Hepatic glutaminase mRNA is confined to part of the urea cycle domain in the adult rodent liver lobule

Antoon F.M. Moorman[a,*], Piet A.J. de Boer[a], Malcolm Watford[b], Maria A. Dingemanse[a], Wouter H. Lamers[a]

[a]*Academic Medical Center, University of Amsterdam, Department of Anatomy and Embryology, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands*
[b]*Department of Nutritional Sciences, Cook College, Rutgers, The State University, New Brunswick, NJ 08903, USA*

Abstract This in situ hybridization study describes the developmental appearance of the lobular distribution of the mRNA encoding hepatic glutaminase in normal rat liver. Glutaminase has been proposed to provide the urea cycle with ammonia [Häussinger and Gerok (1983) Eur. J. Biochem. 133, 269–275]. Hence, the (developmental) pattern of expression of the mRNA would be expected to be closely linked to that of the urea cycle enzymes. From embryonic day 20 onward, hepatic glutaminase mRNA can be detected along the entire porto-central axis, with predominant expression in the portal area. In the adult phenotype, which is acquired at the end of the first postnatal week, glutaminase mRNA is no longer present along the entire porto-central distance but has become confined to a relatively small periportal domain in which the expression decreases in a porto-central direction. Thus, in contrast to the large periportal domain, in which the urea cycle enzymes are expressed, the glutaminase mRNA-expressing domain is much smaller and not contiguous with the glutamine synthase mRNA-expressing pericentral domain, leaving a midlobular area that is devoid of glutaminase mRNA. A similar pattern of distribution was found in adult mouse liver. The significance of these observations is that, within the liver lobules, there is an area in which glutaminase is not expressed and, hence, glutamine can not be the substrate for urea synthesis.

Key words: Glutaminase; In situ hybridization; Liver; Urea cycle

1. Introduction

Hepatocytes in the adult mammalian liver are arranged in regular functional units or 'metabolic lobules' [1]. The presence of an upstream periportal and a downstream pericentral domain in these units suggests that the molecular phenotype of a particular hepatocyte depends on its position on the porto-central axis. However, the confines of these domains are dynamic and depend on the metabolic state of the animal [2].

The homeostatic function of the liver requires that the opposite processes have to be performed within the same organ. In the mammalian liver such opposite processes are spatially separated, occupying either the periportal or the pericentral domain. Thus, gluconeogenesis, urea synthesis and glutamine hydrolysis predominate in the upstream domain, whereas glycolysis and glutamine synthesis are predominant in the downstream region [2,3].

A unique isoform of glutaminase is expressed in the liver, the ammonia-activated glutaminase (EC 3.5.1.2) [4]. This enzyme hydrolyses glutamine into ammonia and glutamate, important substrates for ureagenesis and gluconeogenesis [5]. Hence, it would be expected that hepatic glutaminase is localized to a similar region in the lobule as the urea cycle enzymes, particularly carbamoylphosphate synthase (I) which fixes ammonia into carbamoylphosphate, and phospho*enol*pyruvate carboxykinase, the marker enzyme for gluconeogenesis. Carbamoylphosphate synthase (I) protein [6] and mRNA [7] have been shown to be localized in a large periportal domain that is contiguous with the small glutamine synthetase-expressing pericentral domain. Phospho*enol*pyruvate carboxykinase protein [8] and mRNA [9,10] are also present in a periportal area which is, however, not as large as that of the urea cycle enzymes.

There is functional evidence that glutaminase is predominantly present in the periportal hepatocytes [11], and it has been shown that isolated periportal hepatocytes have a 2–3 fold higher glutaminase activity and mRNA level than pericentral hepatocytes [12]. However, the digitonin/collagenase perfusion technique used to isolate periportal and pericentral hepatocytes does not permit the establishment of the precise intralobular extent of the glutaminase-expressing periportal domain in relation to other marker enzymes/mRNAs. To this end we have carried out an in situ hybridization study to unambiguously localize the glutaminase mRNA-expressing domain in normal adult rat liver in relation to other marker mRNAs encoding glutamine synthase and carbamoylphosphate synthase (I).

2. Materials and methods

2.1. Animals

Wistar rats and Swiss mice were obtained from the HSD animal farm (Zeist, The Netherlands) and maintained on a standard diet and water ad libitum. Fetal and neonatal age were calculated from dated matings and time of birth, respectively. Adult mice and rats were approximately 3 months of age.

2.2. Hybridocytochemical analysis

Formalin fixation, paraffin embedding and sectioning, pretreatment of the sections, hybridization conditions, and signal visualization have been described in detail [13]. At variance to this protocol riboprobes were used rather than cDNA probes. Consequently, hybridization and wash temperatures were raised to 54°C and an additional incubation with RNase A (10 μg/ml) for 30 min at 37°C was included after the second wash.

α-^{35}S-Labelled transcripts were made by in vitro transcription of linearized pBluescript in which the appropriate restriction fragment was subcloned. As probes for the detection of hepatic glutaminase, carbamoylphosphate synthase, glutamine synthase and albumin mRNAs, the following sequences were used, respectively: (i) the entire insert (1000 bp) of the hepatic glutaminase cDNA clone [14], (ii) the 564 bp *Bam*HI–*Sma*I DNA fragment localized at position 719–1283 of the carbamoylphosphate synthase (I) cDNA clone CPS-KdG [15], (iii) the

---

*Corresponding author. Fax: (31) (20) 697 6177.

1025 bp *KpnI–TaqI* DNA fragment localized at position 235–1260 of the glutamine synthase cDNA clone pGS4 clone [16], and (iv) the entire insert (1034 bp) of the albumin cDNA clone ALB1 [17]. To allow efficient hybridization, RNA transcripts longer than 200 nucleotides were subjected to alkaline hydrolysis to yield fragments of a mean size of 100–150 nucleotides as assessed by polyacrylamide gel-electrophoresis. Probe concentration was approximately 30 pg (specific activity 1700 cpm/pg) per μl hybridization solution, and approx. 6 μl hybridization mixture was applied per section. No coverslips were used to improve efficiency of hybridization and to allow easy comparison of serial sections incubated with different probes and controls.

3. Results

Hybridization to α-³⁵S-labelled probes was visualized by bright-field microscopy, revealing positive signals as black silver grains. The distinct patterns of hybridization obtained with the different probes are considered important tissue-intrinsic controls that are imperative for reliable mRNA localizations [13]. Fig. 1 demonstrates that specific hybridization is being achieved. In agreement with previous work [14], glutaminase mRNA could not be detected in fetal liver and other tissues, such as spinal cord, kidney and intestine, whereas albumin mRNA (that served as a positive control) was found to be exclusively expressed in the liver.

3.1. Development appearance

Up until 18 days of development hepatic glutaminase mRNA can not be detected in liver or other organs. At embryonic day 20, glutaminase mRNA can first be detected (Fig. 2a): it is present in all hepatocytes, but the density of grains decreases in the porto-central direction. Glutamine synthase mRNA (Fig. 2b), that at this stage is still expressed in all hepatocytes (cf. [18]), is present in a central-to-portal gradient and as such identifies the central venules. In newborn rat liver glutaminase mRNA is strongly expressed in all hepatocytes and a clear porto-central gradient can be recognized (Fig. 2c). From this

stage onwards glutamine synthase mRNA is strongly expressed in a small pericentral area only (Fig. 2d,f). 5 days after birth the adult pattern of expression has almost been achieved and glutaminase mRNA is no longer expressed in all hepatocytes (Fig. 2e).

3.2. The adult pattern of expression

In adult rat liver, glutaminase mRNA is present in a clearcut porto-central gradient, background levels being reached midway along the porto-central distance (Fig. 3a). Its domain of expression is therefore not contiguous with the pericentral glutamine synthase-expressing area (Fig. 3b) and is distinctly smaller than that of carbamoylphosphate synthase (Fig. 3c). A similar pattern of expression is observed in adult mouse liver (Fig. 3d and e).

4. Discussion

The mammalian liver plays an important role in the regulation of circulating glutamine levels as it has the capacity for both glutamine synthesis and glutamine degradation. In isolated perfused liver, pericentral glutamine synthesis and periportal glutamine degradation take place simultaneously due to the concurrent action of pericentrally localized glutamine synthase and periportally localized glutaminase [11,19]. This study shows that the mRNAs encoding glutaminase and glutamine synthase are expressed in distinct periportal and pericentral populations of hepatocytes, respectively. Previously, it was demonstrated that glutaminase mRNA is two-fold enriched in isolated periportal hepatocytes [12]. However, the techniques used to separate periportal and pericentral hepatocytes do not permit the zonation of a particular mRNA or protein to be accurately established. To this end in situ hybridization or immunohistochemistry is a more suitable technique.

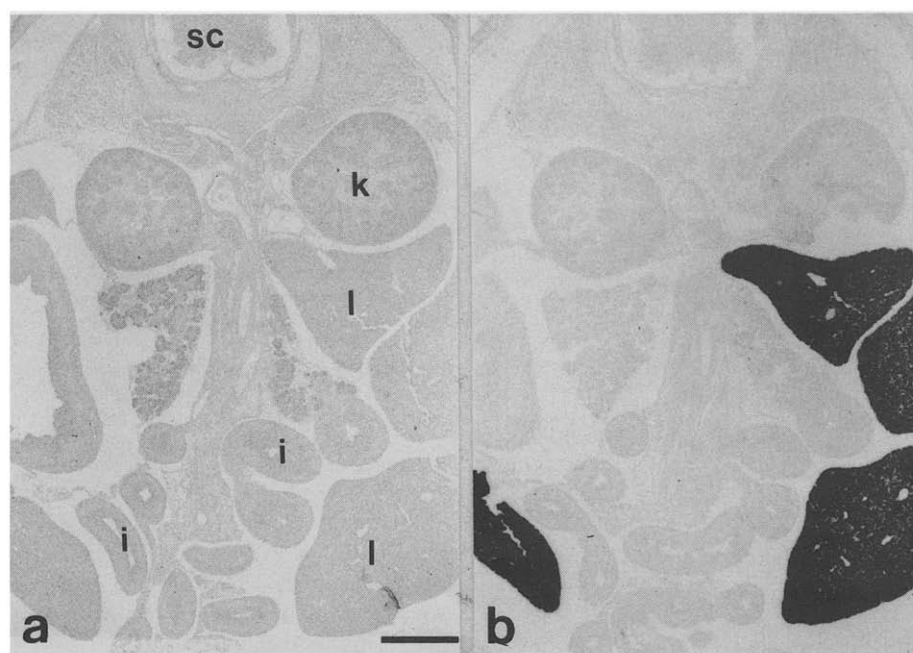This study shows that there is no overlap in the expression

Fig. 1. Absence of hepatic glutaminase mRNA from fetuses of 18 days of development. Serial sections were allowed to hybridize with ³⁵S-labelled probes for hepatic glutaminase mRNA (a) and, as a positive control, albumin mRNA (b). sc, spinal cord; k, kidney; i, intestine; l, liver. Bar = 200 μm.
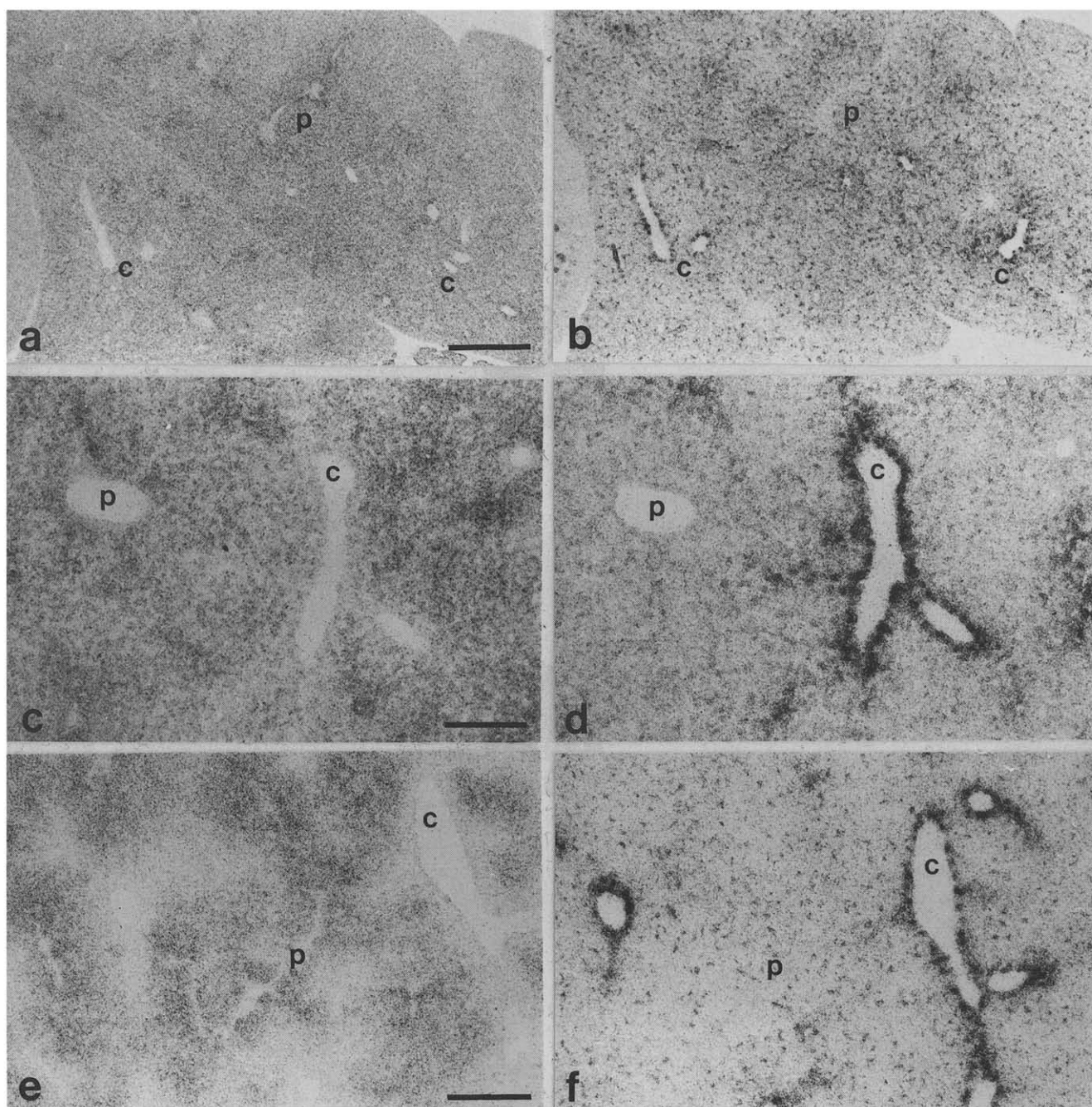
Fig. 2. Ontogenesis of glutaminase mRNA heterogeneity in developing liver. Hybridization analysis was carried out on liver sections of 20-day-old fetuses (a,b), and of neonates at 2.5 h (c,d) and 5 days (e,f) after birth. Sections were allowed to hybridize with ^{35}S-labelled probes for hepatic glutaminase mRNA (a,c,e) and, to identify the central venules, glutamine synthase mRNA (b,d,f). p, portal venule; c, central venule. Bar = 500 μm.

of the mRNAs encoding glutaminase and glutamine synthase. On the contrary, in between the glutaminase mRNA-expressing periportal domain and the glutamine synthase-expressing pericentral domain there is a zone where neither glutaminase mRNA nor glutamine synthase mRNA is expressed but in which the mRNA encoding the ammonia-fixing enzyme carbamoylphosphate synthase (I) is expressed. A similar pattern of expression was found in adult mouse liver. The relatively small periportal zone of glutaminase expression explains the high ratio of glutaminase mRNA concentration that is found in isolated periportal and pericentral cells [12].

For several reasons the observations presented here were unexpected. (i) Hydrolysis of glutamine by the action of glutaminase yields the substrates for the intimately associated gluconeogenic and ureagenic pathways (see [20] for a review). (ii) Glutamine-derived ammonia has been shown to be the preferential substrate for carbamoylphosphate synthase (I) [21]. (iii) Both glutaminase [22] and carbamoylphosphate synthase (I) [23] share a mitochondrial localization and are closely linked with the mitochondrial inner membrane. (iv) The periportally localized glutaminase would raise the intramitochondrial ammonia concentration and, hence, facilitate the channel-
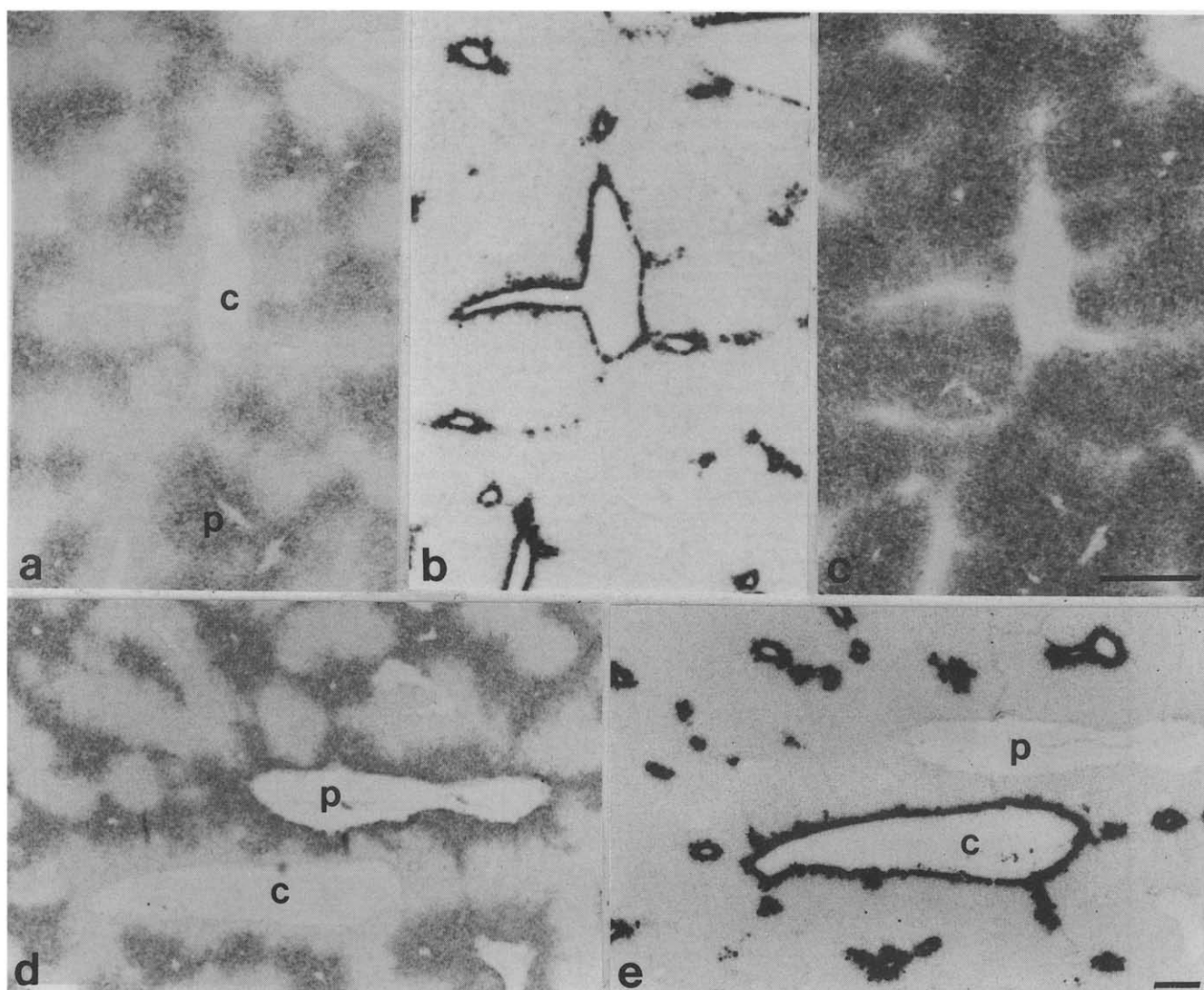
Fig. 3. Adult pattern of glutaminase mRNA (a,d) compared with that of glutamine synthase mRNA (b,e) and carbamoylphosphate synthase mRNA (c) in rat (a,b,c) and mouse (d,e) liver. p, portal venule; c, central venule. Bar = 500 μm.

ing of ammonia into the urea cycle by carbamoylphosphate synthase that has a relatively low affinity for ammonia [19,24]. (v) In line with these ideas, the urea cycle enzymes, gluconeogenic enzymes and hepatic glutaminase share a comparable pattern of regulation by diet and hormones, and all show a strong upsurge in the first postnatal week ([14,25–30] and this study).

Despite this common pattern of regulation, and the common subcellular localization of glutaminase and carbamoylphosphate synthase, the periportal domain expression of hepatic glutaminase mRNA is much smaller than that of carbamoylphosphate synthase and more like that of phosphoenolpyruvate carboxykinase [9,10,31]. The functional significance of this remarkable difference is puzzling, since it raises the question of how the low-affinity carbamoylphosphate synthase is provided with sufficient ammonia. Obviously, metabolic studies are required to elucidate the handling of ammonia in the 'glutaminase-free' midlobular zone of the liver.

References

[1] Lamers, W.H., Hilberts, A., Furt, E., Smith, J., Jones, C.N., van Noorden, C.J.F., Gaasbeek Janzen, J.W., Charles, R. and Moorman, A.F.M. (1989) Hepatology 10, 72–76.
[2] Jungermann, K. and Katz, N. (1989) Physiol. Rev. 69, 708–764.
[3] Häussinger, D., Lamers, W.H. and Moorman, A.F.M. (1992) Enzyme 46, 72–93.
[4] Smith, E.M. and Watford, M. (1988) Arch. Biochem. Biophys. 260, 740–751.
[5] Aikawa, T., Matsutaka, H., Takezwa, K. and Ishikawa, E. (1972) Biochim. Biophys. Acta 279, 234–244.
[6] Gaasbeek Janzen, J.W., Gebhardt, R., ten Voorde, C.H.J., Lamers, W.H., Charles, R. and Moorman, A.F.M. (1987) J. Histochem. Cytochem. 35, 49–54.
[7] Moorman, A.F.M., de Boer, P.A.J., Geerts, W.J.C., van de Zande,

L.P.W.G., Charles, R. and Lamers, W.H. (1988) J. Histochem. Cytochem. 36, 751–755.

[8] Anderson, B., Nath, A. and Jungermann, K. (1982) Eur. J. Cell Biol. 28, 47–53.

[9] Bartels, H., Linnemann, H. and Jungermann, K. (1989) Febs Lett. 248, 188–194.

[10] Moorman, A.F.M., de Boer, P.A.J., Charles, R. and Lamers, W.H. (1990) Febs Lett. 276, 9–13.

[11] Häussinger, D. (1983) Eur. J. Biochem. 133, 269–275.

[12] Watford, M. and Smith, E.M. (1990) Biochem. J. 267, 267.

[13] Moorman, A.F.M., de Boer, P.A.J., Vermeulen, J.L.M. and Lamers, W.H. (1993) Histochem. J. 25, 251–260.

[14] Smith, E.M. and Watford, M. (1990) J. Biol. Chem. 265, 10631–10636.

[15] de Groot, C.J., Zonneveld, D., de Laaf, R.T.M., Dingemanse, M.A., M.A., Mooren, P.G., Moorman, A.F.M., Lamers, W.H. and Charles, R. (1986) Biochim. Biophys. Acta 866, 61–67.

[16] van de Zande, L.P.W.G., Labruyère, W.T., Arnberg, A.C., Wilson, R.H., van den Bogaert, A.J.W., Das, A.T., van Oorschot, D.A.J., Frijters, C., Charles, R., Moorman, A.F.M. and Lamers, W.H. (1990) Gene 87, 225–232.

[17] de Groot, C.J., van Zonneveld, A.J., Mooren, P.G., Zonneveld, D., van den Dool, A., van den Bogaert, A.J.W., Lamers, W.H., Moorman, A.F.M. and Charles, R. (1984) Biochem. Bioph. Res. Commun. 124, 882–888.

[18] Moorman, A.F.M., de Boer, P.A.J., Das, A.T., Labruyère, W.T., Charles, R. and Lamers, W.H. (1990) Histochem. J. 22, 457–468.

[19] Häussinger, D., Gerok, W. and Sies, H. (1984) TIBS 300–302.

[20] Meijer, A.J., Lamers, W.H. and Chamuleau, R.A.F.M. (1990) Physiol. Rev. 70, 701–748.

[21] Meijer, A.J. (1985) Febs Lett. 191, 249–251.

[22] McGivan, J.D., Lacey, J.H. and Joseph, S.K. (1980) Biochem. J. 192, 537–542.

[23] Powers-Lee, S.G., Mastico, R.A. and Bendayan, M. (1987) J. Biol. Chem. 262, 15683–15688.

[24] Häussinger, D. (1990) Biochem. J. 267, 281–290.

[25] Lamers, W.H., Hanson, R.W. and Meisner, H. (1982) Proc. Natl. Acad. Sci. USA 79, 5137–5141.

[26] Meisner, H., Lamers, W.H. and Hanson, R.W. (1983) Trends Biochem. Sci. 8, 165–167.

[27] Iynedjian, P.B. and Salavert, A. (1984) Eur. J. Biochem. 145, 489–497.

[28] Christ, B., Nath, A., Bastian, H. and Jungermann, K. (1988) Eur. J. Biochem. 178, 373–379.

[29] Watford, M. (1993) FASEB J. 7, 1468–1474.

[30] Watford, M., Vincent, N., Zhan, Z., Fannelli, J., Kowalski, T. and Kovacevic, Z. (1994) J. Nutr. 124, 493–499.

[31] Bartels, H., Herbort, H. and Jungermann, K. (1990) Histochemistry 95, 637–644.