Histone Deacetylase Inhibitors Activate p21<sub>WAF1</sub> Expression via ATM

Rong Ju, and Mark T. Muller<sup>1</sup>

Department of Molecular Genetics, The Ohio State University, Columbus, Ohio 43210

ABSTRACT

Histone deacetylase (HDAC) inhibitors are known to induce expression of genes such as p21<sub>WAF1</sub>, thereby, leading to cell cycle arrest. In this work, we show that p21<sub>WAF1</sub> induction by HDAC inhibitors (depsipeptide and trichostatin A) is defective in Ataxia telangiectasia (AT) cells but normal in matched wild-type (WT) cells (human diploid fibroblasts). To verify the role of ATM in this effect, we show that ectopic expression of the WT ATM gene in an AT cell line fully restores p21<sub>WAF1</sub> induction by the HDAC inhibitors. Furthermore, because caffeine and wortmannin attenuate p21<sub>WAF1</sub> induction in WT cells, it is probable that the phosphatidylinositol 3'-kinase activity is essential for this process. Besides the p21<sub>WAF1</sub> promoter, activation of topoisomerase III<sub>B</sub> and SV40 promoters by the HDAC inhibitors are also decreased in the AT cell lines relative to WT cells; thus, these findings pertain to other promoters. Finally, despite the obvious induction deficiency of gene expression, the overall levels of H3 and H4 histone acetylation appear to be the same between AT and normal cells in response to HDAC inhibitor treatments. Taken together, the data indicate that ATM is involved in histone acetylation-mediated gene regulation.

INTRODUCTION

In eukaryotic cells, DNA combines with core histones and other chromosomal proteins to form chromatin (1, 2) where two copies of each of four inner histones are wrapped by 146 bp of DNA to make a nucleosome, the basic repeating unit. With the aid of additional proteins, including histone H1, nucleosomes are additionally organized into higher-order chromatin structures (3). The presence of histones on DNA limits access of nonhistone DNA-binding proteins and thereby represses transcription (4). To date, at least two distinct mechanisms are thought to alter the repressive nature of bulk chromatin vis a vis a vis gene expression (5–7). One mechanism involves ATP-dependent nucleosome remodeling by which chromatin remodelling complexes transfer and slide histone cores, making DNA accessible to transcription factors and DNA binding factors that promote transcription (6, 8, 9). The second mechanism involves factors that directly modify core histone tails through phosphorylation, methylation, and acetylation (10, 11). Acetylation-related factors include HATs<sup>2</sup> and HDACs (12–14), which acylate and deacylate, respectively, lysine residues at the NH<sub>2</sub>-terminal tails of the histone core particle (15). Because the histone tails are located outside of the particles, such modifications are believed to neutralize the positive charge of basic histones and weaken histone-DNA interactions, thus making the nucleosome more “accessible.” Consequently, HATs activate transcription (16, 17), whereas HDACs remove acetyl groups, leading to a more repressive form of chromatin (18). The dynamic equilibrium of HATs and HDACs determines the net level of acetylation and, in turn, regulate transcription. At present, several classes of HATs (12–14) and HDACs (19–22) have been identified in yeast and human cells. In general, transcriptional coactivators recruit HATs to promoter regions to activate the corresponding genes (23, 24), whereas transcriptional corepressors recruit HDACs to shut down expression of the genes (25–27); thus, both function in complexes (13, 28). However, the exact mechanism of how histone acetylation activates transcription remains unclear.

AT is an autosomal recessive disorder in which patients exhibit cerebellar ataxia, dilated blood vessels, immunodeficiency, hypersensitivity to ionizing radiation, and elevated risk of certain cancers (29, 30). Cells derived from AT patients display several cellular defects including chromosome instability (31), defective cell cycle checkpoints (G<sub>S</sub>, S, and G<sub>2</sub>; Refs. 32, 33), and radioreistant DNA synthesis (34). The mechanistic reason is that these cells fail to activate damage response signaling, such as p53-dependent pathway (35). In addition, a study has revealed that a significant decondensation of the nuclear chromatin was associated with the AT disorder (36, 37).

The ATM gene was identified by positional cloning and encodes a 3056-amino acid protein with a calculated molecular weight of M<sub>c</sub> 350,000. ATM shares homology with a gene family in which all of the members have a COOH-terminal 300 amino acid motif that resembles the catalytic domain of PI3k. Members of this gene family are typically involved in DNA damage detection and cell cycle control (38–40); examples include <i>Saccharomyces cerevisiae</i> proteins TEL1 and MEC1 (41, 42), <i>Schizosaccharomyces pombe</i> RAD3 (43), <i>Drosophila</i> MEI-41 (44), ATR (45), and human DNA-pyruvate kinase (46). The cumulative data indicate that the ATM gene product is a protein kinase rather than a lipid kinase (38, 47). Consistent with the central roles of ATM protein in cell cycle control and DNA repair, it has multiple substrates including p53, Chk2, p95/NBS, BRAC1, and MDM2 (48, 49). Although it is reported that ATM phosphorylates HDAC1 (50) and ATR is associated with HDAC2 (51), there have not been any reports that functionally link ATM to histone acetylation-mediated gene expression.

p21<sub>WAF1</sub> is a cyclin-dependent kinase inhibitor that associates with a class of cyclin-dependent kinases and inhibits their kinase activities leading to cell cycle arrest and dephosphorylation of Rb (reviewed in Ref. 52). HDAC inhibitors have been reported to consistently induce p21<sub>WAF1</sub> expression in a p53-independent manner (53). In this study, we used p21<sub>WAF1</sub> as a model system to reveal whether ATM is required for histone acetylation-mediated gene activation. We show that such activation is defective in AT cells and that the PI3k activity appears to be necessary to HDAC inhibitor-induced p21<sub>WAF1</sub> activation in WT cells.

MATERIALS AND METHODS

Cell Cultures. Cells were grown in a humidified incubator at 37°C and 5% CO<sub>2</sub>, GM (90607, GM05849, GM08505, GM00639, and GM00637 were purchased from Coriell Cell Repository (Camden, NJ). They were grown as monolayers in DMEM (Life Technologies, Inc.) containing 15% fetal bovine serum (Life Technologies, Inc.), 2× concentrations of essential amino acid, and 50 μg/ml of gentamicin. AT22IJE pEB57 and AT22IJE pEB57-Y52 cells were generous gifts from Dr. Michael B. Kastan at St. Jude Children’s Research Hospital (Memphis, TN). They were originally constructed in the laboratory of Dr. Yosef Shiloh (Tel Aviv University, Tel Aviv, Israel) by

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<sup>1</sup>To whom requests for reprints should be addressed, at Department of Molecular Genetics, Ohio State University, 484 West 12th Avenue, Columbus, OH 43210. Phone: (614) 292-1914; Fax: (614) 292-4702; E-mail: muller.20@osu.edu.

<sup>2</sup>The abbreviations used are: HAT, histone acetyltransferase; HDAC, histone deacetylase; AT, ataxia telangiectasia; ATM, ataxia telangiectasia-mutated; PI3k, phosphatidylinositol 3'-kinase; ATR, ataxia telangiectasia-mutated-related protein kinase; Rb, retinoblastoma; TSA, Trichostatin A; topo, topoisomerase; PMSF, phenylmethylsulfonyl fluoride; TBST, 0.2 m Tris-HCl (pH 7.5), 150 m NaCl, 0.1% Tween; FR, depsipeptide; WT, wild-type; pol II, polymerase II.
transfecting an immortalized fibroblast line AT221JE with the mammalian expression vector pEB87 and pEB87 plus full-length ATM reading frame, respectively (54). AT221JE/pEB87 and AT221JE pPBS-YZS cells were cultured in the same media as described above plus 100 μg/ml hygromycin B. All of the cells were in exponential growth phase at the time of harvest.

**Drugs and Antibodies.** TSA was purchased from Sigma (St. Louis, MO) and dissolved in DMSO at 100 mg/ml. FR 901228 was a kind gift from Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan) and dissolved in DMSO at 5 mM. Aliquots were stored at −20°C until immediately before the experiments.

Mouse monoclonal p21 WAF1 antibody was from Pharmingen (Los Angeles, CA). Actin antibody was purchased from Sigma. Rabbit anti-acetylated histone H3 and H4 antibodies were from Upstate Biotechnology (Lake Placid, NY) and mouse anti-Rb antibody was purchased from Calbiochem (Los Angeles, CA).

**Isolation of the p21 WAF1 and the Topoisomerase IIIα Promoters.** To amplify the p21 WAF1 promoter, two primers were designed and synthesized according to Richon et al. (55). The sequences of upstream and downstream primers were: 5'-GGT GTC TAG GTG CTC CAG GT-3' and 5'-CCG CTC TAG GTG CTC CAG GT-3'. The PCR reaction was carried out in the following condition: 50°C 45 min, 72°C 30 s, and 94°C 1 min and 30 cycles. The PCR fragment was subcloned into pCR2-TOPO vector (Invitrogen, Carlsbad, CA) to yield pCR-II-21p. All of the orientations were examined by sequencing.

The 659-bp KpnI-XhoI fragment of the p21 WAF1 promoter was cut out of pCR-II-21p and cloned into pG3-Vector (Promega, Madison, WI) and designated p2l-puc. To amplify the top IIIα promoter, two primers were synthesized as following according to the published top IIIα promoter sequence (56): upstream 5'-ATA GGT ACC CAA AAC GGC CTC ACG AAG CCA C-3' and downstream 5'-TCA GTC GAG TCT TCG GGC CGT CGC AGC CAC CGG A-3'. This fragment spanned from +305 to −1, 262 covering Y11, USF, and four Sp1 sites. Genomic DNA was extracted from GM0637 and used as the template. The PCR reaction was carried out in the following conditions: 94°C 1 min, 72°C 1 min, and 60°C 1 min and 30 cycles. Next, the 1.5-kb PCR fragment was digested with KpnI and XhoI and subcloned into Bluescript II SK-phiagemid to create pBS-topoIIIα. After the top IIIα promoter fragment was sequenced, pBS-topoIIIβ was digested with KpnI and XhoI, and the 1.5-kb fragment of topo IIIα promoter was subcloned into pG3 to yield pTopoIII-Luc.

**Transient and Stable Transfection as well as Luciferase Analysis.** For transient assay, 1 × 10^6 GM0637 or GM5849 cells were transfected with p2luc or pTopoIII-Luc (0.5 μg/transfection) using LF2000 (2 μg/μg DNA; Life Technologies, Inc.) in 24-well plates. After 24 h, transfected cells were trypsinized and cultured in the same medium as described above plus 100 μg/ml hygromycin B. For an additional 24 h, cells were lysed and assayed for luciferase activity according to the manufacturer instructions (Bio-Rad, Hercules, CA).

**Western Blot Analysis.** To obtain a whole cell extract, cells were pelleted, washed with PBS, and then suspended in radioluminoprecipitation assay buffer (50 mM Tris-HCl (pH 7.4), 1% NP40, 0.25% sodium deoxycholate, 1 mM EDTA, 1 mM PMSF, 1 μg/ml aprotenin, leupeptin, and peptatin each, 1 mM NaF, 100 mM Tris-HCl (pH 6.8), 1 mM PMSF, and 1 μg/ml aprotenin, leupeptin, pepstatin each), and the insoluble fraction was removed by centrifugation at 12,000 × g for 10 min. The protein concentration of supernatant was determined using a Bio-Rad DC protein assay kit according to the manufacturer instructions (Bio-Rad, Hercules, CA).

**RESULTS**

**Induction of p21 WAF1 by HDAC Inhibitors Is Defective in AT Cells.** FR is a potent HDAC inhibitor (59), and like other HDAC inhibitors, FR induces p21 WAF1 in a p53-independent manner in WT cells (60). To test whether FR can induce p21 WAF1 expression in AT cells, SV40-transformed AT cells (GM5849) were treated with FR at different concentrations and for different intervals. As a control, a matched, SV40-transformed WT cells (GM0637) were treated in the same way. Fig. 1A reveals that in GM637, p21 WAF1 was induced by FR at as low as 5 nM and saturated at 50 nM. In contrast, in the AT cell line (GM5849), p21 WAF1 was barely observed even when the cells were treated with FR at a concentration as high as 500 nM. Furthermore, whereas the GM0637 cells started expressing p21 WAF1 within 12 h after addition of FR (and reached a significant high level at 24 h), p21 WAF1 was hardly detected at 24 h in the GM5849 cells (Fig. 1B).

In both experiments, the β-actin levels that served as a loading control stayed constant. These data indicate that FR cannot effectively induce p21 WAF1 expression in AT cells. On the basis of these results, we performed subsequent experiments by using FR (50 nM) for 24 h except where indicated.

To test whether such an induction deficiency is specifically because of HDAC inhibition of FR, another structurally unrelated HDAC inhibitor, TSA, was used to treat both cell lines. Similar results were obtained as shown in Fig. 1C.

To assess whether p21 induction deficiency only occurs in GM5849 and to exclude the possibility that secondary mutations might be
Fig. 1. Defective p21WAF1 induction by HDAC inhibitors in AT cells. WT (GM0637) or AT (GM5849) cells were cultured with the indicated concentrations, times of exposures, and specific HDAC inhibitor. A, cells were treated with 0–500 nm FR for 24 h; B, cells were treated with 50 nm FR for the indicated times; C, cells were incubated with 0.5 μg TSA/ml for 24 h; D, WT (GM0639) or AT (GM9607) cells were treated with FR (50 nm) or TSA (0.5 μg/ml) for 24 h; E, various cell lines were tested for FR induction (50 nm for 24 h); GM0637 (WT), GM8505 (Bloom’s syndrome), GM0639 (galactosemia cell line), and GM5849 (AT cell line). Cells were lysed and each extract (100 μg) was loaded onto a 15% SDS polyacrylamide gel. P21WAF1 was detected by using a mouse monoclonal anti-p21WAF1 antibody probed as described in “Materials and Methods.” A parallel blot was carried out to detect actin with a corresponding mouse antibody to serve as a loading control. F, Northern blot analysis of p21WAF1 mRNA expression. Total RNA (10 μg) was isolated and fractionated on 1.2% agarose, and transferred to a nylon membrane. The membrane was probed with 32P-labeled human p21WAF1 cDNA. A parallel gel was stained with ethidium bromide for a loading control.

desired (because of an unstable genome caused by loss of ATM), another SV40-transformed AT cell line GM9607 was also incubated with both FR and TSA. Although p21WAF1 induction deficiency in GM9607 (50% induction compared with WT) is not as severe as in GM5849 (10% induction compared with wild type), p21WAF1 induction by both FR and TSA indeed decreased significantly when compared with the WT cells (Fig. 1D). These results suggest that it is unlikely that the impaired p21WAF1 expression is because of the ATM-unrelated secondary mutations. In addition, to confirm that our results were specific to AT cells, SV40-transformed Bloom’s syndrome cell line GM8505 and galactosemia cell line GM6039 were treated with FR. Galactosemia is an autosomal recessive disorder with deficiency of galactose-1-phosphate uridyltransferase (61). Fig. 1E shows that both of these cell lines expressed similar levels of p21WAF1 to the WT cells in response to FR; therefore, p21WAF1 induction deficiency appears to be AT-specific. Finally, to determine whether the induction deficiency occurred at protein levels or mRNA levels, p21WAF1 mRNA from both GM0637 (WT) and GM5849 (AT) cells treated with various concentrations of FR was examined via Northern blot analysis. Fig. 1F indicates that p21WAF1 mRNA levels markedly increased in GM637 cells after cultured with FR at as low as 5 nm and reached the saturated level at 50 nm. On the other hand, although the level of p21WAF1 mRNA in GM5849 elevated in a dose-dependent manner after FR treatments, the degree of induction is only minor compared with the one in GM637.

HDAC Inhibitor-mediated Induction Deficiency Occurs at Promoter Levels and Is Not Confined to p21WAF1. A 650-bp promoter fragment for p21WAF1 was amplified by PCR using DNA from WT cells (GM0637). The PCR product was validated by sequencing (data not shown). To examine whether HDAC inhibitor-mediated induction deficiency was transcriptional or post-transcriptional, the p21WAF1 promoter was fused with the luciferase gene, and the construct was transfected into both the AT and WT cell lines. Fig. 2A shows that in WT GM0637 cells, with increased concentration of FR, the episomal p21WAF1 promoter was activated from 38-fold (5 nm FR) to as much as 80-fold (500 nm FR). In contrast, there was an across the board 20-fold induction of the promoter activity in AT cells that did not respond to increases in FR concentration. A similar profile was observed when the p21WAF1 promoter was integrated into genome, except the differences between the WT and AT cell was more dramatic (Fig. 2C). In AT cells, the integrated p21WAF1 promoter was activated only ~5-fold on average in response to FR of all three concentrations, compared with 20-fold as unintegrated plasmid in transient assays. On the other hand, in the WT cells, both the integrated and episomal p21WAF1 promoter displayed a high level of activation (80-fold by 50 nm of FR). Different from the results of transient assays, the integrated p21WAF1 promoter appeared to be saturated at 50 nm of FR. Thus, the results from stable assays appear to be more consistent with Western blot data shown in Fig. 1. Taken together, these data indicate that the p21WAF1 induction deficiency is because of poor p21WAF1 promoter activation.

HDAC inhibitors affect expression of about 1–2% of all of the genes (62); therefore, it was of interest to see whether loss of ATM affected other genes besides p21WAF1. As shown in Fig. 2B, the topoIIIα promoter appears to be activated up to 6-fold in WT cell line GM0637, whereas in the AT cell line GM5849, little if any activation was seen. A constitutive promoter from SV-40 also revealed similar activation patterns. In addition, after stable transfection of topo IIIα promoter-luciferase gene into normal and AT cell lines, the integrated topo IIIα promoter was activated significantly higher in normal transfectants relative to AT clones in response to FR (Fig. 2D). It is also worth noting that in normal cells, the topo IIIα promoter was activated at different degrees in the various transfected clones.

Ectopic Expression of ATM Restores the Defective p21WAF1 Induction by FR901228 in AT Cells. Genomic instability is a hallmark of ATM deficiency (31) largely because of random secondary mutations. If HDAC inhibitor-mediated p21WAF1 induction deficiency in AT cells is because of these types of mutations, it should be
irreversible. That is, ectopic expression of a WT ATM in AT cells should not restore the defective induction. To test this, we obtained two AT fibroblast lines, AT22IJE pEBS7, derived from an immortalized fibroblast line AT22IJE transfected with an "empty" mammalian expression vector pEBS7, and AT22IJE pFBS-YZ5, derived from the same cell lines but transfected with an ATM cDNA (54). As shown in Fig. 3A, AT cells expressed barely detectable levels of p21 WAF1 after FR treatment. On the other hand, p21 WAF1 expression in ATM-complemented cells was slightly less than in the WT cell line GM0637. The similar result was seen after FR was replaced with TSA (Fig. 3B). The data suggest that expression of the WT ATM gene restores the p21WAF1 induction deficiency in AT cells and that the lack of HDAC inhibitor induction appears to be a direct consequence of the loss ATM activity, as opposed to some downstream secondary mutations.

PI3k Activity and Induction of p21WAF1 by HDAC Inhibitors. PI3k activity of ATM appears to be closely connected to many cellular defects in AT cells (63). For example, expression of a truncated ATM gene lacking the PI3k domain confers the AT phenotype on WT cells (63). To test the role of PI3k activity on ATM-mediated p21WAF1 expression, the PI3k inhibitors caffeine and wortmannin were used to treat the WT cells (GM0637) with FR and TSA. As shown in Fig. 4A, combinations of either caffeine with FR or wortmannin with FR induced less p21 WAF1 relative to FR alone. A similar result was obtained with TSA (Fig. 4B). Moreover, caffeine inhibited FR-mediated p21WAF1 promoter activation (Fig. 4C). Using the pooled clones carrying integrated the p21 WAF1 promoter, FR alone induced the p21 WAF1 promoter activity by ~60-fold at 5 nM and 80-fold at 50 nM FR or higher concentration, whereas addition of caffeine to FR activated the p21WAF1 promoter by only 30-fold at the same concentrations of FR. These results suggest that caffeine and wortmannin repress the FR-mediated p21WAF1 induction at least partially at the transcriptional level.
FR Fails to Induce Rb Dephosphorylation in AT Cells. Dephosphorylation of Rb is one downstream consequence of p21WAF1 in the G1 checkpoint control pathway (reviewed in Ref. 64). To examine the effect of FR on Rb phosphorylation status in AT cells, we carried out Western blotting by using the antibodies against Rb. As shown in Fig. 5, elevated p21WAF1 is correlated with decreased Rb phosphorylation in WT cell line GM637 in the presence of FR; however, in the AT cell lines, GM5849, the levels of hyperphosphorylated Rb remained nearly unchanged in response to FR. Moreover, the AT cells displayed higher levels of Rb than the WT cells, consistent with previous studies (65). The similar results were seen in another AT cell line GM9607 (data not shown).

H3 and H4 Histone Acetylation in WT and AT Cells. To determine whether defective promoter activation was because of alterations in histone acetylation, acid-soluble nuclear proteins were isolated from WT and AT cells after exposure to FR for 0, 2, 4, and 8 h. Western blot data (Fig. 6) show that before incubation with FR (0 h), the levels of H3 and H4 were low in both the normal and AT cells (Fig. 6). After FR addition, acetylated H3 and H4 started to accumulate equally in both cell lines. There was a relatively minor difference in H4 acetylation kinetics in AT cells (see acetylated H4 in AT blot, Fig. 6); however, other differences were not obvious between the two cell lines.

DISCUSSION

Previous studies revealed that both FR and TSA induce the expression of p21WAF1 in ATM WT cells (60, 66, 67). In this study, we found that p21WAF1 induction by HDAC inhibitors (FR and TSA) was defective in the AT cells. Because the defect was observed in three independent AT cell lines and not in the other non-AT mutant lines such as Bloom’s syndrome and galactosemia, it is unlikely that this particular defect is because of random mutations unrelated to loss of ATM gene function. Among these AT cell lines, the degree of the defects appears different (Fig. 1), suggesting that different types of ATM mutations may have distinct effects.

Like the other HDAC inhibitors, FR and TSA affect many biological processes, such as differentiation and apoptosis of transformed cells in culture and inhibition of tumor growth (60, 68–70). The global effects of FR and TSA may reflect the multiple roles of HDACs in chromatin. It is also possible that FR or TSA affects other physiological targets and processes besides HDACs; however, given their complete unrelated structures, any common effects caused by both TSA and FR are likely to be because of an HDAC target. Therefore, we conclude that defective p21WAF1 induction by FR or TSA is associated directly with the expected target for these drugs, HDACs. The fact that ectopic expression of the ATM gene restores defective p21WAF1 induction supports the notion that ATM is associated directly with HDAC inhibitor-mediated p21WAF1 induction. The data also suggest that repression of FR/TSA-induced p21WAF1 expression by caffeine or wortmannin is most likely related to ATM (instead of ATR), although ATR has been reported to associate with HDAC2 (51). Taken together, we conclude that a functional ATM is essential for histone acetylation-dependent p21WAF1 expression. Whereas both caffeine and wortmannin behaved similarly, the former drug was a more efficient inhibitor of the FR/TSA-mediated p21WAF1 induction for reasons that are not clear (note that we cannot rule out stability defects).

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The fact that ectopic expression of the ATM gene restores defective p21WAF1 induction supports the notion that ATM is associated directly with HDAC inhibitor-mediated p21WAF1 induction. The data also suggest that repression of FR/TSA-induced p21WAF1 expression by caffeine or wortmannin is most likely related to ATM (instead of ATR), although ATR has been reported to associate with HDAC2 (51). Taken together, we conclude that a functional ATM is essential for histone acetylation-dependent p21WAF1 expression. Whereas both caffeine and wortmannin behaved similarly, the former drug was a more efficient inhibitor of the FR/TSA-mediated p21WAF1 induction for reasons that are not clear (note that we cannot rule out stability defects).
differences between caffeine and wortmannin over the 24-h incubation in vitro.

Although FR and TSA inhibit most HDACs, they only affect expression of ~2% of mammalian genes (62). Therefore, it was of interest to determine the generality of the response with other promoters besides p21WAF1. To address this, we tested two other promoters, SV40 and topoIIIa. The topoIIIa promoter is TATA-less, sharing similarity to a number of housekeeping genes (56). The SV40 promoter is a constitutive “promiscuous” promoter with the prototypic TATA box and has shown to be activated in normal cells by TSA and FR (59). Despite these differences, both promoters appear to be induced by the HDAC inhibitors in WT human cells in this study. However, activation was clearly diminished in AT cells. From these data we conclude that the defect in acetylation-dependent gene expression in AT cells is not limited to p21WAF1 alone.

Stably transfected WT cells containing integrated topo IIIa promoter-luciferase DNA, it was noted that the different clones exhibited different degrees of activation by FR. From this observation, it appears that positional effects are relevant to the FR activation phenomenon reported here. In contrast to WT cells, stably transfected AT cell exhibited very low levels of FR activation. These results imply that the loss of ATM function may have a global effect on histone acetylation-mediated gene transcription.

On the basis of these observations, decreases in HDAC inhibitor-mediated induction in AT cells might be explained in two ways. One is that the ATM protein directly modulates HAT and HDAC activities, such that overall acetylation is less robust in the absence of a fully functional ATM gene product. Therefore, reducing HDAC activity (by addition of FR or TSA) does not culminate in excess acetylation, because HAT activity is globally less active overall. The other possibility is that the loss of ATM function does not directly influence histone acetylation but influences the events downstream of histone acetylation presumably in chromatin. That is, the AT cell will have the normal levels of acetylation in response to FR or TSA, but somehow the acetylation fails to result in activation of transcription. Assessment of FR-induced histone acetylation (Fig. 6) appears to support the second possibility. There were no substantive differences observed in histone H3 acetylation between the WT cells and AT cells after the FR treatment. Although H4 displays a delay in histone acetylation in response to FR, the final acetylation levels were no different between the wild type cells and AT cells.

The ATM protein is localized in chromatin and the nuclear matrix (71). Because ionizing radiation does not change its localization or amount and because chromatin structure is also abnormal AT cells (71), it is possible that ATM is required to maintain an appropriate chromatin structure as a prerequisite for histone acetylation-dependent gene regulation. Struhl (72) has proposed models for how histone acetylases and deacetylases selectively affect gene expression. One of his ideas is that histone acetylations are generally targeted to promoters, and the selection is because of inherent differences in the promoters. For example, acetylation-sensitive promoters are associated with more tightly packed and/or positioned nucleosomes, and acetylation changes the nucleosome organization to facilitate accessibility of RNA pol II machinery to promoters. In contrast, acetylation-insensitive promoters are located in less tightly packed and/or positioned nucleosome region, and the organization state of nucleosomes does not affect the accessibility of pol II machinery. This model may apply to regulation of p21 or topo IIIa. That is, these promoters are acetylation-sensitive in presence of the normal ATM protein, and loss of ATM turns these promoters into an acetylation-insensitive mode. However, because we have not examined the change of local histone acetylation in response to FR or TSA, for instance, the acetylation levels of nucleosomes at the p21 promoter, we cannot exclude the possibilities in which the loss of ATM results in disassembly of certain HAT complexes on the promoters we tested, which, in turn, fail to acetylate the histones on those promoters. These acetylated histones may only account for a small portion of total acetylated histone; therefore, the change cannot be detected under the conditions of this study.

A large body of evidence supports the idea that acetylation of histones activates transcription; however, exactly how the histone acetylation leads to transcription remains unclear (2). In this study, we show that the ATM gene product plays a role in this process. Additional understanding of this phenomenon will not only provide an insight into the mechanism of histone acetylation and transcription but also open a new angle to understand multiple functions of ATM.

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Rong Ju and Mark T. Muller


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