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Colocalization in pericentral hepatocytes in adult mice and similarity in developmental expression pattern of ornithine aminotransferase and glutamine synthetase mRNA

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ABSTRACT **_In situ_ hybridization showed that the mRNA for ornithine aminotransferase (OAT; ornithine–oxo-acid aminotransferase; L-ornithine:2-oxo-acid aminotransferase, EC 2.6.1.13) colocalized with glutamine synthetase [GS; glutamate–ammonia ligase; L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] in pericentral hepatocytes of the adult mouse liver. In addition to an identical distribution in adult hepatocytes, OAT and GS have very similar expression patterns in fetal and neonatal liver. As was earlier described for GS, there is a low level of OAT mRNA in fetal cells and increasing pericentral levels in neonates that reach adult patterns within 2 weeks. These results suggest that the transcriptional regulation of the two genes is similar in the liver. However, there was a lack of colocalization of the mRNAs for the two enzymes in cells of the kidney, intestine, and brain, suggesting different regulatory decisions for the OAT and GS genes in the cells of these different tissues. The metabolic consequences of these localized expression patterns favor ammonia clearance from the blood by the liver and urea synthesis by the kidney.**

The vertebrate liver performs many vital functions including production of plasma proteins, balancing carbohydrate and fat metabolism, and detoxification of exogenous and endogenous noxious chemicals, one of the most important being ammonia clearance by the action of glutamine synthetase [GS; glutamate–ammonia ligase; L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2], which couples ammonia and glutamate to produce glutamine. Ever since the acinus concept of liver structure was proposed more than 30 years ago (1), there have been numerous studies showing that hepatocytes in different positions in the acinus may perform different functions. For example, qualitative and quantitative histochemistry (2), immunohistochemistry (3), and microdissection with microbiochemistry (4) suggested that the cells in the periportal zone (the cells at the center of the acinus) are specialized to perform gluconeogenesis, lipid oxidation, urea synthesis, and transamination at a higher rate than in pericentral cells where a greater capacity for glycolysis, lipogenesis, and xenobiotic detoxification appeared to be located (5). However, only recently with the availability of monospecific antibodies and cDNA clones to detect various hepatocyte-expressed mRNAs has the degree of such specific localization in hepatocytes become clear. Antibodies against GS localized this enzyme to one or two layers of pericentral hepatocytes in adult rats (6) and mice (7), and GS mRNA was also located only in pericentral cells (8). Because GS was present at high levels in only occasional cultured adult or fetal hepatocytes, we suggested that some mechanism other than circulating metabolic signals triggered differential GS expression (7, 8). In addition, a series of studies involving *in situ* hybridization in fetal, neonatal, and adult liver coupled with nuclear run-on transcriptional analysis led us to suggest further that extracellular position-specific signals are the most logical triggers for a proposed transcriptional regulation (8).

In an effort to learn more about this potential position-specific transcriptional control, we explored the possibility that other enzymes might be coregulated with GS in assisting the liver to control serum ammonia levels (9). We have found that ornithine aminotransferase (OAT; ornithine–oxo-acid aminotransferase; L-ornithine:2-oxo-acid aminotransferase, EC 2.6.1.13), which at elevated concentrations could function to generate glutamate (a GS substrate) from ornithine, is indeed found in a single layer around central veins in the adult liver just as is GS. Moreover, this enzyme has the same pattern of expression during fetal and neonatal development as does GS. We discuss the biochemical logic of this distribution, which facilitates both ammonia clearance by pericentral cells and urea synthesis by nonpericentral cells.

MATERIALS AND METHODS

DNA Isolation and Manipulation. The manipulations of DNA used in cloning and probe preparation for GS were as generally described in standard molecular biology manuals (10, 11) or in our previous publications (8, 12). A human cDNA clone encoding OAT (13) was used to select a mouse cDNA clone that was verified by sequencing.

RNA Isolation and Slot Blot Analysis. RNA isolation, selection of poly(A)-containing mRNA, and detection of specific mRNA by Northern blot or slot blot analysis were described (8, 12).

***In Situ* Hybridization and Microscopy.** The procedures used for examination of adult and fetal tissues by *in situ* hybridization have been described (8). The ^{35}S-labeled probe for GS was 750 nucleotides long and that for the OAT was ≈1 kilobase. After emulsion autoradiography, the slides were stained with hematoxylin and eosin and examined under bright- and dark-field microscopy.

RESULTS

Two major functions of GS are the production of glutamine as an energy source for intestinal epithelial cells and lymphocytes and as the molecular carrier that allows ammonia clearance in the liver (14, 15). The other major biochemical pathway that mammals use to clear ammonia is the urea cycle. Carbamoyl-phosphate synthetase condenses ammonia and CO_2, eventually leading to ornithine synthesis. The conversion of ornithine to citrulline is a major entry point into the urea cycle. When Moorman and his colleagues (3) ex-

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Abbreviations: GS, glutamine synthetase; OAT, ornithine aminotransferase.

amined the cellular distribution of carbamoyl-phosphate synthetase I in liver, they found that it has a reciprocal distribution compared to GS—i.e., it is in all the hepatocytes except those cells that are immediately pericentral and are GS positive. We were interested in other genes whose regulation might be similar to that of GS. An enzyme that might divert ornithine from the urea cycle into glutamate might colocalize in the same cells as GS. Recently, cDNA clones have been obtained (13) that encode OAT, a mitochondrial enzyme that, as we discuss in detail below, can convert ornithine into glutamate, thus increasing GS activity. With a mouse cDNA copy of OAT, we tested for the hepatic localization of OAT in mouse liver by *in situ* hybridization. The signal for both GS and OAT was localized almost exclusively to the hepatocytes lining the central veins (Fig. 1) and, as discussed elsewhere (16), in all larger collecting veins that empty into the hepatic vein. Based on different times of exposure, the OAT mRNA in pericentral cells gave at least a 10-fold stronger signal than that in the remainder of hepatocytes. In most sections, only one layer of OAT-positive cells was seen. However, because of the randomness of the liver sections relative to the direction of the blood vessels, it was difficult to rule out the possibility of two layers of OAT-positive cells at some locations.

The pericentral expression of GS in adult animals is a developmentally regulated phenomenon (3, 8). All fetal hepatocytes express low levels of GS when tested both by antibody staining and by *in situ* hybridization for GS mRNA. Just after birth, pericentral cells begin to exhibit stronger expression. After 2 weeks of life, the nonpericentral cells show no expression above background, while pericentral cells have a very high concentration of GS. We used *in situ* hybridization to test whether the same gene expression pattern occurred for OAT mRNA. Fig. 2 shows a low level of OAT staining in day-17 fetal liver with the same postnatal progression for OAT that had been observed for GS. Thus, both the adult pattern of expression and the developmental pattern preceding it were the same for these two mRNAs.

To obtain a more quantitative impression of adult pericentral hepatocyte expression for OAT compared to the diffuse fetal and neonate hepatocyte levels of the two mRNAs, total

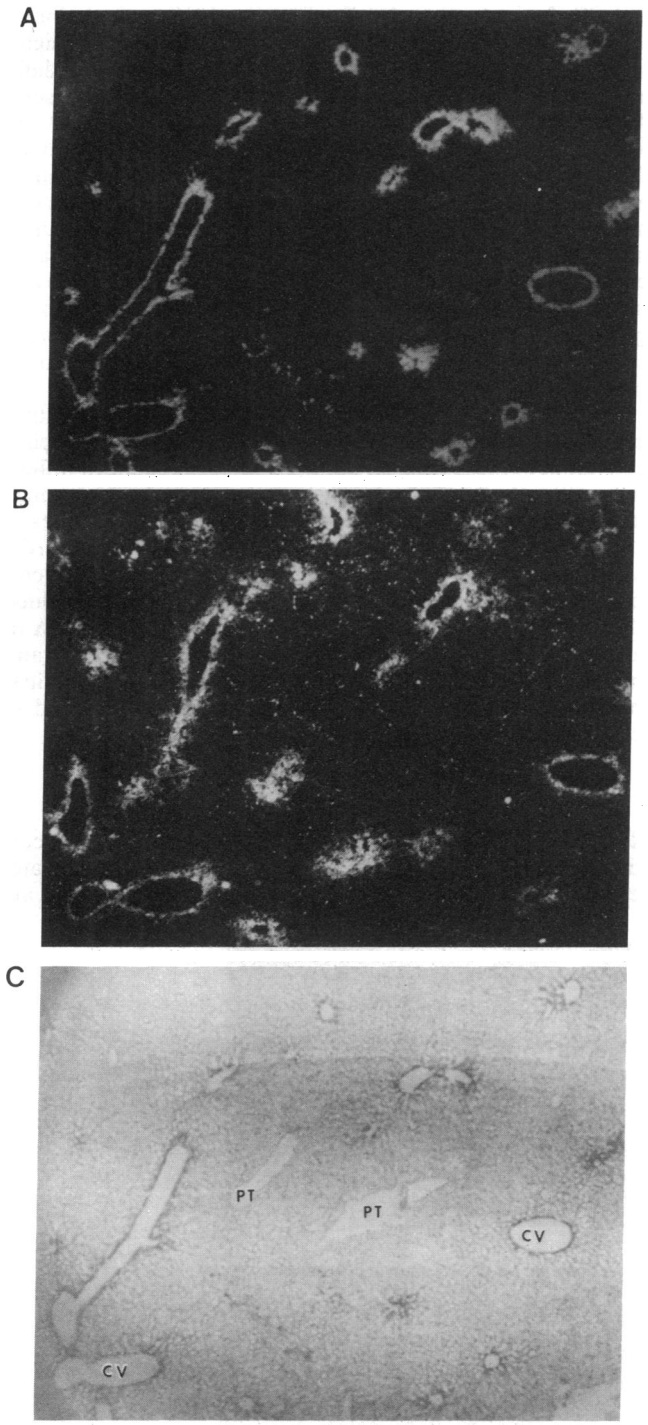

FIG. 1. Colocalization of GS and OAT mRNA in adult pericentral hepatocytes. *In situ* hybridization with antisense ^{35}S-labeled RNA probes. Serial liver sections probed with GS (*A*) and OAT (*B*) probes. Central veins (CV) were surrounded by hepatocytes that contain both GS and OAT mRNA, while none of the portal triad regions (PT) had GS- or OAT-positive cells. (*C*) Bright-field photomicrograph of the section in *A*. (×45.)

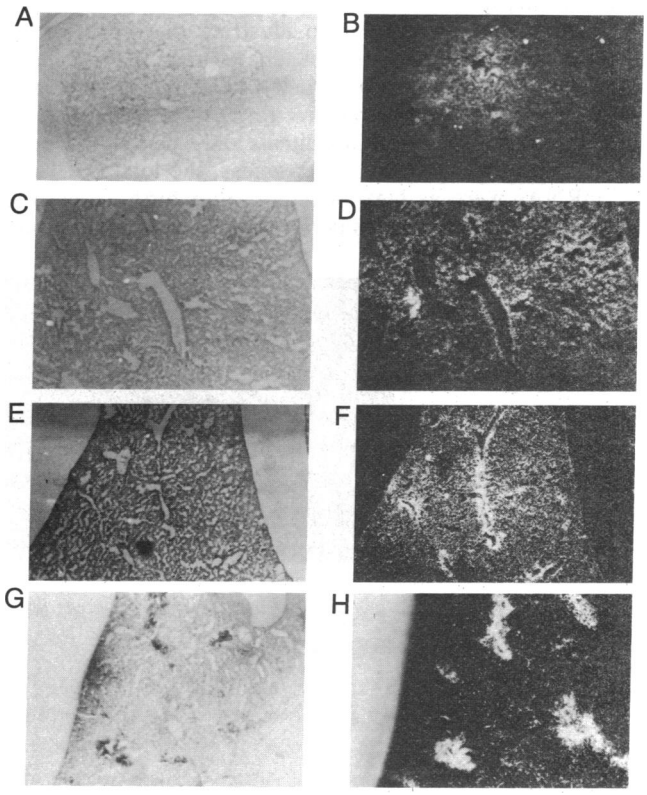

FIG. 2. Changing patterns of OAT expression in developing mouse liver. *In situ* hybridization in different developmental stages in mouse liver using ^{35}S-labeled antisense probe to OAT. (*Left*) Bright-field microscopy. (*Right*) Dark-field microscopy. (*A* and *B*) Day-17 fetus. (*C* and *D*) Day-2 neonate. (*E* and *F*) Day-10 neonate. (*G* and *H*) Adult. (×30.)

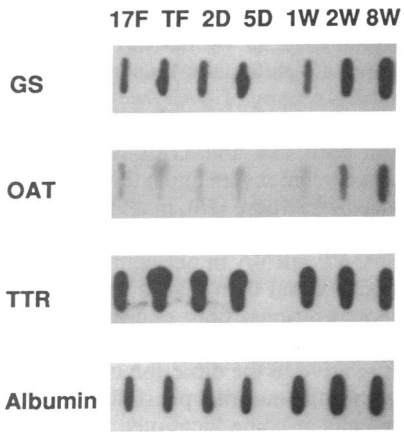

FIG. 3. OAT mRNA during mouse development. Poly(A)-containing mRNA was attached to nitrocellulose filters (2 μg per slot) and probed with the following ^{32}P-labeled mouse cDNA probes: GS, 750 base pairs; TTR, 600 base pairs; albumin, 400 base pairs; OAT, 500 base pairs. 17F, day-17 fetus; TF, term fetus; 2D, day-2 neonate; 5D, day-5 neonate; 1W, day-7 neonate; 2W, day-14 neonate; 8W, adult (8 weeks old).

mRNA was isolated from livers of animals of various ages and samples were attached to nitrocellulose. Different labeled cDNA sequences were then hybridized with the deposited RNA samples followed by autoradiography to obtain a relative measure of mRNA concentration (the so-called slot blot technique). As shown in Fig. 3, GS mRNA was, as previously reported (8), present throughout fetal and neonatal samples as was OAT mRNA. OAT mRNA only reached its maximal peak several weeks after birth. The presence of albumin and transthyretin mRNAs at high and approximately equal concentrations serves to illustrate that hepatocytes exhibit a differentiated pattern of gene expression throughout this time. Note that by *in situ* hybridization all the fetal hepatocytes were weakly positive for OAT and GS mRNAs (Fig. 2), while in the adult animals only pericentral cells expressed these mRNAs. Nevertheless, the total OAT and GS mRNAs were as high or higher after birth. Thus, the content of these mRNAs in pericentral cells, which are only $\approx$5% of the total, must be at least 20-fold higher than in fetal cells.

OAT and GS Patterns Are Not the Same in Other Organs. We previously have found that, like its distribution in the liver, GS mRNA is not equally expressed in all of the cells in other organs of the adult mouse. GS mRNA was present in the convoluted portion of the proximal tubules of the kidney (12) and in the glial cells but not neurons in the brain (12). Therefore, we tested these organs for the distribution of OAT and GS. In contrast to the distribution of GS in the kidney, the majority of the OAT mRNA was localized in the straight portion of the proximal and distal tubules of the kidney (data not shown). In addition, in the brain OAT mRNA was present diffusely and not localized in glial cells (data not shown). In fetal intestine, OAT was distributed throughout the whole thickness of intestinal villi similar to GS except at a much higher level (Fig. 4 *A* and *B*). In the adult intestine, where GS mRNA was not detectable, OAT mRNA was present mainly in epithelial cells in the crypts of Lieberkühn (the most basal cells) and the signal disappeared quickly as the cells moved up along a villus (Fig. 4 *C* and *D*). Thus, a pattern of generalized expression in the fetal cells and localized expression in the adult cells also occurred for OAT in the small intestine.

The pattern of expression of OAT in kidney and intestine clearly shows that this gene is subject to position-specific activation in these tissues, but the expression pattern differs from GS in cells of these organs in contrast to the similar pattern for expression of the two mRNAs in hepatocytes.

Although OAT expression in the eye is of special interest since congenital deficiency in humans results in childhood blindness and retinal atrophy (17), we were unable to reduce the background in that organ to localize the OAT mRNA to a particular cell type. However, by microdissection and microenzyme assay it has been shown that, in the retina, pigmented epithelial cells and even the choroid seemed to contain abundant OAT enzyme activity (18–20).

DISCUSSION

The major result of this paper shows developmentally coordinated position-specific control of OAT and GS in hepatocytes. Possible mechanisms for gene expression patterns are

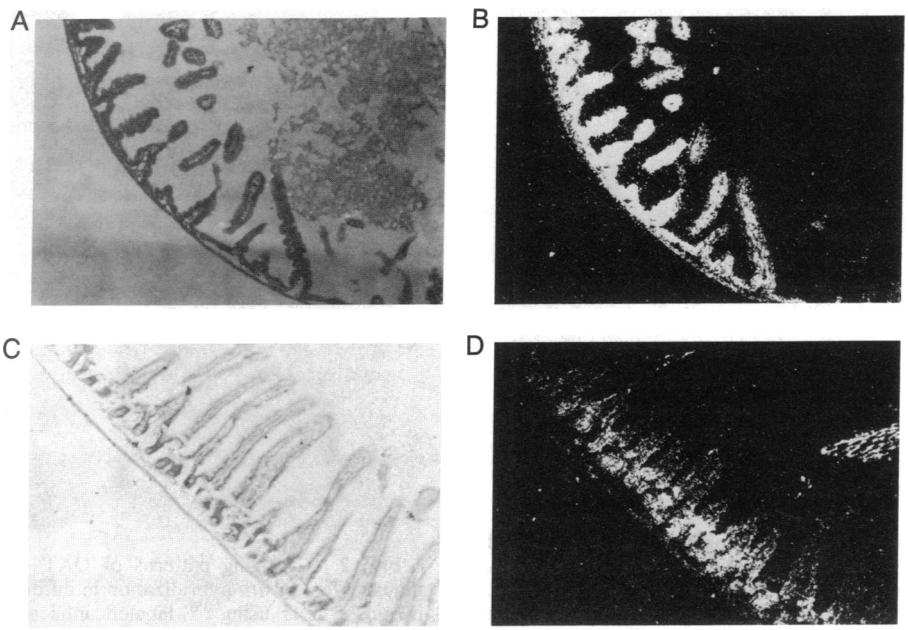

FIG. 4. Changing pattern of OAT expression in developing mouse small intestine. Intestinal sections were probed with OAT ^{35}S-labeled RNA probe. (*Left*) Bright-field microscopy. (*Right*) Dark-field microscopy. (*A* and *B*) Day-2 neonate. (*C* and *D*) Adult. (*A* and *B*, ×30; *C* and *D*, ×70.)

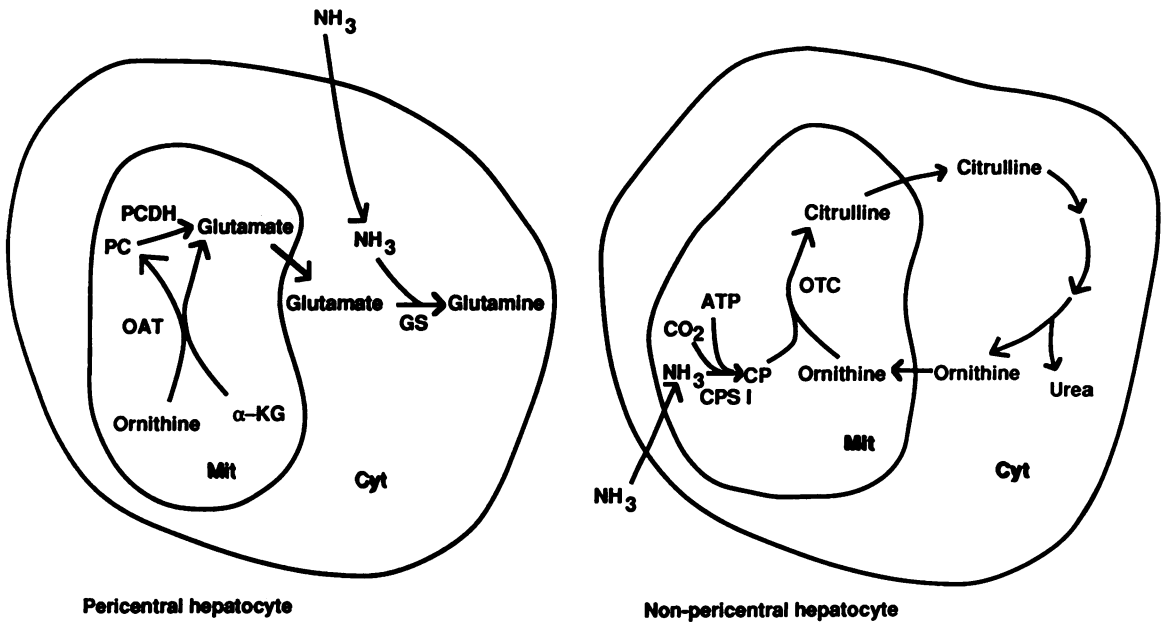

FIG. 5. Proposed effects of pericentral localization of OAT on clearance of ammonia by pericentral and nonpericentral hepatocytes. Diagram depicts the distribution in mitochondria of pericentral hepatocytes (Mit) and cytoplasm (Cyt) of OAT and GS that would facilitate incorporation of NH_3 into glutamine. Carbamoyl-phosphate synthetase is absent; thus, the urea cycle does not occur in these cells. The relative absence of OAT and GS in nonpericentral cells and the presence of carbamoyl-phosphate synthetase (CPSI) and of ornithine carbamoyltransferase allow the urea cycle to function. α-KG, α-ketoglutarate; PC, 1-pyrroline-5-carboxylate; PCDH, PC dehydrogenase.

discussed elsewhere (16). On examination of the biochemical pathways affected by these enzymes and from what is known about the cellular localization of various enzymes, we suggest the following biochemical results would be a logical consequence of our findings (see Fig. 5). First, consider the pericentral hepatocytes, which contain both GS and OAT mRNA. An important proposed role of the pericentral hepatocyte is to serve as a scavenger system to remove ammonia, a toxic compound at high levels, from the blood before it is returned from the liver to the general circulation. In this model, GS, which has a higher affinity for ammonia than the urea cycle enzymes is responsible for clearing the residual ammonia that reaches the central veins. We now add that a high concentration of glutamate would increase the elimination of ammonia. Cells with high OAT concentrations should shunt ornithine to glutamate through L-glutamate 5-semialdehyde [which first rearranges to 1-pyrroline-5-carboxylate before conversion to glutamate through the action of 1-pyrroline-5-carboxylate dehydrogenase (21)]. The glutamate produced by this pathway would then be used as a substrate by GS to incorporate ammonia into glutamine. Thus, the presence of the two enzymes, GS and OAT, in pericentral hepatocytes would lead to more efficient removal of ammonia from the blood. (The location of 1-pyrroline-5-carboxylate dehydrogenase in the liver acinus has not been described but we predict it might also be pericentral in location.)

In the nonpericentral hepatocytes, the urea cycle is the primary metabolic pathway for ammonia clearance. The rate-limiting enzyme of urea synthesis, carbamoyl-phosphate synthetase I, is only present in these cells (3). In the absence of OAT, ornithine carbamoyltransferase would divert ornithine into the urea cycle. Thus, the absence of OAT in nonpericentral cells should favor the participation of ornithine in urea synthesis. The other interesting point about the differential expression of OAT, ornithine carbamoyltransferase, and carbamoyl-phosphate synthetase I is that they are all mitochondrial enzymes encoded by nuclear genes. Thus, different hepatocytes constitute different mitochondrial enzyme arrangements to become most efficient at removing ammonia from the blood.

In the past, metabolic studies of these biochemical pathways have not separated pericentral from other hepatocytes, leading to controversy concerning the role of OAT in the liver (22). We suggest that if pure pericentral cells were available it could be demonstrated that ornithine in those cells would mainly end up as glutamate and not be converted into other amino acids or into urea.

Another very interesting aspect of the present results concerns the gene control elements required to bring about all of the instances of differential expression we have noted. Assuming that transcriptional control is the basis for these differences [and we have argued elsewhere (8, 16) that this is the case for GS], the following regulated states must occur: both GS and OAT are expressed at low levels in all fetal hepatocytes, repressed in all nonpericentral hepatocytes in adults, and subjected to greatly enhanced transcription in pericentral adult hepatocytes. Thus, several shared elements in the genes for these two enzymes might exist. Important differences in regulatory elements might also be expected to explain the lack of coordinated expression of GS and OAT in the kidney, brain, and intestine. Finally, and central to our interest in these regulatory events, we know nothing yet of the signals that cause hepatocytes or other cell types to differentially express GS and OAT genes. Since GS and OAT are expressed similarly in hepatocytes, however, we expect the same signals to be responsible for triggering the regulation for these genes in this tissue. As we have discussed previously (8, 16), the most logical source of the signal(s) upon which pericentral expression depends is the position-specific presence (or absence) of cell or cell matrix ligands that are contacted by hepatocytes in different locations. The finding of two genes that are coordinately controlled in a position-specific manner in cells of the same lineage should aid in identifying such signals.

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