Biochem. J. (1990) 268, 99–103 (Printed in Great Britain)

Specific expression of lactase in the jejunum and colon during postnatal development and hormone treatments in the rat

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The expression of lactase was compared in the jejunum and colon of the rat at the levels of enzyme activity and protein and RNA content. We found that the enzyme proteins and the corresponding mRNAs share common features and are encoded by a single gene in both intestinal segments. In the jejunum, large amounts of lactase mRNA and proteins were detected during postnatal development as well as in adult rats, despite the 10-fold decline in lactase specific activity which occurs at weaning. In contrast, in the colon the expression of lactase was restricted to early postnatal development. In the colon, the enzymic activity of lactase and the amounts of protein and mRNA followed parallel development profiles with a peak at day 4 after birth. Injections of thryoxine or epidermal growth factor into neonates led to small modifications in the expression of lactase in the jejunum. On the other hand, these treatments caused a large decline in lactase activity in the colon that paralleled a decrease in the amount of lactase protein and mRNA. These data indicate that the expression of lactase is mainly regulated at the post-transcriptional level in the jejunum, whereas it is controlled at the pre-translational level in the colon.

INTRODUCTION

Within the intestinal tract, the small intestine and the colon have distinct structural and functional properties (Henning, 1987; Potter & Lester, 1984). In the human, the differentiation of the large intestine is achieved at birth, but the fetal colon transiently exhibits, until late gestation, some features restricted thereafter to the postnatal small intestine, e.g. villi structures and some hydrolase activities (Auricchio *et al.*, 1965; Lacroix *et al.*, 1984; Raul *et al.*, 1986). In rats, the presence of villi characterizes the perinatal colon (Halender, 1973; Eastwood & Trier, 1974), which expresses enzyme activities otherwise displayed in the small intestine, i.e. lactase and aminopeptidase (Foltzer-Jourdainne *et al.*, 1989).

Lactase activity is associated with a bifunctional protein, termed lactase–phlorizin hydrolase (LPH), which is processed intracellularly from a high-molecular-mass precursor (Büller *et al.*, 1987; Mantei *et al.*, 1988). In the proximal part of the small intestine, the jejunum, the expression of lactase is controlled by thyroid hormones (Henning, 1987). Indeed, an increase in the plasma concentration of thyroxine parallels the decline in lactase activity occurring at weaning, and experimental changes in the amount of thyroid hormones, e.g. by thyroxine injection, thyroidectomy or starvation, modulate lactase activity accordingly (Yeh & Moog, 1974; Raul *et al.*, 1982, 1983).

A great number of investigations have been conducted in order to define the nature of the decline in lactase observed at weaning in the jejunum. A correlation has been tentatively established between the drop in enzyme activity and the acceleration of enterocyte renewal in the adult mucosa, which might be interpreted as a decreased time available for the processing of the LPH precursor (Tsuboi *et al.*, 1981; Smith & James, 1987). However, at the molecular level, conflicting results have been reported. Whereas some authors found no differences in the rate of biosynthesis of LPH between neonates and adults (Tsuboi *et al.*, 1985), others propose that the incorporation of the mature protein into the microvillar brush-border membrane decreases at weaning (Jonas *et al.*, 1985). This may reflect, at least in part, the modification in the processing of the LPH precursor that is caused by changes in the concentration of thyroxine in the plasma (Nsi-Emvo *et al.*, 1987). Recently, the regulation of lactase expression has been shown to take place essentially at the post-transcriptional level in the jejunum, since the LPH mRNA accumulates at a constant rate throughout development, irrespective of the specific activity of lactase and of the concentration of thyroid hormones (Freund *et al.*, 1989; Sebastio *et al.*, 1989).

Information about lactase in the colon is scarce, although some modulation of enzyme activity by thyroxine has been reported (Foltzer-Jourdainne *et al.*, 1989). In the present study, we have analysed lactase expression in the rat colon at the enzyme, protein and mRNA levels, and the results have been compared with those obtained in the jejunum. We also detailed the effects of exogeneous administration of thyroxine and epidermal growth factor (EGF), a peptide hormone present in milk (Koldovsky & Thornburg, 1987).

MATERIALS AND METHODS

Animals and tissues

Proximal jejunal and colon segments, including the epitheleum and muscularis, were excised from Wistar rats. Administration of hormones or control NaCl solutions was performed daily from birth until the animals were killed (Foltzer-Jourdainne *et al.*, 1989). Thyroxine (Roche) and EGF (Sigma) were injected subcutaneously at a dose of 1 μg/g and 0.5 μg/g body weight respectively. Samples originating from neonates at day 0 were taken 3–5 h after birth.

Protein analysis

Intestinal brush-border proteins were prepared and analysed by PAGE as described previously (Nsi-Emvo *et al.*, 1987). After electrotransfer on to nitrocellulose filters, the immunodetection of the LPH-type proteins was performed using a rabbit antiserum directed against the brush-border enzyme extracted from the jejunum of suckling rats (anti-LPH antiserum) (Nsi-Emvo *et al.*, 1986). The amount of LPH-type protein contained in the

Abbreviations used: LPH, lactase–phlorizin hydrolase; EGF, epidermal growth factor.

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intestinal samples was determined by e.l.i.s.a., which was performed according to Clark & Adams (1977). Lactase activity was determined in the brush-border membranes at pH 6.5 in the presence of 0.1 mM-*p*-chloromercuribenzoate, which inhibited lysosomal β-galactosidase (Koldovsky *et al.*, 1969). Protein concentration was measured as described by Lowry *et al.* (1951). The specific activity of lactase is expressed as milliunits of enzyme per mg of protein, where one unit corresponds to the amount of material hydrolysing 1 μmol of lactose/min at 37 °C.

Nucleic acid analysis

Intestinal cytoplasmic RNAs were extracted using the LiCl method (Auffray & Rougeon, 1980), separated on 1% agarose/17% formaldehyde gels (Lehrach *et al.*, 1977) and blotted on to nitrocellulose filters (Thomas, 1980). They were hybridized under standard high-stringency conditions [0.2 × SSC (1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate)/0.1% SDS at 62 °C] to a cDNA probe for human α-actin and to the plasmid pRLU6. This plasmid contained a 6 kb cDNA segment corresponding to fetal rat LPH, and has been used previously to detect the related mRNA (Sebastio *et al.*, 1989). Probes were labelled according to Feinberg & Vogelstein (1983). The radioactivity retained on the nitrocellulose filters were quantified by scanning the autoradiograms using a dual-wavelength flying-spot densitometer (Shimadzu, Roucaire, France). Genomic DNA from rat liver was extracted and digested with appropriate restriction enzymes (Gros-Bellard *et al.*, 1973). The DNA fragments were separated by agarose-gel electrophoresis and blotted on to nitrocellulose filters (Southern, 1975). Hybridization to the probes was performed under low-stringency conditions in a solution containing 2 × SSC and 0.1% SDS at 37 °C.

RESULTS

Lactase activity in the jejunum and colon

Lactase activity was determined in the brush-border membranes of the jejunum and colon at different developmental stages (Table 1). The enzyme profiles were similar when the activity was related either to the amount of brush-border protein or to the length of the intestinal segment (results not shown). The data presented here were consistent with those reported previously (Foltzer-Jourdainne *et al.*, 1989). At birth, the specific activity of lactase was low, although detectable, in the colon, but 40-fold higher in the jejunum. It increased transiently in the large intestine during the first 4 days of the postnatal life and rose to a level corresponding to 7% of the activity measured in the jejunum at the same stage. Within the next few days, lactase activity dropped in the colon to negligible levels. In the jejunum, the elevated activity found soon after birth decreased progressively during the suckling period and reached a 10-fold lower level in adults than in neonates. The enzyme activity found in the jejunum of adult animals was thus similar to that displayed in the postnatal colon at 4 days after birth.

LPH-type antigenic determinants in the jejunum and colon

The finding of a transient lactase activity led us to search in the colon for the presence of antigenic determinants related to the jejunal enzyme. After separation by gel electrophoresis, brush-border proteins extracted from the small and large intestines were probed using serum directed against the jejunal LPH (anti-LPH antiserum) (Fig. 1). In the jejunum of suckling rats, this serum detected the mature 140 kDa LPH, as well as two larger proteins with apparent molecular masses around 250 kDa and 200 kDa. These have been designated as different glycosylated forms of the LPH precursor (Büller *et al.*, 1987). In adults, the larger protein was prominent, corroborating the finding of a modification in the processing of the LPH precursor at weaning (Nsi-Emvo *et al.*, 1987). The relative amount of LPH-type proteins in the total protein content of the brush-border membranes was determined in the same samples by e.l.i.s.a. As summarized in Fig. 2(*a*), the proportion of these proteins was elevated during the suckling period, dropped at weaning and remained at a 2-fold lower level in adult animals.

In the colon, we found that the antiserum directed against the jejunal LPH detected three types of brush-border proteins that had electrophoretic mobilities similar to those identified in the jejunum (Fig. 1). As shown by e.l.i.s.a., the overall amount of these LPH-type proteins was low at birth as well as in 10-day-old animals and was negligible in adults, but was elevated transiently between the second and sixth day of postnatal life (Fig. 2*a*). Moreover, the 200 kDa and 250 kDa proteins were abundant, whereas the 140 kDa form was barely detected (Fig. 1). Thus antigenic determinants similar to those of jejunal LPH were expressed transiently in the rat colon at the developmental stages at which lactase activity appeared.

LPH-type mRNA in the jejunum and colon

In order to gain more insight into the molecular basis of lactase expression in the jejunum and in the colon of rats, cytoplasmic RNAs were extracted from both intestinal segments at various developmental stages, and hybridized to the cDNA for the jejunal LPH. They were also probed with the cDNA

Table 1. Lactase specific activity in the jejunum and colon during postnatal development in the rat

The specific activity of lactase was determined in the brush-border membranes of the jejunum and colon. Values are presented as means ± S.E.M. For a given intestinal segment, a ≠ b ≠ c ≠ d at $P < 0.01$, as determined using Wilcoxon's test for unpaired samples.

Age (days)	*n*	Specific activity (munits/mg of protein) Jejunum	Colon
0	5	753 ± 8^a	19 ± 2^a
2	4	1110 ± 13^b	50 ± 6^b
4	4	1088 ± 12^b	72 ± 7^c
6	6	812 ± 9^a	43 ± 5^b
10	5	391 ± 8^c	17 ± 2^a
90	4	81 ± 8^d	2 ± 1^d

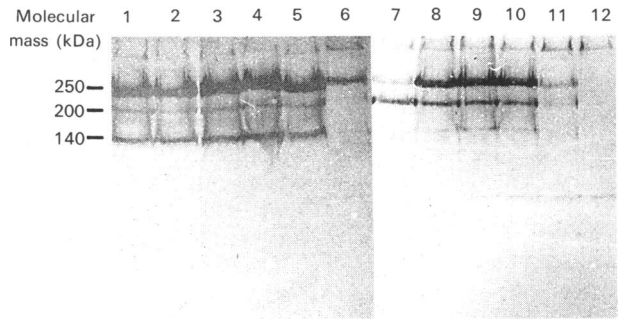

Fig. 1. LPH-type antigenic determinants in the jejunum and colon

Proteins were extracted from brush-border membranes and probed using the anti-LPH antiserum. Lanes 1–6 correspond to jejunal samples originating respectively from 0, 2, 4, 6, 10 and 90 day-old rats; lanes 7–12 correspond to colon samples from the same animals. About 70 μg of protein was loaded in each lane.

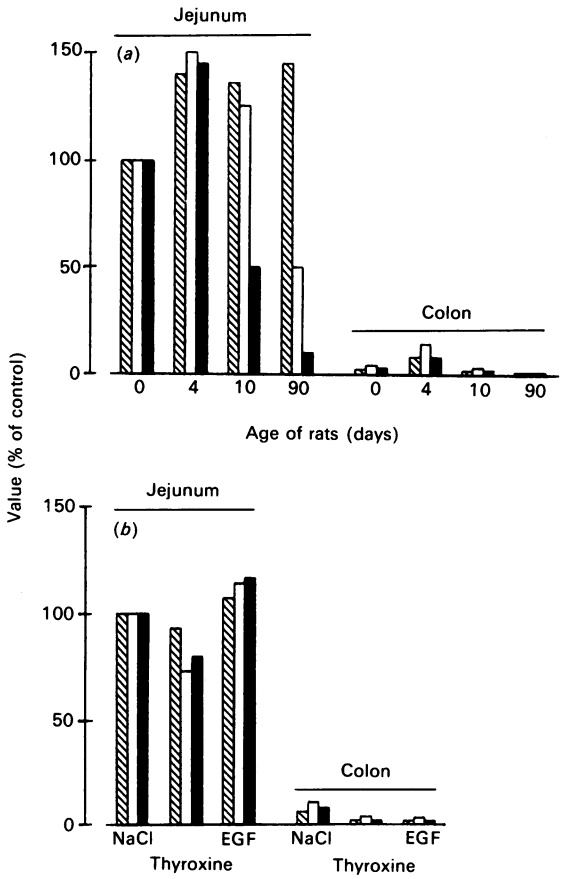

Fig. 2. Lactase expression in the jejunum and colon of the rat

(*a*) The amounts of LPH mRNA (▨), LPH-type proteins (□) and the lactase specific activity (■) were compared in the jejunum and colon during development. The amount of LPH mRNA was obtained by scanning the autoradiograms using a densitometer, and the values were reported to a constant weight assigned to the actin messenger. The content of LPH-type proteins in the brush-border membranes was measured by e.l.i.s.a. The specific activity of lactase was determined in brush-border membranes. All data are expressed as percentages of the values measured at birth in the jejunum. (*b*) Similar representation for 4 day-old rats injected with thyroxine or EGF. The values are percentages of those obtained in the jejunum of NaCl-injected animals.

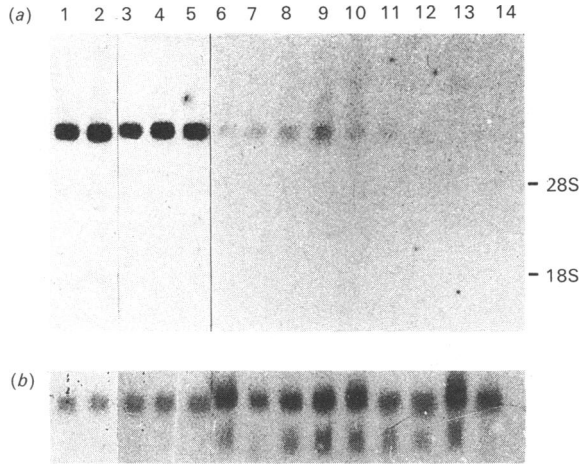

Fig. 3. Jejunal and colon RNA transcripts detected by the cDNAs for LPH and α-actin

(*a*) Cytoplasmic RNAs extracted from the jejunum of 0, 2, 4, 10 and 90 day-old rats (lanes 1–5), and from the colon of 0, 1, 2, 3, 4, 6, 8, 10 and 90 day-old animals (lanes 6–14), were hybridized to the cDNA for jejunal LPH. About 10 μg of jejunal RNA and 20 μg of colon RNA was loaded in the corresponding lanes. The positions of the 28S and 18S ribosomal RNAs are indicated. (*b*) The above samples were hybridized to the cDNA for α-actin and exposed to autoradiography for 10-fold less time than for (*a*).

corresponding to α-actin. The relative amount of LPH mRNA contained in each sample was determined by scanning the autoradiograms with a densitometer and the results were reported to a constant value assigned to the actin RNA transcript. As depicted in Figs. 2(*a*) and 3, the rate of accumulation of the 6.3 kb LPH mRNA reached a plateau soon after birth in the jejunum and remained stable during development, despite the 10-fold difference in lactase activity observed between neonates and adults. In the colon, we found that the LPH probe hybridized to a low-abundance transcript that was similar in size to the small-intestinal LPH mRNA. Yet, in contrast with the situation described in the jejunum, this transcript was only present during the first week of postnatal life, with a peak at 4 days after birth.

Effects of thyroxine and EGF on lactase expression

We have investigated, at the molecular level, the decline in lactase activity that has been reported to follow thyroxine administration in 3–4-day-old animals (Foltzer-Jourdainne *et al.*, 1989). As already reported, thyroxine injections soon after birth led to a greater effect in the colon than in the jejunum (Foltzer-Joudainne *et al.*, 1989; Yeh *et al.*, 1989). Indeed, compared with control neonates (injected with NaCl), hormone-injected rats showed decreases in the lactase specific activity of 20% and 70% in the jejunum and the colon respectively (Table 2). The proteins and RNA of these animals were analysed as described above (Fig. 2*b*). In the jejunum, thyroxine caused a 25% decline in LPH-type proteins and a 15% decrease in the amount of LPH mRNA. In the colon, in contrast, the LPH-type proteins as well as the 6.3 kb mRNA were 3-fold less abundant in thyroxine-injected than in NaCl-injected animals.

Since EGF is a constituent of breast milk (Koldovsky & Thornburg, 1987), we have analysed in neonates the effects of exogeneous administration of this growth factor on various intestinal functions (C. Foltzer-Jourdainne & F. Raul, unpublished work) including lactase expression (Table 2, Fig. 2*b*). In the jejunum, the treatment increased slightly the specific activity of lactase as well as the amounts of LPH-type proteins and mRNA. In the colon, in contrast, we found that EGF injections caused simultaneous 70% decreases in the 6.3 kb RNA tran-

Table 2. Effect of hormone treatments on lactase specific activity in the jejunum and colon of 4-day-old rats

The specific activity of lactase was determined in the brush-border membranes of the jejunum and colon. Values are presented as means ± S.E.M. ($n = 7$). For a given intestinal segment, $*P < 0.01$ compared with control as determined using Wilcoxon's test for unpaired samples.

Treatment	Specific activity (munits/mg of protein)	
	Jejunum	Colon
NaCl	1053 ± 25	60 ± 4
Thyroxine	843 ± 53*	17 ± 2*
EGF	1222 ± 43	15 ± 3*

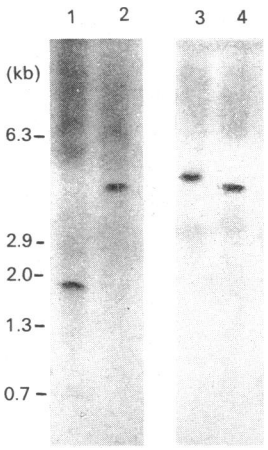

Fig. 4. Detection of genomic sequences hybridizing to rat LPH cDNA

The genomic DNA from rat liver was cut with the restriction enzymes *Eco*RI (lanes 1 and 3) or *Hin*cII (lanes 2 and 4). It was hybridized under low-stringency conditions to the 0.6 kb *Eco*RI–*Hin*dIII restriction fragment (lanes 1 and 2) or to the 0.4 kb *Nae*I–*Eco*RI restriction fragment (lanes 3 and 4) originating respectively from the 5′ and 3′ extremities of the LPH cDNA contained in the plasmid pRLU6.

script, the LPH-type proteins and the specific activity of lactase. Neither EGF nor thyroxine administration modified the electrophoretic properties of the proteins and mRNAs detected in both intestinal segments.

A single gene for jejunal and colon LPH

The final step of this study was conducted in order to elucidate the number of genes encoding the LPH-type proteins in the various segments of the intestine. Genomic DNA was extracted from the liver of an adult rat and hybridized to different regions of the rat cDNA for the jejunal LPH. We used as probes the 0.6 kb *Eco*RI–*Hin*dIII and the 0.4 kb *Nae*I–*Eco*RI restriction fragments originating respectively from the 5′ and 3′ extremities of the cDNA contained in the plasmid pRLU6 (I. Duluc, J.-N. Freund, N. Mantei & F. Raul, unpublished work). Even under low-stringency hybridization conditions, each probe detected a single fragment in the genomic DNA cut with the restriction enzymes *Eco*RI or *Hin*cII (Fig. 4). These data suggested that the LPH gene is single copy in the rat genome.

DISCUSSION

In the present study, we have compared the expression of lactase during the postnatal development of Wistar rats in the proximal parts of the small and large intestines. In a recent paper, Büller *et al.* (1989) confirmed the presence of this enzyme in the rat colon, and Colony *et al.* (1989) correlated these findings with the structure of the intestinal mucosa in Sprague–Dawley rats. The data shown here indicate that the transient expression of lactase activity in the neonatal colon coincides with the presence of proteins with the same molecular mass and antigenic determinants as the LPH-type molecules found in the jejunum. In addition, a transcript similar in size and sequence to the jejunal LPH mRNA accumulates in the colon at this stage. Since genomic restriction mapping suggests that there exists a single gene for LPH in the rat genome, we conclude from these data that lactase in the jejunum and colon is encoded by the same gene.

Investigations of the human colon at the developmental stage at which villi appear, i.e. the fetus, did not reveal significant amounts of brush border lactase, in contrast with other disaccharidases such as sucrase and maltase (Lacroix *et al.*, 1984; Raul *et al.*, 1986). A low lactase activity has however been tentatively described in the human fetal colon, but was not attributed to LPH itself or to cytosolic β-galactosidases (Dahlqvist & Lindberg, 1966; Menard & Pothier, 1987). Thus we propose that the transient expression of lactase in the developing colon may not be a general feature in mammals.

The quantitative data obtained in this study are summarized in Fig. 2. Concerning the jejunum, they corroborate previous findings (Nsi-Emvo *et al.*, 1987; Freund *et al.*, 1989; Sebastio *et al.*, 1989); indeed, in this intestinal segment, the LPH mRNA accumulates at a nearly constant rate whereas lactase specific activity is changed under physiological or experimental constraints. These results confirm that lactase expression is controlled mainly at the post-transcriptional level in the jejunum. In accordance with Yeh *et al.* (1989), we found that the effect of thyroxine injection on jejunal lactase activity was weaker in the neonates analysed here than in older suckling rats (Freund *et al.*, 1989). Since on the other hand EGF administration was without marked effect on lactase expression in the jejunum of newborn animals, this suggests that the jejunum of neonates is hardly sensitive to exogenous hormone treatments. In contrast, the present study points out the similar developmental profiles shown in the colon by lactase specific activity, the amount of LPH-type proteins and the amount of LPH mRNA. Moreover, the fall in activity following thyroxine and EGF treatments parallels the decline of LPH-type proteins and mRNA in the colon. Therefore the parallel established between the accumulation of the LPH transcript, the LPH-type proteins and the specific activity of lactase strongly supports the hypothesis that the transient expression of lactase displayed in the colon is essentially regulated at the pre-translational level, although we cannot discriminate between control of gene transcription and mRNA turn-over.

In the colon, the cellular basis of the regulation process remains unknown; indeed, it may affect the mature epithelial cells lining the villi and/or the stem cells restricted to the crypts, leading to the replacement of lactase-expressing cells with colonocytes lacking this glycosidase. Of note is that the half-life of the intestinal epithelial cells is much longer in neonates than in adults (Eastwood & Trier, 1974), but different results have been obtained concerning cellular proliferation along the developing intestine (Al Nafusi & Wright, 1982; Buts *et al.*, 1983). It seems unlikely that thyroid hormones could actually be responsible for the normal decline of lactase occurring in the large intestine 4 days after birth, since the amount of thyroxine is very low during this period. The situation may be different for EGF, as this factor is contained in milk and has been recovered in the neonatal colon (Schaudies *et al.*, 1989). EGF-binding sites were also displayed in the mouse colon soon after birth (Menard *et al.*, 1987).

Colon LPH-type proteins have similar molecular masses to their jejunal counterparts, but proportions are different. Indeed, the 140 kDa form, which co-migrates with the mature jejunal LPH, was barely detected at steady-state in the colon, in contrast with the higher-molecular-mass proteins. This observation is consistent with the slower rate of processing of the LPH precursor found in the colon as compared with the small intestine (Büller *et al.*, 1989). It raises the question of whether the colon lacks the machinery needed for the efficient processing of the LPH precursor, or whether this precursor has a structure which prevents subsequent processing. In this regard, glycosylation may play a key role in the biosynthesis of the mature enzyme. In the jejunum, for instance, fucosylation has been shown to accompany the fall in lactase activity occurring at weaning when the LPH precursor is no longer processed correctly (Nsi-Emvo

et al., 1987). Changes in the activity of some glycosyltransferases have already been described during intestinal development (Kolinska *et al.*, 1988; Ozaki *et al.*, 1989). In the jejunum, lactase activity correlates with the amount of the mature 140 kDa LPH (Nsi-Emvo & Raul, 1984). In the colon, the finding of an elevated amount of high-molecular-mass forms among the total LPH-type proteins led us to reconsider a possible enzymic function for the LPH precursor itself, at least in this intestinal segment.

In conclusion, the present study offers a first example where a hydrolase encoded by a single gene in two distinct intestinal segments is regulated differently in the two segments during development and hormone treatments. Lactase activity is controlled essentially at the pre-translational level in the colon of the rat, in contrast with the jejunum, where it is controlled at the post-transcriptional level. This may result from different exposure of the two intestinal segments to regulatory factors and/or from a disparity in their cellular receptors and effectors.

We thank Dr. N. Mantei (ETH, Zürich) and Dr. R. Heilig (INSERM U184, Strasbourg) for the gifts of the LPH and actin cDNAs. We are grateful to C. Haffen for the photographs and to B. Lafleuriel and L. Mathern for preparing this manuscript. This work was supported by INRA grant 88/4667. I.D. is a research student funded by the Ministère de la Recherche et de la Technologie.

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Received 27 November 1989/22 January 1990; accepted 31 January 1990