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Pericentral expression pattern of glucokinase mRNA in the rat liver lobulus

Antoon F.M. Moorman, Piet A.J. de Boer, Robert Charles and Wouter H. Lamers

Department of Anatomy and Embryology, University of Amsterdam, AMC, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands

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The spatial distribution of glucokinase mRNA (GK mRNA) in rat liver was studied by in situ hybridization under normal and inducing conditions. GK mRNA was first detectable in the liver parenchyma of neonatal rats of 1.5 days. The density of grains decreases in a central-portal direction. This pattern remains essentially unchanged up to 15 days, after which the adult type of distribution gradually starts to develop, i.e. low density of grains indicating low levels of GK mRNA, in which no gradient of expression could be visualized. Within 2 h after an oral glucose load to starved animals, the GK mRNA expression pattern changed from hardly detectable to a clear gradient with the highest grain density around the terminal central venules. Within 6 h relatively high levels of grains, almost homogeneously distributed across the liver lobule, were observed. Glucocorticosteroid treatment also induced GK mRNA in the pericentral area. It is concluded that the observed induction pattern qualifies GK mRNA as a pericentral mRNA suggesting that the pericentral expression pattern of the protein is primarily regulated at the pretranslational level.

Glucokinase; Enzymic zonation; In situ hybridization; Rat liver

1. INTRODUCTION

The central role of the liver in glucose homeostasis has been recognized for more than a century. Nowadays it is fairly well-established that the enzymes involved in glucose production via gluconeogenesis and glycogenolysis predominate in the upstream, periportal domain of the liver lobule, whereas those enzymes involved in glucose uptake for glycolysis and glycogen synthesis prevail in the downstream, pericentral domain [1]. The borders of both domains are not fixed as they depend on the metabolic state of the animal, in agreement with the concept of metabolic zonation that presumes that the zonal enzymic patterns are indicative for metabolic flux [1,2].

Recent hybridocytochemical analysis have classified the mRNA for phospho*enol*pyruvate carboxykinase (PEPCK) as a periportal mRNA, indicating a pretranslational level of regulation of the pattern of expression of this key enzyme of gluconeogenesis [3,4]. In contrast, a possible regulation at the translational level has been suggested for enzymes involved in glycolysis, as the messenger for pyruvate kinase type L has been reported to be homogeneously distributed in the liver lobule of normal rat [5], whereas the corresponding protein and other enzymes of glycolysis are pericentrally localized [1,6,7]. This conclusion would imply a distinct mechanism in the regulation of these two closely interdependent metabolic processes. Because of this we felt it necessary to establish the dynamics of the spatial expression patterns of the mRNA for a glycolytic key enzyme in order to determine the major level of regulation. To this end we have chosen to study the spatial distribution of the mRNA for the liver-specific enzyme glucokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) under various inducing conditions. Glucokinase plays a pivotal regulatory role in hepatic glucose metabolism and its expression is under multihormonal control with insulin as the dominant inducing factor [8], whereas glucocorticosteroids appeared to be essential in the suckling period [9]. At the time of weaning its RNA level increases up to 40-fold, while glucose refeeding of fasted adult animals results in a nearly 100-fold induction [10].

In this communication we report that the developmentally, dietary, and hormonally regulated expression of GK mRNA across the porto-central distance classifies this RNA among the class of pericentral mRNAs.

Abbreviations: CPS, carbamoylphosphate synthetase; GK, glucokinase; GS, glutamine synthetase; PEPCK, phospho*enol*-pyruvate carboxykinase

Correspondence address: A.F.M. Moorman, Anatomisch-Embryologisch Laboratorium, Academisch Medisch Centrum, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. Fax: (31) (20) 912401.

2. MATERIALS AND METHODS

2.1. *Treatment of animals* Wistar rats were obtained from the TNO animal farm in Zeist, The Netherlands. Starved animals were deprived of food for two days. They were next refed by gastric gavage with 5 g glucose per kg body weight every two hours. Animals were sacrificed for analysis of the liver after 0, 2, 4 and 6 h glucose

refeeding. When appropriate glucocorticosteroid hormone (dexamethasone, 2.5 mg per kg body weight per day) was administered intraperitoneally to rats fed ad libitum and animals were killed 16 h later. These animals and control rats were sacrificed at approx. 9.00 a.m.

2.2. *In situ hybridization*

Livers were fixed in 4% paraformaldehyde buffered in PBS pH 7.4, for 4 h, quickly frozen in liquid Freon-22 that was cooled with liquid nitrogen, and stored at −70°C until use. 7-μm-thick cryostat sections were made and mounted onto 3-aminopropyltriethoxysilane-coated microscope slides [11]. The sections were pretreated, hybridized and processed for autoradiography essentially as described previously [12–14]. Hybridization was carried out overnight at 42°C in 50% (v/v) deionized formamide, 0.1% Triton X-100, 10% (w/v) dextran sulphate, 2 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate (pH 7.0), 0.04% (w/v) each bovine serum albumin, polyvinylpyrrolidone and Ficoll, 10 mM dithiothreitol, 200 ng herring sperm DNA per μl and labelled probe. The probe concentration was approx. 0.1 ng/μl hybridization solution in case of adult liver sections and approx. 0.5 ng/μl in case of neonatal liver sections. Sections were washed twice at 44°C for 15 min in 50% (v/v) formamide, 1 × SSC and twice for 10 min in 1 × SSC and finally for 10 min in 0.1 × SSC. Sections were then dehydrated in graded ethanol series containing 0.3 M ammonium acetate. They were further processed for autoradiography at 4°C for 5 days, using Ilford nuclear research emulsion [13].

Controls included hybridization with probes for glutamine synthetase (GS) and PEPCK mRNA. The distinct hybridization patterns obtained by these probes that are in agreement with literature [3,4,13–15], are indicative for the specificity of the reactions.

2.3. *Preparation and specification of probes*

As probes for the detection of GK, GS and PEPCK mRNA, the 1800 bp *Eco*RI fragment of the pUC-GK1 clone [10], the 663 bp DNA insert of the pGS3 cDNA subclone [16] and the 1081 bp *Pst*I fragment of cDNA clone PCK-10 [17] were used, respectively. Agarose-purified DNA fragments (agarose N.A., Pharmacia, Uppsala) were labelled

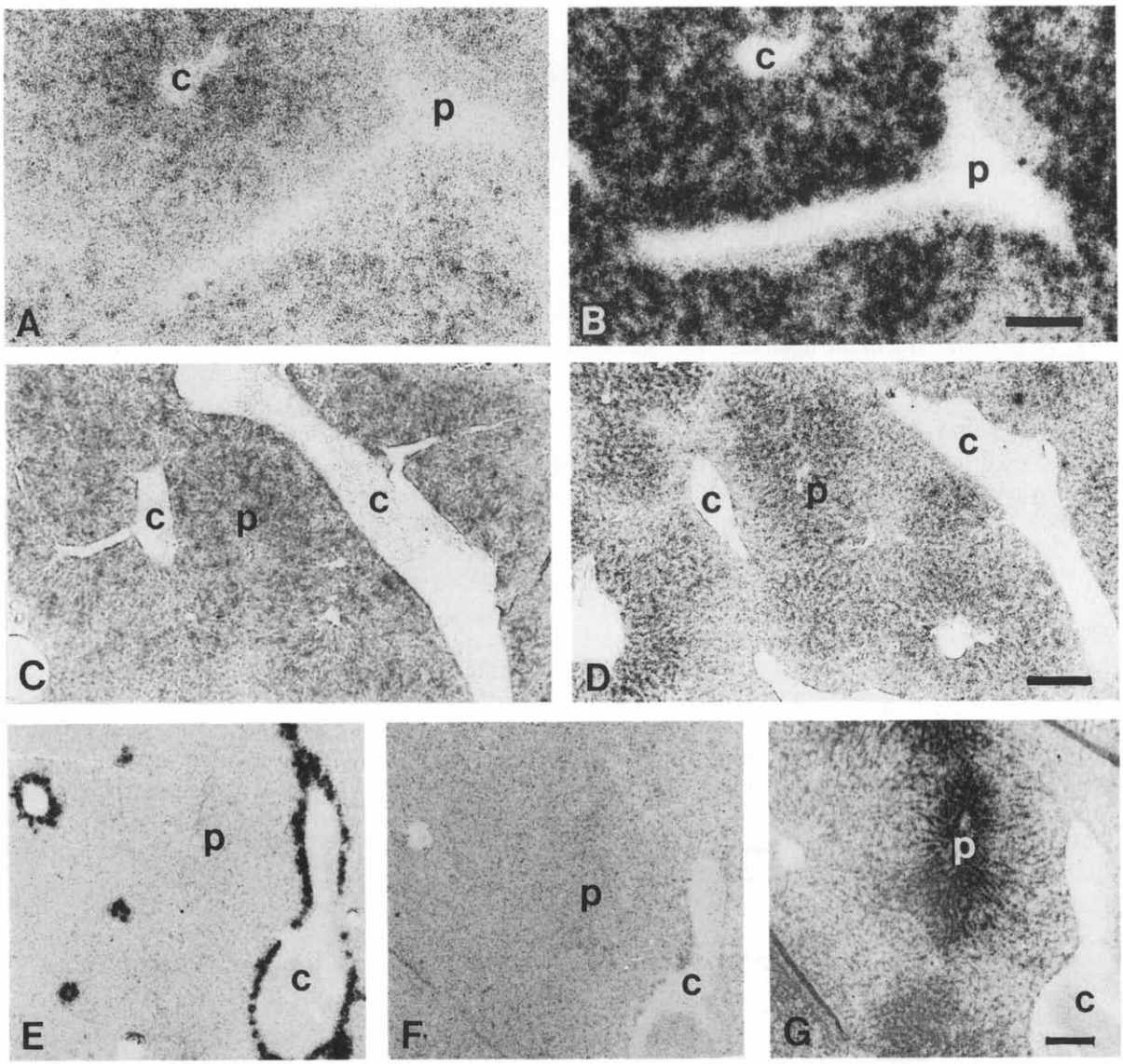

Fig. 1. Developmental appearance of GK mRNA in normal rat liver. In situ hybridization was performed on liver sections of 1.5 days (A,B), 15 days (C,D) and adult (E,F,G) rats, using probes for GK mRNA (A,C,F), PEPCK mRNA (B,D,G) and GS mRNA (E). p, portal tract; c, central venule. Bar = 0.2 mm.

overnight at 15°C with [α-^{35}S]dCTP to a specific activity of approx. 1–5 × 10^8 cpm/μg, according to the multiprime DNA labelling method.

3. RESULTS

3.1. *Developmental appearance of gene expression (Fig. 1)*

GK mRNA can first be detected in neonatal livers at 1.5 days (Fig. 1A). It is clearly present across the entire porto-central axis in a gradient decreasing from the central to the portal venule. PEPCK mRNA is rather homogeneously distributed, but the first indications of a periportal predominance are apparent (Fig. 1B). At 15 days after birth GK mRNA is still expressed according to a pericentral gradient, albeit that the gradient has faded (Fig. 1C). PEPCK mRNA is now expressed in a distinct periportal gradient (Fig. 1D). In adult liver, in which very low levels of GK mRNA are present [18], there is an apparent homogeneous distribution (Fig. 1F). Control sections show a normal distribution of GS mRNA, i.e. a characteristic expression in a small pericentral compartment (Fig. 1E) and of PEPCK mRNA, i.e. a predominant expression in the periportal zone (Fig. 1G).

3.2. *Glucose- and glucocorticoid-induced expression patterns (Figs. 2 and 3)*

Starvation of adult animals has been reported to result in a decrease of GK mRNA [10] and an increase in PEPCK mRNA [3,19]. In agreement with this, high hybridization of PEPCK mRNA is observed in a similar pattern as in control liver (Fig. 2A). Virtually no GK mRNA can be detected (Fig. 2B). After the glucose load PEPCK mRNA levels have drastically dropped (Fig. 2C), whereas a massive increase of GK mRNA levels can be observed. GK mRNA is present in a definite gradient decreasing in a central-portal direction (Fig. 2D). Four hours after the glucose load the pattern of expression of GK mRNA essentially remained unaltered (Fig. 2F), virtually no PEPCK mRNA could be detected (not shown). GS mRNA was used as a control and to unambiguously identify the central venules (Fig. 2E). Six hours after the glucose load GK mRNA is still abundantly present but the gradient of expression has all but disappeared (Fig. 2H).

Fig. 3 shows that administration of glucocorticoids to fed animals results in a decrease of PEPCK mRNA as previously reported [4] and in a pericentral induction of GK mRNA. This treatment did not change the pattern of expression and probably relates to the glucocorticoid hormone-induced increase of insulin [20].

4. DISCUSSION

The enzymes participating in glucose uptake and release are reciprocally distributed in the liver according to a so-called 'gradient type' of zonation [21], that is to say, they are expressed in a gradient across the entire porto–central distance. In normal rat liver, glucose release predominates in the periportal domain and glucose uptake in the pericentral domain [1]. The enzymes involved reveal a dynamic zonation depending on the metabolic state.

The molecular mechanisms underlying the zonation of gene expression are only partly understood. Hybridocytochemical analyses have disclosed that the major site of control is at the pretranslational level. Zonal distributions have been observed of the mRNAs for albumin [15,22], α-fetoprotein [15,22], fibrinogen [23] (Moorman et al., unpublished results), cytochrome P450 [24], carbonic anhydrase type III [25], carbamoylphosphate synthetase (CPS) [4,13,14], glutamine synthetase [13,26], tyrosine aminotransferase [27], and PEPCK [3,4,27]. So far the only exception with respect to the level of control has been suggested to hold for the glycolytic enzyme pyruvate kinase L [5]. The homogeneous distribution of its mRNA in adult rat liver, whereas enzyme protein and activity have a pericentral distribution [28] have led to the conclusion that the zonation of this enzyme and possibly that of other glycolytic key enzymes would be primarily regulated at the translational level [3,5,27]. This would be in contrast to the regulation of the gluconeogenic enzymes and would be remarkable as the regulation of gluconeogenesis and glycolysis are intimately associated to ensure a proper glucose homeostasis.

Similar to pyruvate kinase L, GK enzyme and protein have a pericentral distribution [6,7] and its mRNA seems homogeneously distributed in the liver lobule of fed adult rats (this study). At first glance these observations seem to corroborate the above-mentioned notion. However, the inducing conditions that exist during postnatal development, after an oral glucose load, or upon dexamethasone treatment, definitely demonstrate a transient, predominantly pericentral pattern of expression of GK mRNA, similar to the protein, pointing to a pretranslational level of regulation. In agreement with this, run-on transcription experiments and measurements of mRNA levels in parallel with measurements of enzyme activities, indicate that the developmental, dietary and hormonal regulation is primarily achieved at the transcriptional level [10,18,29].

The apparent difference between the pattern of expression of GK mRNA and that of GK protein in normal adult liver remains incompletely understood. A possible explanation might be that the short half life of the mRNA (1–2 h) [29], resulting in very low GK mRNA concentrations, does not allow reliable detection of subtle gradients in its expression. The normal circadian feeding rhythm will induce GK mRNA in a pericentral fashion resulting in a short-lived pericentral

gradient of GK mRNA, but a relatively long-lived gradient of GK protein, due to the longer half life of the protein (half-life 30 h) [18].

The sensitive detection of GK in neonatal livers deserves some additional comment. We have the impression that embryonic, fetal and neonatal material is

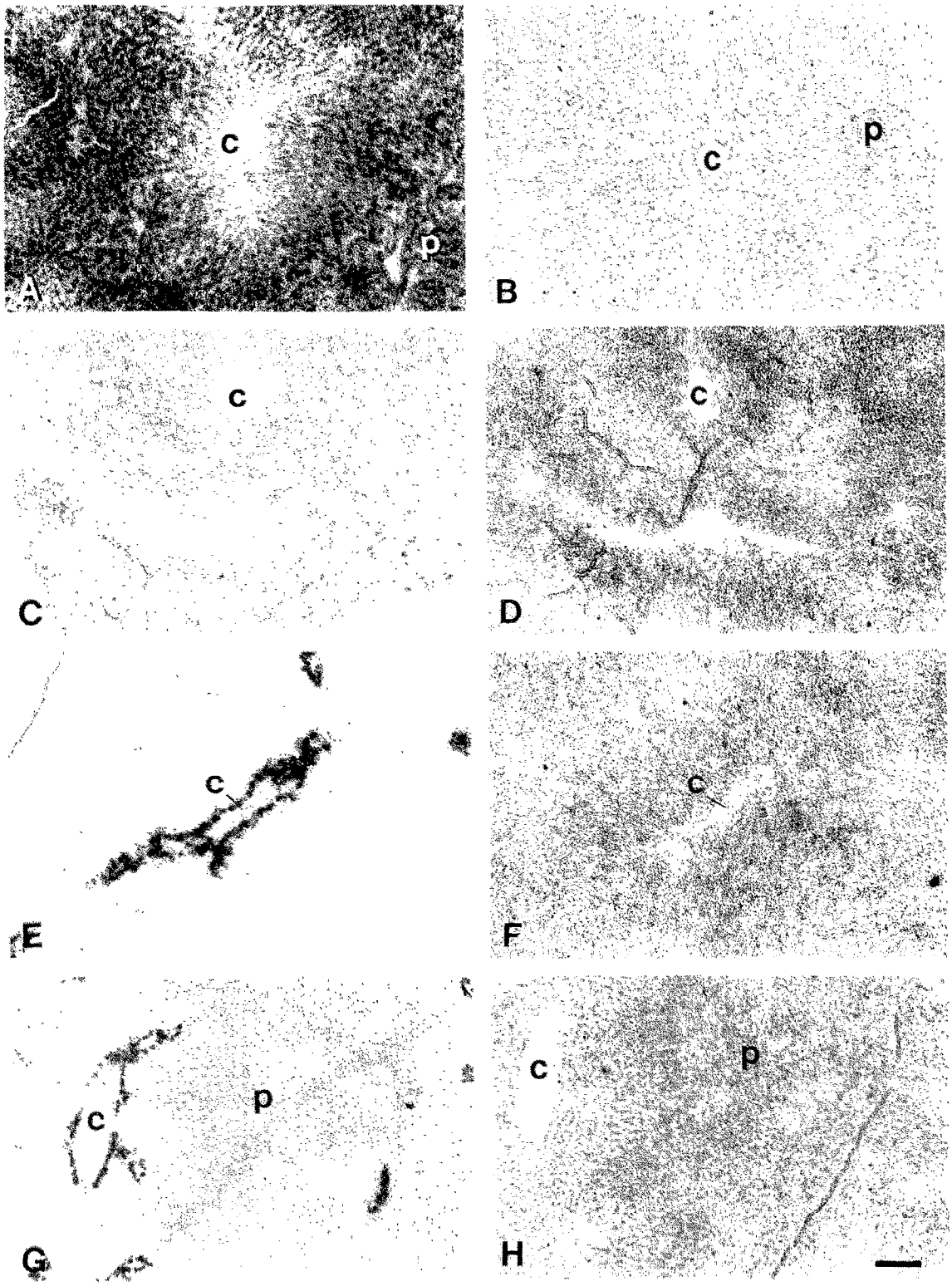

Fig. 2. Glucose-induced pattern of expression of GK mRNA in the liver lobule of starved, adults rats. The hybridocytochemical analysis was carried out on liver sections from starved rats after 0 (A,B), 2 (C,D), 4 (E,F) and 6 (G,H) h glucose refeeding, using probes for PEPCK mRNA (A,C), GK mRNA (B,D,F,H) and GS mRNA (E,G). p, portal tract; c, central venule. Bar = 0.2 mm.

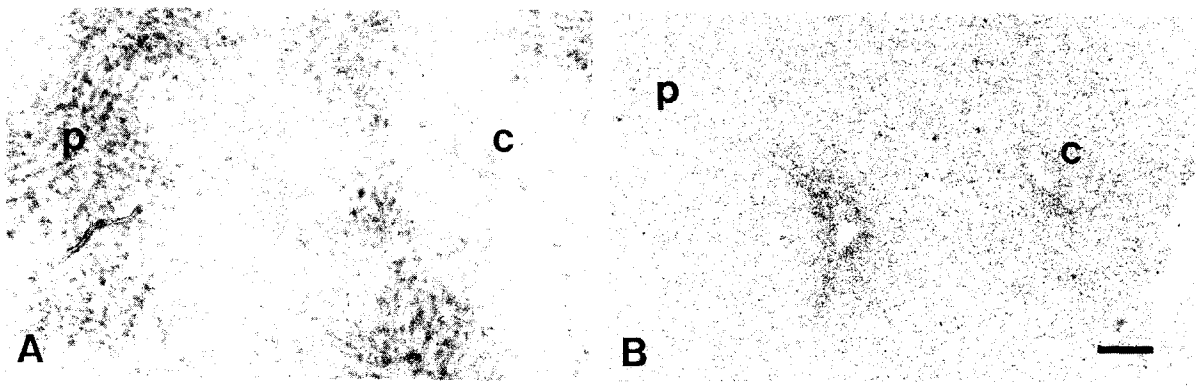

Fig. 3. Dexamethasone-induced pattern of expression of GK mRNA in the liver lobule of adult rats. Sections were hybridized with probes for PEPCK mRNA (A) and GK mRNA (B). p, portal tract; c, central venule. Bar = 0.2 mm.

much more accessible to cDNA probes or antibodies than adult tissues. In neonatal liver both CPS protein [30] and RNA [14] seem to be expressed to a higher level than in adult livers, as determined by immuno- and hybridocytochemistry, respectively, whereas it is known from the respective developmental profiles that it is the other way around [31]. Hence, one should be aware that hybridocytochemistry does not allow quantitative statements on RNA levels in different developmental stages.

Finally, the ultimate factors that determine the enzymic zonation in the liver have to be established yet. This communication strengthens the notion that this zonation basically depends on a topographical modulation of specific gene regulation. It requires the concerted action of many regulatory factors that a bewildering array of sequence motifs within a promoter to achieve fine control of gene expression. As the structure of many liver-characteristic genes has now been determined and many transcription factors have been described, we are in the position to analyze the patterns of expression of these regulatory factors/receptors in the liver in order to define which factors are crucial for the dynamics of enzymic zonation in vivo.

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