Short Chain Fatty Acid Regulation of Signaling Genes Expressed by the Intestinal Epithelium

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ABSTRACT Changes in diet greatly affect the mucosal immune system, particularly in diseases such as Crohn's disease and necrotizing enterocolitis. This review examines the hypothesis that alterations in the luminal environment of the intestine regulate the expression of genes in the epithelium responsible for signaling to immune cells. Increasing chemokine expression in the mouse intestinal epithelium using transgenic techniques enhances the recruitment of neutrophils and lymphocytes into the intestine. Furthermore, SCFA concentrations in the intestinal lumen vary markedly with diet. SCFAs alter chemokine expression by inhibiting histone deacetylase activity in the enterocyte. The review therefore describes a molecular pathway explaining how changes in diet may alter leukocyte recruitment by regulating enterocyte gene expression. It is likely that other similar pathways remain to be discovered. J. Nutr. 134: 2450S–2454S, 2004.

KEY WORDS: • *intestine* • *epithelium* • *butyrate* • *chemokine* • *gene expression* • *histone acetylation*

Genes expressed in the intestinal epithelial cell can be divided into 2 groups. There are the intrinsic proteins, which include those that are important for epithelial function, such as brush border proteins, solute transporters, and brush border enzymes. Proteins necessary for the function of any cell type such as histones, enzymes in the metabolic pathways, and cytoskeletal proteins are also part of this group. The proteins in the second group act as signals between the epithelial cell and other cells of the intestine. These include surface molecules such as class II MHC, and proteins that are released from the epithelium, including chemokines or IGF-binding proteins.

This latter group of signaling proteins enables the epithelial cell to orchestrate events in the intestine. In our research group we have hypothesized that the expression of signaling molecules by the epithelial cell is regulated by changes in the intestinal lumen. By this means, the intestinal lumen acts through the epithelium to alter indirectly events in the intestine, particularly those of the mucosal immune system.

This review will first present evidence that altering the expression of signaling genes in the epithelium affects the mucosal immune system. Secondly, it will describe how changes in the intestinal lumen (influenced by diet) alter the expression of these genes. The effects of the lumen on epithelial cell gene expression can, therefore, be considered as an afferent limb in this process; the effect of the epithelial cell on the mucosal immune system is the efferent limb.

It is also possible that butyrate inhibits cancer invasion by downregulating a series of genes involved in carcinogenesis (1–3). However, the role of butyrate in cancer prevention is beyond the scope of this review. SCFA are considered here as heralds of bacterial activity, to which the intestine responds in an innate manner, signaling to other cell types. It is the efferent limb that constitutes the epithelial response to changes in the intestinal lumen.

The efferent limb

Evidence for the effect of epithelial cell gene expression on the mucosal immune system has come from the ability to selectively alter the expression of genes in the intestinal epithelial cell by transgenic techniques. We have used chemokine expression by the epithelium as a model to show that the epithelium can orchestrate the mucosal immune system. The chemokine IL-8, which in the human results in recruitment of neutrophils, was the first identified chemotatic cytokine. However, IL-8 is not expressed in the mouse. To examine the effects of chemokines on the mucosal immune system, a system was developed whereby the chemokine macrophage inflammatory protein-2 (MIP-2),[3] whose effects are very similar

[1] Presented at the 6th Postgraduate Course on Nutrition entitled "Nutrition and Gene Regulation" Symposium at Harvard Medical School, Boston, MA, March 13–14, 2003. This symposium was supported by Conrad Taff Nutrition Educational Fund, ConAgra Foods, GlaxoSmithKline Consumer Healthcare, McNeil Nutritionals, Nestle Nutrition Institute, The Peanut Institute, Procter & Gamble Company Nutrition Science Institute, Ross Products Division–Abbott Laboratories, and Slim Fast Foods Company. The proceedings of this symposium are published as a supplement to *The Journal of Nutrition*. Guest editors for the supplement publication were: W. Allan Walker, Harvard Medical School, George Blackburn, Harvard Medical School, Edward Giovanucci, Harvard School of Public Health, Boston, MA, and Ian Sanderson, University of London, London, UK.

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[3] Abbreviations used: FABPI, fatty acid binding protein of the intestine; MCP-1, monocyte chemotactic protein-1; MIP-2, macrophage inflammatory protein-2; TSA, trichostatin A.

those IL-8 in the human, was linked to an FABPI (fatty acid binding protein of the intestine) promoter (4). The promoter is only active in the epithelial cells of the small intestine and proximal colon. A construct was developed where the FABPI promoter and MIP-2 cDNA was linked to an intron and a polyadenylation site. This construct was injected into mouse oocytes. The epithelium from the first generation of the founder was shown to express MIP-2 mRNA (**Fig. 1**). Analysis showed effects both on neutrophil and on lymphocyte recruitment. The transgenic mice had an increased recruitment of neutrophils into the lamina propria (**Fig. 2**) and into the epithelial cell fraction (data not shown). The effects of the chemokine could be seen only in those tissues where the FABPI promoter was active. In the small intestine, where the FABPI promoter is active, the neutrophil recruitment, expressed as myeloperoxidase activity (per unit weight of intestine), was significantly greater in the transgenic mice. In the proximal colon, where the FABPI promoter is also active, there was also an increase in neutrophil infiltration. However, in the distal colon, where the FABPI promoter is inactive, there was no effect. In addition, the liver and the spleen showed no increased infiltration in the transgenic mouse over the normal mouse. The FABPI promoter is not active in these organs.

These data show for the first time that the epithelial cell can, through the release of chemokines, alter the mucosal immune function of the intestine in vivo. Interestingly, this effect of epithelial signaling is also seen in a background of inflammation. Wild type mice fed dextran sodium sulfate exhibited less neutrophil recruitment than MIP-2 transgenic mice receiving the same agent (5).

Further analysis of the immune system in the MIP-2 transgenic mice demonstrated that the small intestine had increased lymphocyte infiltration, in addition to neutrophils. Lymphocyte numbers in the lamina propria were significantly increased, and there was also a doubling of the numbers of intraepithelial lymphocytes. The increase of intraepithelial lymphocytes was due to an increase in $\alpha\beta$ lymphocytes and in $\gamma\delta$ lymphocytes. Further examination of the receptors on the surface of the intraepithelial lymphocytes showed that they expressed the CXCR2 which is the receptor responsible for MIP-2 activity. Therefore, these experiments show that altering the expression of only 1 chemokine in the epithelium has

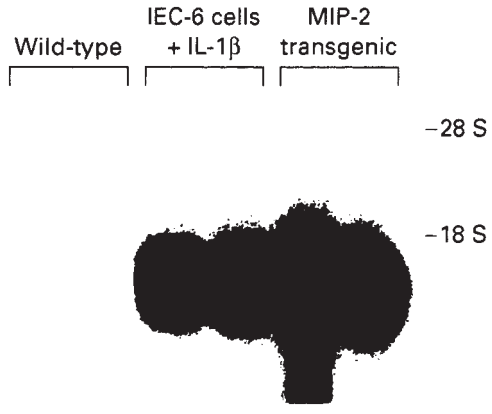

FIGURE 1 Northern blot analysis of MIP-2 mRNA accumulation in the intestinal epithelium of wild-type and transgenic mice. MIP-2 mRNA was detected using an MIP-2 cDNA probe. Epithelial cells were removed from wild-type mice (lanes 1 and 2) and from transgenic mice. MIP-2 mRNA derived from IEC-6 cells stimulated with IL-1β was used as a positive control. Reproduced from (4) with permission.

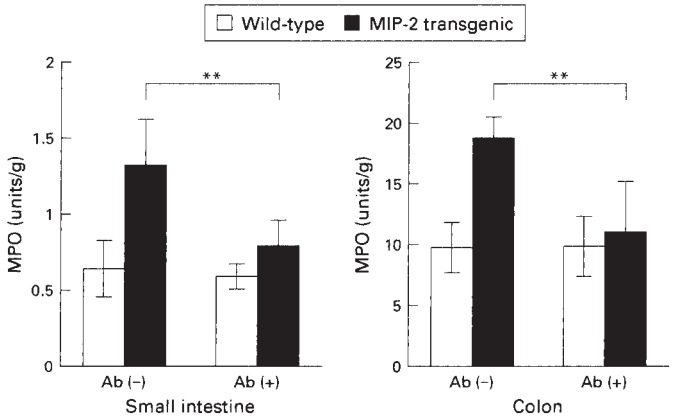

FIGURE 2 Neutrophil recruitment is increased in expressing MIP-2 in the intestinal epithelium. These effects were blocked with a MIP-2 antibody. Data are means ± SD of 8 animals for each group. $^{**}P < 0.01$. Reproduced from (4) with permission

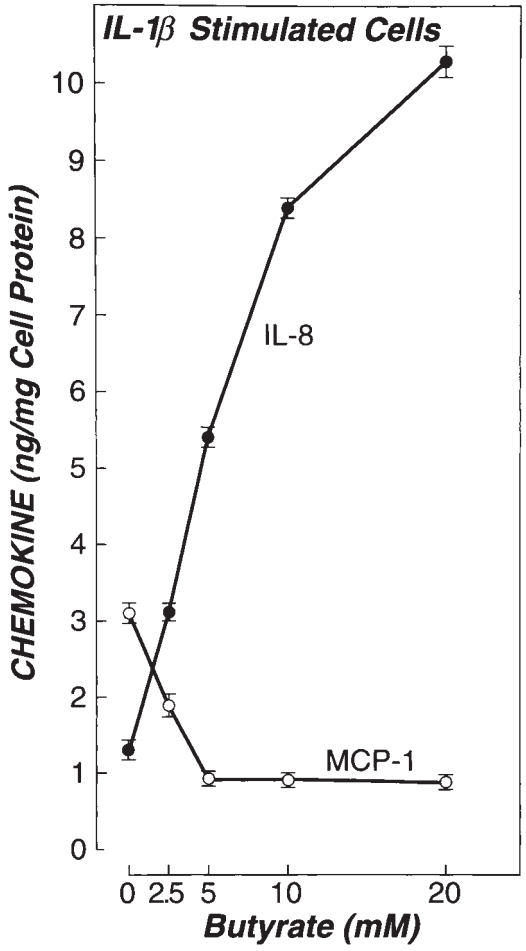

FIGURE 3 Effect of IL-1β and butyrate on IL-8 and MCP-1 secretion by Caco-2 cells. IL-1β alone stimulated the secretion of both IL-8 and MCP-1. Butyrate differentially regulates the pattern of chemokine secretion in IL-1β-stimulated cells. IL-8 secretion was increased ($P < 0.0001$) whereas MCP-1 secretion was decreased ($P < 0.0001$). Bars represent standard deviations of 3 different wells for each point. The data are representative of 5 experiments. Reproduced from (11) with permission.

marked effects on both lymphocyte and neutrophil function. However, changes in the intestinal lumen may affect many chemokines as well as other cytokines that alter immune function. It is likely therefore that the changes in gene expression in the epithelium have far-reaching effects on the rest of the mucosal immune system.

The afferent limb

In the preceding section we gave evidence that the epithelium can orchestrate the events of the mucosal immune system. It is the purpose of this section to show that alterations in the intestinal lumen can affect the expression of these genes. In our laboratory we examined 3 sets of genes in intestinal epithelium (6) and studied how luminal factors can alter their expression. These included the MHC Class II complex (7), the IGF-binding protein complex (8,9), and chemokine expression. In this review we will limit the evidence to the effect of luminal factors on chemokine expression (10,11).

Bacterial fermentation in the small intestine results in SCFA production. Butyrate levels therefore reflect changes in bacterial populations and in the substrates available for bacterial metabolism. Butyrate levels vary greatly in response to external changes. For example, newborn babies have very low butyrate levels in either the small or large intestine. However, within 2 years butyrate levels rise to adult levels (12). Interestingly, butyrate levels are much higher in bottle-fed babies than they are in breast-fed babies during the first 6 months of life (12).

Butyrate levels therefore reflect events in the intestinal lumen and we hypothesized that their concentrations may alter epithelial cell signaling. We therefore examined its effects on IL-8 and monocyte chemotactic protein-1 (MCP-1) expression (11). Increasing the concentration of sodium butyrate increased IL-8 secretion while simultaneously decreasing MCP-1 expression. These effects were seen in resting epithelial cell lines but were much more marked in cells that have been stimulated with a pro-inflammatory agent such as LPS or IL-1β (**Fig. 3**).

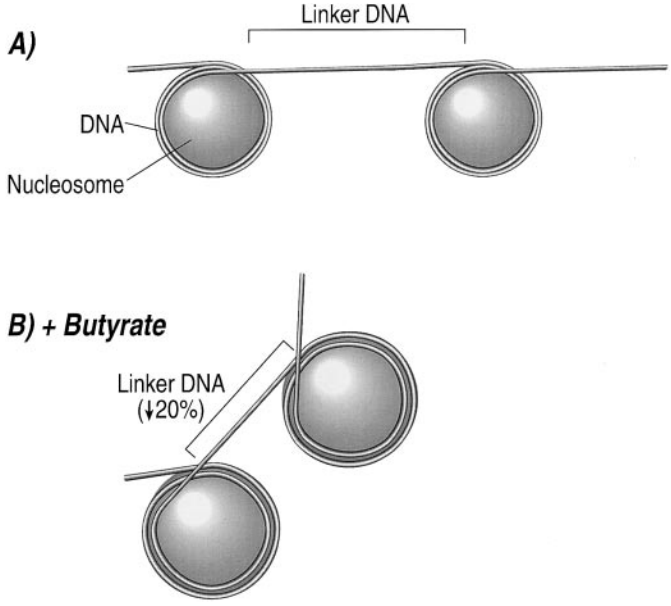

FIGURE 4 Relationship between nucleosomes and DNA. (*A*) DNA is wrapped 2 full turns around nucleosomes made up of unacetylated histones. (*B*) With butyrate-induced acetylation, the nucleosome expands, reducing the number of turns of DNA around the nucleosome to 1.8, with less linker DNA connecting each nucleosome. The result of this is that DNA cannot pass linearly from nucleosome to nucleosome but turns at an angle after every nucleosome, leading to disruption of nucleosome packaging. Reproduced from (6) with permission.

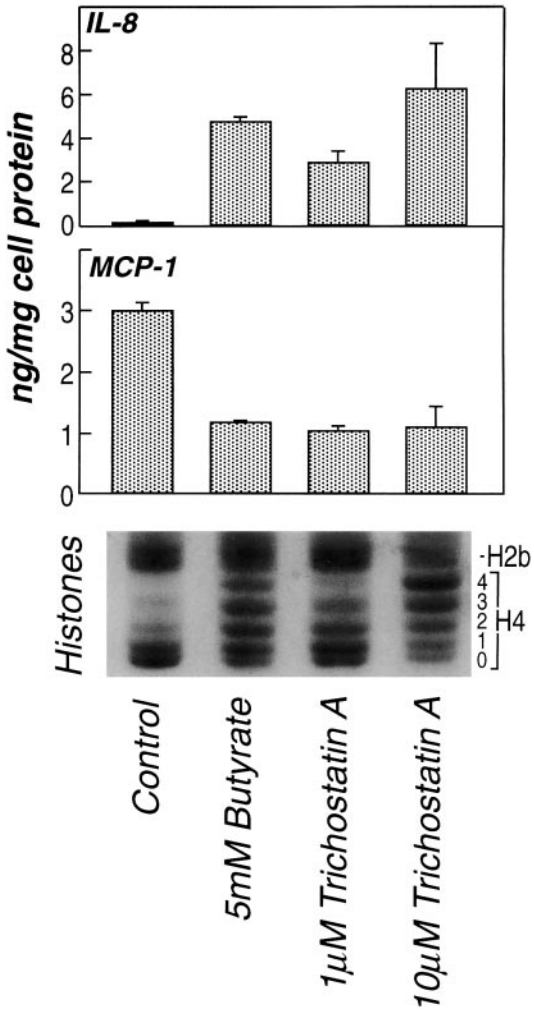

FIGURE 5 Effect of butyrate and trichostatin-A in the induction of histone acetylation and chemokine secretion by Caco-2 cells. Both butyrate and trichostatin A increased the acetylation of histones. IL-8 secretion was simultaneously increased ($P < 0.0001$) and MCP-1 production decreased in Caco-2 cells stimulated with IL-1β ($P < 0.001$). Trichostatin-A, a specific histone deacetylase inhibitor, acted in a manner similar to that of butyrate when given at concentrations that produced a comparable change in histone acetylation. Bars represent standard deviations of 3 different wells for each point. The data are representative of 3 experiments. Reproduced from (11) with permission.

It is known that sodium butyrate alters histone acetylation. The nucleosome consists of a solenoid of histones wrapped around by an integral of 2 turns of DNA (**Fig. 4**). Butyrate increases histone acetylation and this reduces the compactness of the histone. The DNA cannot wrap around the large nucleosome in an integral number of turns. The nucleosome can no longer be packaged into tight bundles. This exposes the DNA and makes it more amenable to transcription factors. We hypothesized that butyrate altered the expression of chemokines by this process. To test this hypothesis we used a fungicide, trichostatin A (TSA), which is 700 times more potent in inducing histone acetylation than butyrate. If the

effects of butyrate on chemokine secretion were due to increased histone acetylation, we would expect them to be reproduced by the TSA. Experiments with TSA (**Fig. 5**) showed that TSA increased IL-8 secretion and decreased MCP 1 secretion. Figure 5 (lower part) shows that both TSA and butyrate increased the acetylation of histone 4. Nonacetylated histones move rapidly through the gel and form a single band, whereas acetylated histones form a ladder depending on the degree of acetylation. The histone 4 has 4 lysine residues that are acetylated and thus, acetylation of histones will result in a ladder of 5 bands. This can be seen in the cells given butyrate or TSA. The upper part of the figure shows that TSA has a similar effect to butyrate. It increases IL-8 secretion and decreases MCP-1 secretion. Furthermore, the degree of this increase in IL-8 varies with the degree of histone acetylation. Moreover, the effect of butyrate on histone acetylation was

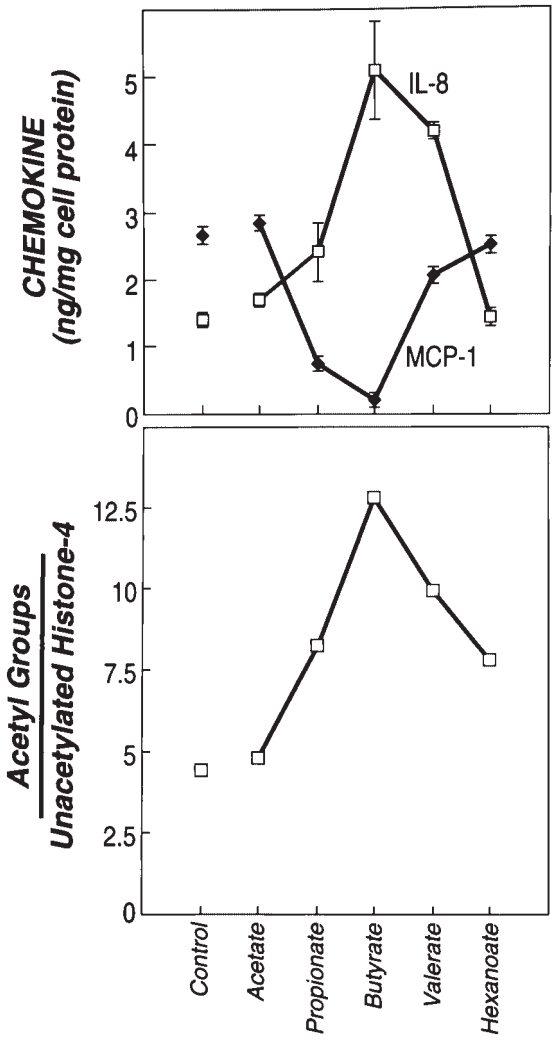

FIGURE 6 SCFA of different chain lengths (C2–C6) alter IL-8 and MCP-1 production according to their effects on histone acetylation. Here 5mM each of acetate (C2), propionate (C3), butyrate (C4), valerate (C5), and hexanoate (C6) were added to Caco-2 cells for 24hr. IL-1β was then given for a further 24hr. Acetate and hexanoate had little effect on acetylation and on chemokine expression (*P*, not significant). Valerate and propionate altered both chemokine secretion ($P < 0.01$) and histone acetylation, but the effects were less than those of butyrate. Bars represent standard deviations of 3 different wells for each point. The data are representative of 3 experiments. Reproduced from (11) with permission.

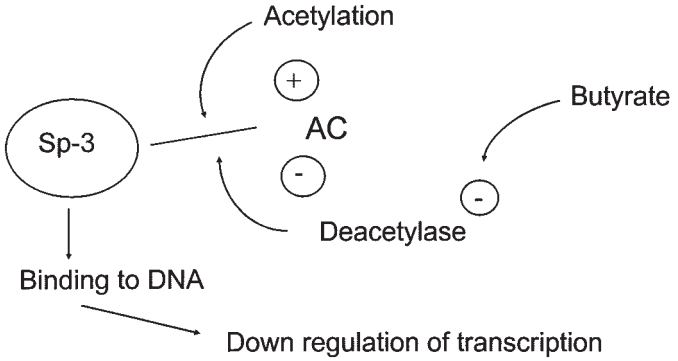

FIGURE 7 Proposed mechanism of butyrate downregulation of gene expression. In this model, butyrate simultaneously inhibits the deacetylation of a transcription factor SP3 whose action is to downregulate gene expression. Thus, butyrate inhibits 2 types of protein deacetylation, one on histones and the other on transcription factors. It is not known, however, whether any single deacetylase can have a dual action, or whether butyrate is acting on different deacetylase at the same time.

reversible (11). After removal of butyrate, histone acetylation returns to normal and the effects of IL-1β on IL-8 secretion and MCP 1 secretion returns to those seen in the untreated cells. Different length SCFAs have differing effects on histone acetylation. Butyrate is the most effective SCFA in inducing histone acetylation while longer and shorter carbon lengths have lesser effects. This effect on histone acetylation is reflected by effects on the expression of IL-8 and MCP 1 (**Fig. 6**). Butyrate has the greatest increase on IL-8 secretion and the greatest decrease on MCP-1 secretion of the various SCFAs used.

The effects on epithelial cell signaling genes correlate with those seen in other genes regulated by butyrate (13,14) where it enhances gene expression through histone acetylation. These experiments, however, do not exclude the possibility that additional effects of sodium butyrate may occur through promoter systems. It is a challenge of future work to examine the interaction between chromosomal regulation, as is seen in these experiments, and promoter based regulation with both butyrate and other luminal molecules. In particular, downregulation of gene expression is difficult to explain by changes in histone acetylation. Loosening protein-DNA interactions would enable DNA to bind to transcription factors and to the machinery of RNA polymerase activity. A possible explanation of how butyrate may downregulate gene transcription is if it were to induce a repressor, to inhibit gene expression indirectly. However, experiments using cycloheximide to inhibit translation did not alter butyrate's ability to downregulate signaling genes, including human IGF binding protein-3 (15). If butyrate were to alter the expression of a repressor though new transcription, interruption of translation of the resulting mRNA would be expected to remove the inhibitor effect of the butyrate. Furthermore butyrate did not change mRNA stability, excluding another possible mechanism. Thus, it must directly act on the target gene itself. One possibility is that it alters the acetylation state of a transcription factor that is already fully synthesized. Certain transcription factors are now known to be capable of acetylation. They include p300 and SP3 (16). It is possible therefore that butyrate may down regulate by also inhibiting deacetylases that act specifically on acetylated transcription factors. Data in our laboratory have shown that butyrate alters the binding of SP3 to DNA, and that this is related to SP3 acetylation. Thus we propose a

model (**Fig. 7**) of butyrate action on downregulation of genes that contain an SP3 site in their promoters.

Conclusion

Our experiments examine the hypothesis that changes in the intestinal lumen can alter the expression of molecules in the intestine epithelium that direct the mucosal immune system. The intestinal epithelium acts as a relay for transducing the information of the intestinal environment to the mucosal immune system. This mechanism has advantages over other forms of immune surveillance in the gut that require the breach of the mucosal barrier. Such breaches can be manipulated by invading organisms to enter the body. The classic of example of this is the polio virus which enters the intestine through the M cell to the immune system of Peyer's patch, which is designed as a sampling system of the mucosal environment.

We believe that these signaling processes are important not only in health but also in the treatment of disease. For example, the primary therapy of children with Crohn's disease in the United Kingdom is treatment with enteral feeds (17,18). Although there are many mechanisms by which enteral feeds may have their activity, we think it possible that one of them is by radically altering the luminal environment to such an extent that it varies the signals from the intestinal epithelium to the mucosal immune system.

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