

Gene Expression and Activity of Enzymes in the Arginine Biosynthetic Pathway in Porcine Fetal Small Intestine

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ABSTRACT

We recently reported the presence of ornithine aminotransferase (OAT) enzymatic activity and mRNA expression in the intestine of fetal pigs from 30 to 110 d of gestation. Here we describe the activities and mRNA expression patterns of other key enzymes in the arginine biosynthetic pathway, specifically carbamoyl phosphate synthase I (CPS-I), ornithine carbamoyl transferase (OCT), and pyrroline-5-carboxylate reductase (P5CR), in the fetal porcine small intestine from 30 to 110 d of gestation. The activities of all three enzymes increased from d 30 to d 110 of gestation, and *in situ* hybridization demonstrates that 1) CPS-I and OCT genes are expressed in distinct patterns and are confined to the mucosal epithelium and 2) P5CR mRNA is present in mucosal epithelium and lamina propria of the fetal porcine small intestine from d 30 to d 110 of gestation. The presence of CPS-I and OCT in conjunction with the presence of

OAT suggests that the fetal porcine small intestine is capable of synthesizing citrulline from P5C. In addition, the presence of P5CR suggests that the fetal porcine small intestine is able to synthesize proline from ornithine *via* OAT. This ability of the fetal small intestine to synthesize amino acids may be important for development and metabolic activity of the intestine during somatic growth of the fetus. (*Pediatr Res* 53: 274–280, 2003)

Abbreviations

OAT, ornithine aminotransferase
CPS-I, carbamoyl phosphate synthase I
OCT, ornithine carbamoyl transferase
P5CR, pyrroline-5-carboxylate reductase
ASS, argininosuccinate synthase
ASL, argininosuccinate lyase

The small intestine of the fetal pig begins its development early in gestation relative to that of the rodent and undergoes extensive morphologic (1) and functional (2–4) changes over the course of gestation. During this time, mRNA and enzymatic activity of ornithine aminotransferase (OAT; 2.6.1.13), a nuclear-encoded, mitochondrial matrix enzyme that catalyzes the interconversion of pyrroline-5-carboxylate and ornithine, are present in the epithelium of the porcine fetal small intestine from early (d 30) in gestation to near term (d 110) (5). Although the functional significance of intestinal OAT during fetal development is not understood, its reversibility and position in the arginine biosynthetic pathway suggest that it plays a central role in amino acid metabolism during gestation, particularly citrulline and proline synthesis.

The small intestine of most neonatal mammals, including the pig, is responsible for maintaining arginine homeostasis and is the source of endogenous arginine for the neonate (6). Because arginine synthesis by the neonatal small intestine depends on glutamine (6), glutamate (6, 7), and proline (8) as substrates, OAT is essential for this process (9). In addition, porcine enterocytes are capable of synthesizing proline from ornithine, which is dependent on the conversion of ornithine to P5C by OAT (10). Although the presence of OAT in the fetal porcine small intestine suggests that it may play a similar role to that in the neonatal small intestine, the function of OAT in fetal porcine small intestine remains unclear. The importance of its expression pattern during gestation is most relevant when considered relative to the expression patterns of other amino acid-metabolic enzymes, in particular, those enzymes that use the products of the OAT catalyzed reaction and result in synthesis of both proline and citrulline. We hypothesized that the fetal porcine small intestine has the capacity to synthesize proline and citrulline, which are important components in the arginine biosynthetic pathway. The first step needed to test this hypothesis was to determine whether the small intestine of fetal

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pigs expresses carbamoyl phosphate synthase I (CPS-I; EC 6.3.4.16), ornithine carbamoyl transferase (OCT; EC 2.11.3.3), and pyrroline-5-carboxylate reductase (P5CR; EC 1.5.1.2), enzymes required for synthesis of citrulline and proline, respectively.

In this study, we investigated activities and compartment-specific expression patterns of CPS-I, OCT, and P5CR in the same tissues in which we previously reported presence of OAT. CPS-I and OCT are nuclear-encoded, mitochondrial matrix enzymes that together catalyze the formation of citrulline from ammonia, bicarbonate, and ornithine. Both enzymes are expressed solely in the liver and small intestine, and recent investigations in the rat revealed that CPS-I and OCT are coexpressed with OAT in enterocytes of the fetal small intestine (11). P5CR is a nuclear-encoded, cytosolic enzyme that catalyzes the conversion of P5C to proline and is found in numerous cell types, including intestinal epithelium (10) and fibroblasts (12). P5CR activity is detectable in enterocytes of newborn pigs (10) and may play a role in extracellular matrix deposition in the developing small intestine. (For diagrammatic representations of these pathways, see 5, 13). Thus, the first objective of this study was to measure enzymatic activities of these three enzymes, in *ex vivo* preparations, to determine whether functional enzymes exist within the porcine fetal small intestine, throughout gestation, to synthesize both citrulline and proline. Because coexpression of these enzymes is needed for completion of the biosynthetic pathways, the second objective of this study was to determine cell compartment-specific expression of the enzymes within the small intestine. Because specific antibodies that recognize porcine CPS-I, OCT, and P5CR were not available, *in situ* hybridization was used to determine expression patterns of mRNAs encoding for these three enzymes. Together, these results will provide needed insight into the potential capacity for amino acid metabolism in the fetal small intestine and, more generally, the potential for fetal contribution to endogenous synthesis of amino acids in the arginine biosynthetic pathway. Results of this study demonstrate that 1) enzymatic activities of CPS-I, OCT, and P5CR in the fetal small intestine increase throughout gestation; 2) CPS-I and OCT genes are expressed in distinct patterns and are confined to the mucosal epithelium; and 3) P5CR mRNA is present in mucosal epithelium and lamina propria of the fetal porcine small intestine from d 30 to d 110 of gestation.

METHODS

Animals and tissue collection. Experimental and surgical procedures were approved by the Texas A&M Laboratory Animal Care Committee. Sexually mature gilts were monitored daily for onset of estrus and were hand-mated to boars after experiencing two estrous cycles. Fetuses were collected on d 30, 35, 40, 45, 60, 90, and 110 of gestation (term is 114 ± 2 d) after hysterectomy of pregnant gilts and killed by decapitation while in a surgical plane of anesthesia, as previously reported (1). For the remainder of the article, days of gestation will be designated as d 30, d 35, d 40, d 45, d 60, d 90, and d 110. The small intestines were divided into duodenum and proximal and distal jejunum as previously described (1); small intestines

from d 30 fetuses were too fragile to divide into segments. For analysis of CPS-I, OCT, and P5CR *via* *in situ* hybridization, tissues were fixed overnight in 4% paraformaldehyde and then stored in 70% ethanol before processing and embedding in paraffin. Paraffin-embedded sections (4 μ m) were adhered to Superfrost Plus-coated slides (Statlab Medical Products, Lewisville, TX, U.S.A.).

Preparation of homogenates for enzyme assay. CPS-I, OCT, and P5CR enzymatic activities were determined in fetal intestines from d 30, 35, 45, 60, 90, and 110 using previously described methods (10, 14). On d 30, 35, and 45, pooled whole intestines from 8–10 fetuses from $n = 6$ litters and on d 60, 90, and 110 individual small intestines from four fetuses per litter ($n = 6$ litters) were used for measurement of enzymatic activities. Briefly, whole intestine (d 30, 35, 35) or small intestine (d 60, 90, 110) was homogenized at 4°C in homogenization buffer [0.25 M sucrose, 1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.5), 2.5 mM DTT, 5 μ g/mL phenylmethylsulfonyl fluoride, 5 μ g/mL aprotinin, 5 μ g/mL chymostatin, and 5 μ g/mL pepstatin] and centrifuged at $600 \times g$ for 15 min at 4°C. The supernatant was centrifuged at $14,000 \times g$ for 15 min at 4°C to obtain cytosolic (supernatant) and mitochondrial (pellet) fractions. The resulting supernatant was used to assay P5CR activity. The resulting mitochondrial pellet was suspended in homogenization buffer and subjected to three cycles of freezing in liquid nitrogen and thawing at 37°C, and the lysates were centrifuged at $10,000 \times g$ for 15 min at 4°C. The resulting supernatant was used to assay activities of OCT and CPS-I.

Measurement of the activities of CPS-I, OCT, and P5CR. The activities of CPS-I, OCT, and P5CR were measured at 37°C and performed using two protein levels and three time points (0, 10, and 20 min) following previously reported protocols (10, 14). The assay mixture for CPS-I was composed of 0.15 M potassium phosphate buffer (pH 7.5), 25 mM ATP, 25 mM $MgCl_2$, 5 mM N-acetylglutamate, 20 mM NH_4Cl , 5 mM ornithine, 100 mM $NaHCO_3$, 10 U of added ornithine carbamoyltransferase (Sigma Chemical Co., St. Louis, MO, U.S.A.), and mitochondrial extracts. The assay mixture for OCT contained 0.1 M potassium phosphate buffer (pH 7.5), 15 mM ornithine, 40 mM carbamoyl phosphate, and mitochondrial extracts. The assay mixture for P5CR consisted of 10 mM DL-P5C, 4 mM NADPH, and cytosolic preparation. All of the above reactions were terminated by addition of 1.5 M $HClO_4$ and neutralized by addition of 2 mM K_2CO_3 .

Subcloning of cDNA. cDNAs for human P5CR (American Type Culture Collection), pCPSr4 (rat carbamoyl phosphate synthase I; provided by Dr. William O'Brien, Baylor College of Medicine, Houston, TX, U.S.A.), and pOCTr-SM (rat ornithine transcarbamylase, provided by Dr. Masataka Mori, Kumamoto University School of Medicine, Kumamoto, Japan) were subcloned for use in ISH. For P5CR and CPS-I, a 1.2-kb *Bam*HI/*Hinc*II fragment from P5CR and a 1-kb *Eco*RI/*Xho*I fragment from pCPSr4 were subcloned into pBluescript (Stratagene, La Jolla, CA, U.S.A.) for generation of sense and antisense probes. For OCT, a 0.3-kb fragment was generated from pOCTr-SM by PCR using specific primers based on the porcine OCT sequence (GenBank accession no. Y13045; for-

ward 5'-CGGGATCCAAGCATGGGACAAGAGGATG; reverse 5'-CGGAATTCGGCATCAGAA-CTTTGGCTTC). PCR conditions were 25 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 40 s. Each PCR product was subcloned into pCR4-TOPO TA-cloning vector (Invitrogen, Carlsbad, CA, U.S.A.). PCR product size was verified by separation on a 2% agarose gel and staining by ethidium bromide, and PCR product identity was verified by sequence analysis.

In situ hybridization. *In situ* hybridization analysis of CPS-I, OCT, and P5CR mRNAs was undertaken subsequent to detection of the respective enzymatic activities in the intestinal tissues. Because the period between d35 and d45 is a time of remarkable morphologic change and differentiation in the porcine small intestine, including formation of villi (1), and one of our objectives was to define compartment-specific distribution of CPS-I, OCT, and P5CR, an additional day of gestation (d 40) was added for this portion of the study. Sense and antisense [α - 35 S]UTP-labeled cRNA probes were generated from the above subcloned cDNAs by *in vitro* transcription, and ISH analysis was performed as reported previously (1, 5). Briefly, slides were deparaffinized and hybridized with sense or antisense probes (5×10^6 cpm/section) in hybridization buffer [50% formamide, 0.3 M NaCl, 20 mM Tris-HCl (pH 8.0), 5 mM EDTA (pH 8.0), 10 mM sodium phosphate (pH 8.0), $1 \times$ Denhardt's solution, 10% Dextran sulfate, 100 mM DTT] at 55°C overnight. Separate experiments were conducted on duodenal, proximal jejunal, and distal jejunal segments. Each experiment included two fetuses from each of three litters for each gestational day studied. All slides were subjected to a 3-wk exposure time after which they were developed using Kodak D-19 (Eastman Kodak, Rochester, NY, U.S.A.) developer and counterstained with hematoxylin. Evaluation of slides and acquisition of images was performed as previously described (5) using a Zeiss Axioplan II Research microscope (Carl Zeiss, Inc., Thornwood, NY, U.S.A.), under bright-field and dark-field illumination, comparing sense and antisense signal for determination of cell-specific expression. Digital images were acquired using a Zeiss Axioplan II Research microscope interfaced with a Hamamatsu color camera (Hamamatsu Corporation, Bridgewater, NJ, U.S.A.) supported by a Power Macintosh G3 Computer (Apple, Cupertino, CA, U.S.A.). Images were captured and processed using Adobe Photoshop 5.0 (Adobe Systems Incorporated, San Jose, CA, U.S.A.).

RESULTS

Enzymatic Activity

Enzymatic activities of CPS-I, OCT, and P5CR in the fetal porcine small intestine (Table 1) were detectable at d 30 of gestation and increased during gestation to levels similar to those observed in the small intestine of neonatal pigs (10, 14). Fetal small intestinal activities of CPS-I, OCT, and P5CR increased 73-, 170-, and 45-fold, respectively, from d 30 to d 110.

mRNA Expression

ISH analysis indicated that CPS-I and OCT mRNA expression was confined to the mucosal epithelium, whereas expression of P5CR mRNA was detected in mucosal epithelium and lamina propria on all days studied. Compartment-specific expression patterns are described below and, unless otherwise noted, were similar in duodenum and the proximal and distal jejunum.

CPS-I. By d 30, the small intestine had retracted into the peritoneal cavity of the fetal pig and consisted of a simple tube lined by a stratified epithelium of endodermal origin (1). At d 30, CPS-I mRNA was detected throughout the stratified epithelium (Fig. 1, *A* and *B*) and remained throughout the stratified epithelium between d 30 and d 40 (Fig. 1, *C* and *D*). By d 45, villi had formed and the epithelium, which had evolved from stratified to simple columnar, was segregated into villus and intervillus compartments (1). CPS-I mRNA was detected in both the villus and intervillus epithelium from d 45 through d 90 (Fig. 1, *E–J*). By d 110, CPS-I mRNA in the duodenum was confined to villus epithelium and was not observed in crypt or villus tip enterocytes, whereas the expression pattern in the proximal and distal jejunum resembled that of d 90 (Fig. 2), with distribution in all compartments of the mucosal epithelium.

OCT. Similar to the expression of CPS-I, OCT mRNA was detected throughout the stratified epithelium between d 30 and d 45 (Fig. 3, *A* and *B*). At d 45 and through d 110, OCT mRNA remained throughout the epithelium; however, stronger signals for OCT were detected in intervillus epithelium (Fig. 3, *C–H*). This change in expression intensity at d 45 corresponded with the time when the intervillus epithelium became simple columnar (1).

Table 1. Activities of arginine-synthetic enzymes in the small intestine of fetal pigs

Age of gestation (d)	CPS-I (nmol·min ⁻¹ ·mg protein ⁻¹)	OCT (nmol·min ⁻¹ ·mg protein)	P5CR (nmol·min ⁻¹ ·mg protein ⁻¹)
30	0.02 ± 0.00f	0.71 ± 0.01f	0.53 ± 0.04f
35	0.08 ± 0.01e	2.24 ± 0.02e	1.29 ± 0.15e
45	0.14 ± 0.02d	3.63 ± 0.31d	2.46 ± 0.18d
60	0.50 ± 0.03c	42.8 ± 2.70c	8.84 ± 0.77c
90	0.82 ± 0.07b	77.2 ± 5.24b	15.1 ± 1.03b
110	1.46 ± 0.12a	121 ± 7.41a	23.9 ± 1.26a

Data are means ± SEM, $n = 6$ litters. For enzyme assays, whole intestines pooled from the fetuses of each litter were used for 30- and 45-d-old fetal pigs, whereas the small intestine of individual pigs was used for 60- to 110-d-old fetal pigs. a–f, within columns means sharing different letters are statistically different ($p < 0.01$), as analyzed by one-way ANOVA and the Student-Newman-Keuls multiple comparison test.

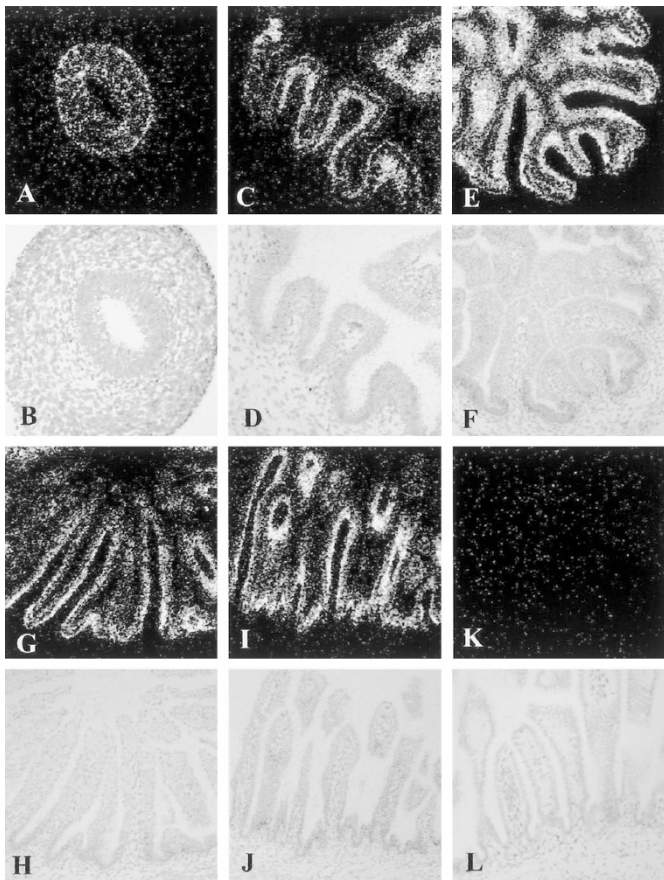


Figure 1. Expression of CPS-I in the fetal porcine proximal jejunum is limited to the mucosal epithelium. Representative dark field and corresponding bright field micrographs for d 30 (A and B), d 40 (C and D), d 45 (E and F), d 60 (G and H), and d 90 (I and J) demonstrate expression of CPS-I mRNA throughout the epithelium. (K and L) Sense control and corresponding bright field image, respectively. Field width of A–F is 270 μm ; field width of G–L is 430 μm .

P5CR. P5CR mRNA was detectable in both the stratified epithelium and the underlying mesenchyme from d 30 to d 45 (Fig. 4, A–F). By d 45, P5CR mRNA remained throughout the epithelium and mesenchymal expression was confined to the lamina propria (Fig. 4, E and F). Similar mRNA patterns were detected through d 110, with no detectable differences in intensity between villus and intervillus cell populations (Fig. 5, A and B). At d 110, P5CR mRNA was also detectable in the developing duodenal glands (Fig. 5, C and D).

DISCUSSION

In a previous report, we described the presence of OAT activity in porcine fetal small intestine from d 30 to d 110 and limitation of the OAT mRNA, also present throughout gestation, to the intestinal mucosal epithelium (5). Within the fetal intestine, the role of OAT, which interconverts P5C and ornithine, was unclear because of the bifunctional nature of OAT, which is driven, in part, by substrate availability (13). OAT substrate concentrations are regulated, in turn, by additional enzymes in the arginine metabolic pathway, including OCT, CPS-I, and P5CR (15). Results of the current study suggest

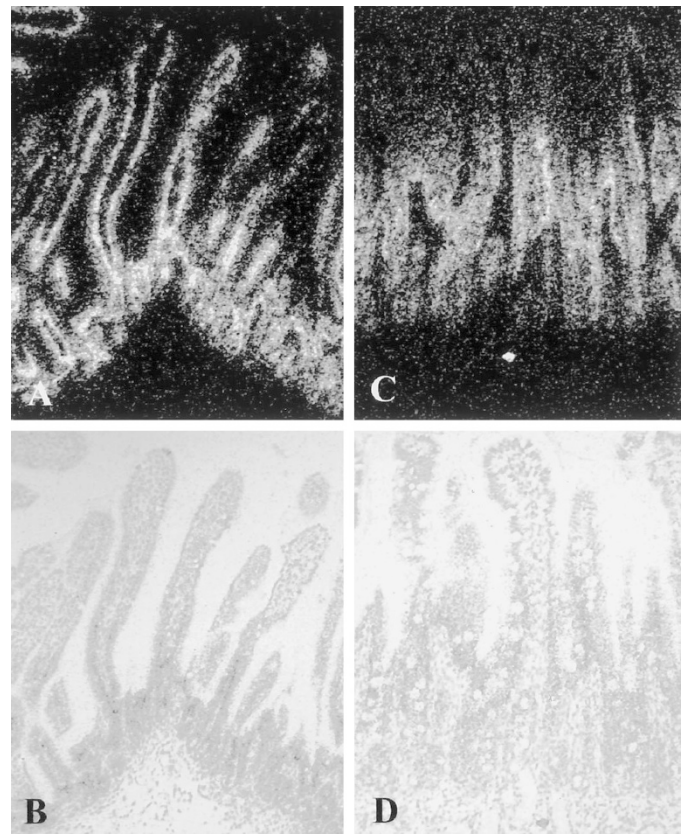


Figure 2. Expression pattern of CPS-I in proximal jejunum and duodenum of d 110 fetal pig small intestine. At d 110, CPS-I expression is throughout villus epithelium of the proximal jejunum but is confined to the epithelium lining the villi in the duodenum. Representative dark and bright field images, respectively, of d 110 proximal jejunum (A and B) and duodenum (C and D). Field width is 640 μm .

that, within the fetal small intestine, OAT may participate in endogenous production of both proline and citrulline.

Results of this study indicate that CPS-I, OCT, and P5CR enzymatic activities are present in the porcine fetal intestine from d 30 to d 110. Because of limitations of the enzymatic analysis with regard to amounts of tissue required, whole intestines were used on d 30–45, whereas only small intestine—the focus of this study—was used on d 60–110. This imposes some limitation on direct quantitative comparison between the two tissue collection groups; nonetheless, increases in enzymatic activities within those two groups (from d 30 to d 45 and from d 60 to d 110) are substantial and suggest an overall increase in CPS-I, OCT, and P5CR activities as gestation progresses. Although *in vivo* activity of the enzymes clearly would be influenced by substrate availability, results presented in this report clearly suggest that the porcine fetal intestine possesses the capability to synthesize citrulline from ornithine (*via* the activities of CPS-I and OCT) and proline from P5C (*via* activity of P5CR).

We demonstrated previously that intestinal protein content on a per wet weight basis remains relatively constant from d 30 to d 110 (5), suggesting that the total amounts of CPS-I, OCT, and P5CR available and potentially active within the porcine fetal intestine increase throughout the gestational period. The observed increases in enzymatic activity are coincident with

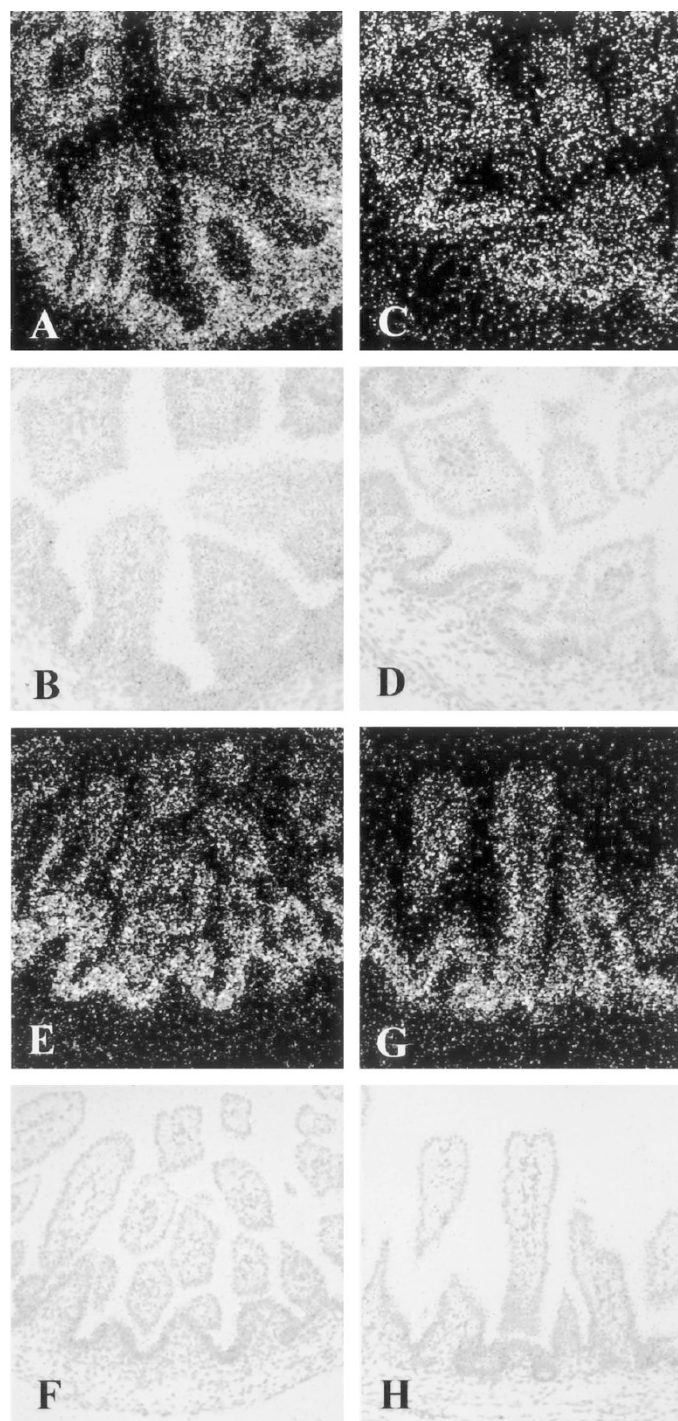


Figure 3. Expression of OCT mRNA in fetal porcine small intestine is limited to the mucosal epithelium. Representative micrographs showing dark and bright field images, respectively, of d 40 (A and B), d 45 (C and D), d 60 (E and F), and d 110 (G and H) proximal jejunum. Field width of A–D is 350 μm ; field width of E–H is 550 μm .

increases in growth and differentiation of the intestinal mucosa during this period (1), and *in situ* hybridization has demonstrated that expression of CPS-I, OCT, P5CR is limited to the mucosa. Whether the observed enzyme increases are merely reflective of an increased mucosal mass or result from additional, more specific regulatory mechanisms cannot be discerned from the current study.

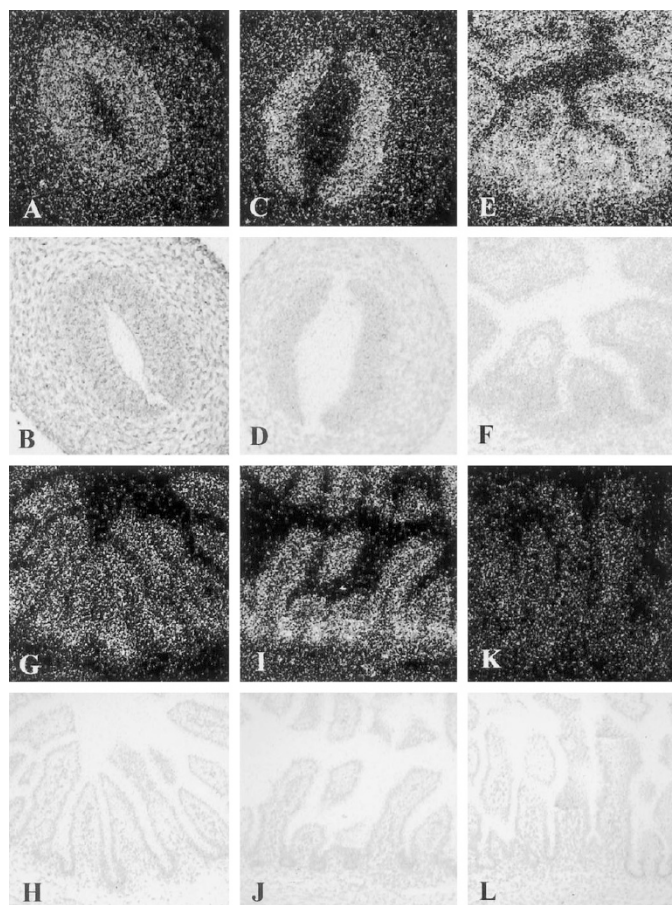


Figure 4. Expression of P5CR in the mucosa of the fetal porcine small intestine. Representative dark and bright field images, respectively, of d 30 (A and B), d 35 (C and D), d 45 (E and F), d 60 (G and H), and d 90 (I and J) proximal jejunum. (K and L) Sense control and corresponding bright field image. Field width of A–F is 270 μm ; field width of G–L is 430 μm .

In neonatal enterocytes, CPS-I and OCT function to produce citrulline from ammonia, bicarbonate, and ornithine (6), and citrulline is subsequently converted to arginine by argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL) (9). Studies in progress in our laboratories indeed indicate that isolated fetal pig enterocytes are able to synthesize citrulline and proline from glutamine and ornithine, respectively, and that enzymatic activities of ASS and ASL are also present in homogenates of fetal porcine small intestine (G. Wu, unpublished data). Taken together with the previously reported presence of OAT mRNA and enzymatic activity (5), current evidence thus suggests that fetal enterocytes can synthesize citrulline from intramitochondrially generated ornithine between d 30 and d 110.

The small intestine of the neonatal pig expresses all of the enzymes necessary for using glutamine, glutamate, and proline as substrates for arginine synthesis and is the primary source of endogenous arginine for the animal (6, 8, 16). The fate of intestinal citrulline and whether porcine fetal enterocytes can further convert citrulline to arginine cannot be stated definitively without further study; however, ASS and ASL mRNA and protein have been detected in fetal rat small intestine (17), suggesting that fetal enterocytes are also capable of synthesiz-

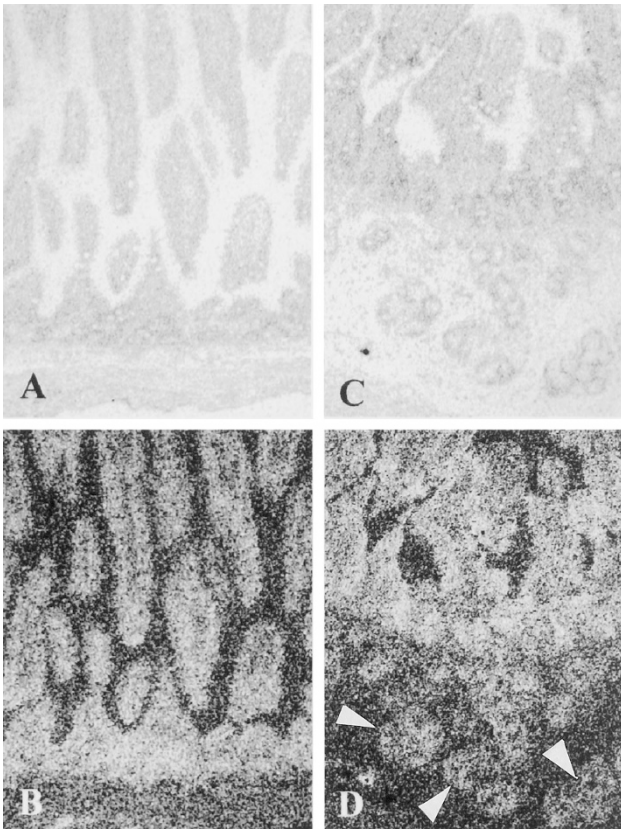


Figure 5. Expression of P5CR in proximal jejunum and duodenum of d 110 fetal pig. Expression is observed in the epithelium and lamina propria of the proximal jejunum (A and B) and duodenum (C and D) bright and dark field, respectively. Expression is also detected in duodenal glands (arrows). Field width is 450 μm .

ing arginine. The functional significance of this capability in the fetus is not yet clear. Arginine has many roles in the intestine, including protein synthesis, cell proliferation (18, 19), and polyamine synthesis (20, 21), and maintenance of the epithelial barrier function (22). As the physiologic precursor of nitric oxide, arginine plays an important role in regulating vascular tone, hemodynamics, and whole-body homeostasis (23). Synthesis of arginine by the fetal small intestine may also play an important role in intrafetal provision of arginine during late gestation as recent studies with the pig suggest that the maternal supply of arginine is inadequate for arginine accretion in fetal pigs between d 110 and d 114 (24).

An important factor that affects the *in vivo* capability of the fetal small intestine to produce the amino acids, as suggested above, is whether the relevant enzymes are coexpressed within cells. Our current results indicated that mRNAs encoding for CPS-I, OCT, and P5CR are coexpressed in cells of the porcine small intestine between d 30 and d 110. Although identification of immunologically identifiable CPS-I, OCT, and P5CR in these specific cell types awaits development of suitable antibodies, presence of the relevant mRNAs further supports the hypothesis that fetal enterocytes have the capacity to synthesize citrulline and proline, as suggested by results of enzymatic activities in tissue homogenates.

The expression patterns of CPS-I and OCT mRNA in the fetal small intestine suggest that enterocytes along the crypt-

villus axis are capable of synthesizing citrulline. As mentioned above, the capability of porcine fetal enterocytes to synthesize arginine within the same mucosal compartment has yet to be determined. De Jonge *et al.* (17) recently reported compartmentalization of several urea cycle enzymes, including OCT, CPS-I, ASS, ASL, and OAT, in the epithelium of fetal and neonatal rat small intestine and concluded that enterocytes along the crypt-villus axis were capable of synthesizing citrulline, whereas enterocytes on villus tips were capable of synthesizing both citrulline and arginine. The reasons for the confinement of CPS-I mRNA to enterocytes lining the lateral portions of duodenal villi on d 110 in the pig are not known. Although it is possible that only cells in this location participate in production of citrulline, another, perhaps more likely, scenario is that the CPS-I protein is maintained in the enterocytes as they migrate up to the villus tip, precluding a requirement for additional mRNA synthesis in cells that are destined for extrusion into the intestinal lumen.

Development of the small intestine of the fetal pig begins early in gestation relative to the rodent and involves extensive morphologic (1) and functional (2–4) changes that require cell proliferation and changes in extracellular matrix components (25). The presence of P5CR mRNA in the epithelium and lamina propria of the fetal small intestine suggests that cells of these compartments can synthesize proline from P5C, which is a product formed by OAT-catalyzed degradation of ornithine. In the case of enterocytes, P5CR expression in conjunction with OAT expression and activity (5) suggests that these cells can synthesize proline from ornithine. Proline is a major component of collagen and other extracellular matrix proteins that play significant roles in the morphologic and functional changes that occur in the small intestinal mucosa during gestation (25–27) and also contribute to formation of the basement membrane, which is directly involved in differentiation of mucosal epithelium (28). A novel finding of this study was the presence of P5CR in the duodenal glands. Expression of P5CR in duodenal glands is limited to d 110 of gestation and suggests that cells of the duodenal glands, like cells of the small intestinal villus epithelium, are capable of synthesizing proline from P5C. Unlike intestinal epithelial cells, however, cells of the duodenal glands do not express OAT (5), suggesting that they are unable to synthesize proline from ornithine. Therefore, cells of the duodenal glands must rely on alternative sources of P5C, such as glutamine from amniotic fluid or the fetal circulation (29, 30), for proline synthesis. Although the fate of proline within the porcine duodenal glands during late gestation is unknown, we speculate that this amino acid may be incorporated into the duodenal gland secretions and perhaps play a role in perinatal functions of the small intestine. Duodenal glands produce mucin glycoproteins, which contain large amounts of proline (nearly 20%) and, in other species, mucins are secreted by the small intestine during gestation (31, 32).

In summary, the present study characterized developmental changes in CPS-I, OCT, and P5CR gene expression in the small intestine of fetal pigs. CPS-I and OCT mRNAs were confined to the mucosal epithelium throughout gestation, whereas P5CR mRNA was detectable in mucosal epithelium and lamina propria. These results, combined with the measured

enzymatic activities of these enzymes, suggest the capacity for multiple cell-specific pathways for amino acid metabolism in the porcine small intestine during gestation. Furthermore, depending on substrate availability, these results suggest that reactions may be driven toward the production of proline, perhaps for utilization in production of structural and extracellular matrix proteins within the intestine, or toward citrulline and arginine, for local use within the small intestine and/or secretion into the fetal circulation for somatic growth and development. Results of this study establish the basis for additional studies that are needed to determine definitively the fate of the endogenously synthesized products and the source(s) of the substrate amino acids for the fetal small intestine (e.g. maternal circulation, fetal fluids, synthesis by other organs). This line of investigation will lead to a better understanding of the role and importance of the fetal small intestine in amino acid metabolism during gestation and the necessary provision of specific substrate amino acids that are required for normal fetal growth and development.

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