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Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation

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Short-chain fatty acids (SCFA), particularly butyrate, were shown to regulate cell proliferation *in vitro* and *in vivo*. Indeed, butyrate is the major fuel for colonic epithelial cells, and it can influence cell proliferation through the release of growth factors or gastrointestinal peptides such as gastrin, or through modulation of mucosal blood flow. Lastly, SCFA can act directly on genes regulating cell proliferation, and butyrate is the main SCFA to display such an effect. Butyrate inhibits histone deacetylase, which will allow histone hyperacetylation. Such hyperacetylation leads to transcription of several genes, including p21/Cip1. Moreover, it will allow cyclin D3 hyper-expression by inhibiting its degradation. The induction of the cyclin-dependent kinase inhibitory protein p21/Cip1 accounts for cell arrest in the G1 phase of the cell cycle. However, in the absence of p21 other mechanisms are initiated, leading to inhibition of cell proliferation.

Butyrate: Colon: Cell cycle: p21/Cip1: Cyclin D3

Short-chain fatty acids (SCFA) are the by-products of dietary fibre fermentation by the colonic flora. As a result of the work of Roediger (1980) and the biochemical studies performed in several laboratories (Riggs *et al*. 1977; Sealy & Chalkley, 1978), the SCFA, particularly butyrate, have interested scientists and clinicians all over the world. The potential involvement of SCFA in several diseases (including inflammatory bowel diseases and colon cancer), associated with the *in vitro* differentiating properties of butyrate, has led to a great many scientific contributions. The present review will focus on the effect of SCFA on colonic cell proliferation, particularly the mechanisms involved at the molecular level.

Short-chain fatty acids and colonocyte proliferation

In the case of colonocytes, two concepts seem to have emerged from the literature: SCFA exert a trophic effect on the intestinal mucosa *in vivo*, whereas *in vitro* they have an inhibitory effect on colonic neoplastic cells. Thus, it seems that there is a paradox between their role in normal colonic cells *v*. neoplastic cells.

The first report of a stimulatory effect of SCFA on the intestinal mucosa in non-ruminant mammals was published about 20 years ago by Sakata & Von Engelhardt (1983). The authors filled colonic pouches with a SCFA solution (acetate 75 mM, propionate 35 mM, butyrate 20 mM) or a saline (9 g NaCl/l) solution. After 1 h, tissue samples were taken and analysed for mitotic index and cell DNA synthesis using [^{3}H]thymidine incorporation. The authors reported an increase in the labelling and mitotic indexes in exposed, but also non-exposed, proximal colon. Such an effect was abolished in vagotomised and sympathectomised rats, suggesting the involvement of the autonomic nervous system. Later, Sakata (1987, 1989) described this phenomenon in more detail. Moreover, Rombeau and his team (Kripke *et al*. 1989; Frankel *et al*. 1994; Reilly *et al*. 1995) performed similar studies in order to understand the mechanism of action of this stimulatory effect. It is noteworthy that in most experiments SCFA or butyrate were compared with non-energetic saline solution. Recently, Hass *et al*. (1997) showed that lack of butyrate leads to massive apoptosis of mucosal epithelial cells from guinea-pig proximal colon, associated with the induction of a pro-apoptotic protein, Bax. However, the

Abbreviations: cdk, cyclin-dependent kinases; HDAC, histone deacetylase; SCFA, short-chain fatty acids; TSA, trichostatin A.

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authors did not analyse the effect of other SCFA with potent energetic capacities (i.e. propionate and acetate). Finally, in rats fed increasing amounts of wheat bran, Boffa *et al*. (1992) measured the proliferation index of colonic mucosa and butyrate concentrations in the colonic lumen. These authors found an inverse correlation between these two variables. Indeed, when butyrate was found at the highest concentration, the proliferation index was the lowest. Thus, different conclusions may be drawn from *in vivo* experiments, depending on the experimental procedure.

In *in vitro* experiments when freshly-isolated cells or biopsies were used similar discrepancies were found between studies. For example, a stimulation of proliferation was observed in colonic biopsies (Bartram *et al*. 1993); in other experiments no effect was seen (Scheppach *et al*. 1996), whereas an inhibitory effect was described elsewhere (Sakata, 1987). However, for established colonic epithelial cell lines, most of which originated from human adenocarcinoma, the situation is more straightforward. Indeed, a dose-dependent inhibition of cell proliferation has been observed for all tumour cell lines tested (Gamet *et al*. 1992; Siavoshian *et al*. 1997*b*). Moreover, using the non-tumoural rat intestinal epithelial cells IEC-6 a similar dose-dependent inhibitory effect has been observed (Fig. 1). Butyrate is the most efficient SCFA in inhibiting cell proliferation. However, propionate and valerate also display inhibitory properties, but to a lesser extent. Indeed, a 5 mM concentration of these acids gave the same inhibition as 2 mM-butyrate (Siavoshian *et al*. 1997*b*). The other SCFA (acetate, caproate and the branched-chain acids, isobutyrate and isovalerate) were devoid of effect. For acetate, no effect was observed on cell proliferation even when used at a concentration of 20 mM.

To summarise, these results indicate three different mechanisms, depending on the experimental system. First, SCFA, particularly butyrate, are the main energy source for colonic epithelial cells. They will, therefore, stimulate proliferation when compared with a non-energetic (or less-energetic) solution. Second, it has been shown that SCFA are able to stimulate the release of gastrointestinal peptides or growth factors which may affect cell proliferation depending on the receptor and the second messenger involved; moreover, such factors may act on vascular tone modulating blood flow (Mortensen *et al*. 1990). Third, SCFA, and again butyrate particularly, can exert molecular effects, and by acting on particular genes will inhibit cell proliferation, stimulate cell differentiation, but also induce apoptosis. The latter effects are readily observed *in vitro*, where the first two effects are mostly abolished; these models are therefore well suited to the study of the molecular mechanisms implicated.

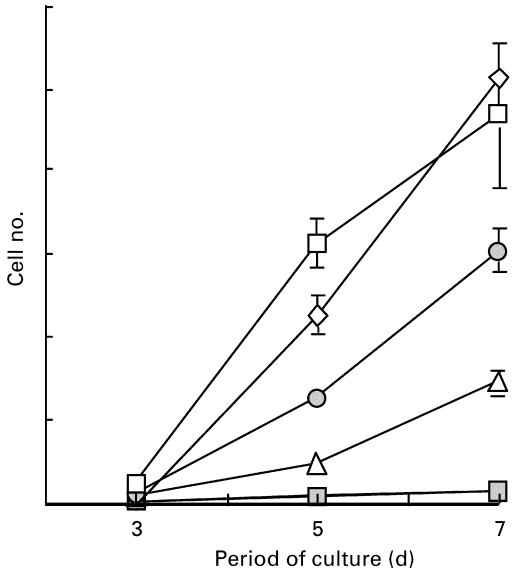

Fig. 1. Dose-dependent inhibition of rat non-tumoural intestinal epithelial cells (IEC-6) by butyrate as assessed *in vitro*. Cells were cultured without (◇; control) or with butyrate (□, 0·5 mM; ●, 1 mM; △, 2 mM; ▩, 5 mM) and cell number was measured after 3, 5 and 7 d.

Short-chain fatty acids and other colonic cell proliferation

Cells other than colonocytes, mainly smooth muscle cells, fibroblasts and immune cells, are able to proliferate in the gut mucosa and may therefore be influenced by SCFA. In the case of colonic smooth muscle cells, the effect of SCFA was investigated in primary culture as well as in an established cell line (Le Blay *et al*. 2000). A dual effect was observed in primary culture. Indeed, at low concentrations (0·05 and 0·1 mM) butyrate mildly stimulated cell proliferation. At 0·5 mM no modulation was observed, and ≥ 1 mM-butyrate dose-dependently inhibited cell proliferation. In an established cell line ≥ 0·05 mM-butyrate inhibited cell proliferation. Propionate was also effective but to a lesser extent, and acetate showed no effect. In the case of immune cells and fibroblasts inhibition of proliferation has been observed both in primary culture and in established lines (Gilbert & Weigle, 1993; Buquet-Fagot *et al*. 1996; HM Blottière and B Buecher, unpublished results).

Mechanisms of action of short-chain fatty acids: implication of p21/Cip1

In most cell lines tested butyrate has been shown to cause an accumulation in the G1 phase of the cell cycle (Darzynkiewicz *et al*. 1981; Archer *et al*. 1998; Siavoshian *et al*. 2000). As illustrated in Fig. 2, cell analysis using flow cytometry after DNA staining with propidium iodide shows a strong increase in the number of cells in the G1 phase in the presence of butyrate, together with a diminution of the S phase. However, in some cell lines butyrate was also shown to induce a block in the G2/M phase, as observed in Caco-2 cells (Harrison *et al*. 1999) or in SW1116 cells (HM Blottière and B Buecher, unpublished results). It is noteworthy that in SW1116 cells, alternatively a G1 or a G2/M block is obtained depending on the experimental conditions. The cell cycle is under the control of three families of proteins, i.e. cyclins, cyclin-dependent kinases (cdk) and inhibitors of the cdk (Sherr, 1996). As shown in Fig. 3, specific kinases are involved depending on the phase of the cell cycle. These cdk are activated when conjugated with a particular cyclin, e.g. cyclin D in early G1. Moreover, inhibitors can also regulate the cdk activity. They are from

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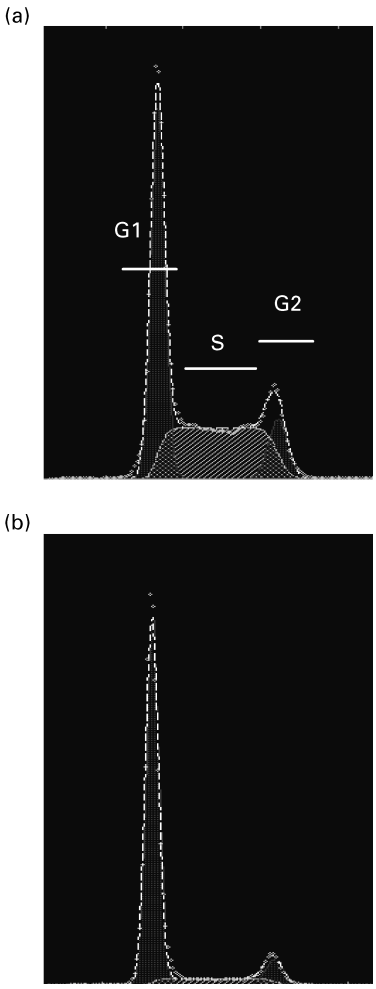

Fig. 2. Typical histograms of cell cycle (G1, S and G2/M) analysis by flow cytometry. HT-29 colon adenocarcinoma cells were cultured for 24 h without (a) or with (b) 5 mM-butyrate. DNA was then stained with propidium iodide before analysis. The relative no. of cells in each phase of the cycle (% total) was: (a) G1 46, S 43, G2 10; (b) G1 80, S 11, G2 8.

two families: the Cip/Kip family represented by p21/Cip1, p27/Kip1 and p57/Kip2 which bind to the cyclin-cdk complex to inhibit its activity; the INK4 family, especially p16/INK4a, which bind to cdk4 and cdk6 and prevent the binding of cyclin D. In the early G1 cdk4 and cdk6 are activated by cyclin D, and phosphorylate the retinoblastoma protein, thus enabling the transcription factor E2F to activate the transcription of key genes allowing the cycle to continue. Three cyclins D have been identified in man, D1, D2 and D3.

In order to understand the mechanism of action of butyrate in cell proliferation, the expression of these cell-cycle regulatory proteins has been studied (Siavoshian *et al*. 1997*a*; Archer *et al*. 1998). In colonic epithelial cells butyrate induces the expression of p21/Cip1 both at the protein and mRNA levels, but does not modulate p16/INK4a and p27/kip1. Moreover, cyclin D3 is markedly up regulated, while cyclin D1 is not affected by butyrate (Siavoshian *et al*. 2000). In fibroblasts a marked down regulation of cyclin D1 has been reported (Lallemand *et al*. 1996). Butyrate does not affect the expression of other cell-cycle regulatory proteins such as cdk4, cdk6, cyclin B and cyclin E, and a mild decrease in cdk2 expression has been observed (Siavoshian *et al*. 1997*a*). Moreover, the key transcription factor c-myc is also down regulated (Heruth *et al*. 1993).

The role played by p21/Cip1 was addressed using cells in which p21/Cip1 had been deleted by homologous recombination (Archer *et al*. 1998). It was shown that this cdk inhibitor is of critical importance in butyrate-mediated G1 growth arrest. Indeed, using [^{3}H]thymidine incorporation it was observed that in the absence of p21/Cip1 cells entered the S phase. However, using the same cells, in the absence of p21/Cip1 butyrate still induced inhibition of cell proliferation (S Siavoshian and HM Blottière, unpublished results). In the presence of butyrate these cells accumulated in G2/M, but a strong apoptotic effect was also induced. The G2/M block may be due to a decrease in cdk1 activity, similar to that observed in Caco-2 cells (Harrison *et al*. 1999). Moreover, the mechanism by which butyrate induces epithelial cell apoptosis is now well documented (Medina *et al*. 1997; Mandal *et al*. 2001). The fact that retinoblastoma protein is found hypophosphorylated in the presence of butyrate (Gope & Gope, 1993) is consistent with a key role played by p21/Cip1. This cdk inhibitor binds to cyclin D-cdk4 and cdk6 complexes, therefore preventing the phosphorylation of retinoblastoma protein.

Mechanism of induction of p21/Cip1 by butyrate

It is clear that the effect of butyrate on p21/Cip1 expression is a transcriptional effect. Indeed, butyrate induces its expression both at the protein and mRNA levels (Archer *et al*. 1998; Siavoshian *et al*. 2000). Moreover, in the presence of cycloheximide, an inhibitor of protein synthesis, the effect of butyrate is not blocked indicating that it is a direct effect. Using gene reporter technology, Nakano *et al*. (1997) showed that two specific GC-rich SP1 binding sites on the promoter region of the p21 gene are involved in the butyrate effect. These two sites are at positions – 82 and – 69 relative to the transcription start site. The effect of butyrate on p21/Cip1 expression is independent of p53, since butyrate still induces its expression in p53-deficient cells, and since the deletion of the p53 binding site on the promoter does not prevent its effect.

Thus, the butyrate-responsive elements are identified in the p21 gene; however, how does butyrate act on p21 expression? In the late 1970s, butyrate was shown to induce histone hyperacetylation through inhibition of histone deacetylase (HDAC) (Riggs *et al*. 1977; Sealy & Chalkley, 1978). It is generally assumed that histone hyperacetylation results in relaxation in chromatin structure, thereby making DNA more accessible to transcription factors. In man at least eleven HDAC have been identified so far, and the equilibrium between histone acetyltransferase and HDAC appears to be a key element in the regulation of gene expression (Bertos *et al*. 2001). HDAC have been shown to be involved in the transcriptional machinery as a part of co-repressor complexes controlling the transcription of genes. Using trichostatin A (TSA), a specific inhibitor of HDAC

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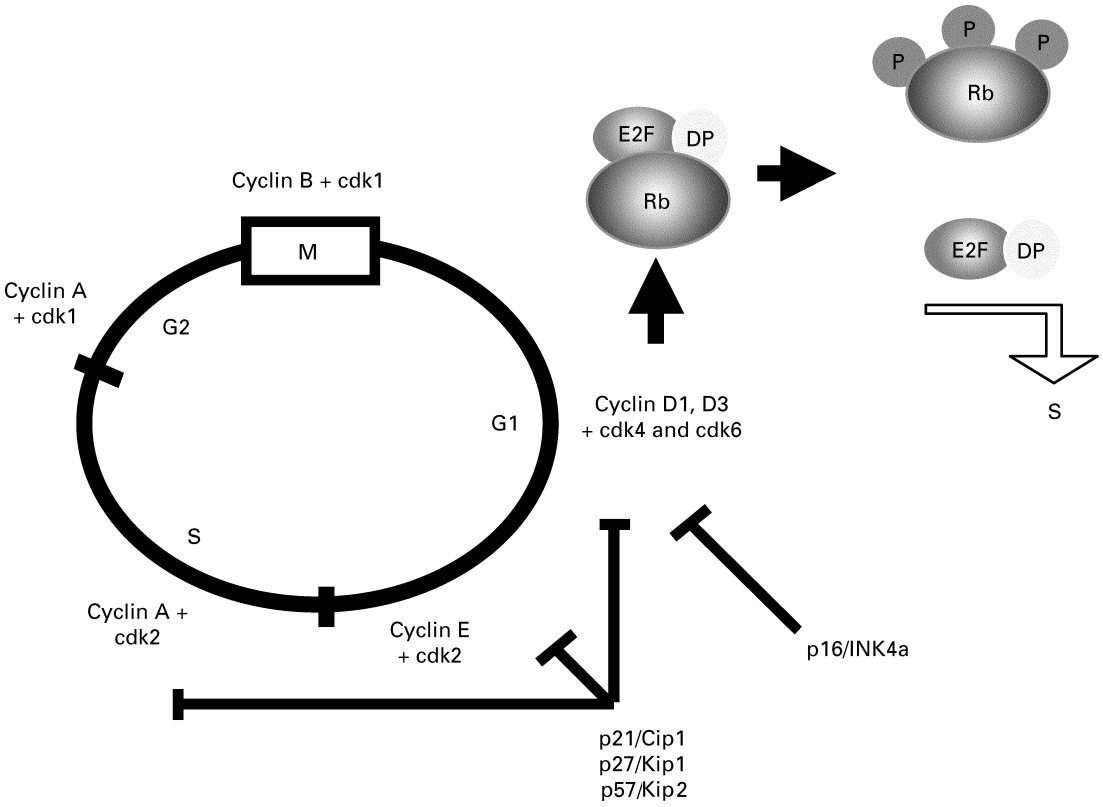

Fig. 3. The main cell cycle regulatory proteins involved in cell proliferation. Cyclin-dependent kinases (cdk) are activated when bound to a particular cyclin. These cyclins are expressed sequentially during the cell cycle. Inhibitors belonging to two families, INK4 and Cip/Kip, also control the activity of the cdk. In early G1 the binding of cyclin D with cdk4 and 6 leads to phosphorylation of retinoblastoma (Rb) protein enabling the release and activation of E2F transcription factor.

structurally not related to butyrate, it was shown that the induction of p21/Cip1 was clearly due to HDAC inhibition (Archer *et al*. 1998; Siavoshian *et al*. 2000). Moreover, the overexpression of HDAC abolished most of the butyrate effect (Archer *et al*. 1998). Recently, an analysis of genes modulated early by HDAC inhibitors, butyrate and TSA, using DNA expression array technology, allowed the identification of around twenty genes that were regulated by butyrate (Della Ragione *et al*. 2001). Interestingly, these genes were identically regulated by TSA, and no major difference was seen between the two HDAC inhibitors. It is noteworthy that the analysis was performed after 5 h incubation and in the presence of cycloheximide. We have shown that TSA and butyrate displayed a different kinetic of action (Siavoshian *et al*. 2000). After 6 h, both drugs induced histone hyperacetylation, whereas after 24 h, butyrate effect was sustained and TSA was no more effective.

The link between SP1, HDAC and p21/Cip1 was elucidated by Bai & Merchant (2000). Two other actors were added in the system, a transcriptional co-activator p300, which actually is a histone acetyltransferase, and another transcription factor ZBP-89, which is a Krüppel-like Zn finger protein and binds to GC-rich DNA sequences such as SP1. These authors showed that SP1 binds HDAC and ZBP-89, the latter being linked to p300. Therefore, SP1/ZBP-89 complex binds to GC-rich sites on the promoter of the p21 gene. p300 acts as a co-activator by acetylating histones and butyrate through inhibition of the HDAC bound to SP1 allowing a sustained activation of the gene.

Mechanism of up regulation of cyclin D3 by butyrate

Interestingly, the mechanisms leading to cyclin D3 up regulation are different from the one described for p21/Cip1. Indeed, no modulation is observed at the mRNA level, indicating that transcriptional activation is not implicated (Siavoshian *et al*. 2000). Moreover, at the protein level, the effect of butyrate is not blocked by cycloheximide suggesting that stabilisation of the protein occurs rather than stimulation of translation. Metabolic labelling using [^{35}S]methionine followed by a chase confirmed this hypothesis (HM Blottière and B Buecher, unpublished results). Cyclins are degraded by the proteasome 26S after ubiquitinylation of the protein. Investigation of the mechanism involved is in progress in our laboratory.

Overview: other mechanisms

p21/Cip1 was shown to be the main effector involved in the butyrate-induced G1 block in colon epithelial cells. However, it is not the only one (Fig. 4). In contrast with what is observed in colon epithelial cells, fibroblasts deficient in p21/Cip1 are still arrested in G1 by butyrate (Vaziri *et al*. 1998). In these cells butyrate inhibited the

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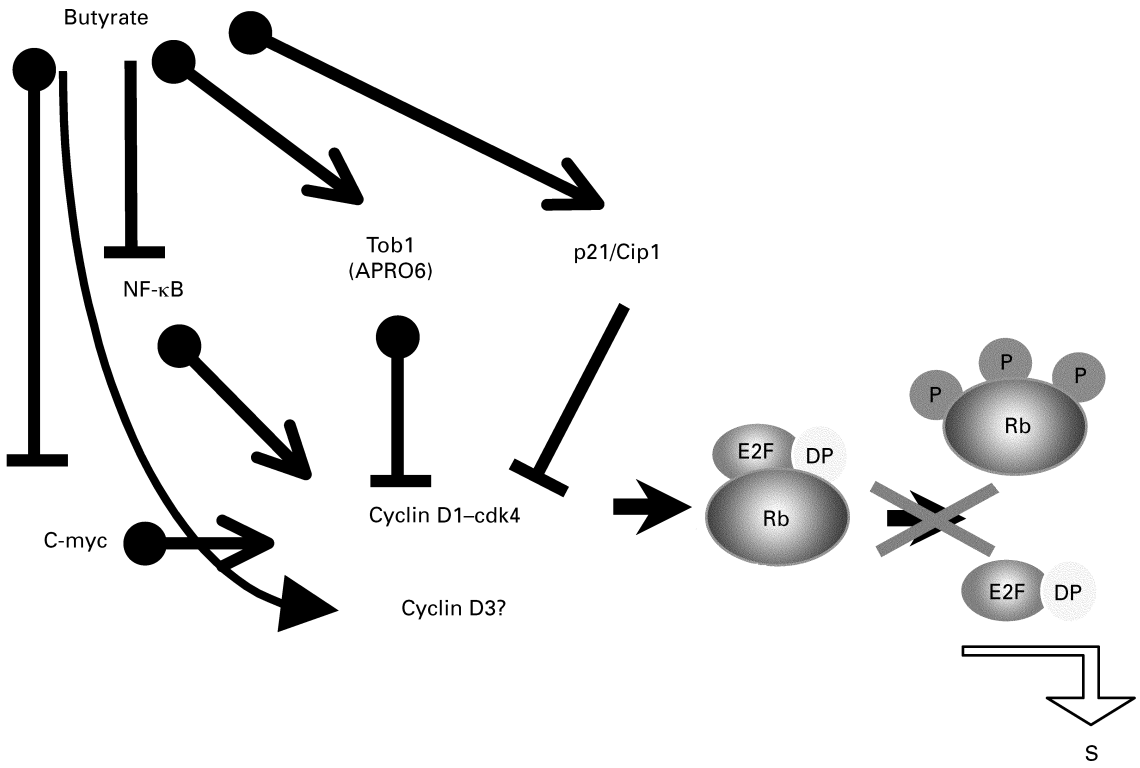

Fig. 4. Overview of the different pathways leading to inhibition of cell proliferation and the blocking of the G1 stage of the cell cycle (for details, see p. 104). NF-kB, nuclear factor kappa B; Rb, retinoblastoma protein; cdk, cyclin-dependent kinases; p21/cip1, a member of the Cip/Kip family which bind to the cyclin–cdk complex to inhibit its activity; Tob1 (APRO6), a member of the anti-proliferative family APRO, members of which control cyclin D1 transcription.

mitogen-dependent transcriptional induction of cyclin D1, thus inhibiting retinoblastoma protein phosphorylation and blocking cells in G1. Several pathways may lead to cyclin D1 inhibition of transcription. It has been shown that butyrate down regulates c-myc transcription factor (Heruth *et al*. 1993), and one of the foremost targets of c-myc is cyclin D1. Moreover, it has been shown that butyrate inhibits the translocation of nuclear factor kappa B transcription factor to the nucleus, thereby preventing the activation of pro-inflammatory genes (Inan *et al*. 2000; Segain *et al*. 2000). Cyclin D1 is also under the control of nuclear factor kappa B. Also, using DNA expression array technology, another gene has been found to be markedly up regulated by butyrate (Della Ragione *et al*. 2001), i.e. Tob1 (APRO6), a member of the anti-proliferative family APRO, members of which control cyclin D1 transcription (Guardavaccaro *et al*. 2000; Matsuda *et al*. 2001). Lastly, it has been shown that cyclin D3 is up regulated in colon epithelial cells by butyrate through inhibition of its degradation. It is not known what role cyclin D3 plays in butyrate-induced inhibition of cell proliferation; however, marked expression of cyclin D3 has been found in quiescent differentiated cells at the top of the colon crypt, suggesting a potential role (Bartkova *et al*. 2001).

In conclusion, the present paper, which is not an exhaustive review of the literature, attempts to show how SCFA resulting from the fermentation of dietary fibre can act on cell proliferation and, through complex molecular regulation, lead to cell cycle arrest.

References

Archer SY, Meng S, Shei A & Hodin RA (1998) p21(WAF1) is required for butyrate-mediated growth inhibition of human colon cancer cells. *Proceedings of the National Academy of Sciences USA* **95**, 6791–6796.

Bai L & Merchant JL (2000) Transcription factor ZBP-89 co-operates with histone acetyltransferase p300 during butyrate activation of p21waf1 transcription in human cells. *Journal of Biological Chemistry* **275**, 30725–30733.

Bartkova J, Thullberg M, Slezak P, Jaramillo E, Rubio C, Thomassen LH & Bartek J (2001) Aberrant expression of G1-phase cell cycle regulators in flat and exophytic adenomas of the human colon. *Gastroenterology* **120**, 1680–1688.

Bartram HP, Scheppach W, Schmid H, Hofmann A, Dusel G, Richter F, Richter A & Kasper H (1993) Proliferation of human colonic mucosa as an intermediate biomarker of carcinogenesis: effects of butyrate, deoxycholate, calcium, ammonia, and pH. *Cancer Research* **53**, 3283–3288.

Bertos NR, Wang AH & Yang XJ (2001) Class II histone deacetylases: structure, function, and regulation. *Biochemistry and Cell Biology* **79**, 243–252.

Boffa LC, Lupton JR, Mariani MR, Ceppi M, Newmark HL, Scalmati A & Lipkin M (1992) Modulation of colonic epithelial cell proliferation, histone acetylation, and luminal short chain fatty acids by variation of dietary fiber (wheat bran) in rats. *Cancer Research* **52**, 5906–5912.

Buquet-Fagot C, Lallemand F, Charollais RH & Mester J (1996) Sodium butyrate inhibits the phosphorylation of the retinoblastoma gene product in mouse fibroblasts by a transcription-dependent mechanism. *Journal of Cell Physiology* **166**, 631–636.

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Darzynkiewicz Z, Traganos F, Xue SB & Melamed MR (1981) Effect of n-butyrate on cell cycle progression and in situ chromatin structure of L1210 cells. *Experimental Cell Research* **136**, 279–293.

Della Ragione F, Criniti V, Della Pietra V, Borriello A, Oliva A, Indaco S, Yamamoto T & Zappia V (2001) Genes modulated by histone acetylation as new effectors of butyrate activity. *FEBS Letters* **499**, 199–204.

Frankel WL, Zhang W, Singh A, Klurfeld DM, Don S, Sakata T, Modlin I & Rombeau JL (1994) Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. *Gastroenterology* **106**, 375–380.

Gamet L, Daviaud D, Denis-Pouxviel C, Remesy C & Murat JC (1992) Effects of short-chain fatty acids on growth and differentiation of the human colon-cancer cell line HT29. *International Journal of Cancer* **52**, 286–289.

Gilbert KM & Weigle WO (1993) Th1 cell anergy and blockade in G1a phase of the cell cycle. *Journal of Immunology* **151**, 1245–1254.

Gope R & Gope ML (1993) Effect of sodium butyrate on the expression of retinoblastoma (RB1) and P53 gene and phosphorylation of retinoblastoma protein in human colon tumor cell line HT29. *Cellular and Molecular Biology* **39**, 589–597.

Guardavaccaro D, Corrente G, Covone F, Micheli L, D'Agnano I, Starace G, Caruso M & Tirone F (2000) Arrest of G(1)-S progression by the p53-inducible gene PC3 is Rb dependent and relies on the inhibition of cyclin D1 transcription. *Molecular Cell Biology* **20**, 1797–1815.

Harrison LE, Wang QM & Studzinski (1999) Butyrate-induced G2/M block in Caco-2 colon cancer cells is associated with decreased p34cdc2 activity. *Proceedings of the Society for Experimental Biology and Medicine* **222**, 150–156.

Hass R, Busche R, Luciano L, Reale E & Von Engelhardt W (1997) Lack of butyrate is associated with induction of Bax and subsequent apoptosis in the proximal colon of guinea pig. *Gastroenterology* **112**, 875–881.

Heruth DP, Zirnstein GW, Bradley JF & Rothberg PG (1993) Sodium butyrate causes an increase in the block to transcriptional elongation in the c-myc gene in SW837 rectal carcinoma cells. *Journal of Biological Chemistry* **268**, 20466–20472.

Inan MS, Rasoulpour RJ, Yin L, Hubbard AK, Rosenberg DW & Giardina C (2000) The luminal short-chain fatty acid butyrate modulates NF-kappaB activity in a human colonic epithelial cell line. *Gastroenterology* **118**, 724–734.

Kripke SA, Fox AD, Berman JM, Settle RG & Rombeau JL (1989) Stimulation of intestinal mucosal growth with intracolonic infusion of short-chain fatty acids. *Journal of Parenteral and Enteral Nutrition* **13**, 109–116.

Lallemand F, Courilleau D, Sabbah M, Redeuilh G & Mester J (1996) Direct inhibition of the expression of cyclin D1 gene by sodium butyrate. *Biochemical and Biophysical Research Communications* **229**, 163–169.

Le Blay G, Blottiere HM, Ferrier L, Le Foll E, Bonnet C, Galmiche JP & Cherbut C (2000) Short-chain fatty acids induce cytoskeletal and extracellular protein modifications associated with modulation of proliferation on primary culture of rat intestinal smooth muscle cells. *Digestive Diseases and Sciences* **45**, 1623–1630.

Mandal M, Olson DJ, Sharma T, Vadlamudi RK & Kumar R (2001) Butyric acid induces apoptosis by up-regulating Bax expression via stimulation of the c-Jun N-terminal kinase/activation protein-1 pathway in human colon cancer cells. *Gastroenterology* **120**, 71–78.

Matsuda S, Rouault J, Magaud J & Berthet C (2001) In search of a function for the TIS21/PC3/BTG1/TOB family. *FEBS Letters* **497**, 67–72.

Medina V, Edmonds B, Young GP, James R, Appleton S & Zalewski PD (1997) Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (inhibitors of histone deacetylase): dependence on protein synthesis and synergy with a mitochondrial/cytochrome c-dependent pathway. *Cancer Research* **57**, 3697–3707.

Mortensen FV, Nielsen H, Mulvany MJ & Hessov I (1990) Short chain fatty acids dilate isolated human colonic resistance arteries. *Gut* **31**, 1391–1394.

Nakano K, Mizuno T, Sowa Y, Orita T, Yoshino T, Okuyama Y, Fujita T, Ohtani-Fujita N, Matsukawa Y, Tokino T, Yamagishi H, Oka T, Nomura H & Sakai T (1997) Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line. *Journal of Biological Chemistry* **272**, 22199–22206.

Reilly KJ, Frankel WL, Bain AM & Rombeau JL (1995) Colonic short chain fatty acids mediate jejunal growth by increasing gastrin. *Gut* **37**, 81–87.

Riggs MG, Whittaker RG, Neumann JR & Ingram VM (1977) n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. *Nature* **268**, 462–464.

Roediger WEW (1980) The colonic epithelium in ulcerative colitis: an energy-deficiency disease? *Lancet* **ii**, 712–715.

Sakata T (1987) Stimulatory effects of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal factors. *British Journal of Nutrition* **58**, 95–103.

Sakata T (1989) Stimulatory effect of short-chain fatty acids on epithelial cell proliferation of isolated and denervated jejunal segment of the rat. *Scandinavian Journal of Gastroenterology* **24**, 886–890.

Sakata T & Von Engelhardt W (1983) Stimulatory effect of short chain fatty acids in the epithelial cell proliferation in rat large intestine. *Comparative Biochemistry and Physiology* **74**, 459–462.

Scheppach W, Dusel G, Kuhn T, Loges C, Karch H, Bartram HP, Richter F, Christl SU & Kasper H (1996) Effect of L-glutamine and n-butyrate on the restitution of rat colonic mucosa after acid induced injury. *Gut* **38**, 878–885.

Sealy L & Chalkley R (1978) The effect of sodium butyrate on histone modification. *Cell* **14**, 115–121.

Segain JP, Raingeard de la Bletiere D, Bourreille A, Leray V, Gervois N, Rosales C, Ferrier L, Bonnet C, Blottiere HM & Galmiche JP (2000) Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut* **47**, 397–403.

Sherr CJ (1996) Cancer cell cycles. *Science* **274**, 1672–1677.

Siavoshian S, Blottiere HM, Cherbut C & Galmiche JP (1997*a*) Butyrate stimulates cyclin D and p21 and inhibits cyclin-dependent kinase 2 expression in HT-29 colonic epithelial cells. *Biochemical and Biophysical Research Communications* **232**, 169–172.

Siavoshian S, Blottiere HM, Le Foll E, Kaeffer B, Cherbut C & Galmiche JP (1997*b*) Comparison of the effect of different short chain fatty acids on the growth and differentiation of human colonic carcinoma cell lines in vitro. *Cell Biology International* **21**, 281–287.

Siavoshian S, Segain JP, Kornprobst M, Bonnet C, Cherbut C, Galmiche JP & Blottiere HM (2000) Butyrate and trichostatin A effects on the proliferation/differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression. *Gut* **46**, 507–514.

Vaziri C, Stice L & Faller DV (1998) Butyrate-induced G1 arrest results from p21-independent disruption of retinoblastoma protein-mediated signals. *Cell Growth and Differentiation* **9**, 465–474.

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