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In vivo immediate early gene expression induced in intestinal and colonic mucosa by feeding

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Since the gut responds rapidly to food intake, the levels of expression of several immediate early genes were measured in mucosa from small and large intestine of rats starved for 3 days or refed. Within 1 h of refeeding, jejunal and ileal *c-fos*, *jun* B and *zif/268* mRNA and colonic *zif/268* dramatically increased. The *zif/268* gene in jejunum corresponded in size to the full-length cDNA but, in ileum, an RNA band of about 1.2 kb in size increased greatly after feeding. This represents a physiologic in vivo model for the study of gene regulation associated with intestinal epithelial cellular responses to feeding.

Starvation; Refeeding; Gene activation; Immediate early gene; Intestinal gene regulation

1. INTRODUCTION

A fundamental property of gastrointestinal (GI) epithelial cells is their ability to respond rapidly to changing physiological needs, such as altered food intake. Adaptation of the stomach, small intestine and colon has been studied in vivo, using short-term starvation and refeeding [1]. Changes occur in epithelial cell mass, in cell proliferation [2] and cell enzymes [3]. Starvation induces a progressive reduction in cell mass, in proliferation [4] and in enzyme activities [5]. Refeeding after starvation rapidly returns the gut epithelium to a normal fed condition.

3T3 fibroblasts in culture respond to nutrient deprivation, such as removal of serum, by entering a quiescent or G_0 state, followed by cell division 12–16 h after addition of serum [6]. Within 30 min, transcription of a set of 'immediate early' genes increases [7]. These immediate early genes encode transcription factors such as *c-jun, jun* B, *zif/268* [8], *c-fos*, and other nuclear proteins such as *nup/475* [9] which may play a role in the genetic program resulting in cell division. Gut adaptation following starvation or refeeding resembles that of 3T3 fibroblasts or intestinal cancer cells [10] in that many metabolic processes are regulated by nutrient/growth factor manipulation.

Alterations in in vivo gene expression have been observed following partial hepatectomy [11], but gene expression in the GI tract with changing nutrient intake has not been studied.

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2. MATERIALS AND METHODS

2.1. *Animals*

Control male Wistar rats (Taconic Farms, Long Island, NY), weighing about 185 g, were fed until the morning of sacrifice, starved animals received water only for 3 days, and refed animals were given pelleted chow for the times indicated.

2.2. *RNA isolation*

Rats were killed between 09.00 and 11.00 h and intestinal segments were flushed with ice-cold saline, rapidly removed and the mucosa scraped into a solution of 4 M guanadinium thiocyanate, with or without freezing in liquid nitrogen, and immediately sonicated for 1 min at full power. Colonic segments were removed from 1 cm above the cecum to 2 cm above the anus, approximately 20 cm of jejunum was taken, starting 10 cm beyond the ligament of Treitz, and a 20-cm ileal segment was removed, starting 7 cm above the ileocecal valve. Total mucosal RNA was isolated using a modification of the procedure of MacDonald et al. [12].

2.3. *Northern blot analysis*

Fifteen μg of total RNA was denatured in 50% formamide/6% formaldehyde and electrophoresed on a 1.5% agarose gel in MOPS buffer [13]. The gel was transferred to nitrocellulose in 20× SSC and probed with cDNA fragments which were labeled with ^{32}P by nick translation [14]. Hybridization was carried out in sealed bags with 2×10^6 cpm/ml of hybridization solution at 42°C overnight. The blots were then washed 3 times in 0.25 × SSC with 0.2% SDS and exposed to XAR-5 film. Control hybridizations were carried out using a constituative aldolase probe [15] to verify identical RNA loading and confirm a differential response (data not shown).

3. RESULTS

Fig. 1 depicts a compilation of numerous Northern blots of RNA taken from various regions of the GI

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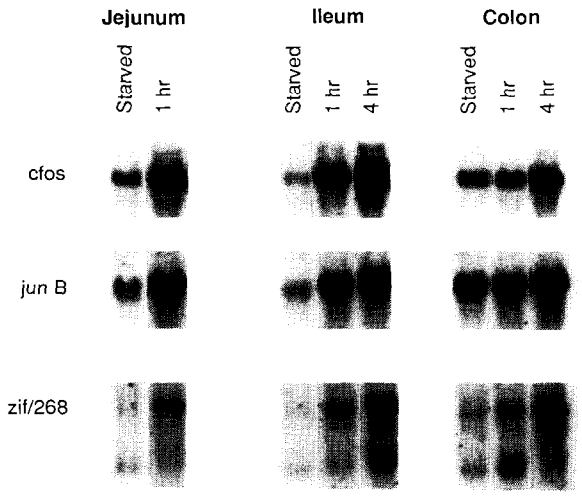

Fig. 1. Northern blots of RNA extracted from jejunal, ileal or colonic mucosa from rats submitted to 3 day starvation or refeeding (after starvation) for the time periods shown. Note increased RNA for *c-fos*, *jun* B and *zif/268* following refeeding. Also note the presence of a 1.2 kb band hybridizing with the *zif/268* cDNA probe.

tract, showing pronounced increases in the levels of some immediate early RNAs following refeeding. Specifically, in the jejunum, *c-fos*, *jun* B and *zif/268* RNA increases dramatically by 1 h. It is noteworthy that the RNA band for the *zif/268* gene corresponds in size to the full-length cDNA. In ileum, the RNA for the 3 immediate early genes shown increases dramatically by 1 h and increases further to 4 h after refeeding. On the Northern blot probed with the *zif/268* cDNA, there are two bands which hybridize with the probe, one corresponding in size to the full-length cDNA and the other band corresponding to an RNA of about 1.2 kb which dramatically increases following refeeding. The colon Northern blot analysis demonstrates that starvation does not decrease the level of RNA for *c-fos* and *jun* B RNA, and no significant changes occur following refeeding. Analysis of the colon also demonstrates that RNA increases 1 h after refeeding and that the smaller *zif/268* RNA species increases by 1 h and decreases significantly by 4 h. Colonic *zif/268* RNA also increased markedly 2 h after refeeding a non-residue liquid elemental meal to starved rats.

4. DISCUSSION

The genetic regulation of cell growth has been intensively studied during the past decade. Polypeptide growth factors generate cellular signals and activate a series of growth-related [16] and other [17] genes prior to the onset of DNA synthesis. Several immediate early genes are activated shortly after serum stimulation of quiescent fibroblasts in culture [7]. The immediate early genes are superinduced by the addition of cycloheximide, suggesting that the inhibition of the synthesis of some cellular protein stabilizes the immediate early mRNA [7]. Although preliminary studies of growth responses of immediate early genes have been performed in immortalized gut epithelial cells [18], very little work has been reported on the genetic regulation of cell growth in intact organisms.

This report analyzes the expression of selected immediate early genes in an in vivo model. *C-fos* and *jun* B RNA increased greatly in jejunum and ileum following refeeding of starved rats. *C-fos* RNA increased within 30 min in jejunum, and a major increase was evident in *c-fos* and *jun* B mRNA at 1 h in jejunum and ileum. The level of RNA for *c-fos* and *jun* B is not affected by starvation in the colon and there is very little change following refeeding. However, the RNA encoding *zif/268* decreases with starvation and increases abruptly following refeeding in jejunum and ileum and in colon. In addition, a smaller RNA species was seen in the colon and ileum which increased following refeeding. The stimulus for the increased expression of these mRNAs might be the presence of food in the lumen of the intestinal segments. However, colonic *zif/268* RNA also increased after rats were fed a liquid elemental diet which would not be expected to reach the colonic lumen, suggesting the influence of a systemic signal to the colon.

Starvation and refeeding of animals represent a physiologic in vivo model system for the study of gene regulation associated with cell growth. Extensive previous studies of starvation, feeding, and the introduction of specific food products have demonstrated the importance of nutrients as stimuli of epithelial cell proliferation throughout the GI tract [1]. However, the epithelial cellular systems that respond to luminal or systemic signals are unknown. The rapidity of the transcription responses of immediate early genes suggests that they are primed for activity and that transcription occurs shortly after a putative growth signal interacts with these epithelial cells. We hypothesize that immediate early gene activation is a part of the very early cellular response to feeding.

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