Cloning of the Rat Ecotropic Retroviral Receptor and Studies of Its Expression in Intestinal Tissues (43875)

MÔNICA PUPPI* AND SUSAN J. HENNING*,†,[1]

Departments of Cell Biology and Pediatrics,† Baylor College of Medicine, Houston, Texas 77030-3498*

Abstract. **A long-term goal of our laboratory is to establish a rat model to study the feasibility of using the intestinal tract as a site for somatic gene therapy. As a step toward that goal, the current study reports the cloning of the rat ecotropic retroviral receptor (EcoR) cDNA and the study of various aspects of its expression in the intestinal tissues. The cDNA for rat EcoR was cloned by screening a size-selected rat intestinal cDNA library with mouse EcoR cDNA. A clone of approximately 7 kb, designated MP10, was obtained. Partial sequencing of MP10 from the 5′ end revealed a level of similarity of 92% compared with mouse EcoR. The presence of a 5′ untranslated region and a 3′ poly(A) tract, together with the overall size of the cDNA, suggest that is very close to being a full-length cDNA for this large transcript. Northern blots with MP10 showed an RNA of approximately 7.9 kb present along the entire length of the small intestine and somewhat less abundant in the colon. Developmental studies showed high levels of EcoR in fetal rat intestine, a decline in the early postnatal period, then a gradual rise to adulthood. Caco-2 cells were used to assess the expression of EcoR in proliferating compared with differentiated intestinal epithelial cells. EcoR mRNA was found to be very much more abundant in nondifferentiated cells and declined to low levels as the cells underwent spontaneous differentiation. These patterns of EcoR expression indicate that ecotropic retroviruses should be suitable vectors with which to attempt gene transfer into the intestinal epithelium. In addition, since the endogenous role of EcoR is as the y^+ cationic amino acid transporter, these data have significance for understanding patterns of amino acid transport in the intestinal epithelium.** [P.S.E.B.M. 1995, Vol 209]

The advantages of somatic gene therapy for both genetic and acquired diseases are widely recognized (1–4). Although numerous vector systems are currently under investigation, recombinant derivatives of murine retroviruses remain the most widely used vectors in both human and animal studies (5). For any tissue there are two prerequisites for successful gene transfer by retroviral vectors: (i) the appropriate retroviral receptor must be expressed to allow cellular entry; and (ii) target cells must be actively dividing to allow synthesis and integration of proviral DNA. With respect to the former, early studies documented the presence of retroviral receptors using binding assays with purified envelope glycoprotein (6). More recently, the cloning of receptor cDNA has allowed receptor expression to be studied at the molecular level. The first such cDNA was cloned from mouse fibroblast cells by Albritton *et al.* (7). This cDNA, denoted EcoR, encodes a protein that determines the host range of the ecotropic subclass of murine leukemogenic retroviruses (MuLV). The predicted protein, EcoR, of 622 amino acid residues permits infection by functioning as a receptor that binds specifically to ecotropic MuLV envelope glycoprotein (8, 9).

We have proposed (10, 11) that the epithelia of the small and large intestine may be excellent target tissues for somatic gene therapy. Attractive features of

[1] To whom requests for reprints should be addressed at Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030-3498.

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the intestinal epithelium are its ease of access via the lumen; its large mass; its metabolic similarity to the liver (thus allowing it to serve as an alternative site for correction of various hepatic deficiencies); and its ability to secrete foreign proteins into the blood stream (10, 11). Binding studies with purified envelope glycoprotein have established the presence of ecotropic retroviral receptors in the small intestine of adult mice (6). However, to date there have been no studies with rat intestine. Moreover, many proteins exhibit dramatic gradients of expression along the length of the intestinal tract (commonly known as longitudinal gradients). These may take various forms (e.g., peaks seen in either duodenum, jejunum, or ileum). Likewise, many proteins show marked ontogenic changes, particularly during the suckling/weaning transition. In rodents, some proteins (e.g., lysosomal enzymes, Fc receptors) are abundantly expressed during the first two postnatal weeks (the suckling period), then decline markedly (12). On the other hand, proteins such as sucrase-isomaltase are undetectable during the suckling period, appear during the third postnatal week, and rise rapidly to adult levels (12). If the viral receptor displays either longitudinal or ontogenic gradients of expression, it would clearly be to our advantage to be aware of these gradients before proceeding with the use of retroviral vectors to transfer genes into the intestinal epithelium.

Another issue related to the use of retroviruses as vectors for somatic gene therapy is that synthesis and integration of the proviral DNA into the host chromosome can occur only in actively dividing cells (13–15). This makes the intestinal epithelium, which is continuously renewed even in the adult, a logical target for retroviral vectors. However, since proliferating cells are confined to intestinal crypts (16), a critical question is whether EcoR is expressed in these particular cells as compared with the more differentiated villus cells. Previous studies have suggested that gene expression along the crypt-villus axis falls into three categories, with some genes being expressed constitutively, some being highly expressed in the crypt and repressed in the villus, and, conversely, many (e.g., characteristic of the differentiated phenotype) being repressed in the proliferating cells of the crypt and activated as these cells leave the proliferative sac and migrate onto the villus (reviewed in Ref. 17). The goals of the current study were 2-fold: (i) to clone the cDNA for the rat homolog of mouse EcoR; and (ii) to study its expression along the longitudinal axis of the small and large intestine, during ontogeny and in proliferating versus differentiated intestinal epithelial cells. Apart from the relevance to the use of ecotropic retroviruses as vectors for gene transfer in the intestinal tract, an added impetus for these studies was the fact that the endogenous role of EcoR is as the y^+ cationic amino acid transporter (8, 9). Although y^+ transport activity has been demonstrated in the rat intestine (18, 19), its longitudinal, ontogenic, and cellular patterns have not been previously documented.

Materials and Methods

Cloning of the EcoR cDNA. A size-fractionated (>4.5 kb) adult rat intestinal cDNA library was kindly provided by Dr. Lawrence Chan (Baylor College of Medicine). The library was constructed by oligo dT priming and was unidirectionally cloned at the EcoRI/XhoI sites of the poly-linker region of the vector λZAP. The library was plated at about 20,000 plaques/plate, and lifts were probed with the 2.3-kb insert of the plasmid pJet. The latter (kindly provided by Dr. Lorraine Albritton) represents the open reading frame of the mouse EcoR clone (7). After secondary and tertiary screening, the positive λ clones were excised into Bluescript phagemids (as recommended by Stratagene). The phagemids were partially sequenced using the Applied Biosystems, Inc., (ABI) Model 373A DNA sequencer (Molecular Genetics Core Facility, Department of Microbiology, University of Texas Health Sciences Center at Houston, Houston, TX). Assembly of the DNA sequence and comparison analysis were performed using the Intelligenetics Suite software, release 5.4 (Intelligenetics, Inc., Mountain View, CA).

Animals. Timed-pregnant rats of the Sprague-Dawley strain (Charles River Crl:CD[SD]BR) were received from Charles River Laboratories, Inc. (Portage, MI) on the 14th day of gestation. They were maintained at 21° ± 1°C in separate opaque polystyrene cages under a 12:12-hr light:dark cycle. Food (Rodent Laboratory Chow 5001; Ralston Purina, St. Louis, MO) and deionized water were supplied *ad libitum*. The day of birth was considered Day 0. Each litter was culled to nine pups 1 day after birth. Pups remained with their dams until Day 21, when weaning was completed by removal of the dam. Adult male rats of the same strain from the same supplier were used to provide testis which was used as the control tissue.

Tissue Collection. To study expression of EcoR along the length of the intestinal tract, rats were sacrificed and the entire intestine was removed and divided into segments corresponding to the duodenum, proximal and distal jejunum, proximal and distal ileum, and colon. Liver and testis also were collected as negative and positive control samples. Intestinal segments were flushed with cold saline (0.9% NaCl) and immediately frozen in liquid nitrogen. The liver was rinsed in cold saline then frozen. Testis was detunicated, rinsed in cold saline, then frozen.

To study expression of EcoR during development, fetal intestines were collected at 18, 19, 20, and 21 days of gestation following cesarean section under an-

esthesia. The fetal small and large intestine was removed with forceps and frozen in liquid nitrogen. For each dam, intestines from all her fetuses were pooled to represent a single sample. At postnatal Day 0 (before first suckling), 2 and 4, duodenum and jejunum were pooled from two pups. At postnatal Day 6, 11, 15, 17, 18, 20, 24, and 36, jejunum was collected from individual pups. All postnatal intestinal segments were flushed and frozen as described above.

Cell Culture. Caco-2 cells purchased from the American Type Culture Collection (Rockville, MD) were grown in Dulbecco's minimum essential medium (DMEM) supplemented with nonessential amino acids (0.1 m*M*), Hepes (10 m*M*), L-glutamine (2 m*M*), penicillin (50 U/ml), streptomycin (50 μg/ml), and fetal bovine serum (10%). Harvesting of cells was performed at t_0 (confluence), t_7, and t_{14} (7 and 14 days postconfluence, respectively). Medium was removed, cells were rinsed with phosphate buffered saline at 37°C, and 4 *M* guanidine isothiocyanate solution was added. The viscous mix was scraped and transferred to a tube using a 1-ml syringe with a 21-gauge needle to draw in and expel the mixture four to five times. The sample was then frozen at −70°C.

Determination of Sucrase Activity. Caco-2 cells were collected at t_0, t_7, and t_{14} for determination of sucrase activity. Medium was removed from the plates and the cells were rinsed with phosphate-buffered saline at 4°C. A fresh aliquot of this buffer was added and cells were scraped, transferred to a conical tube and centrifuged. The cell pellet was resuspended in 1:3 weight:volume of 0.15 *M* KCl and homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, NY) for 30 seconds on stop 7. Sucrase activity was determined as previously described (20). Glucose was measured with a glucose oxidase kit (Glucose Trinder 100; Sigma Chemical Co., St. Louis, MO). Protein was measured in duplicate according to the method of Lowry *et al.* (21). Sucrase activity was expressed as micromoles of glucose produced per hour per milligram protein.

Northern Blot Analysis. Total cellular RNA was prepared from rat tissues or Caco-2 cells as described by Leeper and Henning (20). Northern blots were generated as described previously (20). The probes used were: mouse EcoR (pJet; 7); mouse elongation factor-1α (EF-1α; Sunitha and Slobin, unpublished); rat EcoR (clone MP10, described in this paper); and human sucrase-isomaltase (SI) (22). These probes were radioactively labeled by random-primed oligo labeling (23) of either excised inserts (pJet and MP10) or linearized plasmids (mouse EF-1α and human SI). Blots were cut in half for hybridization with EcoR and EF-1α, and two identical Northerns were prepared for hybridization with human SI and EcoR (Caco-2 samples). Hybridization conditions were as described previously (20). The blots were washed in a standard manner (20), with the final wash temperature being 55°C for heterologous probes (i.e., mouse/rat and mouse/human) and 65°C for homologous probes (rat/rat and human/human). Blots were exposed to Kodak XAR-5 film (Eastman Kodak, Rochester, NY) for autoradiography at −70° using two intensifying screens.

Results

Cloning of the Rat EcoR cDNA. Screening of a total of approximately 200,000 plaques of the rat intestinal cDNA library with the mouse pJet insert (7) yielded seven positive clones after plaque purification. Restriction analyses indicated that these represented only two independent clones. One of the clones (MP10) with the largest insert was chosen for further studies. This clone has an insert of approximately 7.0 kb which can be excised intact by EcoRI/XhoI digestion. Because the mouse EcoR cDNA has an extremely long 3′ untranslated region (7), our sequencing efforts were directed primarily to the 5′ end of MP10. Sequencing of 747 nt at this end of the cDNA revealed an 87 nt untranslated region followed by an ATG translational start site and a total of 660 nt of open reading frame (ORF). The latter showed great similarity to the mouse homolog EcoR. At the nucleotide level, the mouse and rat sequences within the ORF were 92% identical, and at the amino acid level the identity reached 96%. While this work was in progress, two other groups reported cloning rat EcoR cDNA (24, 25). Comparison of those sequences with that generated for MP10 showed 98% identity at the nucleotide level, indicating that it is probably the same cDNA. Limited sequencing from the 3′ end of MP10 revealed a poly(A) tract (16 nt) and a putative polyadenylation signal AATAAA 17 nt upstream, suggesting that our cDNA includes the 3′ end of the EcoR transcript. As this 3′ untranslated region is not represented in either of the other two rat EcoR DNA's (24, 25) and was not sequenced in the mouse EcoR cDNA (7), no sequence comparisons are possible in this region.

Longitudinal and Ontogenic Expression of EcoR mRNA in Rat Intestine. The expression of EcoR mRNA along the adult rat intestine is shown in Figure 1. The Northern blot included rat testis RNA (last lane) as a positive control, because previous studies in the mouse reported high levels of EcoR RNA in this tissue (8). Hybridization with MP10 revealed a strong signal which represents an mRNA of approximately 7.9 kb (Fig. 1A). As reported previously (8, 25), EcoR expression was not detectable in the liver. Within the intestinal tract, the expression level was approximately constant along the small intestine (duodenum, jejunum, and ileum), but decreased in the colon. To assess the integrity of the RNA, the North-

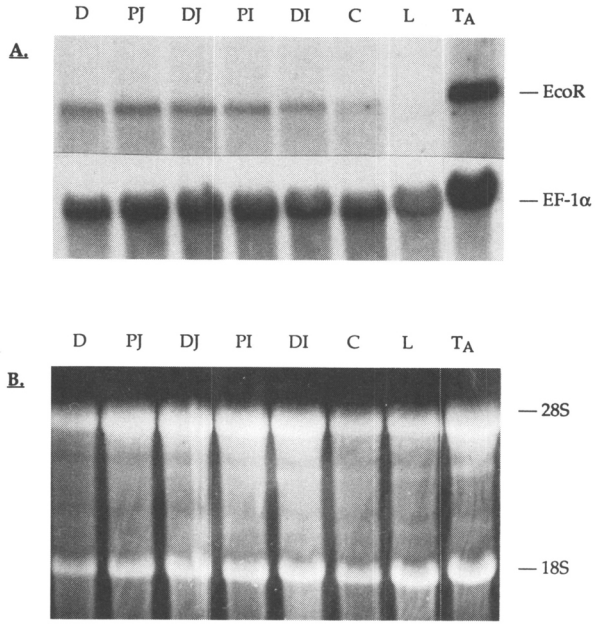

Figure 1. Longitudinal distribution of the EcoR mRNA in adult rat intestine. (A) Northern blot of 20 μg of total cellular RNA prepared from the following regions of the intestinal tract: duodenum (D), proximal and distal jejunum (PJ and DJ), proximal and distal ileum (PI and DI), and colon (C). Liver (L) and testis (T_A) RNA were included as negative and positive control samples, respectively. Data shown are from a single animal, but the same general pattern was observed with RNA from two other animals. The probes used were clone MP10 (EcoR) and EF-1α. The autoradiographs shown are the result of 20 days' exposure for EcoR and 18 hr for EF-1α. (B) Ethidium bromide staining of the gel to assess RNA loading. The 18S and 28S ribosomal RNA bands appear dense and integral.

ern blot was also hybridized to EF-1α, which has proven to be a useful constitutive marker in the intestine (17). EF-1α seems to be less abundant in rat liver and more abundant in testis. Ethidium bromide staining of the gel (Fig. 1B) showed that there was a slight variation in RNA loading, but it did not affect overall conclusions regarding levels of EcoR mRNA along the length of the intestinal tract.

The profile of EcoR mRNA expression during rat intestinal development is presented in Figure 2A. The EcoR signal was somewhat stronger in the fetus, declined at birth, and increased again after the second week of life. Although the EF-1α signal was also stronger in the fetal samples, the ethidium bromide staining of rRNA (Fig. 2B) indicates that the fetal samples were not overloaded.

The Effect of Differentiation Status on the Expression of EcoR in Caco-2 Cells. In order to address the question of how EcoR is expressed during cellular differentiation, we chose to use Caco-2 cells. These are epithelial cells derived from a human colon carcinoma. They grow as a monolayer and display spontaneous enterocytic differentiation during the 2 weeks following confluence. During this period, cells become polarized, elongate, express small intestinal markers, and acquire microvilli (26). Figure 3 shows the results of analyses of sucrase activity in confluent and post-confluent Caco-2 cells. This enterocytic marker was undetectable at the time of confluence and then gradually increased over the next 14 days to levels comparable to those seen in the mature small intestine (12), thus confirming the differentiation process.

EcoR mRNA expression during differentiation of Caco-2 cells is shown in Figure 4A. Duplicate blots were probed for sucrase-isomaltase mRNA. Just as in the rat tissues, in these human cells the EcoR probe detected a single transcript of approximately 7.9 kb. As can be seen, the expression of EcoR was inversely proportional to that of sucrase-isomaltase. EcoR mRNA was substantially more abundant in nondifferentiated Caco-2 cells (t_0) than in differentiated cells (t_7, t_{14}). On the other hand, sucrase-isomaltase mRNA displayed a pattern very similar to that of sucrase activity (Fig. 3).

Discussion

Studies in other tissues have shown a very strong correlation between levels of EcoR mRNA and infectivity by ecotropic retroviruses. Expression of exogenous EcoR *in vitro* confers infectivity on cells that are normally nonpermissible for ecotropic retroviruses (7). Moreover, expression of endogenous EcoR in liver cells explains the unique patterns of infectability of these cells. Specifically, normal adult rat liver does not express EcoR and is not infectable by ecotropic retroviruses (25, 27). In contrast, cultured rat hepatocytes and murine hepatoma cells express EcoR and are infectable (27). Likewise, *in vivo* studies have shown that both infectivity and EcoR expression are induced following partial hepatectomy, with the time course of infection paralleling that of the EcoR mRNA (25). Thus, expression of EcoR appears to be a sensitive and useful predictor of ecotropic retroviral infectivity. Although EcoR mRNA expression in various tissues of both mouse (8) and rat (25, 27) has been reported, there have been no previous studies of its expression in tissues of the gastrointestinal tract. Clearly, such expression is relevant to the use of retroviral vectors for gene transfer in this organ system.

Our efforts to clone the rat EcoR apparently proceeded in parallel with those of two other laboratories (24, 25). Stoll *et al.* (24) employed a PCR strategy using primers based on mouse EcoR (7) to clone an 852-nucleotide cDNA from rat brain. Wu *et al.* (25) used the open reading frame (ORF) of the mouse EcoR cDNA to screen a rat hepatoma cDNA library and generated three overlapping clones totalling 2.85 kb. The latter was the same strategy we chose, except that we used a size-selected intestinal cDNA library. This

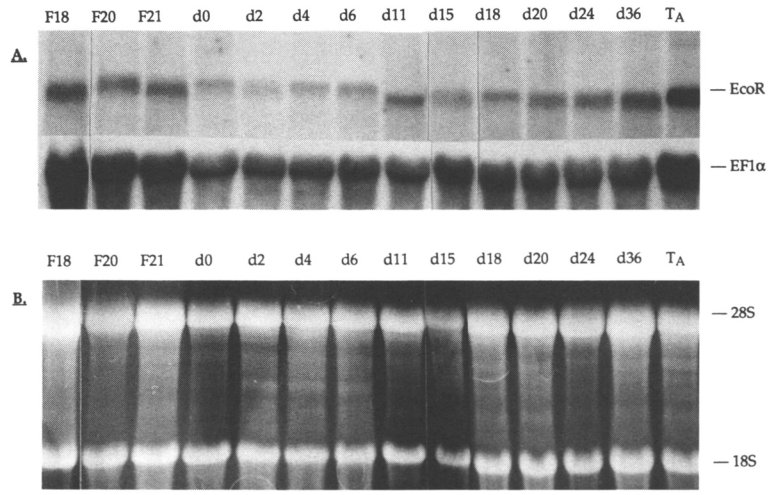

Figure 2. Developmental expression of EcoR mRNA in rat intestine. (A) Northern blot of 20 μg of total cellular RNA prepared from the entire intestine of rat fetuses at 18, 20, and 21 days of gestation; from duodenum and jejunum of rat pups at postnatal Day 0, 2, and 4; from jejunum of rat pups at postnatal Day 6, 11, 15, 18, 20, 24, and 36; and from the adult testis (T_A). The hybridization probes and the exposure times were as described in Figure 1. (B) Ethidium bromide staining of the same gel. Data shown are from two separate Northern blots, one which contained samples F18–d11 and the other, samples d15–T_A. To enable splicing into a single figure, the two blots were hybridized and exposed simultaneously. In addition, the T_A reference sample was included on both blots and showed identical intensity. Two samples (F19 and d17) were eliminated from the final figure, because ethidium bromide staining indicated significant underloading.

allowed us to clone a >7.0-kb cDNA (MP10), which probably represents the full-length transcript. The overall size of our cDNA is very similar to that contained in the three overlapping cDNA clones originally generated for mouse EcoR cDNA by Albritton *et al.* (7). Of all EcoR cDNA's generated to date (7, 24, 25), ours is by far the biggest single cDNA. This cDNA should be valuable in future studies designed to investigate the role of the very long 3′ untranslated region of the EcoR transcript. Based on a mouse open reading frame (ORF) of 1866 nt (7) and a rat ORF of 1872 nt (25), the 3′ untranslated region is predicted to be approximately 5 kb.

When MP10 was used to probe Northern blots of RNA from rat intestine, a single mRNA species of approximately 7.9 kb was observed. This agrees with findings in adult rat kidney and spleen, as well as in fetal rat liver (25, 27). In contrast, mouse tissues appear to express two large transcripts of 7.0 and 7.9 kb (8, 27). The significance of a smaller transcript of 3.4–4.0 kb in various cultured rat cells (25, 27) is not known. Such a transcript was not detected in our studies or in other studies of rat tissues (24, 25) with the exception of brain, where it has been detected, although at greatly reduced levels compared with the 7.9-kb transcript (25). Given that the 2.85-kb rat EcoR cDNA cloned from hepatoma cells included a poly(A) tract and a polyadenylation signal (25), it would appear that there may be an alternative splicing phenomenon in certain cell lines and tissues.

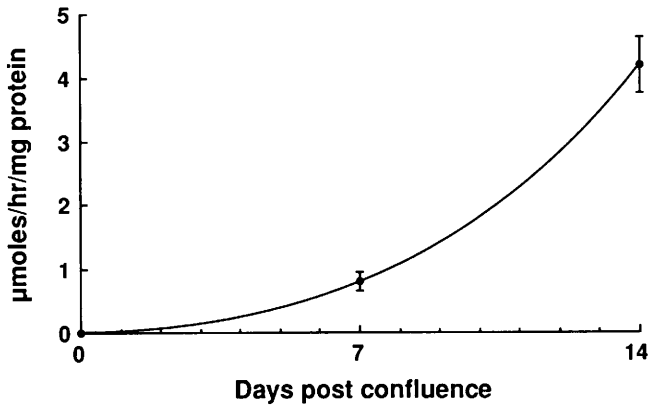

Figure 3. Sucrase activity in Caco-2 cells. Enzyme activity was determined in cellular homogenates as a function of time after cells reached confluence. Data are shown as means ± SEM (n = 5).

For mouse EcoR, expression studies in Xenopus oocytes (8, 9) have made a convincing case that, in addition to being a retroviral receptor, the protein encoded by this cDNA functions as the y^+ cationic amino acid transporter. This is a Na-independent transporter that is stereospecific for lysine, arginine, and ornithine (28). The y^+ system is the principal cationic amino acid transporter for most mammalian cells, with the exception of hepatocytes (28). In the intestine, a high-affinity, Na-independent transport system has long been recognized as the major transporter of lysine (18, 19, 29). Recently, Harvey *et al.* (30) have demonstrated that microinjection of Xenopus oocytes with total poly A^+ RNA from rat jejunum results in the expression of three types of lysine transport, one being Na dependent and two, Na independent. Although one of the latter appeared to have the general characteristics of y^+, the data did not allow definitive conclusions. Our cloning of EcoR cDNA

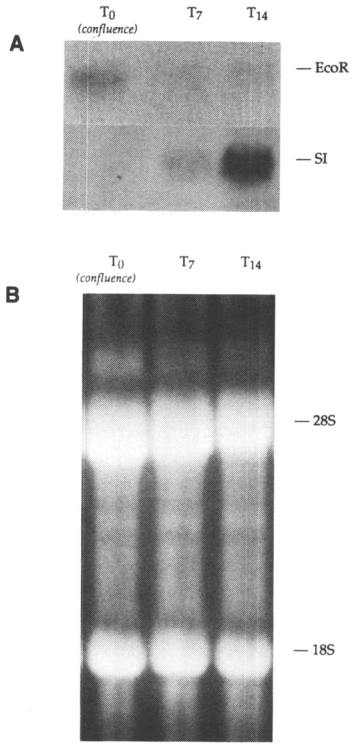

Figure 4. Expression of EcoR mRNA in Caco-2 cells. (A) Northern blot of 20 μg of total cellular RNA prepared from cells at confluence (T_0), 7 days postconfluence (Day 7), and 14 days postconfluence (Day 14). Two separate identical Northern blots were hybridized, one with EcoR and the other with the human sucrase-isomaltase (SI). Exposure times were 29 hr for EcoR and 4 days for SI. (B) Ethidium bromide staining of the gels (gels were identical, only one shown).

from a rat intestinal cDNA library, together with our Northern blots, indicates that the mRNA for the y^+ transporter is present in rat intestine.

Our investigation of the expression of EcoR mRNA along the length of the rat intestine showed it to be readily detectable in all regions of the small intestine and considerably less abundant in the colon. Its presence in the distal ileum is consistent with the recent demonstration of retrovirally mediated gene transfer in this region (31). The current studies would suggest that gene transfer in more proximal regions of the small intestine should be equally feasible. The approximately equivalent expression of EcoR mRNA in all regions of the rat small intestine is also consistent with previous amino acid transport studies which have shown lysine transport capacity to be uniformly distributed (32). Although the latter study did not distinguish y^+ transport activity from other modes, the bulk of evidence from intestine (18, 19, 29) and other tissues (28) suggests that y^+ is likely to be the major transporter of lysine under physiological conditions.

The study of EcoR expression during rat ontogeny indicated substantially higher levels of the mRNA in fetal intestine as compared with postnatal ages. This would suggest that fetal intestine may be even more infectable by ecotropic retroviruses than its adult counterpart. If this proves true, it will enhance the usefulness of the rat as a model system to study intestinal gene therapy for various genetic diseases in which the phenotype becomes manifested at birth.

Our data on EcoR mRNA expression in the developing rat also shed some light on the maturation of cationic amino acid transport. Although there are several published studies of lysine transport in developing rat intestine (33–35), none of these distinguished Na-independent transport from Na-dependent transport. Overall, the lysine transport pattern reported by Toloza and Diamond (35) is similar to that for EcoR mRNA, with levels being high at birth, then declining in the postnatal period and rising again between 21 and 40 days of age. In contrast, other studies (33, 34) have reported that lysine transport continues to decline through weaning to adulthood. The reason for this discrepancy is not immediately apparent. Further functional studies distinguishing y^+ from other cationic amino acid transporters are clearly warranted.

In order to investigate the effect of differentiation status on expression of EcoR mRNA, we used the Caco-2 cell line. Although this cell line is derived from a colon carcinoma, its pattern of differentiation postconfluence mimics that occurring along the crypt-villus axis of the small intestine (26). Sucrase-isomaltase is an excellent marker protein for this differentiation process, because, in the normal adult animal, its expression is confined to the enterocytes of the intestinal villi (17). After first using sucrase activity measurements to verify that Caco-2 cells were behaving as expected in our hands (Fig. 3), we studied the effect of differentiation status on EcoR mRNA expression. Although the mRNA was detectable at all stages it was substantially more abundant in the undifferentiated cells. By analogy, in the small intestine we would predict EcoR expression to be maximal in the undifferentiated, proliferating cells of the intestinal crypts. Such a pattern would be consistent with the findings that liver cells (25, 27) and lymphoid cells (36) express EcoR mRNA only when stimulated to divide.

Our findings with Caco-2 cells also raise interesting questions regarding cationic amino acid transport along the crypt-villus axis. If the y^+ transporter is abundant in crypt but not villus cells, one of the other cationic amino acid transporters must account for lysine uptake by villus epithelial cells. Using an autoradiographic method to study Na^+-independent lysine transport in rabbit ileum, King *et al.* (37) showed that high-affinity uptake is more pronounced in villus tip cells than in cells at the villus base. Unfortunately, their methods did not allow assessment of transport by the crypt epithelial cells. Nevertheless, the data suggest that there may be distinct patterns of expression of different cationic amino acid transporters along the

crypt-villus axis, which greatly complicates interpretation of transport experiments performed with intact tissue.

In summary, we have demonstrated that EcoR, the gene encoding a protein that functions as both the ecotropic retroviral receptor and the y^+ cationic amino acid transporter, is expressed in the rat intestine. EcoR mRNA is present in all regions along the length of the small intestine and at all stages of development, from fetal life through adulthood. Studies with Caco-2 cells indicate that EcoR mRNA is probably more abundant in undifferentiated intestinal epithelial cells than in their differentiated counterparts. Taken together, these findings should encourage further exploration of the intestine as a site for retrovirally mediated gene transfer and of the contribution of various individual transporters to overall cationic amino acid transport in the intestine.

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