# The expression of epidermal growth factor and transforming growth factor-α mRNA in the small intestine of suckling rats: organ culture study

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**Abstract Epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) are associated with regulation of various gastrointestinal functions. In order to better understand their role in developing small intestine EGF, TGF-α and EGF-R steady-state mRNA levels and transcript stability were determined. Reverse transcription (RT) competitive-polymerase chain reaction (PCR) revealed that intestinal TGF-α mRNA levels were 10-fold higher in comparison with EGF mRNA. The primary intestinal culture technique was used to evaluate mRNA stability. The stability of TGF-α mRNA was remarkably lower than the stability of EGF mRNA. High levels of TGF-α mRNA accompanied by high degradation rate of this mRNA suggested a rapid turnover of intestinal TGF-α mRNA.**



*Key words:* Epidermal growth factor; Transforming growth factor-α; Reverse transcription competitive-polymerase chain reaction; Gastrointestinal tract

## 1. Introduction

Epidermal growth factor and transforming growth factor-α are biologically potent polypeptides that produce a variety of biologic responses, most of which involve enhanced proliferation and/or differentiation of epithelial cells [1,2]. Both growth factors belong to the family of EGF-related ligands, sharing amino acid sequence homology [3] and high affinity to the same receptor – epidermal growth factor receptor [4,5]. EGF-R is a transmembrane glycoprotein composed of an extracellular peptide binding domain, a transmembrane domain, and an intracellular domain possessing tyrosine-specific protein kinase activity which is believed to be the primary effector of EGF or TGF-α responses [6,7]. It is likely, therefore, that regulation of EGF-R expression has a significant influence on the control of cell growth and differentiation.

Locally derived, paracrine/autocrine-acting peptide growth factors play crucial roles in the control and maintenance of growth and differentiation of epithelial tissue, such as mucosa of gastrointestinal tract. In suckling rats, significant amounts of EGF and TGF-α peptides have been reported throughout the gastrointestinal tract [8–18], however, the origin of these growth factors is not clear. High concentrations of EGF peptide were found in rat amniotic fluid and rat milk [19], suggesting that during development the major part of intestinal EGF is delivered orogastrically. In contrast to this, TGF-α peptide was not detected in rat milk and the source of intestinal TGF-α in neonates is probably pancreas and intestinal tissue itself [18]. In addition, the presence of specific EGF-receptor throughout the gastrointestinal tract of suckling rats and mice has been reported, suggesting that EGF-related peptides play an important physiological role in this tissue during development [20–23].

Intestinal EGF, TGF-α and EGF-R mRNA levels have not been clearly quantified yet. Previously, we have reported cellular localization of EGF and TGF-α mRNA transcripts in developing small intestine of rats using an in situ hybridization technique. However, an accurate quantification of these two mRNAs was not performed [24]. Since gene expression of these transcripts in developing small intestine is very low, the reverse transcription (RT) competitive-polymerase chain reaction (PCR) assay has been established to amplify the messages and measure mRNA levels quantitatively. Recently, we have used this approach to measure intestinal IGF-I mRNA level [25]. A similar technique has been published for detection of EGF mRNA in developing mouse reproductive tract [26].

The main goal of this study was to detect and quantify EGF and TGF-α steady-state mRNA levels in developing small intestine from rats. Furthermore, to better understand the role of these growth factors in this tissue, gene expression of EGF receptor was measured. Significant differences in mRNA levels raised the question about the transcript stability. Steady-state mRNA levels result from a balance between the rates of mRNA transcription and mRNA degradation. The mRNA stability can be studied by using transcription inhibitor such as actinomycin D, however, direct in vivo application of actinomycin D to the animal can affect several physiological functions of whole organisms. Therefore, we decided to use the primary organ culture method. Intestinal organ culture from fetal and neonatal rats, has been described several times in studies evaluating the direct effect of biologically active substances on the maturation of small intestine [27–37]. Based on our previous experience with this method [32,37], the serum-free organ culture method was used in these studies. The combination of in vitro organ culture method and RT competitive-PCR enabled us to measure more accurately EGF, TGF-α and EGF-R transcripts stability in the small intestine from neonatal rat.

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## 2. Material and methods

### 2.1. Materials

Sodium phosphate, NaCl, F-12/DMEM medium (1:1 mixture), kanamycin, dimethyl sulfoxide (DMSO), actinomycin D, MOPS, sodium acetate, EDTA were purchased from Sigma (St. Louis, MO, USA). Formaldehyde solution (37%, w/v), ethidium bromide were from UBS (Cleveland, OH, USA). RNeasy Mini Kit and QIAamp Tissue Kit were from Qiagen (Santa Clarita, CA, USA). RNase-free DNase enzyme was purchased from Invitrogen (San Diego, CA, USA). SeaKem GTG Agarose was purchased from FMC BioProducts (Rockland, ME, USA). All other chemicals and enzymes used for RT competitive-PCR were purchased from Perkin Elmer (Norwalk, CT, USA).

### 2.2. Organ culture technique

Thirty 10-day-old male and female Sprague-Dawley rats (25–30 g), originating from six different litters, were used in this study. Primary culture of intestinal explants was performed using a modification of a previously described method [32,37]. Briefly, rats were killed by decapitation, the abdomen was aseptically opened and the small intestine was flushed with sterile phosphate buffer saline (PBS; 0.05 M sodium phosphate, pH 7.4, 0.15 M NaCl) injected into the duodenal lumen. The proximal part of jejunum (first third of the jejunoileum) was aseptically removed and cut with scissors into 1–2-cm long segments. One segment serving as an uncultured control was snap-frozen in liquid nitrogen, and stored at −75°C until processed for RNA extraction. Other segments were randomized into 5 groups, cut open longitudinally, and trimmed into 2–5-mm explants. The explants were cultured in sterile 6-cm Petri dishes (Becton-Dickenson Labware, Lincoln Park, NJ, USA) in 4 ml of serum-free F-12/DMEM medium supplemented with kanamycin (100 μg/ml) at 37°C in a humidified atmosphere of 95% $O_2$ and 5% $CO_2$ (v/v). The first 4 h of culture served to adapt the intestinal explants to the in vitro conditions. After this period, the organ culture was supplemented either with actinomycin D (final concentration 10 μg/ml) dissolved in DMSO (5 μg/ml) or with DMSO only (control), and cultured for an additional 16 h. Randomized explants from each jejunum were split and served for both actinomycin D-treated and control groups. Intestinal explants were harvested at 0, 4, 8 or 16 h of culture, centrifuged at $16\,000\times g$ for 30 s, snap-frozen in liquid nitrogen, and stored at −75°C.

### 2.3. RNA extraction

Total RNA was isolated from organ culture or tissue using the RNeasy Mini Kit as described in the manufacturer's protocol, then all samples were incubated with RNase-free DNase (20 U per reaction) for 10 min at 37°C to avoid DNA contamination. The RNA concentration was quantified by UV spectrophotometry at 260 nm ($A_{260}$) and the purity was determined by the $A_{260}/A_{280}$ ratio (SPECTRAmax PLUS, Molecular Devices, Sunnyvale, CA, USA). The integrity of RNA samples was verified by electrophoresis on 1.2% agarose gel containing formaldehyde (2.2 M) and ethidium bromide in 1× MOPS buffer (40 mM MOPS (pH 7.0), 10 mM sodium acetate and 1 mM EDTA (pH 8.0)).

### 2.4. RT competitive-PCR assay

Due to the low abundance of EGF, TGF-α, and EGF-R mRNAs in the small intestine of suckling rats, an RT competitive-PCR assay was established. DNA fragments containing either rat EGF or EGF-R or TGF-α primer sequences were constructed using the PCR MIMIC Construction Kit (Clontech, Palo Alto, CA, USA) as previously described for rat IGF-I DNA competitor [25]. In order to be able to separate and quantify PCR products from competitive PCR on agarose gel, the sizes of competitor (standard) DNAs were designed 130–150 base pair (bp) longer than the actual sizes of PCR products amplified from rat EGF, TGF-α or EGF-R mRNAs. The competitor DNA was prepared in a two-step PCR reaction according to the standard manufacturer's protocol (Clontech), and purified on a CHROMA SPIN column (Clontech). The concentration of newly synthesized competitor DNA was measured by spectrophotometry, verified by densitometry, and stored in aliquots at −75°C.

### 2.5. Reverse transcription (RT)

Single-stranded complementary DNA (cDNA) was reverse-transcribed from 1 μg of total RNA in a 10-μl reaction mixture containing 25 U of murine leukemia virus reverse transcriptase, 2.5 μM random hexamers, 10 U of RNase inhibitor, 1 mM of each dNTP, 5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris-HCl (pH 8.3). The reaction mixture was incubated for 20 min at room temperature, 15 min at 42°C, and then the reaction was terminated at 99°C for 5 min. The reaction mixture was kept at 4°C until the start of PCR amplification. The amounts of total RNA used in the RT reactions were calculated from the absorbency at 260 nm, and verified by densitometry of the 28S ribosomal RNA band separated on denaturing agarose gels (by Gel Doc 1000 Documentation System with Molecular Analyst/PC software, Bio-Rad, Hercules, CA, USA).

### 2.6. Competitive PCR amplification

The reverse transcriptase product (10 μl) was mixed with a 40-μl PCR reaction mixture containing 0.2 μM of each of the sense and antisense primer, 1 U of Taq DNA polymerase, 1 mM $MgCl_2$, 50 mM KCl and 10 mM Tris-HCl (pH 8.3). One μl of a defined concentration of competitor DNA was added to each tube. The mixture was subjected to 30–35 cycles of PCR amplification on a Perkin Elmer DNA Thermal Cycler 480. The PCR cycle conditions included: melting for 1 min at 94°C, annealing for 1 min at 64°C and primer extension for 2 min at 72°C. The sequences of primers used are as follows: rat EGF sense primer (5′-GTGGCGTGTGCATGTATGTT-3′ corresponding to nucleotides 3357–3376), and antisense primer (5′-CTCACGTTGC-TGCTTGACTC-3′ corresponding to nucleotides 3614–3633) [38]; rat TGF-α sense primer (5′-ATGGTCCCCGCGGCCGGACAG-3′ corresponding to nucleotides 145–165), and antisense primer (5′-GGCC-TGCTTCTTCTGGCTGGCA-3′ corresponding to nucleotides 418–438) [39]; rat EGF-R sense primer (5′-CGGCCTGAACATAA-CATCGC-3′ corresponding to nucleotides 1476–1485), and antisense primer (5′-GGTGGCAGACGTTATTGGCA-3′ corresponding to nucleotides 1989–2008) [40].

Twenty μl of the amplification products were separated on 2% agarose gel in 40 mM Tris-acetate, 1 mM EDTA (pH 8.0) buffer, and stained with ethidium bromide. The band densities were evaluated by using Gel Doc 1000 system with Molecular Analyst/PC software (Bio-Rad). In order to assess relative amounts of natural PCR product, the density ratios between the competitor and the target bands were calculated. All PCR products were sequenced on both strands using the same primers used for the PCR amplification and an automated sequencing system with fluorescent dye terminators (DNA Sequencing Service, University of Arizona, Tucson, AZ, USA). Control reactions without reverse transcriptase or without total RNA yielded no PCR product bands.

### 2.7. Statistical analysis

Statistical differences between groups were determined utilizing a two-way analysis of variance (ANOVA) and were considered significant at $P < 0.05$.

## 3. Results

### 3.1. Intestinal EGF, TGF-α and EGF-R mRNA levels in suckling rats

To examine intestinal EGF, TGF-α and EGF-R mRNA levels, a sensitive RT competitive-PCR assay was developed and used. Fig. 1 shows a typical picture resulting from RT competitive-PCR used for the quantification of TGF-α mRNA in the jejunum from 10-day-old suckling rats. Total RNA extracted from the small intestine was reverse-transcribed into cDNA. A constant amount of cDNA was then co-amplified with a five-fold serial dilution of the competitor DNA by using a set of specific primers. The amount of total RNA from each intestinal sample served for individual evaluation of all EGF, TGF-α and EGF-R mRNA levels; samples were not pooled. The differences in nucleotide length between the target band and competitor band were corrected by multiplication of smaller band by a correction factor ($C$), defined as a $C$ = larger band bp size/smaller band bp size. Correction factors were as follows: EGF = 1.54 (i.e. 424/

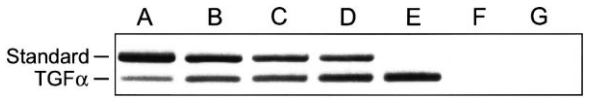


Fig. 1. TGF-α mRNA intestinal level quantified by RT competitive-PCR. Total RNA extracted from jejunum of 10-day-old suckling rats was reverse-transcribed into cDNA, and then co-amplified with serial dilution of the TGF-α competitor DNA. The natural 294- and standard (competitor) 427-bp-long PCR products after amplification for 30 cycles were subjected to electrophoresis on 2% agarose gel stained with ethidium bromide. Lanes A–D contain a constant amount of intestinal cDNA co-amplified with decreasing amounts of standard DNA, 5.0, 1.0, 0.5, and 0.1 amol, respectively. Lanes E–G represent control reactions; lane E: simple RT-PCR without addition of competitor DNA to detect possible cross-contamination of sample with DNA competitor; lane F: no total RNA used in simple RT-PCR to detect possible contamination from RT or PCR reaction mixtures; lane G: no RT enzyme added in the reverse transcription reaction to detect possible TGF-α DNA contamination in the RNA sample.

276), TGF-α = 1.45 (i.e. 427/294), and EGF-R = 1.25 (i.e. 532/424). The logarithm of the density ratio of the target band to the competitor band was then plotted against the logarithm of the starting concentration of competitor DNA (Fig. 2). At the competition equivalence point (log density ratio = 0), the original concentration of target (EGF or TGF-α or EGF-R) cDNA in the PCR reaction corresponds to the initial concentration of competitor (EGF or TGF-α or EGF-R) DNA. The values obtained for the equivalence point were multiplied by a factor of 2, because the competitor DNA was double-stranded and the target cDNA was single-stranded at the beginning of the PCR reaction. To verify the identity of transcripts, PCR products from RT-PCR (without any competitor) were sequenced on both strands. The sequences of the PCR products were identical to those of rat EGF [38] or rat TGF-α [39] or rat EGF-R [40].

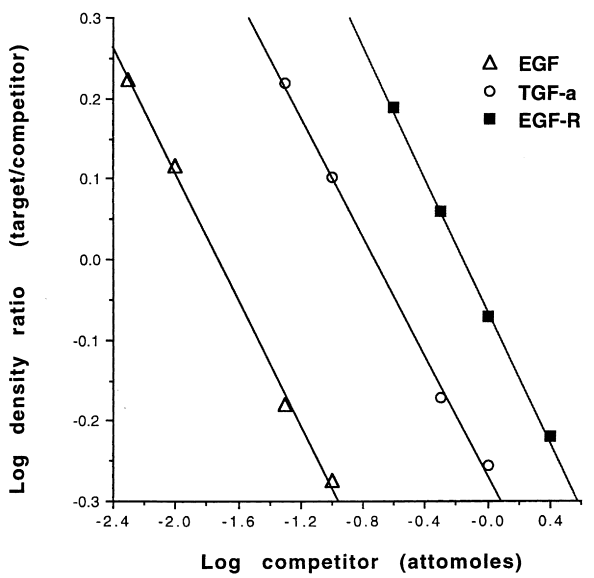


Fig. 2. Quantification of EGF, TGF-α and EGF-R mRNAs in total RNA extracted from jejunum of 10-day-old rats by using RT competitive-PCR. Competition between natural (target) PCR product and competitor PCR product (used in four different concentrations) was determined by computer imaging of the optical intensity of DNA bands on agarose gel. The logarithms of the density ratio of natural (target) band to competitor band (after correction for the difference in size) were plotted as a function of the logarithm of the amount of competitor added to the reaction. The total amounts of EGF, TGF-α and EGF-R mRNAs in the sample were calculated from the competitor values at the equivalence points (log density ratio = 0).

The steady-state levels of EGF, TGF-α and EGF-R mRNAs in the jejunum from 10-day-old suckling rats measured by RT competitive-PCR are shown in Fig. 3. The intestinal TGF-α mRNA level was about 10-fold higher than the EGF mRNA level. The expression of EGF-R mRNA in suckling rat jejunum was the highest, about 6-fold more than TGF-α mRNA, and about 60-fold higher than the EGF mRNA level.

### 3.2. *In vitro transcript levels and mRNA stability in intestinal organ culture*

In order to determine the degradation rate of the mRNA transcripts, proximal jejunum from 10-day-old suckling rats was transferred into the in vitro system. Jejunal explants were first pre-cultured for 4 h to equilibrate the organ culture, then explants were divided into two groups and cultured for up to an additional 16 h in culture medium supplemented either with or without actinomycin D – an inhibitor of mRNA synthesis. The explants were harvested at time 0, 4, 8 and 16 h of culture, and EGF, TGF-α and EGF-R transcript levels were measured by RT competitive-PCR assay. In order to compare EGF, TGF-α and EGF-R mRNA levels, the amount of mRNA in samples harvested at time 0 was expressed as 100%. Messenger RNA levels from control culture without addition of actinomycin D are shown in Fig. 4. The results showed significant differences between EGF and TGF-α mRNA levels. During the first 4 h of culture, TGF-α mRNA levels were markedly reduced to 45% of the starting amount. However, after 4 h the TGF-α mRNA production was dramatically increased, reaching 140% at time 8 h and 400% at time 16 h of culture in comparison to the starting value. In contrast to this, EGF mRNA levels decreased for the first 8 h of culture (60% and 55% of the starting value

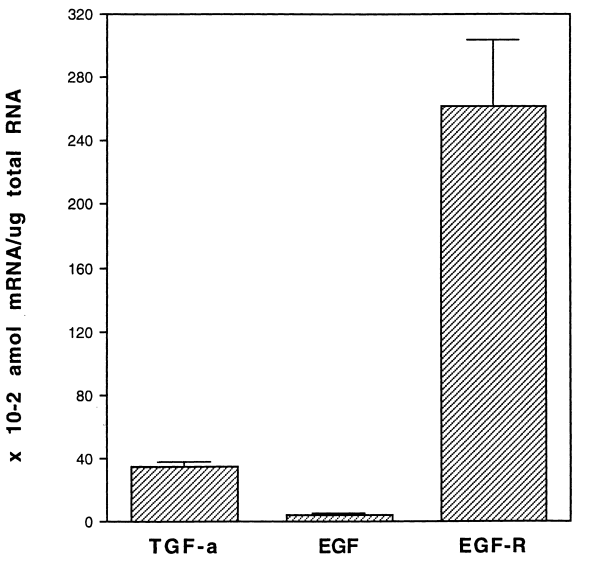


Fig. 3. Expression of TGF-α, EGF and EGF-R mRNAs in the small intestine of suckling rats. The graph shows steady-state levels of intestinal mRNAs in 10-day-old rats quantified by using RT competitive-PCR assay. Data represent means ± S.E.M., $n = 15$.

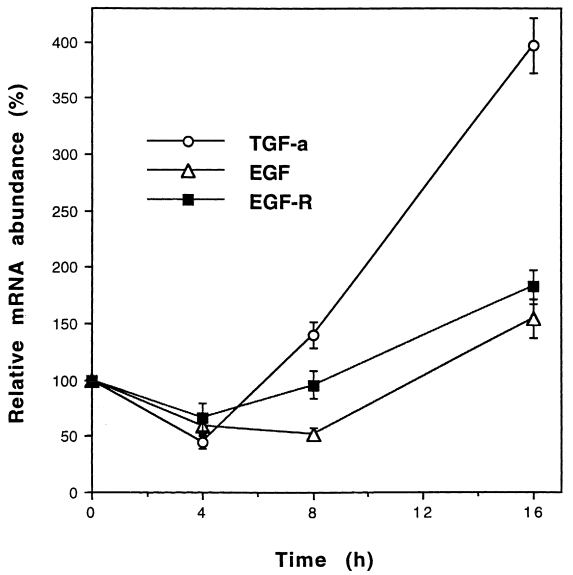


Fig. 4. Expression of TGF-α, EGF and EGF-R mRNAs in the in vitro intestinal culture. Intestinal explants from 10-day-old suckling rats were incubated in culture medium for up to 16 h and total RNA was extracted at the times shown. Transcript levels were measured by RT competitive-PCR assay. Combined data from four experiments are shown. Individual points are the means ± S.E.M. from 8–10 samples.

after 4 h and 8 h, respectively). Finally, at 16 h of culture, EGF mRNA levels were increased to approximately 150% of the starting level. The abundance of EGF-receptor transcripts in organ culture exhibited a pattern similar to EGF mRNA levels. After 4 h of culture, transcript level was about 67% of the starting value, then the production of EGF-R mRNA was steadily increased, reaching levels of about 95% at 8 h and 180% at 16 h of in vitro culture.

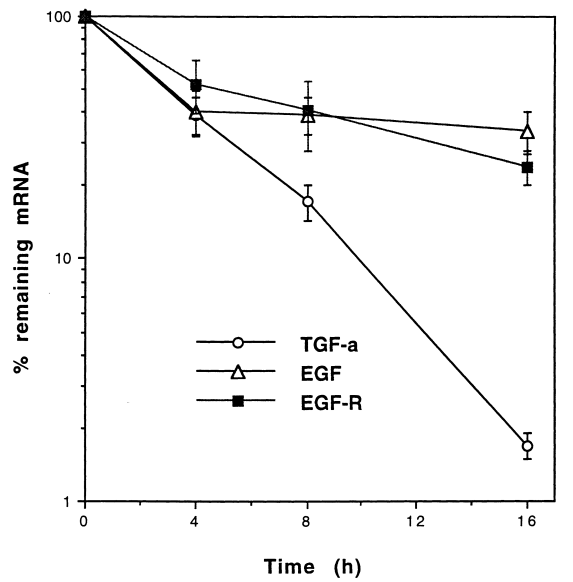


Fig. 5. The stability of TGF-α, EGF and EGF-R transcripts in the in vitro intestinal culture. Intestinal explants from 10-day-old suckling rats were incubated in culture medium supplemented with actinomycin D (10 μg/ml) for up to 16 h and total RNA was extracted at the times shown. Transcript levels were measured by RT competitive-PCR assay. Combined data from four experiments are shown. Individual points are the means ± S.E.M. from 8–10 samples.

The stability of EGF, TGF-α and EGF-R mRNAs was evaluated by using intestinal organ cultures supplemented with actinomycin D. Addition of actinomycin D to the culture led to a significant reduction of the abundance of all three measured transcripts, but the patterns of transcript stability were markedly different (Fig. 5). After 4 h of culture, all three measured mRNAs were reduced by about 40–50% with no statistically significant differences between them. However, additional culture of intestinal explants revealed significant differences between EGF and TGF-α mRNA levels. The degradation of TGF-α mRNA was markedly faster in comparison with EGF mRNA. Transcript levels of TGF-α were reduced to 15% of starting value at 8 h of culture, and an additional 8 h of culture (total 16 h) brought TGF-α transcript levels to 2% of the starting level. In contrast, EGF mRNA degradation was much slower. Transcript levels were reduced after the first 4 h of culture to 40% of starting values, then remained almost unchanged during the culture (39% and 34% of starting value after 8 and 16 h of culture, respectively). The stability pattern of EGF-R mRNA revealed a gradual decrease of EGF-R transcripts during in vitro culture. Transcript levels were 53%, 40% and 25% of the initial amount at 4 h, 8 h and 16 h of culture, respectively

## 4. Discussion

The presence of EGF and TGF-α peptides in the gastrointestinal tract of suckling rats is well established. However, the origin, physiological role and interactions of these growth factors are not completely understood. Previously, we have shown that total content of TGF-α in the small intestine of suckling rats is similar to the total content of EGF, however, the distribution is different. About 90% of total TGF-α peptide is detected in intestinal mucosa and only 10% in intestinal luminal contents [18]. In contrast to this, luminal EGF level in suckling rats represents about 40–70% of the total intestinal EGF content [16,17]. The major source of intestinal EGF content is milk-borne EGF, whereas TGF-α is not detectable in rat milk and intestinal TGF-α content is probably the result of endogenous synthesis by the small intestine and pancreatic excretion. Indeed, fasting (up to 18 h) of suckling rats reduced intestinal TGF-α content by only 25%, whereas total EGF content is decreased by almost 90% [16–18]. In spite of this significant decrease, small amounts of EGF peptide are still detectable in the intestinal mucosa of fasted suckling rats. These observations prompted the question of whether developing small intestine has the potential to synthesize not only TGF-α but also EGF peptide. The major goal of this study was to quantify and compare the steady-state levels of EGF, TGF-α and EGF-R mRNAs in the small intestine of suckling rats. Moreover, results from mRNA stability studies in intestinal organ culture are presented.

Transcript levels of EGF and TGF-α in developing small intestine have not been well characterized. Originally, the presence of TGF-α mRNA transcripts was found in the gastrointestinal tract from adult rodents only, and the expression of EGF mRNA was not detected in either adult [41–44] or suckling gastrointestinal tract [45] at all. In all these reports, EGF and TGF-α gene expression was studied using Northern blot analysis. Miettinen [46] detected the presence of TGF-α

and EGF transcripts in human fetal intestine by using a more sensitive ribonuclease protection assay and reverse transcription-polymerase chain reaction (RT-PCR) technique. Her observation suggested a higher abundance of TGF-α transcripts in human fetal intestine in comparison with EGF mRNA. However, no accurate quantification was performed. Earlier studies from our laboratory have shown the presence and cellular localization of both EGF and TGF-α mRNAs in the small intestine of suckling and adult rats [24]. Using an in situ hybridization technique, we have shown that the intestinal crypt epithelium is the major site of EGF and TGF-α transcripts in both suckling and adult rats. Despite the same histological localization of EGF and TGF-α mRNAs, there are significant differences in the intensity of the detected signals. Strong signals for the presence of both EGF and TGF-α mRNAs were observed in the small intestine from adult rats. In suckling rats, however, the EGF mRNA signal was very low or absent, whereas TGF-α mRNA signal was detected in all samples with markedly higher intensity.

Based on these semiquantitative observations, we decided to clarify the steady-state levels of EGF and TGF-α transcripts in the small intestine of suckling rats. As mentioned above, the abundance of EGF mRNA in developing small intestine is extremely low, therefore it was necessary to establish a very sensitive method to detect and quantify EGF mRNA in this tissue. PCR based detection of the message seems to be a very useful approach; however, simple RT-PCR is not accurate enough for quantification of mRNA levels. The introduction of RT competitive-PCR assay enabled us to measure gene expression of low abundant messages in all samples with high accuracy and good reproducibility. Results from these measurements showed that in suckling rats, intestinal TGF-α mRNA level is about 10-fold higher in comparison with EGF mRNA level. Despite low transcript level, EGF mRNA is detectable in all intestinal samples, suggesting the potential of developing small intestine to produce EGF peptide. High expression of TGF-α mRNA, interestingly, corresponds with previously measured high levels of TGF-α peptide level in this tissue, thus supporting the hypothesis that the developing jejunum is an important source of TGF-α [18]. The presence of TGF-α transcripts in intestinal crypt epithelium followed by translation into peptide in these cells suggests that TGF-α is involved in control of intestinal proliferation and migration during development, rather than in maturation of intestinal epithelium. The amount of EGF mRNA in the jejunum of suckling rats is about 10 times lower in comparison with TGF-α mRNA, suggesting that intestinal EGF gene expression under normal conditions may be downregulated in the presence of milk-borne EGF.

The presence of a specific EGF-receptor is a prerequisite for any action of either EGF or TGF-α peptides. In the developing small intestine, the presence of EGF receptor molecule has been reported, but its gene expression was not studied in rats. In the suckling mouse, an EGF-receptor was detected in the small intestine throughout the entire postnatal period [21]. In developing rats, immunoreactive EGF-receptors have been localized to the basolateral membrane of enterocytes from both crypt and villus in fetal, suckling and adult jejunum. The abundance of a 170-kDa EGF-receptor band, measured by Western blot, was highest in fetal jejunum and decreased after the suckling period [23]. In our current study we also determined the EGF-R mRNA level in jejunum of suckling rats. Intestinal EGF-R mRNA levels were significantly higher than transcript levels of both growth factors.

Significant differences in EGF and TGF-α mRNA gene expression prompted further study of the regulation of mRNA level of these two structurally related growth factors in developing small intestine. The abundance of mRNA is controlled by both transcription and mRNA stability. In our study, we used the combination of primary intestinal culture technique with the RT competitive-PCR assay to evaluate EGF and TGF-α mRNA stability. Results from these measurements revealed considerable differences between these mRNAs. Stability of the TGF-α transcript is remarkably lower than the stability of EGF mRNA. High degradation rate of TGF-α mRNA correlates with significant increase of TGF-α mRNA production during in vitro culture of intestinal explants, suggesting rapid turnover of intestinal TGF-α mRNA. Interestingly, a significantly lower EGF mRNA steady-state level in the small intestine of suckling rats is accompanied by markedly higher mRNA stability and lower in vitro transcription.

In conclusion, the present data demonstrate significant differences in gene expression of EGF and TGF-α mRNAs in the developing small intestine of suckling rats. Moreover, high abundance of EGF-R mRNA is described. EGF and TGF-α are structurally related mitogenic factors that coordinate growth and renewal of the gastrointestinal tract. The mechanism of action of these two growth factors in developing small intestine is not clear but considerable differences in the origin and distribution of EGF and TGF-α are apparent. Markedly high intestinal TGF-α mRNA levels accompanied by the presence of mature TGF-α peptide in intestinal mucosa, support the hypothesis that TGF-α may be the primary ligand of EGF-receptor in the developing intestine. Milk-borne EGF likely plays an important role in the apparent protective effect of breast milk. After the weaning period, endogenous EGF production is encreased in submandibulary glands, duodenum and jejunum. These changes may contribute both directly and via adrenal and thyroid glands to maturation of gastrointestinal functions.

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