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Monensin and forskolin inhibit the transcription rate of sucrase–isomaltase but not the stability of its mRNA in Caco-2 cells

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Treatment of Caco-2 cells with forskolin (25 μM) or monensin (1 μM) has previously been shown to cause a marked decrease in the level of sucrase–isomaltase (SI) mRNA, without any effect on the expression of dipeptidylpeptidase IV (DPP-IV). In the present work, we report that there is no significant difference in the stability of SI mRNA between control and treated cells. On the other hand, we demonstrate a decrease in the transcription rate of SI mRNA which is sufficient to account for the decrease in the steady-state level of SI mRNA both in forskolin- and monensin-treated Caco-2 cells.

Sucrase–isomaltase; Caco-2 cell; Forskolin, Monensin; Transcription; Glucose repression

1. INTRODUCTION

We have previously shown that, in Caco-2 cells, forskolin (25 μM) and monensin (1 μM), in addition to their classical effects which are, respectively, an activation of adenylate cyclase [1] and an alteration of distal Golgi functions [2], cause a modification of glucose metabolism and a decreased expression of the intestinal hydrolase, sucrase–isomaltase (SI) [3–7]. The repression of SI characterized by a significant reduction in its biosynthesis is due to a dramatic decrease of its mRNA level [4–8]. This effect appears to be specific to SI: the mRNA level and the biosynthesis of another brush border hydrolase, dipeptidylpeptidase IV (DPP IV), is not affected [4,7]. Although the human SI gene has been cloned [8,9], very little is known about its regulation. Regulation of expression of eukaryotic genes at the level of mRNA can occur in various ways, of which the more common are: trancsription rate [10], alternative splicing [11], and stability of mRNA [12]. Alternative splicing of the SI gene in forskolin- or monensin-treated Caco-2 cells seemed very unlikely since we have never observed differences in the size of SI mRNA in these cells [5–8]. The aim of this work was, therefore, to investigate whether the decrease of SI mRNA in forskolin- and monensin-related Caco-2 cells was due to a decrease of the stability of SI mRNA, or a decrease of the transription rate of the SI gene, or a combination of both processes.

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2. MATERIALS AND METHODS

Control Caco-2 cells or Caco-2 cells treated for 48 h with 25 μM forskolin or 1 μM monensin were assayed at day 16 after plating as previously reported [5,6]. Northern blot analysis, stability and nuclear run-on studies were performed on triplicate cultures of Caco-2 cells. Extraction of RNA and Northern blot analysis were as described previously [5]. For the Northern blot and run-on studies probes were used which cover most of the cDNA, i.e. for SI, a mix of cDNA clones SI2 [13] and I1 [8], and for dipeptidylpeptidase IV, the rat liver cDP37 clone [14], which was the only cDNA probe then available containing the whole coding region of DPP IV and which showed a high level of homology to sequences available for human DPP IV [15]. When necessary, the cDNA probes were ^{32}P-labelled according to Feinberg and Vogelstein [16] using a multiprime DNA labelling Kit (Amersham International, UK). For the stability studies of the mRNA, 5,6-dichloro-1-β-D-ribofuranosyl benzimidiazole (DRB) (Calbiochem, France) was added on day 13 of culture to a final concentration of 0.1 mM from a stock solution of 0.1 M in dimethyl sulfoxide as described in [17]. At various times after the addition of DRB, total RNA was extracted from the cells and immobilized on filters using a slot-blot manifold (Schleicher and Schuell, Dassel, Germany). After hybridization with ^{32}P-labelled SI2 and I1 probes, the resulting radioactivity was quantified by Cerenkov counting. Isolation of nuclei from control, forskolin- or monensin-treated Caco-2 cells, transcriptional 'run-on', extraction and quantification of the ^{32}P-labelled RNA products were as described by Marzluff and Huang [18]. Each transcription mixture containing 10^7 nuclei was divided into two samples which were either supplemented or not with α-amantin (final concentration, 1 μg/ml). The ^{32}P-labelled products from each transcription reaction sample were hybridized to two identical filters containing 13 μg of immobilized denatured linear plasmid DNA containing the relevant probes (either SI or DPPIV). After 48 h of hybridization at 52°C, the filters were washed three times in 5 × SSC, 0.1% SDS, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5, for 30 min at 52°C, once in 2 × SSC, 10 μg/ml

RNase A for 10 min at 37°C and once in 2 × SSC for 10 min at room temperature.

3. RESULTS

Based on the growth-related variation of sucrase activity [5] and SI mRNA levels (Fig. 1) experiments were performed between days 13 and 15, i.e. when the level of SI mRNA increases dramatically.

Northern blot analysis of RNA (Fig. 2) confirmed the previously observed decrease of SI mRNA level after 48 h of treatment with forskolin or monensin [5–8], and showed that this decrease is already detectable after a 24 h treatment. A similar level of a 4 kb DPP IV message was detected in both the control and treated cells, as previously reported using a 700 bp human DPP IV cDNA probe [7].

To determine the effectiveness of DRB in blocking the RNA polymerase II transcription, we monitored incorporation of [^{3}H]uridine into RNA from control, forskolin- or monensin-treated Caco-2 cells. Up to 24 h after addition of DRB, no [^{3}H]uridine incorporation into RNA was detected whatever the treatment (not shown). The cells cultured 24 h in the presence of DRB were tested for their viability: after re-plating under standard culture conditions, no cellular mortality was observed. The time-course of the decay of SI mRNA was measured by following the hybridization signal for 24 h after DRB treatment (Fig. 3). The SI mRNA stability is about the same in the absence or in the presence of forskolin or monensin, and the half-life of SI mRNA was estimated as 30 h.

Preliminary transcription experiments on isolated nuclei were performed using SI2 alone as the SI cDNA probe, and the filters were washed at high stringency. In these experiments no hybridization of labelled RNA

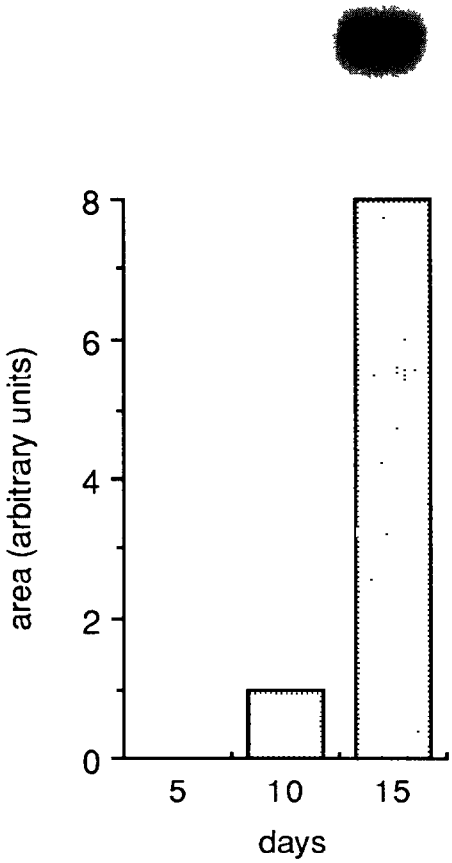

Fig. 1. Growth-related variations of SI mRNA levels in Caco-2 cells. (Upper panel) Northern blot analysis. Total RNA (25 μg) were extracted from Caco-2 cells after 5, 10 and 15 days in culture, separated on a 1% agarose gel and hybridized with SI2. (Lower panel) Densitometric analysis of the fluorogram. Note the large increase of SI mRNA beween day 10 and day 15.

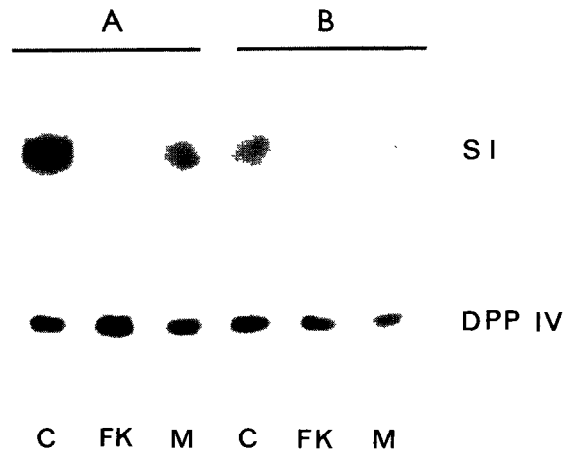

Fig. 2. Northern blot analysis. Total RNA (10 μg) from Caco-2 cells (C) or Caco-2 cells treated with forskolin (FK) or monensin (M) for 24 h (day 15) (A) or 48 h (day 16 (B) was fractionated on a 1% agarose gel. The membrane was probed with SI2 (SI) and cDP37 (DPP IV)

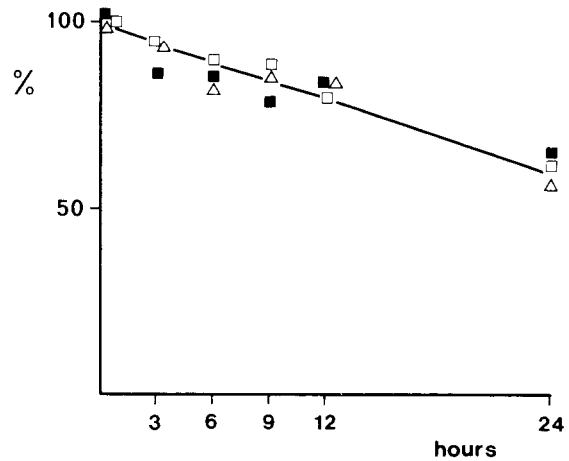

Fig. 3. Measurement of the stability of the SI mRNA. Total RNA (10 μg) were isolated from Caco-2 cells (■) or Caco-2 cells treated with forskolin (□) or monensin (△) at the indicated times after DRB addition. After hybridization to the SI2 probe, the resulting signals were measured and expressed as the percentage of the initial value for each condition

Fig. 4. Nuclear run-on experiments. Transcription of SI (A) or DPP IV (B) in control (C), forskolin (FK)- or monensin (M)-treated cells analyzed after 48 h treatment (day 16) with (1) or without (2) α-amanitin.

to the SI2 probe could be detected. This was probably due to the small size of SI2 as compared with the estimated length of the primary transcripts (55 kb) [8]. Two different modifications were then introduced into the protocol: (i) the use of a mixture of SI2 and I1 as SI probes; (ii) the use of RNase A to reduce the non-specific hybridization of the ^{32}P-labelled RNA products.

The results of the nuclear run-on experiments shown in Fig. 4 demonstrate a reduction in the transcription rate of SI in both the forskolin- and monensin-treated Caco-2 cells. In the absence of α-amanitin there is 4- to 5-fold less signal in the treated cells than in the control cells, whereas the signals obtained for]the DPP IV, used as an internal control, show the same intensity in each case. In the presence of α-amanitin at 1 μg/ml, a concentration which specifically inhibits RNA polymerase II (Fig. 4A(1) and Fig. 4B(1)), only trace amounts of ^{32}P-labelled RNA hybridized with the SI or DPP IV probes.

4. DISCUSSION

We show in the present work that the variations in the steady-state level of SI mRNA between control and forskolin- or monensin-treated Caco-2 cells cannot be attributed to differences in mRNA stability but result from a decrease in transcription rate. It must be noted that, considering the estimated half-life of SI mRNA to be 30 h in all the conditions (Fig. 3), one would expect that the SI mRNA would decrease no more than about 50% within 24 h of treatment with forskolin or monensin. This apparent discrepancy is most likely due in part to the fact that the beginning of the treatments were performed at a period of cell growth when the level of SI mRNA is considerably increasing in control cells (Fig. 1) thus exaggerating the differences. Furthermore it cannot be excluded that DRB treatment results in a stabilization of SI mRNA, through inhibiting RNase synthesis. If so, the half-life of SI mRNA could be overestimated. The effect of both drugs appears to be specific for SI, since the transcription rate of DPP IV, another brush border hydrolase, is not affected.

The classical effect of monensin is post-translational and characterized by an impairment of the N-glycosylation process [2]. It has never previously been reported to have an effect on the regulation of genes at the transcriptional level. Forskolin is commonly used to increase the intracellular cAMP via an activation of adenylate cyclase [1]. Such an increase in the cAMP level can in turn regulate the transcription of a number of genes through the consensus sequence, CRE [19]. The absence of this sequence in the 5′ region of the human gene [8,9], makes it unlikely that the transcription of SI is directly regulated via cAMP.

Thus another mechanism is likely to explain the common and apparently specific effect of forskolin and monensin on the transcription of the human SI gene in Caco-2 cells. It has been demonstrated that the inhibiting effect of these drugs on the expression of SI is always associated with increased glucose utilization in these cells: forskolin induces a decrease in intracellular glycogen content and an increased glucose consumption [3,5,7], and monensin induces an increase in the rate of glucose consumption [4,6,7]. Our previous results led us to hypothesize that glucose negatively interferes with the expression of SI at the transcriptional level. This was further supported by the results obtained with another human colon carcinoma cell line, HT-29, in which the SI mRNA level is detectable only when the cells are cultured in medium totally deprived of glucose [8].

Regulatory elements potentially responsible for the tissue-specific expression of SI have been identified [9,20] but nothing is known about the mechanisms involved in this hypothetical metabolic regulation. The results of the present work lend support to the idea that metabolic glucose repression of SI in Caco-2 cells is indeed achieved through regulatory elements in the SI gene, in a manner similar to that demonstrated for the human glucose-regulated proteins [21].

That SI might be regulated by one of the products of the hydrolysis of sucrose is not entirely surprising, but whether the metabolic repression of SI observed in Caco-2 and HT-29 cells is a consequence of their malignant state remains to be elucidated. One piece of evidence that suggests that this mode of regulation might

be relevant in normal tissue is the observation that the disappearance of SI in human fetal colon at mid-gestation occurs at the same time as the decrease in accumulation of intracellular glycogen in intestinal epithelial cells [22].

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