Butyrate-Induced G_2/M Block in Caco-2 Colon Cancer Cells is Associated with Decreased $p34^{cdc2}$ Activity (44438)

LAWRENCE E. HARRISON,*[,1] QING MEI WANG,* AND GEORGE P. STUDZINSKI†

*Departments of *Surgery, †Pathology and Laboratory Medicine, UMDNJ–New Jersey Medical School, Newark, New Jersey 07103*

Abstract. **Butyrate, a short-chain fatty acid, has been reported to inhibit proliferation and stimulate differentiation in multiple cancer cell lines. Whereas the effects of butyrate on cellular differentiation are well documented, the relationship between butyrate-induced differentiation and its effect on cell cycle traverse is less well understood. The purpose of this study was to investigate the effects of butyrate on the regulatory proteins of the G_2/M traverse in the Caco-2 colon cancer cell model. We demonstrated that the inhibition of proliferation and increased cellular differentiation after treatment of Caco-2 cells with butyrate were associated with a significant G_2/M cell cycle block. Although protein levels of the major G_2/M regulatory protein, $p34^{cdc2}$, were unchanged, a decrease in $p34^{cdc2}$ activity was noted. Despite this decrease in activity, the inhibitory tyrosine phosphorylation of $p34^{cdc2}$ was decreased, suggesting that other factors are responsible for the decreased kinase activity. The reduced activity of $p34^{cdc2}$ provides a possible mechanism for the accumulation of Caco-2 cells in the G_2/M cell cycle compartment following exposure to butyrate. This cell system provides a new model for studies of G_2/M cell cycle perturbations.**

[P.S.E.B.M. 1999, Vol 222]

Many epidemiological and experimental studies suggest that dietary fiber is protective against the development of colon carcinoma (1). The mechanism by which fiber achieves this protective effect remains essentially unknown. Recently, the salutary effects of dietary fiber have been attributed in part to the production of short chain fatty acids (SCFA). SCFAs are natural constituents of the colonic lumen produced during anaerobic fermentation of dietary fiber by endogenous intestinal bacteria (2). Butyrate has been identified as one such SCFA and has been reported to inhibit proliferation and stimulate differentiation in multiple cancer cell lines (3, 4). Specifically, exposure of colon carcinoma cells to butyrate results in growth arrest and cellular differentiation as evidenced by morphologic changes and increased cellular expression of alkaline phosphatase (5), CEA (6), and villin (7). Furthermore, increased colonic butyrate levels, as a result of dietary fiber supplementation, correlate with reduced colon cancer cell proliferation in a rodent model (8).

Although the effects of butyrate on cellular differentiation are well documented, the relationship between butyrate-induced differentiation and its effect on cell cycle traverse is less well understood. Various groups have reported a G_1/S and/or a G_2/M block associated with butyrate treatment of neoplastic cells (9–11). Whereas a mechanism for the butyrate-induced G_1/S block has been suggested (11, 12), less is known about the G_2/M block, also associated with this SCFA. Entry into M phase for all eukaryotic cells depends on activation of the protein kinase $p34^{cdc2}$ complex and its regulatory subunit, cyclin B1. Additional levels of regulation of $p34^{cdc2}$ activity include: 1) availability of the G_2/M cyclin, cyclin B1; 2) phosphorylation of $p34^{cdc2}$ tyrosine 15 and threonine 14 by the inactivating kinases, wee-1 and $p53/56^{lyn}$ and dephosphorylation by the activating phosphatase cdc25c; and 3) activating phosphorylation of threonine 161 by cyclin-activating kinase (CAK), which consists of two subunits, cyclin H and cdk7 (13).

This research was funded by the Department of Surgery, UMDNJ–New Jersey Medical School.

[1] To whom requests for reprints should be addressed at Department of Surgery, Division of Surgical Oncology, UMDNJ–New Jersey Medical School, 185 South Orange Avenue, MSB G588, Newark, NJ 07103. E-mail: Harris11@umdnj.edu

Received March 23, 1999. [P.S.E.B.M. 1999, Vol 222]
Accepted July 9, 1999.

0037-9727/99/2222-0150$14.00/0

The purpose of this study was to investigate the effects of butyrate on the regulatory proteins of the G_2/M traverse in the Caco-2 cell model. We demonstrated that the differentiating and antiproliferative effects of butyrate after exposure to Caco-2 colon cancer cells are associated with a significant G_2/M cell cycle block. We also showed a marked decrease in $p34^{cdc2}$ kinase activity, despite its protein levels being unchanged. Whereas the inhibitory tyrosine phosphorylation of $p34^{cdc2}$ was decreased after butyrate exposure, cyclin B1 activity may account for the observed decrease in $p34^{cdc2}$ activity and G_2/M arrest.

Materials and Methods

Materials. Unless otherwise indicated, all chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). All antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), with the exception of the phosphotyrosine-$p34^{cdc2}$ specific antibody (New England Biolabs, Beverly, MA).

Cell Culture. Caco-2 cells were maintained in RPMI 1640 supplemented with 10% complement-inactivated bovine calf serum. Three × 10^5 cells were seeded and treated with 3 m*M* butyrate for 0 (control), 24, and 48 hr. Cells were harvested and their viability checked by trypan blue (0.25%) exclusion.

Alkaline Phosphatase Activity. Alkaline phosphatase (ALP) activity was measured by a commercially available kit (Sigma). Briefly, total cellular lysates were prepared (in the absence of NaF and $NaVO_4$), and aliquots of 100 μg of protein were assayed for ALP activity by the addition of *P*-nitrophenyl phosphate substrate. Following incubation at 37°C for 15 min, absorbance was read at 410 nm.

Cell Proliferation Assay. Three × 10^4 cells were plated in 96-well plates and treated with 3 m*M* butyrate for 0, 24, and 48 hr. MTT (0.5 mg/ml) was added to each well, and cells were incubated for 3 hr at 37°C and 5% CO_2. Formazan crystals were dissolved with DMSO, and color intensity was measured using an ELISA reader at 570 nm (reference filter, 690 nm).

Flow Cytometry. For DNA content evaluation, 3 × 10^6 cells were fixed in 75% ethanol at –20°C for 24 hr. After washing in ice-cold PBS, the cells were incubated with 0.5 ml of propidium iodide stain (10 μg/ml) in the presence of RNase (500 μg/ml) for 2 hr at 4°C. The DNA content was determined using the Epics Profile II Flow cytometer (Coulter, Hialeah, FL), and cell cycle distribution was analyzed by the Multicycle software package (Phoenix Flow Systems, San Diego, CA).

For evaluation of bromodeoxyuridine (BrdU) incorporation, 10^6 cells were pulsed with BrdU at a final concentration of 10 μ*M* for 3 hr in a 4% CO_2 incubator at 37°C, resuspended in fresh medium and cultured for an additional 6 hr. The cells were then centrifuged and fixed in 75% ethanol at –20°C overnight. To produce the single-stranded DNA, the cells were incubated with 2 *N* HCl/Triton X-100 for 30 min at 25°C, and the cell suspension was neutralized using 0.1 *M* sodium tetraborate. The cells were incubated with FITC-conjugated anti-BrdU antibody (Becton Dickinson, San Jose, CA) for 30 min, and resuspended in propidium iodide (final concentration: 5 μg/ml). Fluorescence intensity was determined using a FACScan flow cytometer (Becton Dickinson) and analyzed by CellQuest software (Becton Dickinson).

Protein Preparation, Immunoblotting, and Immunoprecipitation. Whole cell extracts were prepared by lysing cell pellets with a Dounce microtip homogenizer in lysis buffer (50 m*M* Tris-HCl, 150 m*M* NaCl, 0.5% NP40, 50 m*M* NaF, 0.2 m*M* $NaVO_4$, 1 m*M* DTT, 1 m*M* phenylmethylsulfonyl fluoride, 25 μg/ml leupeptin, 25 μg/ml aprotinin, 25 μg/ml pepstatin A). Cell debris was removed by centrifugation at 14,000*g* for 20 min at 4°C, and the supernatants stored at –80°C.

Samples for immunoblotting were prepared by mixing aliquots of the protein extracts with 3X SDS sample buffer (150 m*M* Tris [pH 6.8], 30% glycerol, 3% SDS, bromophenol blue dye 1.5 μg/100 ml, 100 m*M* DTT) and denatured by heating to 100°C for 4 min. Protein samples were then separated by SDS-PAGE, electrotransferred to a nitrocellulose membrane (Amersham, Arlington Heights, IL), and incubated in 5% nonfat milk blocking buffer (Tris-buffered saline, 5% dry milk, and 0.05% Tween-20) for 1 hr. The membrane was subjected to immunoblot analysis with the appropriate antibody, and proteins were visualized by the chemiluminescence method of detection (Amersham).

For immunoprecipitation, cells were lyzed in lysis buffer and cell debris removed by centrifugation at 14,000*g* for 20 min at 4°C. Five hundred μg of total protein were incubated with the appropriate antibody for 2 hr at 4°C, followed by incubation with protein A-G agarose beads for 1 hr. The protein complexes were washed three times with immunoprecipitation buffer and released from the beads by boiling in 3X SDS sample buffer for 5 min, and separated by 13% SDS-PAGE.

Kinase Reaction Assays. Total cellular lysates were prepared, and $p34^{cdc2}$ was immunoprecipitated as described above. As a control to establish specificity, a blocking peptide to the carboxy terminus of the $p34^{cdc2}$ antibody (Santa Cruz Biotechnology, Inc., 954-P) was first incubated with the $p34^{cdc2}$ antibody for 1 hr prior to immunoprecipitation. The agarose beads were washed with immunoprecipitation buffer and subsequently washed with kinase reaction buffer (50 m*M* HEPES, 10 m*M* $MgCl_2$, 5 m*M* $MnCl_2$, 1 m*M* DTT, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin A, 0.2 m*M* $NaVO_4$, 1 m*M* phenylmethylsulfonyl fluoride and 50 m*M* NaF). The kinase reaction was carried out at 37°C for 30 min in 40 μl of kinase reaction buffer containing 10 μ*M* ATP, 0.4 mCi/ml[γ-^{32}P] ATP (specific activity = 3000 Ci/mmol), and 2 μg of histone H1. The reaction was stopped by adding 3X SDS sample buffer, and the supernatant was separated on a 13% SDS-

PAGE gel. The radioactivity of the ^{32}P-labeled histone was detected by autoradiography.

Statistics. All experiments were run in triplicate. Densitometric quantitation (NIH-Image, (Scion, Frederick, MD)) of protein immunoblotting and kinase assays were expressed as mean ± SEM. Comparison between groups were determined by ANOVA and Bonferroni *post hoc* analysis. A P-value ≤ 0.05 was defined as significant.

Results

Butyrate-Induced Differentiation of Caco-2 Cells Is Associated with a G_2 Cell Cycle Block. Initial experiments were performed to confirm the antiproliferative and differentiating effects of butyrate. Butyrate treatment resulted in a significant decrease in cell proliferation, as measured by MTT uptake (Fig. 1A). A 36% ± 5% ($P < 0.01$) and a 67% ± 8% ($P < 0.001$) decrease in proliferation was noted at 24 and 48 hr, respectively, after exposure to 3 m*M* butyrate. In addition, a significant increase in cellular differentiation, as measured by increased ALP activity was also noted by 48 hr (a 4.5-fold increase, $P < 0.001$; Fig. 1B).

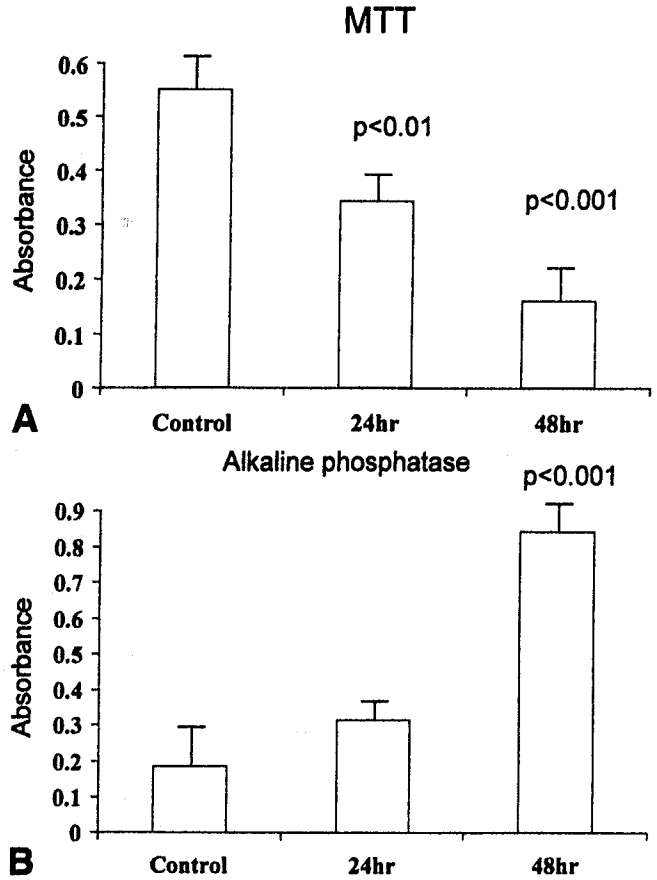

Figure 1. The effect of butyrate on Caco-2 cell proliferation and differentiation. (A) Three × 10^4 cells were plated in 96-well plates and after 24 hr, treated cells were exposed to 3 m*M* butyrate for 24 and 48 hr. Cells grown for the entire 72 hr in the absence of butyrate constitute the control cells. Butyrate treatment resulted in a significant decrease in cell proliferation, as measured by MTT uptake. (B) For ALP activity, 100 µg of protein lysate were added to the substrate, *P*-nitrophenyl. A significant increase in cellular differentiation as measured by a 4.5-fold increase in ALP activity was noted after exposure to butyrate after 48 hr.

The effect of butyrate on cell cycle traverse was analyzed by flow cytometry of propidium iodide–stained Caco-2 cells (Fig. 2A). A marked G_2/M block was apparent, with an increase in the proportion of cells in the G_2/M compartment and a concomitant decrease in the proportion of cells in the G_1 compartment in cultures treated with 3 m*M* butyrate for 24 and 48 hr ($P < 0.01$). We confirmed this isolated G_2/M block by BrdU pulse-chase experiments. Butyrate exposure resulted in a higher percentage of cells in G_2/M (Region 5, 9.9% vs 16.4%) and a lower percentage in G_1 (Region 4, 34.1% vs 24%) as compared to control cells (Fig. 2B, 3 hr pulse). The absence of a G_1 block is also further demonstrated here (Region 4, Fig. 2B)

Butyrate Decreases $p34^{cdc2}$ Kinase Activity. To initiate a study of the mechanisms of the G_2/M block, protein levels and kinase activity of $p34^{cdc2}$ were examined. Immunoprecipitated $p34^{cdc2}$ was analyzed for kinase activ-

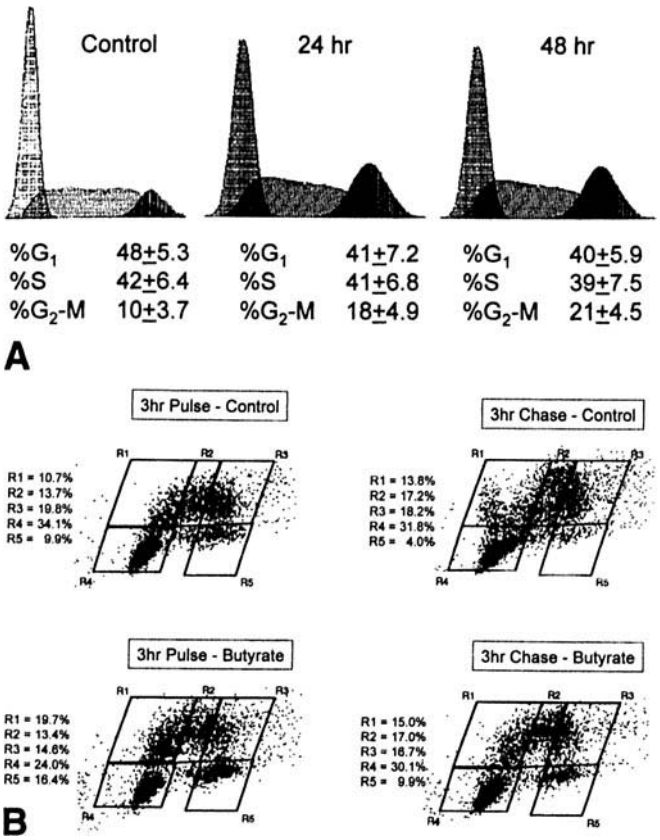

Figure 2. The effect of butyrate on cell cycle distribution of Caco-2 cells. (A) An example of the effect of butyrate on cell cycle distribution of Caco-2 cells as determined by DNA content. Caco-2 cells were treated with 3 m*M* butyrate for 24 and 48 hr, and a significant G_2/M block is evident after treatment at each time point ($P = 0.001$). (B) Pulse-Chase BrdU labeling: Caco-2 cells treated for 48 hr with 3 m*M* butyrate or equivalent volume of the vehicle were pulsed with bromodeoxyuridine (BrdU), washed, and cultured for an additional 3 hr (chase). Cell populations in Panels R1, R2, and R3 represent BrdU-positive cells in early S, mid-S, and late S phases, respectively. Panels R4 and R5 represents cells in G_1 and G_2/M, respectively. Butyrate exposure resulted in a higher percentage of cells in G_2/M (Region 5, 9.9% vs 16.4%) and lower percentage in G_1 (Region 4, 34.1% vs 24%) as compared to control cells. The repopulation of Panel R1 in the control cells after 3 hr chase shows normal traverse of G_2/M.

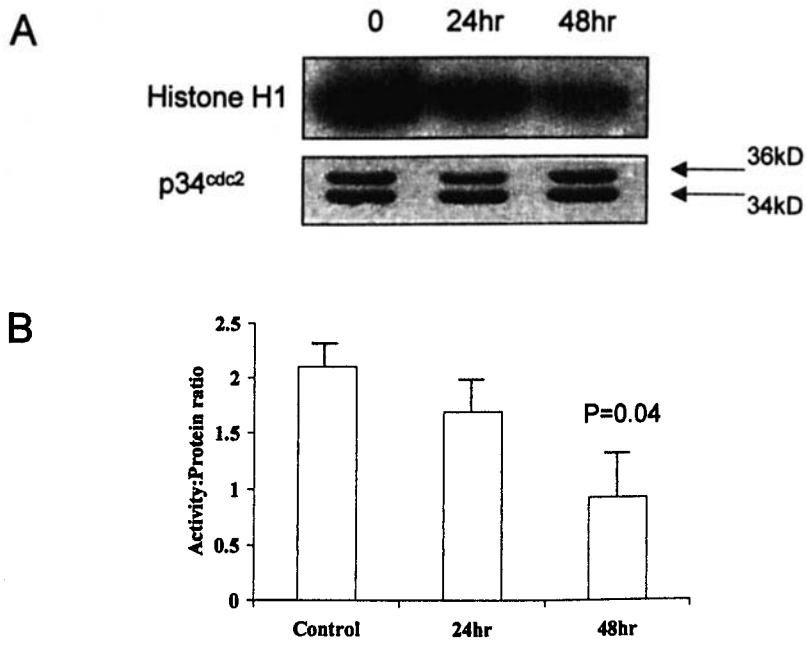

Figure 3. The effect of butyrate on $p34^{cdc2}$. (A) The $p34^{cdc2}$ kinase activity was decreased after exposure to 3 m*M* butyrate for 24 and 48 hr. Total cellular lysates were prepared, and $p34^{cdc2}$ was immunoprecipitated. The kinase reaction was carried out at 37°C for 30 min in 40 µl of kinase reaction buffer containing 10 µ*M* ATP, 0.4 mCi/ml[γ-^{32}P] ATP, and 2 µg of histone H1. The supernatant was separated on a 13% SDS-PAGE gel, and the radioactivity of the ^{32}P-labeled histone was detected by autoradiography. Immunoblot analysis of Caco-2 cells for $p34^{cdc2}$ demonstrates a major band at 34 kDa and a minor band at 36 kDa. There was no difference in $p34^{cdc2}$ protein levels after butyrate treatment. (B) Densitometric quantitation of $p34^{cdc2}$ protein levels and kinase activity is expressed as an activity-:protein ratio. There is a greater than two-fold decrease in the specific activity after 48 hr of exposure to butyrate ($P < 0.01$).

ity using histone H1 as a substrate. Butyrate-treated cells exhibited decreased $p34^{cdc2}$ kinase activity compared to untreated controls. However, treatment of Caco-2 cells with butyrate did not change the protein levels of $p34^{cdc2}$ (Fig. 3A). Equal loading of protein on the gel was documented by Ponceau red staining (not shown). The ratios of kinase activity and protein levels were calculated following densitometry measurements and are shown in Figure 3B. A significant decrease in specific activity of $p34^{cdc2}$ was detected after 48 hr of exposure to butyrate ($P = 0.04$).

The extent of tyrosine phosphorylation of $p34^{cdc2}$ in control and butyrate-treated cells was determined by immunoblotting with a phosphotyrosine $p34^{cdc2}$-specific antibody (Fig. 4A). These results were confirmed by immunoprecipitating $p34^{cdc2}$ followed by immunoblotting with a phosphotyrosine-specific antibody (Fig. 4B). Both methods revealed a decrease in $p34^{cdc2}$ tyrosine phosphorylation. Membranes were re-probed for total $p34^{cdc2}$, demonstrating equal amounts of overall $p34^{cdc2}$ protein levels in control and butyrate-treated cells (Figs. 4A and 4B).

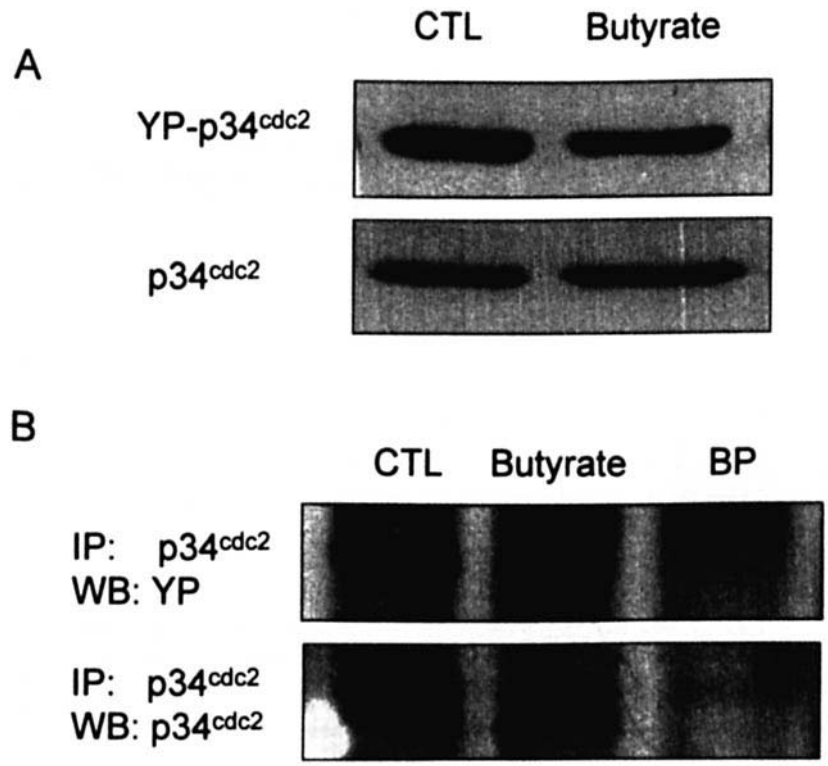

Figure 4. The effect of butyrate on $p34^{cdc2}$ tyrosine phosphorylation. Caco-2 cells were treated with 3 m*M* butyrate for 48 hr. (A) Immunoblotting with a phosphotyrosine $p34^{cdc2}$-specific antibody revealed a decrease in $p34^{cdc2}$ tyrosine phosphorylation. (B) This was confirmed by immunoprecipitating $p34^{cdc2}$ and immunoblotting with a phosphotyrosine-specific antibody. Reprobing both membranes for total $p34^{cdc2}$ demonstrates equal amounts of overall $p34^{cdc2}$ protein.

Butyrate's Effect on the Regulators of $p34^{cdc2}$ Activity. The effects of butyrate on protein levels of the regulators of $p34^{cdc2}$ activity, and therefore of the G_2/M traverse, were also studied. While butyrate had no effect on the protein levels of the protein kinase wee-1, it increased the protein expression of the inhibitory tyrosine kinase $p53/56^{lyn}$ and decreased the levels of the activating phosphatase, cdc25c. The subunits of CAK, cyclin H and cdk 7, were unaffected by butyrate exposure (Fig. 5A). Whereas overall protein expression of cyclin B1 was unaffected, exposure to butyrate resulted in reduced intensity of the retarded cyclin B1 band, consistent with a decrease in cyclin B1 phosphorylation (Fig. 5B). Interestingly, the cyclin-dependent kinase inhibitors, p21 and p27, were increased after exposure to butyrate (Fig. 5C).

Discussion

Butyrate, a four-carbon fatty acid produced by colonic fermentation of fiber, has been shown to inhibit cell proliferation, induce apoptosis, and promote differentiation of multiple cancer cell lines (3, 5, 12, 14). Although the effects of butyrate on cell differentiation are well documented, the relationship between butyrate-induced differentiation and butyrate's effect on cell cycle traverse is less well understood. Various groups have reported a G_1/S and/or a G_2/M block associated with butyrate treatment of neoplastic cells. Siavoshian *et al.* reported that butyrate inhibits cellular proliferation by blocking HT-29 colon cancer cells in G_1. This G_1/S block was associated with increased protein expression of the cyclin-dependent kinase inhibitor (CDKI), $p21^{Waf/Cip1}$ and decreased levels of CDK2 (10). Similar increases in $p21^{Waf/Cip1}$ and $p27^{Kip1}$ have also been reported using the butyrate/Caco-2 cell model (12). Others have reported blocks at both the G_1/S and G_2/M checkpoints after butyrate exposure to colon cancer cell lines (7, 11). In this study, we observed that the principal cell cycle effect of treatment of Caco-2 colon cancer cells with butyrate is a G_2/M block. Although these cells do undergo differentiation after butyrate exposure, we did not detect the G_1/S block previously reported in other colon cancer cell lines by either propidium iodide staining or BrdU incorporation (Figs. 2A and 2B). This lack of a detectable G_1/S block may be the result of either unique laboratory conditions or the use of a Caco-2 subclone. Therefore, our Caco-2/butyrate system provides an excellent model to investigate the effects of this differentiating compound on the G_2/M traverse without a confounding G_1/S block observed in other cell lines.

To initiate a study of the possible mechanisms of this G_2/M block, the protein levels and kinase activity of $p34^{cdc2}$ were examined. Although protein levels of the major G_2/M

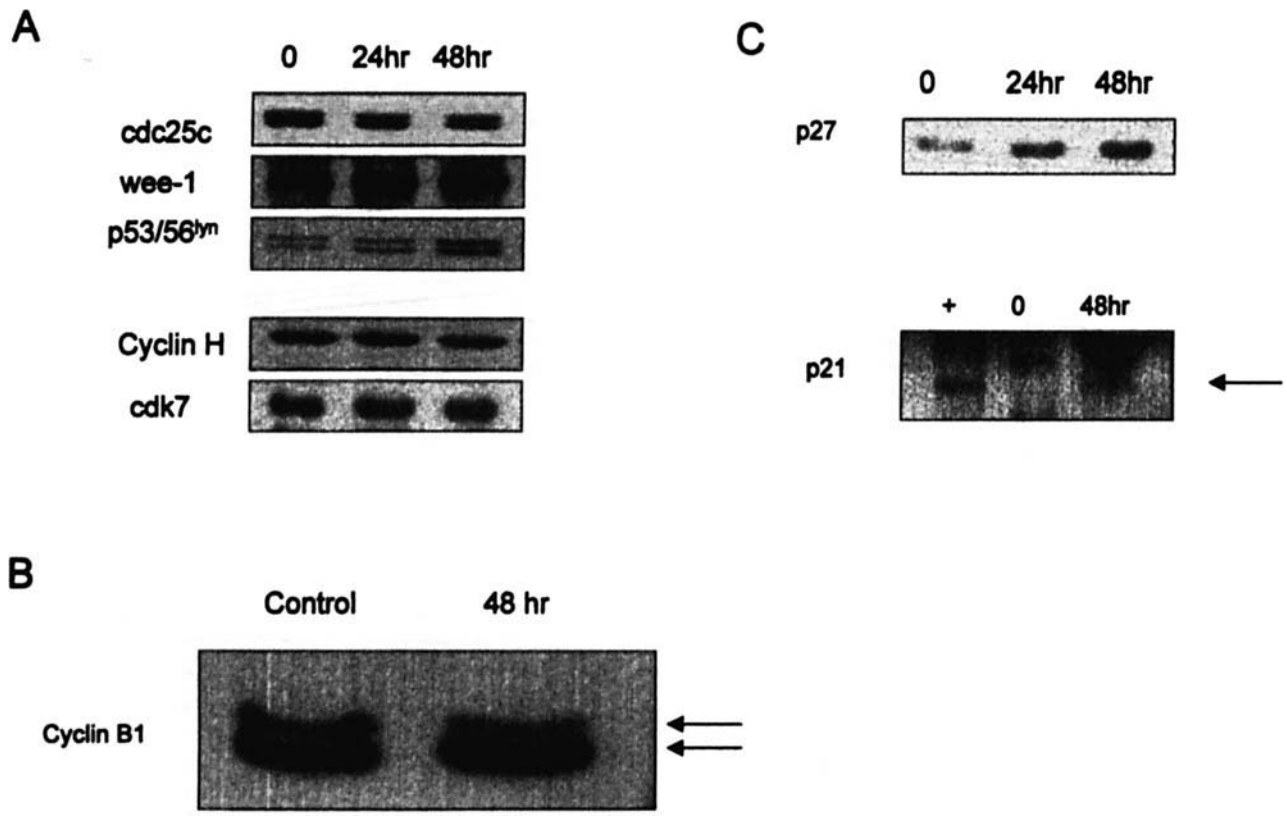

Figure 5. Immunoblot analysis of the regulatory proteins of G_2/M traverse. (A) Caco-2 cells were treated with 3 m*M* butyrate for 24 and 48 hr. Butyrate increased $p53/56^{lyn}$ and decreased cdc25c protein levels. However, protein levels of wee-1, cdk 7, and cyclin H were unaffected by butyrate treatment. (B) Caco-2 cells were treated with 3 m*M* butyrate for 48 hr and subjected to western analysis for cyclin B1. Whereas overall protein expression of cyclin B1 was unaffected, exposure to butyrate resulted in reduced intensity of the retarded cyclin B1 band, consistent with a decrease in cyclin B1 phosphorylation. (C) The protein levels of the cyclin-dependent kinase inhibitors, p21 and p27, were increased with exposure to butyrate. The " + " denotes a positive control for p21 (TPA-treated HL60 cells).

regulatory protein $p34^{cdc2}$ were unchanged, a reduction in $p34^{cdc2}$ activity was noted. Although we expected this decrease in activity to be associated with an increase in tyrosine phosphorylation $p34^{cdc2}$ as reported in other models of chemical or radiation-induced G_2 arrest (15–19), we observed a decrease in the inhibitory tyrosine phosphorylation after butyrate exposure. Tyrosine phosphorylation of $p34^{cdc2}$ is regulated by the activating phosphatase cdc25c and the inhibitory kinases, wee-1 and $p53/56^{lyn}$. Cdc25c promotes the traverse through G_2 by cleaving the phosphate group on tyrosine 15 and threonine 14 residues on $p34^{cdc2}$, whereas wee-1 and $p53/56^{lyn}$ are inhibitory by phosphorylating these same sites (13). Therefore, to study the mechanism of the decreased tyrosine phosphorylation of $p34^{cdc2}$ after butyrate exposure, protein levels of wee-1, $p53/56^{lyn}$, and cdc25c were measured. The decreased tyrosine phosphorylation of $p34^{cdc2}$ in our system could not be explained by changes in protein levels of wee-1, since immunoblot analysis demonstrated no change after treatment with butyrate. Because wee-1 activity is regulated by both protein amount and the level of an inactivating phosphorylation (20), it is possible that the activity of wee-1 was decreased by butyrate exposure in the absence of overall protein changes. While this is possible, the p98/100-phosphorylated form of wee-1 was not detected on western blot analysis (Fig. 5A). On the other hand, our data demonstrated a paradoxical increase in expression of $p53/56^{lyn}$ and a decrease in protein levels of cdc25C after butyrate treatment. This pattern of protein expression of these two regulators of $p34^{cdc2}$ tyrosine phosphorylation could not explain our observed results, and it is possible that protein levels of these regulators do not directly correlate with their activity.

One rationale for the decreased activity is based on the increased expression of the cyclin-dependent kinase inhibitors, p21 and p27. Although originally described to regulate the G_1/S traverse, some authors have recently demonstrated that p21 (21, 22) and p27 (23, 24) also participate in regulating the G_2/M checkpoint. Another possibility in explaining this paradox of decreased $p34^{cdc2}$ activity in the face of decreased inhibitory tyrosine phosphorylation, is based on the regulatory subunit of $p34^{cdc2}$, cyclin B1. Whereas we found no significant change in the overall protein expression of cyclin B, our results suggested a decrease in the phosphorylation status of cyclin B1 after butyrate exposure. It has been reported that cyclin B1 requires phosphorylation to be active (25, 26), and this decrease in phosphorylated cyclin B1 after butyrate exposure could account for the observed decreased $p34^{cdc2}$ activity, despite the decrease in tyrosine phosphorylation.

In conclusion, we propose that the G_2/M block observed following butyrate treatment of Caco-2 cells is associated with an inactivation of $p34^{cdc2}$. The inactivation of $p34^{cdc2}$ does not appear to be the result of decreased protein expression or an increased level of tyrosine phosphorylation of $p34^{cdc2}$, but potentially the upregulation of the cyclin-dependent kinase inhibitors, p21 and p27, and/or the inactivation of the regulatory subunit, cyclin B1.

1. Trock B, Lanza E, Greenwald P. Dietary fiber, vegetables, and colon cancer: Critical review and meta analysis of the epidemiological evidence. J Natl Cancer Inst **82:**650–661, 1990.
2. Cummings JH. Short-chain fatty acids in the human colon. Gut **22:**763–779, 1981.
3. Heerdt B, Houston M, Augenlicht L. Potentiation by specific short-chain fatty acids of differentiation and apoptosis in human colonic carcinoma cell lines. Cancer Res **54:**3288–3294, 1994.
4. Barnard J, Warwick G. Butyrate rapidly induces growth inhibition and differentiation in HT–29 cells. Cell Growth Differ **4:**495–501, 1994.
5. Gum J, Kam W, Byrd J, Hicks J, Sleisenger M, Kim Y. Effects of sodium butyrate on human colonic adenocarcinoma cells. J Biol Chem **262:**1092–1097, 1987.
6. Saina K, Steel G, Thomas P. Induction of carcinoembryonic-antigen-gene expression in human colorectal carcinoma by sodium butyrate. Biochem J **272:**541–544, 1990.
7. Hodin R, Meng S, Archer S, Tang R. Cellular growth state differentially regulates enterocyte gene expression in butyrate-treated HT-29 cells. Cell Growth Differ **7:**647–653, 1996.
8. Boffa L, Lupton J, Mariani M, Ceppi M, Newmark H, Scalmati A, Lipkin M. Modulation of colonic epithelial cell proliferation, histone acetylation, and luminal short-chain fatty acids by variation of dietary fiber (wheat bran) in rats. Cancer Res **52:** 5906–5912, 1992.
9. Darzynkiewicz Z, Traganos F, Xue S, Melamed M. Effect of n-butyrate on cell cycle progression and *in situ* chromatin structure of L1210 cells. Exp Cell Res **136:**279–293, 1981.
10. Siavoshian S, Blottiere HM, Cherbut C, Galmiche J. Butyrate stimulates cyclin D and p21 and inhibits cyclin-dependent kinase expression in HT-29 colonic epithelial cells. Biochem Biophys Res Commun **232:**169–172, 1997.
11. Heerdt B, Houston M, Augenlicht L. Short-chain fatty acid–initiated cell cycle arrest and apoptosis of colonic epithelial cells is linked to mitochondrial function. Cell Growth Differ **8:** 523–532, 1997.
12. Litvak D, Evers BM, Hwang KO, Hellmich MR, Ko TC, Townsend CM. Butyrate-induced differentiation of Caco-2 cells is associated with apoptosis and early induction of $p21^{Waf1/Cip1}$ and $p27^{Kip1}$. Surgery **124:**161–170, 1998.
13. Nurse P. Universal control mechanism regulating the onset of M-phase. Nature **344:** 503–507, 1998.
14. Hague A, Manning A, Hanlon K, Huschtscha L, Hart D, Paraskeva C. Sodium butyrate induces apoptosis in human colonic tumour cell lines in a p53-independent pathway: Implications for the possible role of dietary fibre in the prevention of large bowel cancer. Int J Cancer **55:**498–505, 1993.
15. Herzinger T, Funk J, Hillmer K, Eick D, Wolf D, Kind P. Ultraviolet β irradiation–induced G_2 cell cycle arrest in human keratinocytes by inhibitory phosphorylation of the cdc2 cell cycle kinase. Oncogene **11:**2151–2156, 1995.
16. Jin P, Gu Y, Morgan D. Role of inhibitory CDC2 phosphorylation in radiation-induced G_2 arrest in human cells. J Cell Biol **134:**963–970, 1996.
17. Lock R, Ross W. Inhibition of p34cdc2 kinase activity by etoposide or irradiation as a mechanism of G_2 arrest in Chinese hamster ovary cells. Cancer Res **50:**3761–3766, 1990.
18. Draetta G, Beach D. Activation of cdc2 protein kinase during mitosis in human cells: Cell cycle–dependent phosphorylation and subunit rearrangement. Cell **54:**17–26, 1988.
19. Barth H, Kinzel V. Phorbol ester TPA rapidly prevents activation of p34cdc2 histone H1 kinase and concomitantly the transition from G2 phase to mitosis in synchronized HeLa cells. Exp Cell Res **212:**383–388, 1994.

20. Watanabe N, Broome M, Hunter T. Regulation of the human Wee-1Hu CDK tyrosine 15-kinase during the cell cycle. EMBO J **14:** 1878–1891, 1995.
21. Dulic V, Stein G, Far D, Reed S. Nuclear accumulation of $p21^{cip1}$ at the onset of mitosis: A role at the G_2/M-phase transition. Mol Cell Biol **18:**546–557, 1998.
22. Niculescu A, Chen X, Smeets M, Hengst L, Prives C, Reed S. Effects of $p21^{cip1/waf1}$ at both G_1/S and the G_2/M cell cycle transitions: pRb is a critical determinant in blocking DNA replication and in preventing endoreduplication. Mol Cell Biol **18:**629–643, 1998.
23. Uren A, Jakus J, deMora J, Yeudall A, Santos E, Gutkind S, Heidaran M. Carboxyl-terminal domain of $p27^{kip1}$ activates cdc2. J Biol Chem **272:**21669–21672, 1997.
24. Graig C, Wersto R, Kim M, Ohri E, Li Z, Katayose D, Lee S, Trepel J, Cowan K, Seth P. A recombinant adenovirus expressing p27kip1 induces cell cycle arrest and loss of cyclin-cdk activity in human breast cancer cells. Oncogene **14:**2283–2289, 1997.
25. Mejier L, Azzi L, Wang J. Cyclin B targets $p34^{cdc2}$ for tyrosine phosphorylation. EMBO J **10:**1545–1554, 1991.
26. Li J, Meyer A, Donoghue D. Nuclear localization of cyclin B1 mediates its biological activity and is regulated by phosphorylation. Proc Natl Acad Sci U S A **94:**502–507, 1997.