing a similar punctate staining pattern in the nucleus (Fig. 1B). The marked difference between the staining patterns of phospho-histone H3 and total histone H3 in arsenite-stimulated cells strongly suggests that arsenite induces the phosphorylation of histone H3 at a subset of nuclear loci (Fig. 1B).

To examine further the effect of arsenite on histone H3 modification, histone proteins were extracted with sulfuric acid from either untreated or arsenite-treated cells. Histone H3 modification was examined by Western blotting using antibodies specific for either phospho-histone H3 (Ser(P)-10) or dually modified histone H3 (Ser(P)-10/Ac-Lys-14). Arsenite treatment led to increased histone H3 phosphorylation in a time-dependent manner. This modification was visible within 10 min after the addition of arsenite into the medium and continued to increase over the period studied (Fig. 2A). Phosphoacetyl-histone H3 increased in a time-dependent fashion with kinetics similar to that of histone H3 phosphorylation (Fig. 2A), supporting the notion that phosphorylation and acetylation of histone H3 are tightly coupled events (21). In contrast, bulk levels of acetylated H3 did not significantly change after arsenite treatment (Fig. 2A), presumably because acetylated H3 is generally associated with actively transcribing chromatin, whereas arsenite-regulated genes constitute only a small subset of the genome, so arsenite treatment did not greatly affect total levels of acetylated H3, whereas TSA induced hyperacetylation of histone H3 (Fig. 3A). To confirm the specificity of the phosphoacetyl-H3 antibody on Western blots, lysates from either untreated or arsenite-treated cells were included as positive controls for both phospho-histone H3 and phosphoacetyl-H3 antibodies (Fig. 2A), and only acetylation of histone H3 induced by TSA was not recognized by the anti-phosphoacetyl-H3 antibody. These results demonstrate the presence of H3 histone that is dually modified by phosphorylation and acetylation (at Ser-10 and Lys-14, respectively) in arsenite-treated cells. Furthermore, to investigate whether arsenite-induced histone phosphorylation is specific for H3, a rabbit polyclonal antibody recognizing phospho-H1 (Upstate Biotechnology, Inc.) and a mouse monoclonal antibody recognizing histone phospho-H2AX (phosphorylated at Ser-139, Upstate Biotechnology, Inc.) were used for Western blot analysis. Stauroporine-treated Jurkat cells (0.5 μM, 4 h) were included as positive control of phospho-H2AX. Arsenite treatment did not have any effect on either histone H1 phosphorylation (data not shown) or histone H2A phosphorylation at Ser-139 (Fig. 2A).

Because arsenite is a well known activator of MAP kinases ERK and p38, we sought to assess whether activity of MAP kinases was required for the observed histone H3 modifications. Phosphoacetylation of histone H3 in arsenite-treated cells was moderately inhibited in cells that were pretreated with either the MEK inhibitor U0126 or the p38 inhibitor SB203580 (Fig. 2B). Combination of both U0126 and SB203580 resulted in a more significant inhibition of histone modifications (Fig. 2B). These observations suggest that arsenite-triggered MAP kinase activation contributes to the implementation of histone H3 modifications.

Protein kinase MSK1 lies downstream of p38 and ERK and can be activated by both mitogenic stimulation and stress (36). Recent studies (32) suggest that MSK1 may play an important role in mediating the phosphorylation of histone H3, thus serving as a link between the MAP kinase cascades and nucleosomal alterations. To examine whether MSK1 could be involved in histone H3 phosphorylation, we examined the kinetics of MSK1 activation following arsenite treatment. MSK1 was im-
Arsenite significantly activated MSK1 in a time-dependent manner (Fig. 2C), but this activation was significantly inhibited in cells pretreated with either U0126, SB203580, or a combination of both inhibitors, with the combined inhibitory treatment showing the greatest effect (Fig. 2D). The similarity between the kinetics of histone H3 phosphorylation and MSK1 activation as well as the comparable susceptibility profiles toward the two MAP kinase inhibitors support the hypothesis that MSK1 plays a significant role in
modulating histone H3 modification in response to arsenite.

In Human Diploid Fibroblasts, Arsenite Induces c-fos and c-jun Expression through Mechanisms Partially Dependent on the ERK Pathway—To investigate whether histone H3 modification in response to arsenite may play a role in the induction of proliferation-associated genes, we first examined the effect of arsenite on c-fos and c-jun expression in WI-38 cells by Northern blotting (Fig. 3A). Basal c-fos and c-jun mRNA levels were very low in unstimulated cells, and arsenite treatment potently increased their abundance in a time- and dose-dependent fashion (Fig. 3A). The kinetics of induction of mRNAs encoding c-fos and c-jun was delayed in comparison with that of histone H3 modification (compare Figs. 1 and 2 with Fig. 3).

The effects of arsenite on various MAP kinases in WI-38 cells were examined by Western blot analysis and immunocomplex kinase assays. Arsenite treatment resulted in a transient activation of the ERK MAP kinases, as indicated by the time-dependent increase in phosphorylated ERK (Fig. 3B). This observation was confirmed by immunocomplex kinase assays, showing that ERK activity reached peak levels at about 10 min, rapidly declining thereafter (Fig. 3B). In contrast to the early and transient ERK activation, p38 activation was delayed, and its activity was sustained throughout the period examined (Fig. 3B). This observation was confirmed by immunocomplex kinase assays, showing that MAPKAPK2/3 activity was sustained throughout the period examined (Fig. 3B).

To investigate the role of ERK and p38 MAP kinases in the induction of c-fos and c-jun, inhibitors U0126 and SB203580 were used, respectively. U0126 completely abolished phosphorylation of the ERK MAP kinases (Fig. 4A) but had no effect on p38 activation by arsenite (Fig. 4B). Interestingly, SB203580 significantly augmented ERK activity induced by arsenite (Fig. 4A), suggesting that the p38 pathway antagonized the ERK pathway. The inhibition of the p38 pathway by SB203580 was confirmed by the marked attenuation of arsenite-stimulated MAPKAPK2/3 activity (Fig. 4B). U0126 substantially inhibited c-fos and c-jun mRNA induction by arsenite, reducing c-fos mRNA levels by greater than 70% and c-jun mRNA levels by ≈50% (Fig. 4C). In contrast, the p38 inhibitor SB203580 neither inhibited c-fos expression nor affected c-jun induction by arsenite (Fig. 4C). Interestingly,
SB203580 actually further enhanced arsenite-mediated induction of c-fos (Fig. 4C), consistently with the observation that SB203580 enhanced ERK activation by arsenite (Fig. 4A). The combined effect of both U0126 and SB203580 on c-fos and c-jun mRNA induction by arsenite was similar to that of U0126 alone (Fig. 4C). Taken together, these results indicate that ERK plays a prominent role in the induction of c-fos and c-jun expression by arsenite in normal human fibroblasts.

The observation that SB203580 significantly augmented arsenite-induced ERK activity was confirmed by immunocomplex kinase assays using MBP as a substrate (Fig. 5A), and by using a panel of p38 inhibitors (SB202190, PD169316, and p38 INH), which also augmented arsenite-induced ERK activity (Fig. 5A). Moreover, like SB203580, the other p38 inhibitors were also capable of further enhancing arsenite-mediated induction of c-fos but did not affect arsenite-mediated induction of c-jun (Fig. 5B). These observations further suggest that the p38 pathway antagonizes the ERK pathway and ERK-mediated c-fos induction by arsenite in normal human fibroblasts.

Transcriptional Induction of c-fos and c-jun Is Associated with Phosphoacetylation of Histone H3 at the c-fos and c-jun Chromatin Sites—To examine whether arsenite stimulates histone H3 modification at the c-fos and c-jun loci, control and arsenite-treated cells were treated with formaldehyde to crosslink chromatin and genomic DNA. Following solubilization with SDS and sonication, ChIP assays were carried out using antibodies that specifically recognize either phospho-histone H3 or phosphoacetyl-histone H3. Genomic DNA present in the immunoprecipitates was extracted and analyzed by real time PCR using primers specific for the amplification of the genes of interest. Real time PCR assays revealed a considerable increase in the levels of phosphorylated histone H3 on the c-fos chromatin after arsenite treatment, reducing the Cq value (which represents the number of PCR cycles required to reach a threshold set arbitrarily) from 33.1 to 29.4, which corresponds to a 9.8-fold increase in the amount of phospho-histone H3 (Fig. 6A). Consistent with the notion that acetylation and phosphorylation of histone H3 are tightly coupled events, arsenite treatment also resulted in a substantial increase in the levels of phosphoacetyl-histone H3 on the c-fos chromatin, reducing Cq from 32.2 to 28.7, which corresponds to a 8.6-fold increase in the amount of phosphoacetyl-histone H3 (Fig. 6A). Interestingly, U0126 alone substantially inhibited both phosphorylation and phosphoacetylation of histone H3 at the c-fos chromatin. SB203580 alone had no significant effect on histone H3 modification at the c-fos locus (Fig. 6A). Combination of SB203580 with U0126 did not further reduce histone H3 modification at the c-fos locus, underscoring the notion that p38 does not play a significant role in arsenite-induced c-fos expression in these cells. The specificity of this assay was demonstrated both by the absence of c-fos amplification in the mock immunoprecipitation samples (Fig. 6, B and C, Ab−) and by the inability of arsenite to stimulate histone H3 modification at the transcriptionally inactive β-globin locus (Fig. 6, B and C). These results support the view that arsenite promotes chromatin remodeling only at a subset of genes.

Similarly, histone H3 modification at the c-jun locus was examined. In response to arsenite treatment, the levels of phosphorylated and phosphoacetylated histone H3 on the c-jun chromatin increased 14.9- and 6.9-fold, respectively (Fig. 7A). U0126 strongly inhibited these modifications at the c-jun locus, whereas SB203580 had no such effect (Fig. 7A). Moreover, pre-immune serum did not bring down any c-jun chromatin, as indicated by the absence of c-jun amplification from the mock ChIP samples (Fig. 7B, Ab−). Taken together, our results indicate that arsenite potently enhances histone H3 modification at both the c-fos and c-jun loci through an ERK MAP kinase-mediated pathway.

**DISCUSSION**

Epidemiological studies have clearly established that elevated arsenic levels in drinking water is associated with an increase in the incidence of cancer of the skin, lung, bladder,
kidney, nasal passages, liver, and prostate (4). Although the carcinogenic mechanisms of arsenic are not fully understood, recent studies (3, 6, 9, 10, 15) strongly suggest that arsenic may exert its carcinogenic effects by perturbing key signal transduction pathways. In this regard, the c-fos and c-jun proto-oncogenes have been shown to be inducible by arsenite treatment, both in vitro and in vivo (9, 11, 15). We have shown previously that arsenite treatment can activate various MAP

![Graphs showing the effects of arsenite on c-fos and c-jun expression](image)

**Fig. 6. Arsenite stimulates histone H3 phosphorylation on the c-fos chromatin.** A, analysis of phosphorylated histone H3 (Ser(P)-10) or phosphoacetylated histone H3 (Ser(P)-10Ac-Lys-14) at the c-fos chromatin by real time PCR after ChIP. WI-38 cells were treated with 400 μM arsenite (Ars) for 1 h in the presence or absence of either U0126 (U), SB203580 (SB), or a combination of both inhibitors (each 10 μM). ChIP assays were performed using an antibody recognizing either phospho-histone H3 (α-Phos-H3), phosphoacetyl-histone H3 (α-Phos-Ac-H3), or pre-immune serum (Ab-). DNA recovered from the antibody-bound fractions as well as the DNA from input chromatin (Input) were analyzed for the presence of c-fos promoter and β-globin sequences through real time PCR. Graphs represent the amplification curves for the c-fos promoter using the following DNA as templates: top left graph, DNA isolated using anti-phospho-histone H3 antibody; top right graph, DNA isolated using anti-phosphoacetyl-histone H3 antibody; middle left graph, input DNA; middle right graph represents the relative target DNA copy number compared with control. The cycle numbers to reach a threshold of 0.5 (Ct) are indicated. Final DNA products were separated on 2% agarose gels after the PCRs in A were completed. DNA was stained with ethidium bromide. B, final PCR products of c-fos and β-globin amplified from input DNA (Input), DNA isolated using anti-phospho-histone H3 antibody (Phos-H3), or pre-immune serum (Ab-). C, final c-fos and β-globin PCR products amplified from input DNA (Input), DNA isolated using anti-phosphoacetyl-histone H3 antibody (Phos-Ac-H3), or pre-immune serum (Ab-).
kinase cascades and that arsenite-triggered ERK activation is initiated through the EGF receptor and mediated by oncoproteins Shc and Ras (15, 22). To gain insight into downstream mechanisms of action of this carcinogen, we have examined arsenite-triggered signaling events at the chromatin level. We found that arsenite treatment caused rapid phosphoacetylation of histone H3, which preceded the induction of c-fos and c-jun.

By using pharmacological agents that selectively inhibit either the ERK or the p38 MAP kinase cascades, we demonstrate that the ERK pathway plays a primary role in mediating the induction of c-fos and c-jun by arsenite. Through ChIP and quantitative real time PCR analyses, we show that arsenite treat-
c-fos and c-jun Chromatin Remodeling by Arsenite

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