Inhibition of Histone Deacetylase Activity by Butyrate[1,2]

James R. Davie[3]

Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada

ABSTRACT This article reviews the effects of the short-chain fatty acid butyrate on histone deacetylase (HDAC) activity. Sodium butyrate has multiple effects on cultured mammalian cells that include inhibition of proliferation, induction of differentiation and induction or repression of gene expression. The observation that butyrate treatment of cells results in histone hyperacetylation initiated a flurry of activity that led to the discovery that butyrate inhibits HDAC activity. Butyrate has been an essential agent for determining the role of histone acetylation in chromatin structure and function. Interestingly, inhibition of HDAC activity affects the expression of only 2% of mammalian genes. Promoters of butyrate-responsive genes have butyrate response elements, and the action of butyrate is often mediated through Sp1/Sp3 binding sites (e.g., $p21^{Waf1/Cip1}$). We demonstrated that Sp1 and Sp3 recruit HDAC1 and HDAC2, with the latter being phosphorylated by protein kinase CK2. A model is proposed in which inhibition of Sp1/Sp3–associated HDAC activity leads to histone hyperacetylation and transcriptional activation of the $p21^{Waf1/Cip1}$ gene; $p21^{Waf1/Cip1}$ inhibits cyclin-dependent kinase 2 activity and thereby arrests cell cycling. Pending the cell background, the nonproliferating cells may enter differentiation or apoptotic pathways. The potential of butyrate and HDAC inhibitors in the prevention and treatment of cancer is presented. J. Nutr. 133: 2485S–2493S, 2003.

KEY WORDS: • *sodium butyrate* • *histone deacetylase* • *$p21^{Waf1/Cip1}$* • *histone acetylation* • *gene expression* • *Sp1* • *Sp3*

Butyrate is a short-chain fatty acid that is produced by anaerobic bacterial fermentation of dietary fibers. It was suggested (1,2) that butyrate may inhibit the development of colon cancer. This article reviews the action of butyrate in altering gene expression and arresting cell proliferation by inhibition of the chromatin-remodeling activity of histone deacetylases (HDAC).[4]

MATERIALS AND METHODS

Cell culture

Human breast cancer cell lines MCF-7 (T5) [estrogen-receptor (ER) positive and estrogen dependent] and MDA MB 231 (ER negative and estrogen independent) were cultured as described previously (3).

Pulse-chase labeling cells for analyses of histone acetylation rates

Human breast cancer cells and avian immature erythrocytes were pulse-labeled with [^{3}H]acetate and subsequently incubated in the absence of radiolabel and with sodium butyrate (10 mmol/L) as described previously (3,4). Rates of histone acetylation were determined as previously described (5,6).

Immunoprecipitation

The following is an efficient method to solubilize nuclear proteins. MCF-7 (T5) human breast cancer cells were lysed in immunoprecipitation buffer (50 mmol Tris-HCl/L, pH 8.0, 150 mmol NaCl/L, 0.5% Nonidet P-40 and 1 mmol EDTA/L) that contained 1 mmol phenylmethylsulfonyl fluorid/L, phosphatase inhibitors and protease-inhibitor cocktail. The cells were sonicated twice for 15 s. The cell lysate was collected by centrifugation at 10,000 × *g* for 10 min and incubated with anti-HDAC1, anti-HDAC2, anti-Sp1 or anti-Sp3 antibodies for 16 h at 4°C (7).

Sequential immunoprecipitations

Sequential immunoprecipitations were done as previously described (7). Briefly, cell lysates were incubated with anti-Sp1 antibodies. The immunoprecipitated and immunodepleted (supernatant) fractions were collected. Secondary immunoprecipitations were performed with the Sp1-immunodepleted (supernatant) fraction and anti-Sp3 antibodies, and the immunoprecipitated and immunodepleted fractions were collected.

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[3] To whom correspondence should be addressed. E-mail: davie@cc.umanitoba.ca.

[4] Abbreviations used: AUT, acetic acid–urea–Triton X-100; Cdk, cyclin-dependent kinase; ER, estrogen receptor; HAT, histone acetyltransferase; HDAC, histone deacetylase; NuRD, nucleosome-remodeling histone deacetylase complex; Rb, retinoblastoma protein; TSA, trichostatin A.

RESULTS

Effects of butyrate on cell proliferation and HDAC

In the mid-1970s several research groups reported that sodium butyrate halts DNA synthesis, arrests cell proliferation, alters cell morphology and increases or decreases gene expression (8). Treatment of erythroleukemic cells with butyrate was shown to be very effective in inducing differentiation in these cells (9). A turning point in understanding the mechanism of butyrate action was the observation by Ingram and colleagues (10) that butyrate increased the level of acetylated histones in cultured HeLa and Friend erythroleukemic cells. Several chromatin investigators interested in histone acetylation recognized that to increase histone acetylation, either the activity of histone acetyltransferases (HAT) was increased, or conversely, the activity of HDAC was inhibited. The latter, inhibition of HDAC activity, was found to be the mode of butyrate action (11–14).

Histone acetylation: a dynamic process that regulates chromatin structure

HDAC catalyzes the removal of acetate from modified lysine residues located in the N-terminal tail region of the core histones H2A, H2B, H3 and H4 (**Fig. 1A**). These core histones form a histone octamer around which is wrapped 146 bp of DNA. The four core histones have a similar structure that consists of a basic N-terminal domain, a central histone-fold domain (which mediates histone-histone and histone-DNA interactions) and a C-terminal tail (15). The crystal structure of the nucleosome shows that the N-terminal tails emanate from the nucleosome in all directions (**Fig. 1B**) (16). Reversible acetylation occurs on specific lysines that are located in the N-terminal tail domains of the core histones (Fig. 1A). With the exception of H2A, the core histones are acetylated at four or five sites; thus a nucleosome has potentially 28 or more sites of acetylation. In addition to acetylation, the core histones are modified by methylation, phosphorylation and ubiquitination (17).

Although we have known since the 1960s that histone acetylation has a role in chromatin structure and function, we still know little about what this modification does to remodel chromatin structure (18). However, one function of histone acetylation is to alter the compaction of chromatin. Acetylation of the histone tails disrupts higher-order chromatin folding (19) and promotes the solubility of chromatin at physiological ionic strength (20). Nucleosomes do not have to be maximally acetylated to prevent chromatin compaction. Hansen and colleagues (21) demonstrated that acetylation to 46% of maximal site occupancy is sufficient to prevent higher-order folding and stimulation of transcription by RNA polymerase III. It was proposed (22,23) that acetylation of core histone tails interferes with interactions with proteins and/or DNA and thereby destabilizes higher-order chromatin organization. These combined effects of histone acetylation on the destabilization of chromatin structure facilitate transcription (21,24) (**Fig. 1C**).

Enzymes catalyze dynamic histone acetylation

The steady state of acetylated histones in a eukaryotic cell and at a specific gene locus is governed by the net activities of histone acetyltransferases (HATs) and HDAC (**Fig. 2**). HATs often have transcriptional coactivator activity and when recruited to a gene promoter by a transcription factor will increase the level of acetylated histones and enhance transcriptional activity of the promoter (17,25). The most potent HAT in

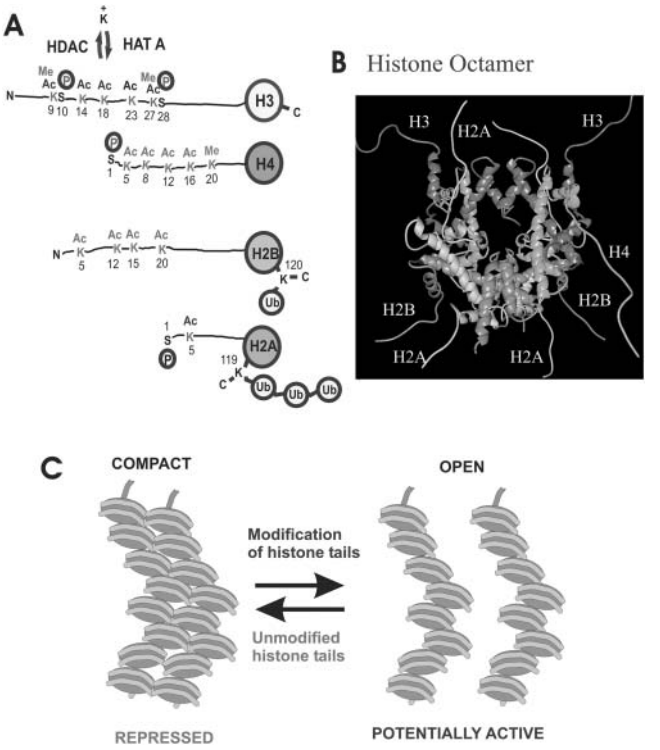

FIGURE 1 (*A*) Sites of postsynthetic modifications on the core histones. Structures of the core histones H2A, H2B, H3 and H4 and the sites of modification are indicated. Modifications shown are acetylation (Ac), phosphorylation (P), ubiquitination (Ub) and methylation (Me). Enzymes that catalyze reversible acetylation and phosphorylation are also shown. (*B*) Crystal structure of the nucleosome [adapted with permission from Dr. Timothy Richmond (15)]. (*C*) Chromatin fibers bearing unmodified tails interact; however, these interactions are disfavored when the tails are modified. HAT, histone acetyltransferase; HDAC, histone deacetylase.

mammalian cells are the following (17,26): cAMP response-element binding protein (CREB) binding protein (CBP), p300, p300/CREB binding protein-associated factor (PCAF) and HIV Tat interactive 60-kDa protein (Tip60). Steroid receptor coactivators 1 and 3 (SRC-1 and -3, respectively) are HATs recruited by steroid receptors (17).

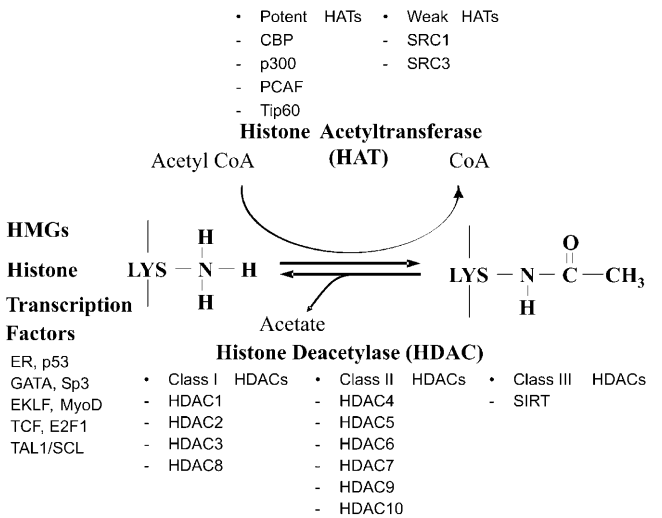

FIGURE 2 Dynamic histone acetylation is catalyzed by HAT and HDAC.

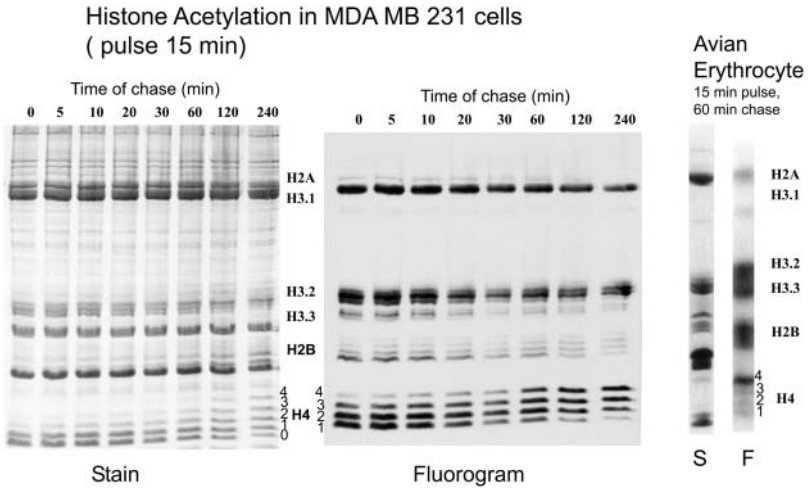

FIGURE 3 Effects of sodium butyrate on dynamic histone acetylation in MDA MB 231 human breast cancer cells and avian immature erythrocytes. MDA MB 231 cells were pulse-labeled with [^{3}H]acetate for 15 min and then chased for 0–240 min in the presence of 10 mmol sodium butyrate/L. Histones were resolved by acetic–acid urea–Triton X-100 polyacrylamide gel eletrophoresis (AUT-PAGE; 60 μg of protein/lane). The Coomassie blue–stained gel (S, *left panel*) and accompanying fluorogram (F, *right panel*) are shown. The two lanes at far right contained histones from avian immature erythrocytes that were pulse-labeled with [^{3}H]acetate for 15 min and chased for 60 min in the presence of 10 mmol sodium butyrate/L. The acetylated species of H4 are denoted numerically as 0, 1, 2, 3 and 4, which represent the un-, mono-, di-, tri- and tetra-acetylated species, respectively (3). (J. Biol. Chem. 276: 49435–49442, with permission.)

Three classes of HDAC are known. Class I HDAC consist of the mammalian HDAC1, -2 (mammalian homolog of yeast RPD3), -3 and -8. Class II HDAC include mammalian HDAC4, -5, -6, -7, -9 and -10 (27–32). Class III HDAC are members of the sirtuin family of HDAC, among which yeast Sir2 is the founding member (30).

Butyrate inhibits most HDAC except class III HDAC and class II HDAC6 and -10. During inhibition of HDAC activity, HAT activity continues, which results in histone hyperacetylation. Histones, however, are not the only substrates of these enzymes. High-mobility group proteins are acetylated. This modification has a wide range of effects on the function of the high-mobility group proteins in remodeling chromatin structure and regulating gene expression (33–35). Multiple transcription factors are acetylated (36) (Fig. 2). Acetylation of a transcription factor may alter its properties (37). For example, CBP acetylates p53 and GATA-1 and potentiates the activities of these transcription factors (36,38).

Dynamic histone acetylation: rates of acetylation and deacetylation

Histone acetylation is a dynamic process that occurs at different rates. In mammalian cells, one population of core histones is characterized by rapid hyperacetylation and rapid deacetylation ($t_{1/2}$ = 3–7 min). This highly dynamic acetylation-deacetylation process is limited to 10–15% of the core histones (3). A second population is acetylated and deacetylated at a slower rate ($t_{1/2}$ = 30 min) (39). Approximately 60–70% of the histones of cultured mammalian cells participate in reversible acetylation. The remainder of the histones is "frozen" in low- or nonacetylated states (25).

Incubation of human breast cancer cells (MDA MB 231) with sodium butyrate for 2 h has a major impact on the steady-state levels of acetylated histones (see acetylated H4 levels in **Fig. 3**). Histones were electrophoretically resolved on an acetic acid–urea–Triton X-100 (AUT) 15% polyacrylamide gel, which resolves histones according to size, charge and hydrophobicity. Thus, this gel system is ideal for separating modified histones and histone variants. For example, H3 has three variants (H3.1, H3.2 and H3.3) that are resolved with this gel system (40).

To study the fast rate of histone acetylation, cells were pulse-labeled with [^{3}H]acetate for 15 min, the label was removed and the cells were incubated with sodium butyrate to drive the dynamically acetylated histones into highly acetylated isoforms (Fig. 3, fluorogram). The fluorogram clearly shows the movement of the label in H4, H2B and H3 moving into the highly acetylated isoforms.

In contrast with mammalian cells in culture, only 2% of the histones in terminally differentiated avian immature erythrocytes participate in dynamic acetylation. Also, only the fast rate of histone acetylation is observed in these cells (6). Thus, a shift in the steady state of acetylated histone is not observed on the

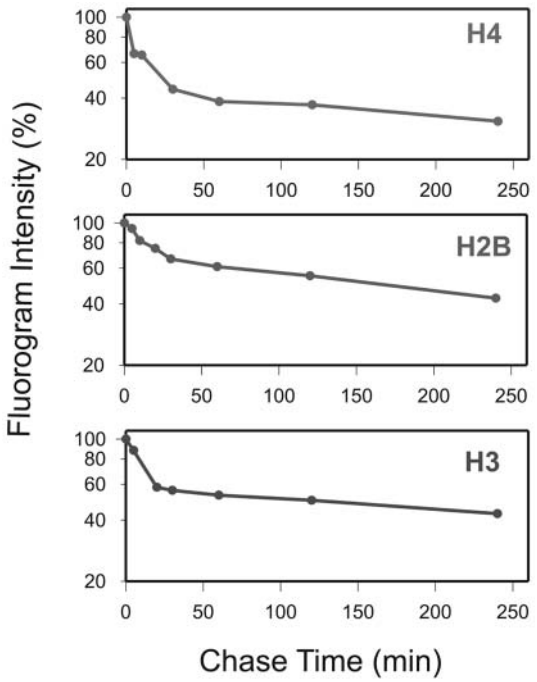

FIGURE 4 Analysis of the rates of histone acetylation in MCF-7 (T5) human breast cancer cells that were cultured under estrogen-replete conditions. MCF-7 (T5) cells were pulse-labeled with [^{3}H]acetate for 15 min and then chased for 0–240 min in the presence of 10 mmol sodium butyrate/L. Histones were resolved by AUT-PAGE (60 μg of protein/lane). Proportions of total radiolabeled H4, H2B and H3 associated with the monoacetylated form were determined by scanning the fluorograms. Proportion of labeled monoacetylated isoforms (H4-Ac1, H3.2-Ac1 and H2B-Ac1) present in total labeled H4, H3 and H2B at zero time was arbitrarily set at 100. The rapid rate of acetylation was determined using the data obtained from the 0–20 min butyrate-chase period, whereas the slower rate of acetylation was determined using data from the 60–240 min butyrate-chase period (3). (J. Biol. Chem. 276: 49435–49442, with permission.)

stained AUT gel pattern when avian cells are incubated with sodium butyrate for 1 h. Pulse-labeling of the cells with [^{3}H]acetate for 15 min followed by a chase for 60 min in the presence of butyrate rapidly drives the histones participating in dynamic acetylation into the highest acetylated isoforms. The dynamically acetylated histones are limited to transcriptionally active and competent regions of the avian erythrocyte genome (41). In mammalian cells, the bulk of the dynamically acetylated histones may serve a surveillance function (42).

By measurement of the rate of loss of label in the monoacetylated histone isoform (e.g., H4, H2B and H3.2 in Fig. 3), the rate of histone acetylation is determined. **Figure 4** shows that H4, H3.2 and H2B have two rates of acetylation in human breast cancer cells [MCF-7 (T5)]. For H4, the fast rate of acetylation has a $t_{1/2}$ time of 8 min, but the slower rate of acetylation has a $t_{1/2}$ time of 200–350 min (3,39).

Butyrate: the mode of action

Recently, the crystal structure of an HDAC-like protein from the hyperthermophilic bacterium *Aquifex aeolicus* with the HDAC inhibitor trichostatin A (TSA; **Fig. 5**) was reported (43). The structure shows the position of the essential zinc atom that is involved in catalysis of class I and II HDAC. HDAC-like protein shares 35.2% similarity over a 390-residue region with mammalian HDAC1; this region constitutes the deacetylase core. The aliphatic chain of TSA occupies a hydrophobic cleft on the surface of the enzyme (Fig. 5). Possibly two butyrate molecules also could occupy the hydrophobic pocket and inhibit the enzyme. However, butyrate was found to be a noncompetitive inhibitor of HDAC, which argues that butyrate does not associate with the substrate-binding site (44). The binding site and mechanism by which butyrate inhibits HDAC activity remain unknown.

HDAC complexes and transcription

Mammalian HDAC1 and -2 exist in large multiprotein complexes, Sin3 complex and nucleosome-remodeling histone deacetylase complex (NuRD; **Fig. 6**). The Sin3 complex, which has an estimated size of 1–2 MDa, contains mammalian (m)Sin3, Sin3-associated proteins of 18 and 30 kDa and retinoblastoma-associated proteins (RbAp)46 and -48 (25,30,45, 46). The Sin3 complex is directed to its target chromatin location by sequence-specific DNA binding proteins that interact directly with mSin3 and other components of Sin3 complex. Some examples of DNA binding proteins that recruit the Sin3 complex include the Mad family proteins, unliganded hormone receptors, methyl cytosine guanine dinucleotide (CpG) binding protein, p53, repressor element (RE)-1 silencing transcription factor and the Ikaros family proteins (25,30,46,47).

The complex called NuRD is ~2 MDa in size and consists of metastasis-associated protein 2 (which is highly related to metastasis-associated protein 1), Mi2, RbAp46 and -48 and methyl CpG binding domain-containing protein 3 (MBD3). NuRD has both ATP-dependent chromatin remodeling and HDAC activities (25,30).

When ER are bound to hydroxytamoxifen, ER recruit nuclear receptor corepressor/silencing mediator for retinoic and thyroid hormone receptors and HDAC to the promoter of an estrogen-responsive promoter and thereby repress promoter activity. However, when estradiol is bound to the ER, ER recruit coactivator/HAT and chromatin-remodeling complexes to the promoter to enable transcription (48,49). It is important to note that the steady-state level of acetylation at a regulatory element (e.g., promoter) or along the coding region of a gene is dictated by the balance of HAT and HDAC recruited to those sites. Because the occurrence of histone acetylation at transcriptionally active genes is a dynamic and rapid process, alterations in recruitment of factors for HAT or HDAC quickly change the balance of these two activities toward increasing or decreasing the steady-state level of acetylated histones. For example, a ligand-binding steroid receptor or a phosphorylated transcription factor (e.g., nuclear factor-κB) can be quickly changed to recruiting HDAC to HAT and vice versa (50,51).

Effects of estradiol on global histone acetylation dynamics in human breast cancer cells

This investigation is an example of studies that use sodium butyrate to determine histone acetylation and deacetylation rates. In this study, we investigated the effects of estradiol on

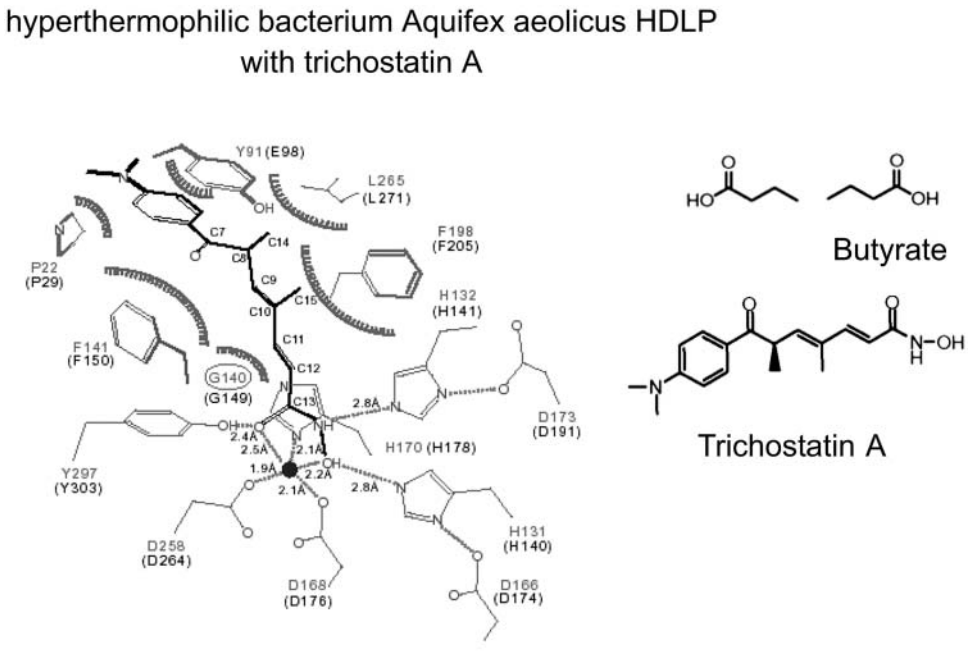

FIGURE 5 Schematic representation of histone deacetylase-like protein (HDLP, gray) interactions with trichostatin A (TSA, black) in the crystal structure of an HDAC homolog from the hyperthermophilic bacterium *Aquifex aeolicus*. HDLP residues are labeled in gray and their counterparts in HDAC1 are indicated in black [adapted with permission from Dr. Nikola P. Pavletich (43)]. Structures of TSA and butyrate are shown. (Nature 401: 188–193, with permission.)

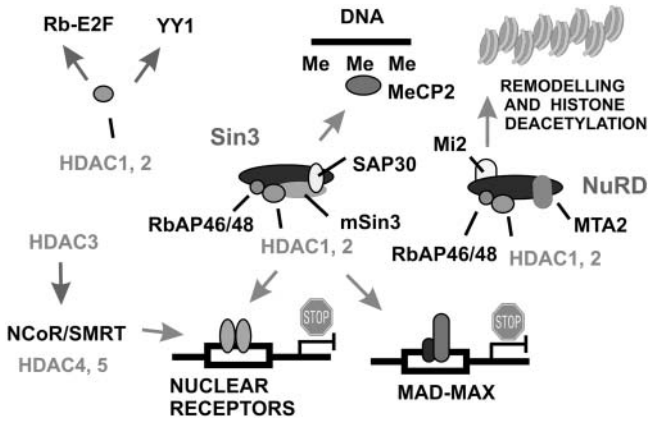

FIGURE 6 HDAC multiprotein complexes are recruited to specific genomic sites by regulatory proteins. HDAC1 and HDAC2 together with retinoblastoma-associated proteins (RbAp)46 and -48 are components of two large multiprotein complexes [Sin3 and nucleosome-remodeling histone deacetylase complex (NuRD)] that contain mSin3A/B or chromodomain helicase DNA binding protein 3/-4, respectively (25).

global dynamic histone acetylation in hormone-responsive human breast cancer cells (3). Histone acetylation–labeling experiments and immunoblot analyses of dynamically acetylated histones show that estradiol rapidly increases histone acetylation in ER-positive hormone-dependent MCF-7 (T5) human breast cancer cells. The effects of estradiol on the rates of histone acetylation and deacetylation in MCF-7 (T5) cells were determined. Estradiol increased the level of acetylated histones by reducing the rate of histone deacetylation, whereas the rates of histone acetylation were not altered.

Butyrate response element and gene expression

Studies reveal that among the fatty acids, butyrate is the most effective in inhibiting HDAC activity and arresting cell proliferation (52). Butyrate also is the most effective fatty acid in stimulating or repressing the expression of specific genes (**Table 1**). Considering the actions of butyrate to inhibit HDAC activity and promote histone hyperacetylation (see Fig. 3), it is surprising to learn that expression of only 2% of the mammalian genes is affected when HDAC activity is inhibited (53,54).

Within the promoter of butyrate-responsive genes is found a butyrate response element (55–59). It appears that these butyrate elements may be separated into different groups depending on the DNA sequence of the butyrate response element (**Table 2**). One group of genes that are either induced or repressed by butyrate has a common DNA sequence in the butyrate response elements, which suggests that a common transcription factor binds to this site. Another group, which includes the cyclin-dependent kinase 2 (Cdk2) inhibitor p21$^{Waf1/Cip1}$, shares an Sp1/Sp3 binding site in the butyrate response elements.

TABLE 2

Butyrate response elements (55–59)[1]

Gene	Butyrate response	Butyrate response element
Group 1		
Cyclin D1	Repression	AGCCACCTCCA
Intestinal trefoil factor	Repression	.....AG .....
Calbindin-D28k	Induction	..... A.G .....
Metallothionein IIA	Induction	.....C...T.....
Group 2		
Galectin 1	Induction	Sp1/Sp3 binding site
$G\alpha_i2$	Induction	Sp1/Sp3 binding site
IGF-binding protein 3	Induction	Sp1/Sp3 binding site
Cdk2 inhibitor p21$^{Waf1/Cip1}$	Induction	Sp1/Sp3 binding site

[1] IGF, insulin-like growth factor; Cdk2, cyclin-dependent kinase 2.

Sp1, Sp3 and recruitment of HDAC

Sp1 and Sp3 are ubiquitously expressed mammalian transcription factors that function as activators or repressors. Sp1 and Sp3 bind with equivalent affinity to GC boxes via their three zinc fingers located in the C-terminal region of the protein (60). Activation domains A and B (Gln- and Ser/Thr-rich regions, respectively) are located in the N-terminal part of the protein, whereas the D domain, which is found in the C-terminal region, is involved in multimerization (61,62). Synergistic transcriptional activation is mediated through the capacity of the Sp1 D domain to form multimers (61,62). Scanning transmission electron microscopy provides evidence (61) that Sp1 first forms a tetramer and then assembles multiple stacked tetramers at the DNA binding site. The interesting feature of this structure is that an Sp1 multimer presents several interacting surfaces to proteins that associate with Sp1 [e.g., p300/CBP, HDAC1, transcriptional activator factor II subunits of transcription factor IID, cofactor required for Sp1 activation, E2F transcription factor 1, and ER (63–66)]. The net activity of these factors to promote or hinder transcription depends on the abundance, affinity and residence time of these factors on the Sp1 multimer.

Sp3 has three isoforms, a long (L-Sp3) and two short (M1- and M2-Sp3) forms that are the products of differential translational initiation (67,68). The protein structure of L-Sp3 is very similar to that of Sp1 except that Sp3 has a repression domain located at the N-terminal to the zinc-finger DNA binding domain (60). The factors that regulate the translational initiation of Sp3 mRNA are currently unknown.

TABLE 1

Effects of fatty acids on mammalian cells in culture (52)

No. of carbons in fatty acid	Effect on fibroblast growth *% control*	Induction of alkaline phosphatase (HeLa) *% control*	Inhibition of estradiol-induced synthesis of transferring mRNA	Inhibition of histone deacetylase (calf thymus)
C2, acetate	82	170	18	10
C3, propionate	45	160	77	60
C4, butyrate	0	630	95	80
C5, valeroic	71	420	—	65
C6, caproate	—	120	—	30

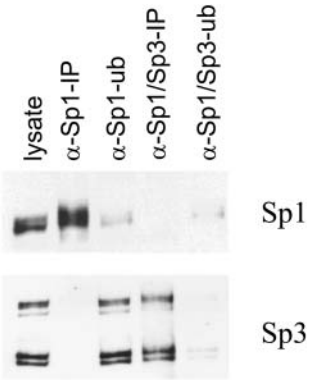

FIGURE 7 Sp1 is not associated with Sp3. MCF-7 (T5) cell lysate was incubated with anti-Sp1 antibodies, and the immunoprecipitation (IP, lane 2) and immunodepletion (ub, lane 3) fractions were collected. The immunodepleted fraction was then incubated with anti-Sp3 antibodies, which yielded IP (lane 4) and ub (lane 5) fractions. Proteins of the cell lysate (lane 1), IP and ub fractions were loaded onto a sodium dodecyl sulfate (SDS) 10% polyacrylamide gel, transferred to nitrocellulose membranes and immunochemically stained with anti-Sp1 and -Sp3 antibodies. Long (L) and short (M1 and M2) forms of Sp3 are identified (7). (J. Biol. Chem. 277: 35783–35786, with permission.)

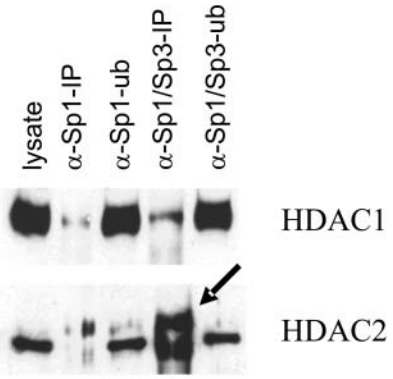

FIGURE 8 Sp1 and Sp3 are associated with HDAC1 and HDAC2. MCF-7 (T5) cell lysate, IP and ID fractions were prepared as described for Fig. 7 and were loaded onto an SDS 10% polyacrylamide gel, transferred to nitrocellulose membranes and immunochemically stained with anti-HDAC1 and -HDAC2 antibodies. Arrow indicates the HDAC2 species with reduced mobility (7). (J. Biol. Chem. 277: 35783–35786, with permission.)

Although Sp1 and Sp3 share a common D domain that is involved in forming multimers, we reported that Sp1 and Sp3 form separate complexes in estrogen-dependent human breast cancer cells (7). In performing these studies, we wanted to ensure efficient solubilization of nuclear proteins, because Sp1 and Sp3 are tightly bound to the nucleus of MCF-7 (T5) breast cancer cells (see Materials and Methods). Sequential immunoprecipitations were done first with anti-Sp1 antibodies and then with anti-Sp3 antibodies (see Materials and Methods). **Figure 7** shows that Sp1 and Sp3 form separate complexes.

Next, we determined whether Sp1 and Sp3 were associated with HDAC activity. A previous report showed that Sp1 was associated with HDAC1 (64). Both Sp1 and Sp3 were associated with HDAC activity in human breast cancer cells (7). In immunoblot analyses of the Sp1 and Sp3 immunoprecipitated complexes, we observed that HDAC1 and -2, but not HDAC3, were associated with Sp1 and Sp3 (**Fig. 8**). However, it was very interesting to find a major enrichment of a slower migrating HDAC2 species associating with Sp1 and Sp3. Additional investigation revealed that this slower migrating species was protein kinase CK2–phosphorylated HDAC2 (7,69). Alkaline phosphatase treatment of HDAC2, Sp1 and Sp3 complexes reduced the associated HDAC activity.

The protein kinase CK2 is a tetramer that consists of two α- (or α'-) and two β-subunits (70). In immunoprecipitation experiments, we found that CK2 was associated with HDAC2 and to a lesser extent with HDAC1 (**Fig. 9**). Although we found that HDAC2 is associated with MBD3 (a component of the NuRD HDAC complex) and Sin3A (a component of the Sin3 complex; see Fig. 6), it remains to be determined whether CK2 or HDAC2 is associated with either of these complexes. CK2 is upregulated in several cancers including breast cancer, and there is evidence that CK2 may promote breast cancer by deregulating key transcription processes (71–74).

A model for butyrate induction of p21$^{Waf1/Cip1}$ gene expression and inhibition of cell cycle

The p21$^{Waf1/Cip1}$ promoter has six Sp1 binding sites (the butyrate response element). Evidence has been presented that Sp3 and not Sp1 is associated with this promoter (75). Also, the

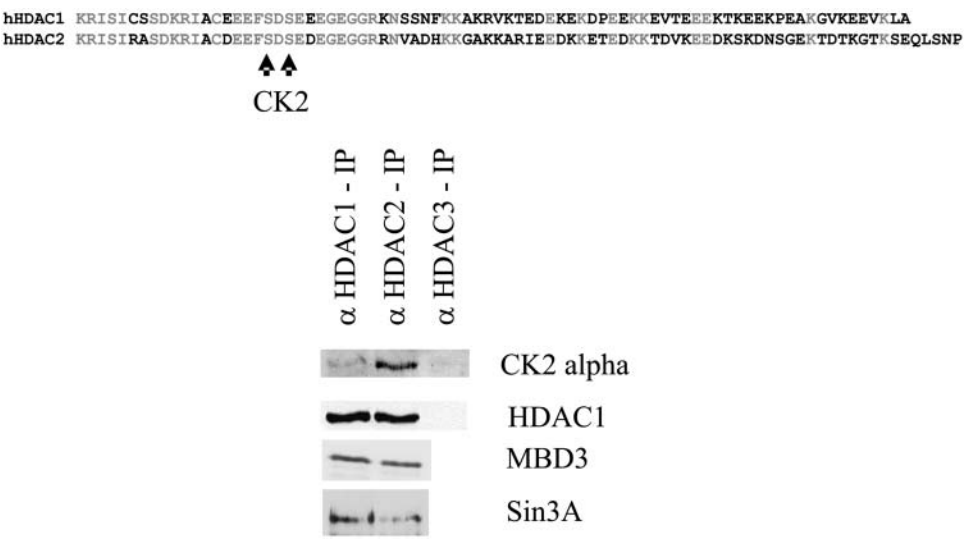

FIGURE 9 HDAC2 is associated with and phosphorylated by protein kinase CK2. The C-terminal amino acid sequences of mammalian HDAC1 and HDAC2 and the sites of protein kinase CK2 phosphorylation are shown. Equal amounts of MCF-7 (T5) cell lysate were immunoprecipitated with anti-HDAC1, -HDAC2 or -HDAC3 antibodies. Immunoprecipitated samples and nuclear extracted protein (10 μg) were loaded onto an SDS 10% polyacrylamide gel, transferred to a nitrocellulose membrane and immunochemically stained with anti-CK2α, -HDAC1, -MBD3 and -Sin3 antibodies (7). (J. Biol. Chem. 277: 35783–35786, with permission.)

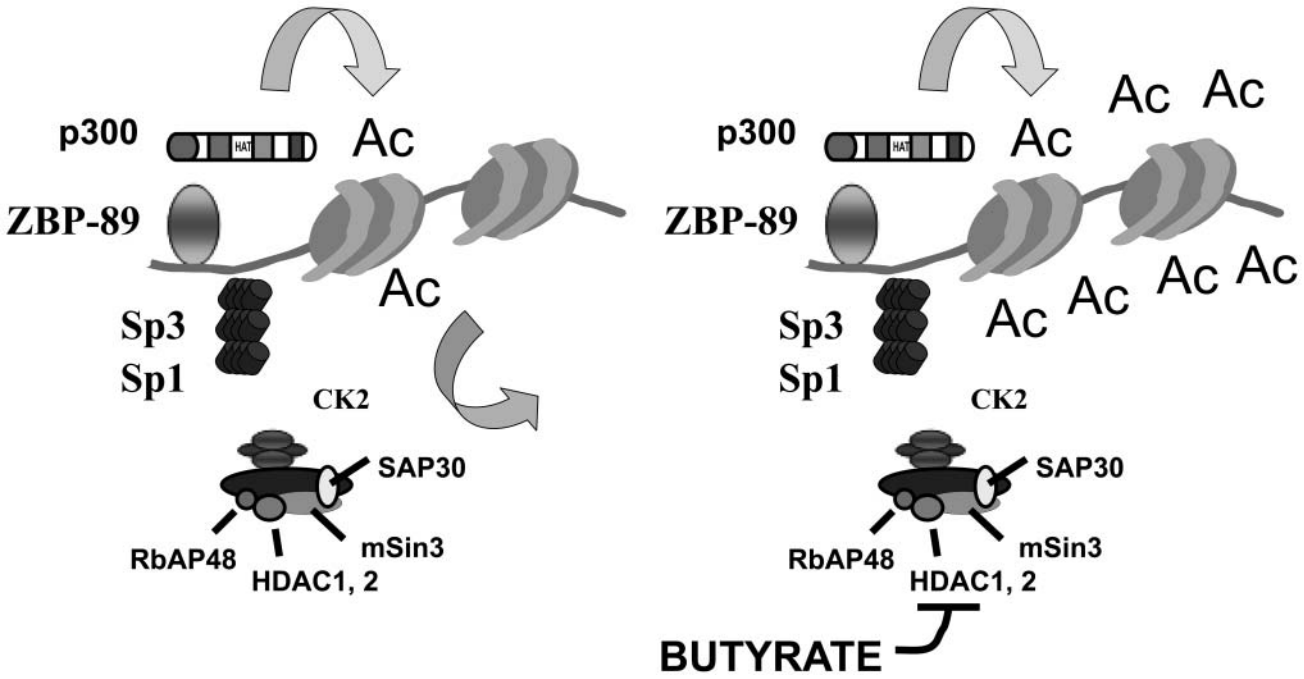

FIGURE 10 Model for the butyrate induction of the Cdk2 inhibitor p21$^{Waf1/Cip1}$. In the absence of butyrate, zinc-finger DNA binding protein 89 and Sp3/Sp1 recruit the p300 and HDAC1,2/CK2 complex, respectively. The steady-state level of histone acetylation is low and not supportive of transcription (*left panel*). When butyrate is present, HDAC activity is inhibited, which allows the histones to become hyperacetylated. The modified chromatin then supports transcription (*right panel*).

Sp1-like protein zinc-finger DNA binding protein 89 (ZBP-89) is associated with one or more of the Sp1 sites. ZBP-89 recruits p300, which is a coactivator/HAT (76). Thus, ZBP-89 would recruit HAT activity to the promoter, whereas Sp3 would recruit HDAC activity to the p21$^{Waf1/Cip1}$ promoter and result in dynamic histone acetylation (**Fig. 10**). The steady-state level of acetylated histones associated with the p21$^{Waf1/Cip1}$ promoter is low, which favors a condensed chromatin structure and inactive promoter (77). Inhibition of HDAC activity with sodium butyrate allows the HAT activity of p300 to increase the histone acetylation levels at the promoter and nearby regions (77). Hyperacetylation of the histones would support chromatin opening and induction of p21$^{Waf1/Cip1}$ gene expression.

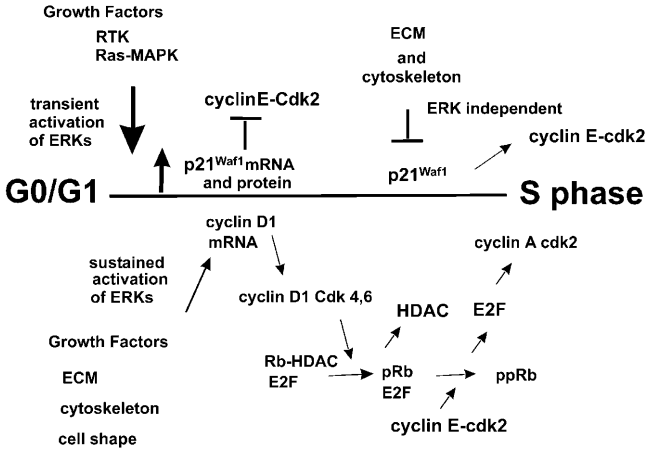

FIGURE 11 Induction of p21$^{Waf1/Cip1}$ gene expression by transient activation of extracellular signal-related kinase activity. Elevated levels of p21$^{Waf1/Cip1}$ inhibit cyclin E–Cdk2 activity and promote the assembly of stable cyclin D1–Cdk4/6 kinase complexes. The p21$^{Waf1/Cip1}$ gene is repressed and p21$^{Waf1/Cip1}$ protein levels decline, which allows activation of cyclin E–Cdk2. Both cyclin D1–Cdk4,6 and cyclin E–Cdk2 are involved in the phosphorylation of Rb and the release of E2F, which activates the promoters of genes involved in progression through the S phase of the cell cycle. Butyrate would induce expression of p21$^{Waf1/Cip1}$ and inhibit cyclin E–Cdk2 activity and thereby arrest cell cycle progression. ECM, extracellular matrix; RTK, receptor tyrosine kinase.

In the transition from the G1 to the S phase of the cell cycle, p21$^{Waf1/Cip1}$ has a key role (**Fig. 11**). Initially, there is an increase in p21$^{Waf1/Cip1}$ expression after the transient activation of the extracellular signal-related kinase and the Ras mitogen-activated protein kinase pathway (78). The p21$^{Waf1/Cip1}$ inhibits the activity of cyclin E–Cdk2 kinase and promotes the assembly of stable cyclin D1–Cdk4/6 kinase complexes (79). Subsequently, p21$^{Waf1/Cip1}$ gene expression is repressed, which results in the lowering of p21$^{Waf1/Cip1}$ protein levels and the activation of cyclin E–Cdk2. Cyclin E–Cdk2 activity is required for the final-stage phosphorylation of Rb and the release of the transcription factor E2F, which induces the expression of genes that are involved in taking cells through the S phase (DNA synthesis phase) of the cell cycle (25,79–81). Butyrate induces expression of p21$^{Waf1/Cip1}$ and thereby inhibits cyclin E–Cdk2 activity and halts the subsequent events that are required for cells to enter S phase. The cell cycle–arrested cells may differentiate or undergo cell death by apoptosis.

Butyrate and HDAC inhibitors in prevention and treatment of cancer

By inhibiting the HDAC activity recruited to the p21$^{Waf1/Cip1}$ promoter by Sp1 or Sp3, butyrate induces the expression of p21$^{Waf1/Cip1}$ and thereby stops cell proliferation. This is a p53-independent process (82). Several studies (1,2) suggest that the production of butyrate in the colon may be protective against colon carcinogenesis. Current studies and clinical trials (83–88) strongly suggest that HDAC inhibitors such as TSA and sub-

eroylanilide hydroxamic acid, which also induce $p21^{Waf1/Cip1}$ expression, are effective in arresting cancer cell proliferation and lead to cells undergoing differentiation (as in acute promelocytic anemia) or apoptosis. These new strategies for prevention and treatment of cancer have been termed "gene-regulating chemoprevention," "gene-regulating chemotherapy" and "transcription therapy" (88,89). No matter which term wins the day, these are exciting times for the dietary micronutrient butyrate and HDAC inhibitors in the challenge of preventing and treating cancer.

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