The EMBO Journal vol.4 no.10 pp.2487–2491, 1985

Dependence of hepatocyte-specific gene expression on cell-cell interactions in primary culture

Jean-Marc Fraslin, Bernard Kneip, Sophie Vaulont[1], Denise Glaise, Arnold Munnich[1] and Christiane Guguen-Guillouzo

INSERM U 49, Hôpital Pontchaillou, 35011 Rennes Cédex, and [1]INSERM U 129, C.H.U. Cochin, 75654 Paris, France

Communicated by D.Louvard

In co-culture with non-parenchymal liver epithelial cells, rat hepatocytes show a marked increase in albumin and total protein synthesis when compared with cells maintained as pure populations in which an early decline in albumin secretion takes place. Analysis of the relative amounts of different mRNA sequences, determined by hybridization, indicated that the increase in protein synthesis resulted essentially from an increased level of the corresponding mRNAs. In addition, when cell-cell contacts were established between the two cell types several days after the seeding of hepatocytes, the stimulation of albumin secretion was similarly observed with a significant increase of the corresponding mRNA on days 10–14 of culture. Transcriptional assays, in which isolated nuclei were used for the study of RNA synthesis, showed that liver-specific gene transcription was significantly increased and maintained for at least 2 weeks. These results demonstrate for the first time long-term stabilization and reversibility of various specific mRNAs at high levels by adult hepatocytes in primary culture. They suggest that establishment of cell-cell contacts between hepatocytes and liver epithelial cells are essential for the maintenance of a high rate of transcription of the liver-specific genes.

Key words: cell-cell interactions/differentiation/hepatocyte culture/*in vitro* transcription/RNA blot analysis

Introduction

Non-proliferating adult rat hepatocytes maintained under standard conditions for primary culture rapidly lose most of their differentiated functions within a few days, undergoing a shift towards a more fetal-like stage (Bissell and Guzelian, 1980; Guguen-Guillouzo and Guillouzo, 1983). The normal phenotype does not persist even in the presence of substances known to improve hepatocyte survival such as certain hormones and nutrients, collagen or more complex insoluble matrices (Michalopoulos and Pitot, 1975; Sirica *et al.*, 1979; Rojkind *et al.*, 1980). Furthermore, phenotypic changes are accelerated and amplified under certain culture conditions, especially those which favor cell attachment and spreading (Guguen-Guillouzo *et al.*, 1982). A prompt decrease in liver-specific gene transcription has been reported to be associated with this loss of tissue specificity in isolated adult mouse hepatocytes maintained as pure populations in primary culture (Clayton and Darnell, 1983), and recently Jefferson *et al.* (1984) have shown that adult rat hepatocytes maintained in a hormonally-defined medium contained high levels of liver-specific mRNAs by day 5 of culture. However, this maintenance was the result of a dramatic post-transcriptional stabilization in the absence of a transcriptional activity superior to that observed in cells cultured in a serum supplemented medium.

These findings suggest that all these culture conditions do not constitute an appropriate environment for the maintenance of liver-specific gene transcription. Therefore, we postulated that a signal, possibly mediated by interactions of hepatocytes with non-parenchymal liver cells, is required. We have recently demonstrated that, when adult hepatocytes were co-cultured with rat liver epithelial cells (RLEC), presumably derived from terminal biliary ductular cells, they survived >2 months and, in addition, specific functions including albumin secretion continued to be expressed at high levels (Guguen-Guillouzo *et al.*, 1983). This implies that specific cell-cell interactions might provide a stimulus responsible for the long-term maintenance of liver specificity. The present study supports this notion. We have identified changes in the amounts of a number of liver-specific proteins and of their corresponding mRNAs in rat hepatocyte co-culture. In addition, we have shown that the level of transcription for these mRNAs was stabilized at a higher rate in co-culture than under standard conditions.

Results

Hepatocytes attached to the substratum within 3 h and formed monolayers of parenchymal cells within 24 h. When cultured under conventional conditions, hepatocytes could not be maintained for >10 days and soon lost their characteristic morphology. By contrast, when co-cultured with RLEC most of the hepatocytes remained viable for at least 8 weeks. They maintained close contacts with RLEC and exhibited limited morphological changes. Bile canaliculi were visible throughout the culture time (Figure 1).

No mitotic figures were observed in parenchymal cells whether cultured alone or with RLEC. However, while in control cultures 50% of hepatocytes were labeled with [methyl^{3}H]thymidine on days 2 and 3, <5% co-cultured hepatocytes entered into S phase during the same period.

Total protein and albumin synthesis in co-cultured hepatocytes

Hepatocytes of pure and co-cultures were allowed to incorporate [^{3}H]leucine for 2 h at various times after cell seeding. Media and hepatocytes selectively detached from RLEC were collected. Cellular incorporation of [^{3}H]leucine in total proteins was similar in both control and co-cultures on day 2 (Figure 2). It was slightly diminished in controls on days 3 and 4 and became undetectable during subsequent days. In contrast, a 2-fold increase of incorporation was observed in co-cultured cells on days 3 and 4. This high level was maintained for at least 15 days.

The level of the labeled proteins secreted into the medium was higher in co-cultures than in controls and remained high during the culture period (Figure 2). Secreted albumin in media from co-cultures was found to increase, whereas it declined dramatically in control cultures within 5 days. The presence of labeled albumin in the medium provided evidence for active synthesis of this protein by co-cultured hepatocytes (Figure 2, inset).

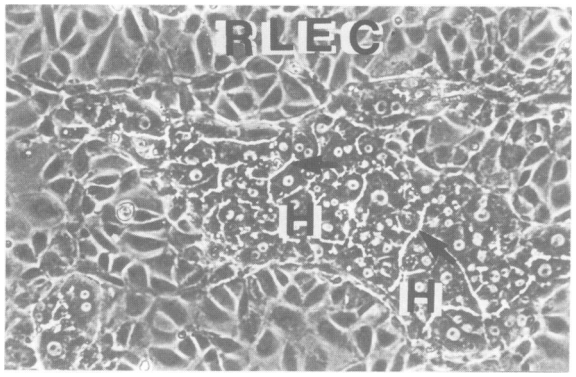

Fig. 1. Phase contrast micrograph of adult rat hepatocytes (H) co-cultured with rat liver epithelial cells (RLEC) for 20 days. Arrows: bile canaliculi (× 140).

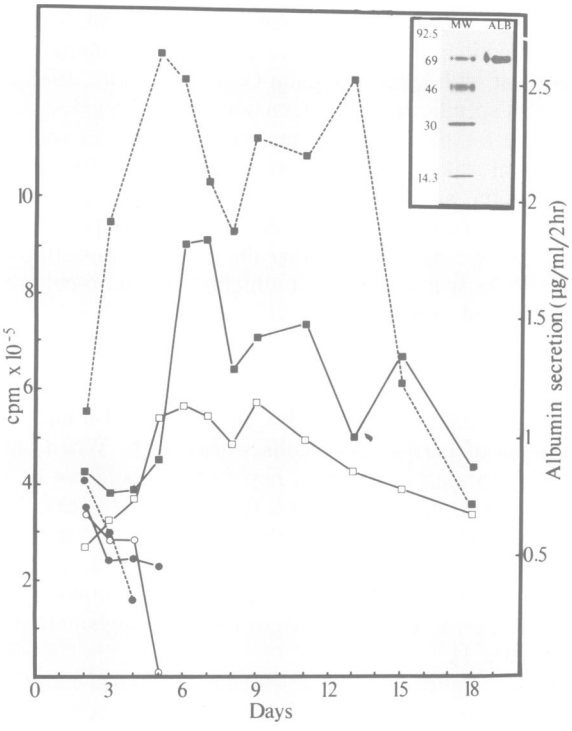

Fig. 2. Total protein synthesis and albumin secretion in conventional and mixed culture conditions. Co-culture (squares) and conventional culture (circles). Total protein synthesis (solid line) was measured by [^{3}H]leucine incorporation for 2 h. Incorporation is expressed either in c.p.m./mg of cellular protein (intracellular labeled proteins: ○,□,) or in c.p.m./ml medium (secreted labeled proteins: ●,■,). Albumin secretion (dotted line) measured by laser immunonephelometry, is expressed in µg/ml medium/2 h. **(Inset).** Immunoprecipitation of labeled albumin in co-culture medium (one-dimensional electrophoretic gel). (MW) Standard proteins with their mol. wt. in kd; (ALB) immunoprecipitation of albumin.

Long-term presence of liver-specific mRNAs in co-cultured hepatocytes

To provide evidence for long-term maintenance of specific mRNAs during co-culture, we determined the relative concentration of liver-specific mRNA sequences in cultured hepatocytes as a function of time. Equal aliquots of RNA samples, prepared at various times of culture, were hybridized to nick-translated cDNA probes (Figure 3). Albumin mRNA was high during the first 2 days in both control and co-cultured hepatocytes. This mRNA dramatically declined in controls within 3 days and disappeared after 5 days, whereas in co-culture it gradually increased until day 8 and remained unchanged up to day 12.

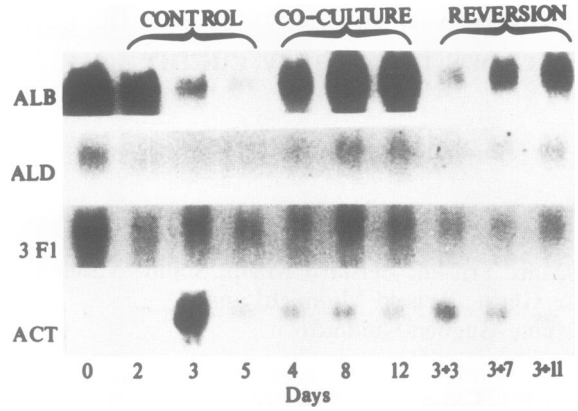

Fig. 3. Northern blot analysis of different mRNA concentrations in hepatocyte cultures as a function of time. (0) freshly isolated hepatocytes; (control) hepatocytes under conventional culture conditions; (co-culture) hepatocytes under co-culture conditions; (reversion) hepatocytes maintained for 3 days under conventional culture, then co-cultured with RLEC for different times. Specific RNAs: albumin (ALB), aldolase B (ALD), liver-specific mRNA (3F1). Common RNAs: actin (ACT).

In addition, equal amounts of RNA were blotted onto nitrocellulose filters and hybridized to labeled cDNA probes (Figure 4a). In co-culture, albumin mRNA showed a transient decrease until day 4 of co-culture, then increased and remained at a high level. By comparing the radioactive counts we estimated that the amount of albumin RNA sequences decreased first to ~25% of its initial level and then increased 2-fold (Figure 4b). In contrast, albumin mRNA from the controls decreased dramatically and was undetectable by day 5.

Two other liver-specific mRNAs, aldolase B and 3F1, changed concomitantly with albumin mRNA in co-cultured hepatocytes and remained at a high level (Figure 3). In control cells they showed an early decrease. The concentration of transferrin mRNA decreased less dramatically in control cells but remained very stable in co-culture. More striking was the complete disappearance of pyruvate kinase L mRNA in the controls as early as day 2 while being maintained at a high level in co-culture (Figure 4).

By contrast, the non tissue-specific actin mRNA was found to be undetectable in freshly isolated cells but strongly elevated in control cells with a maximum on day 3, after which it declined. In co-culture it remained expressed at a low rate (Figure 3). In RLEC, albumin and 3F1 mRNA sequences were not found, but actin mRNA was present at the same level as in cultured hepatocytes (data not shown).

Transcription of liver-specific mRNA sequences in co-cultured hepatocytes

The experiments described above do not indicate whether the increase in mRNA which accompanied active albumin synthesis in co-cultured cells resulted from an increase of the mRNA stability or whether a change of the transcriptional activity took place. To answer this question we carried out two types of experiments.

First, we performed 'reversion' experiments to examine the effect on the level of different mRNA sequences of delayed addition of RLEC to hepatocyte cultures. Northern blots and radioactive counts of the dots showed that on day 3, at the time of RLEC addition, albumin mRNA was reduced to 10% of its

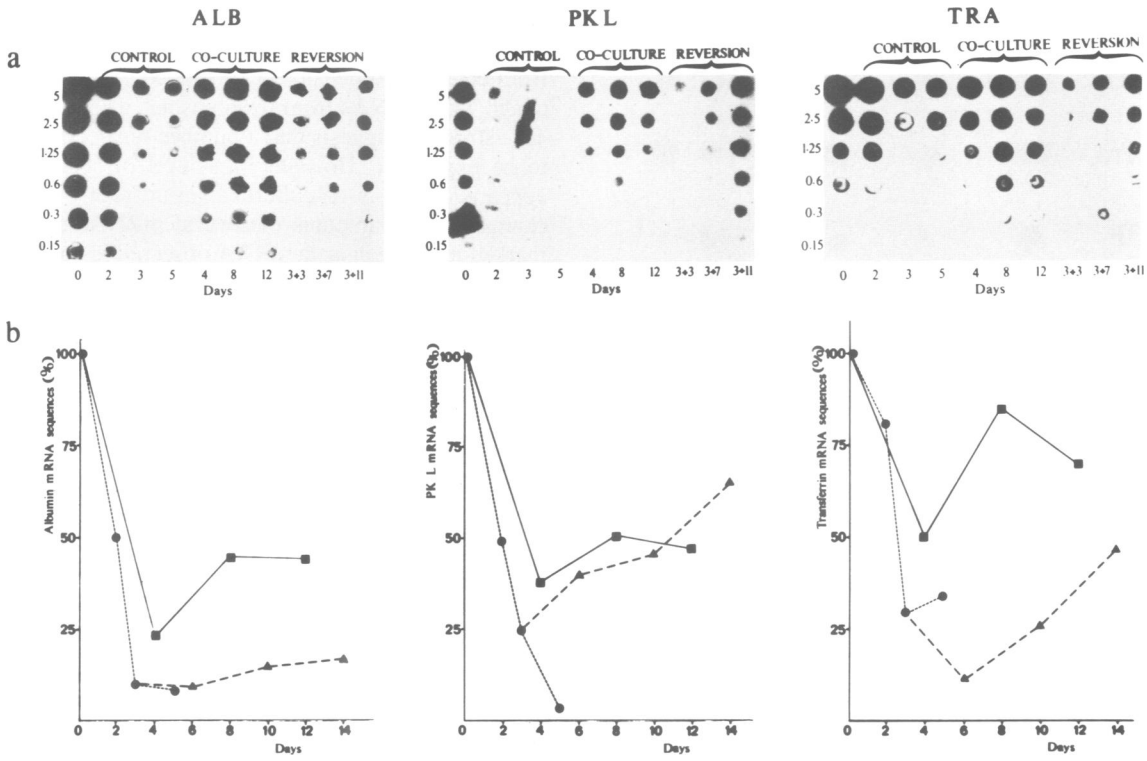

Fig. 4. Dot blot autoradiographs **(a)** and graphic representation of radioactive counts **(b)** of specific mRNA concentrations isolated from the different sources described in the legend to Figure 3. ●: control culture, ■: co-culture, ▲: reversion. Specific RNAs: albumin (ALB), pyruvate kinase L (PKL), transferrin (TRA). Data are given as a percentage of the freshly isolated hepatocyte values.

initial value (Figures 3,4). This level remained low during the following 3 days, but had doubled by day 14. Assays of the other liver-specific mRNAs gave similar results even more striking in the case of pyruvate kinase L.

Secondly, we carried out *in vitro* transcription assays in isolated nuclei from control and co-cultured cells. Nuclei were isolated at 0°C and the previously initiated RNA chains were allowed to elongate in the presence of [α-^{32}P]UTP. Elongated transcripts were then isolated and hybridized to 'gene screen plus' filters containing DNA from various liver-specific genes. Equal amounts of labeled nuclear RNA were used so that the differential transcription rates could be compared. Figure 5 shows the results of such an assay: (i) a rate of transcription in co-cultures twice that of pure cultured hepatocytes on day 4; (ii) no significant change of this level of transcriptional activity for at least 2 weeks in co-cultured cells; (iii) a variation in transcripts from one gene to another when compared with freshly isolated hepatocytes. In co-cultured cells, the transcription of the transferrin gene continued at a high rate corresponding to 60−80% of the initial value, whereas the transcription of the more specific genes such as albumin was retained at a rate of 20−40%.

Discussion

Our experiments show that after establishing cell-cell interactions with a non-parenchymal liver cell type, adult rat hepatocytes retained the ability to synthesize high levels of liver-specific proteins for several weeks. This event was due to a corresponding maintenance of mRNA levels and the data suggest that the mechanism responsible for this functional stability is, at least in part, of transcriptional origin. Moreover, mRNAs, especially those corresponding to specific functions, reverted to high levels following addition of liver epithelial cells to 3-day-old hepatocyte cultures.

Previous observations with pure hepatocytes cultured in various media and on several substrates revealed rapid phenotypic changes and loss of specific transcriptional activity. A dramatic decline of liver-specific mRNAs was found as early as day 2 or 3 in pure cultures of adult mouse and rat hepatocytes (Clayton and Darnell, 1983; Jefferson *et al.*, 1984). By contrast, in co-cultured hepatocytes, an early increase in protein synthesis and albumin secretion was observed by day 4 reaching a maximum on day 6, and large amounts of various specific mRNA sequences were still present by day 12. Furthermore, there was a good correlation between the increase in protein synthesis and the level of corresponding mRNAs. Therefore, the principal factor, if not the only one, in determining the rate of protein synthesis during co-culture appears to be the mRNA level. The increased amounts of liver-specific mRNAs following late addition of RLEC during reversion also supports this conclusion.

Hybridization of equal amounts of total labeled RNA from isolated nuclei revealed a 2-fold increase in the transcription level of various liver-specific genes in co-cultures compared with controls on day 4. This corresponded to the critical time beyond which some cells detached when maintained under standard conditions. Thus, the level of transcription in co-cultured cells was retained at a rate which varied from 20 to 80% of that found in freshly isolated cells, depending on the specific genes studied. Furthermore, since the level of specific mRNAs was increased in hepatocyte cultures by late addition of RLEC, it is likely that a transcriptional event is also involved in these 'reverted' cultures. By contrast, Jefferson *et al.* (1984) demonstrated that even in a hormonally-defined medium, in which specific functional activities are better maintained, the transcription level of specific genes remained very low. Moreover, switching the basal culture medium to the hormonally defined one at 72 h resulted only in stabilization of the remaining mRNAs.

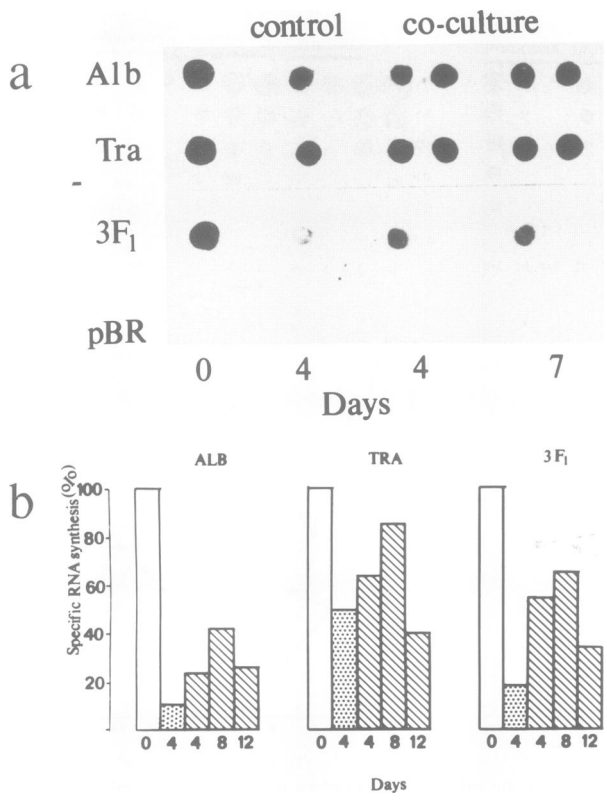

Fig. 5. Transcription rate analysis of specific mRNAs in hepatocyte cultures as a function of time. Typical autoradiograph **(a)** and graphic representation of radioactive counts **(b)**. Equal amounts of labeled RNAs, transcribed *in vitro* in the 'run-off' transcription system, were hybridized with an excess of specific cDNAs. Albumin (ALB), transferrin (TRA) and liver-specific mRNA (3F1). Radioactivity was eluted and measured by liquid scintillation. Data are given as a percentage of the freshly isolated hepatocyte values. □: freshly isolated hepatocytes; ▨: control cultures; ▧: co-cultures.

Therefore, part of the mechanism involved in long-term functional stability in co-culture is apparently of transcriptional origin and this increased transcription is sufficient to allow the cells to survive for several weeks and to express differentiated functions. This efficient liver-specific transcription could be dependent upon a factor provided by the rat liver epithelial cells.

However, the increase of transcription cannot be established as the major basis for persistence of the mRNAs. The maintenance of albumin, transferrin and 3F1 mRNAs may partly be due to a stabilization of these mRNAs. In addition, it is very likely that co-culture also induces other molecular event(s) essential for hepatocyte survival and functions as assessed by (i) cell survival which reaches 2 months; (ii) the reversion which implies an increase of specific mRNA sequences as shown for PKL; (iii) the frequent correlation between the rate of transcription and the level of mRNAs; (iv) the existence of a critical state, at day 4 of culture, beyond which the reversion is no longer possible. This state indicates that the events that occur during the first days of culture involve molecular alterations rather than profound irreversible cellular damage. This is consistent with the important modifications of chromatin phosphoproteins at this time of culture (Guguen-Guillouzo *et al.*, 1978). It may be noted that even if RLEC were added as early as 3 h after seeding of hepatocytes, a transient decline of mRNAs was observed.

Since only a few specific RNAs were studied, we cannot affirm that the total transcriptional apparatus was preserved in co-culture. However, various liver-specific functions, including secretion of several plasma proteins (Guillouzo *et al.*, 1984) and drug metabolism (Bégué *et al.*, 1984) have already been found to remain active. In addition, the translational products of mRNAs extracted from co-cultured hepatocytes included most of the proteins obtained with mRNAs from freshly isolated cells (data not shown). This strongly suggests that a number of specific genes continue to be expressed. However, the level of specific transcription appeared lower in co-cultures than in freshly isolated cells and changes in the amounts of several mRNAs might occur. The marked increase in actin mRNA observed in co-culture, although less than under standard conditions, could correspond to a general change of cell morphology during attachment and spreading. This might be considered as an obligatory adaptation of hepatocytes to the culture environment. An increase of actin mRNA was also observed in cultured mouse hepatocytes (Clayton and Darnell, 1983). A significant change in the amount of extractable actin has also been found in adipocytes accompanying their transformation from fibroblastic to a spherical shape (Spiegelman and Green, 1980).

Maintenance or reversion of specific gene transcription when hepatocytes were co-cultured with a non-parenchymal cell type provide evidence that factors other than nutrients may act on expression of liver-specific genes. Cell-cell contacts appeared essential since RLEC could not be replaced by either a conditioned medium or a cell extract (Guguen-Guillouzo *et al.*, 1983). The need for such cell-cell contacts suggests a key role for plasma membranes rather than for a soluble factor. However, the nature of the signal remains unknown. It may involve specific cell adhesion *via* a molecule similar to the L-Cam (Bertolotti *et al.*, 1980; Gallin *et al.*, 1983) and/or extracellular matrix components (Sirica *et al.*, 1979; Rojkind *et al.*, 1980; Guguen-Guillouzo *et al.*, 1983). Cell-cell contacts have already been reported as being necessary for liver differentiation during embryogenesis (Houssaint, 1980; Thiery *et al.*, 1984).

Hepatocyte co-cultures represent an attractive model for understanding the mechanisms which control liver functions. Moreover, since this system can be applied to human hepatocytes (Clément *et al.*, 1984), it affords a unique tool for studying liver function in man, in normal and pathological conditions.

Materials and methods

Isolation of hepatocytes and culture procedures

Hepatocytes from adult male Sprague-Dawley rats weighing 180–200 g were isolated by canulating the portal vein and perfusing the liver with a collagenase solution according to the method of Seglen (1973). Cells were collected in L-15 medium enriched with 0.1% bovine albumin and 5 μg/ml bovine insulin, and left to sediment for 20 min. After two centrifugations they were counted and tested for viability. The cells were suspended in HAM F12 medium containing 0.1% bovine albumin, 5 μg/ml bovine insulin, and 10% fetal calf serum. Usually, 1.6×10^7 cells were plated in 30 ml of medium in 180 cm^2 Nunclon flasks. The medium was changed 3–4 h later and for co-cultures 1.4×10^7 RLEC were seeded per flask in order to reach confluence within 24 h. The medium was thereafter supplemented with 7×10^{-5} M and 3.5×10^{-6} M hydrocortisone hemisuccinate for controls and co-cultures, respectively, and changed every day. RLEC were originally isolated by trypsinization of 10-days old rat livers and maintained by serial subculture in Williams' E medium supplemented with 10% fetal calf serum (Williams and Gunn, 1974).

For biochemical assays, co-cultured hepatocytes were selectively detached from RLEC by incubation in a calcium-free Hepes-buffered (pH 7.6) collagenase solution for 10 min.

Determination of DNA synthesis was performed by radioautography after incubating cell cultures with 1 μCi per ml of [methyl-^{3}H]thymidine (25 Ci/mmol, Amersham) for 16 h every day.

Quantitation of radioactivity of newly synthesized proteins

Synthesis of intra- and extracellular proteins was measured by incubating hepatocytes under conventional or co-culture conditions with a medium containing 10 μCi/ml [4,5-^{3}H]leucine (25 Ci/mmol, Amersham) for 2 h. Trichloroacetic acid-

precipitable material was deposited on Millipore HA 0.45 μm filters and the radioactivity measured.

Albumin was quantified from culture media by laser immunonephelometry (Ritchie, 1975). Albumin immunoprecipitation was performed from the culture medium essentially as described by Walsh and Crumpton (1977). The immunoprecipitated protein was analyzed by SDS-polyacrylamide gel electrophoresis as described by Laemmli (1970). The labeled albumin was detected by radioautography on the dried gel.

Preparation of RNA from hepatocytes

The extraction procedure of Glisin *et al.* (1974) was used to prepare total RNA from hepatocytes, with the modifications developed by Chirgwin *et al.* (1979) and Raymondjean *et al.* (1983). Typically, the content of four 180 cm^2 flasks was pooled for each preparation.

Electrophoresis of RNA

Gels buffered with 10 mM sodium phosphate (pH 7.4) and containing 1.1 M formaldehyde were cast from 1.5% agarose using a method adapted from Lehrach *et al.* (1977). 5–20 μg of total RNA was dissolved in 25 μl of buffer containing 10 mM sodium phosphate, 2.2 M formaldehyde, 0.5 mM EDTA, 50% formamide, heated at 65°C for 5 min and then 1/5 volume of 0.5% SDS, 25% glycerol, 25 mM EDTA, 0.025% bromophenol blue was added. Electrophoresis was carried out at 80 V for 4 h.

Northern and dot blot hybridization assays

5–20 μg of RNA were separated by electrophoresis and transferred onto a nitrocellulose filter as described by Thomas (1980). After baking for 3 h at 80°C, the Northern blot was prehybridized according to Andrews *et al.* (1982). Hybridization with 3×10^6 c.p.m./ml of [α-^{32}P]nick-translated DNA probe (Rigby *et al.*, 1977) was carried out for 18 h. Post-hybridization washing was performed at 65°C in excess 1 × SSC, 0.1% SDS.

Aliquots of 0.15–5 μg RNA were spotted onto a nitrocellulose filter that had been pre-treated with 20 × SSC (Thomas, 1980). The filter was baked at 80°C for 3 h; pre-hybridization and hybridization were as described above.

Preparation of nuclei

Cultured hepatocytes ($3-7 \times 10^7$) were collected and lysed with a Teflon Potter homogenizer (Braun), and the nuclei were isolated and washed as described by Clayton and Darnell (1983). They were then stored at −70°C according to Schibler *et al.* (1983).

Transcription rate assays

RNA synthesis *in vitro* using [α-^{32}P]UTP, extraction of labeled nuclear RNAs and hybridization were performed as described by Vaulont *et al.* (1984) with slight modifications adapted from Clayton and Darnell (1983): 50×10^6 nuclei instead of 8×10^6 were used and the reaction time for transcription was reduced to 20 min at 30°C. Typically, 2×10^7 c.p.m. of the labeled RNA sequences were hybridized in 200 μl of hybridization mixture (Vaulont *et al.*, 1984) to 6 μg of recombined pBR plasmids immobilized on 5 mm diameter 'gene screen plus' (New England Nuclear) filters. After washing and radioautographic exposure, radioactivity was eluted from the filters and counted by liquid scintillation. Data are given as a percentage of the values obtained from freshly isolated hepatocytes. These cells have been shown to synthesize mRNA-specific sequences in the same ratios as did nuclei from whole liver (Clayton and Darnell, 1983). The blank (non-recombined plasmids), usually <50 c.p.m., was subtracted.

Plasmid DNA samples

The clones were generous gifts from: A.Kahn for rat aldolase B and pyruvate kinase L (Simon *et al.*, 1983) actin, transferrin (Uzan *et al.*, 1984) and liver-specific 3F1, J.Sala-Trépat for rat serum albumin (Sargent *et al.*, 1979).

Acknowledgements

We gratefully acknowledge the substantial contribution of Dr A.Kahn. We thank Drs J.Kruh and D.Webb for their thoughtful criticisms of the paper and Mrs A.Vannier for typing the manuscript. This work was supported by the Institut National de la Santé et de la Recherche Médicale.

References

Andrews,G.K., Dziadek,M. and Tamaoki,T. (1982) *J. Biol. Chem.*, **257**, 5148-5153.

Bégué,J.M., Guguen-Guillouzo,C., Pasdeloup,N. and Guillouzo,A. (1984) *Hepatology*, **4**, 839-842.

Bertolotti,R., Rutishauser,U. and Edelman,G.M. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 4831-4835.

Bissell,D.M. and Guzelian,P.S. (1980) *Ann. N.Y. Acad. Sci. USA*, **349**, 85-98.

Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry (Wash.)*, **18**, 5294-5299.

Clayton,D.F. and Darnell,J.E., Jr. (1983) *Mol. Cell. Biol.*, **3**, 1552-1561.

Clément,B., Guguen-Guillouzo,C., Campion,J.P., Glaise,D., Bourel,M. and Guillouzo,A. (1984) *Hepatology*, **4**, 373-380.

Gallin,W.J., Edelman,G.M. and Cunningham,B.A. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1038-1042.

Glisin,V., Crkvenjakov,R. and Byus,C. (1974) *Biochemistry (Wash.)*, **13**, 2633-2637.

Guguen-Guillouzo,C., Tichonicky,L., Szajnert,M.F., Schapira,F. and Kruh,J. (1978) *Biol. Cell.*, **31**, 225-234.

Guguen-Guillouzo,C., Seignoux,D., Courtois,Y., Brissot,P., Marceau,N., Glaise, D. and Guillouzo,A. (1982) *Biol. Cell.*, **46**, 11-20.

Guguen-Guillouzo,C., Clément,B., Baffet,G., Beaumont,C., Morel-Chany,E., Glaise,D. and Guillouzo,A. (1983) *Exp. Cell. Res.*, **143**, 47-54.

Guguen-Guillouzo,C. and Guillouzo,A. (1983) *Mol. Cell. Biochem.*, **53/54**, 35-56.

Guillouzo,A., Delers,F., Clément,B., Bernard,N. and Engler,R. (1984) *Biochem. Biophys. Res. Commun.*, **120**, 311-317.

Houssaint,E. (1980) *Cell Differ.*, **9**, 269-279.

Jefferson,D.M., Clayton,D.F., Darnell,J.E., Jr. and Reid,L.M. (1984) *Mol. Cell. Biol.*, **4**, 1929-1934.

Laemmli,U.K. (1970) *Nature*, **227**, 680-685.

Lehrach,H., Diamond,D., Wozney,J.M. and Boedtker,H. (1977) *Biochemistry (Wash.)*, **16**, 4743-4751.

Michalopoulos,G. and Pitot,H.C. (1975) *Exp. Cell Res.*, **94**, 70-78.

Raymondjean,M., Kneip,B. and Kruh,J. (1983) *Biochim. Biophys. Acta*, **741**, 30-37.

Rigby,P.W., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.

Ritchie,R.F. (1975) in Putman,F.W. (ed.), *The Plasma Protein: Structure, Function and Genetic Control*, Vol. **II**, Academic Press, NY, pp. 375-425.

Rojkind,M., Mackensen,S., Giambrone,M.A., Ponce,P. and Reid,L.M. (1980) *J. Cell. Biol.*, **87**, 255-283.

Sargent,T.D., Wu,J.R., Sala-Trépat,J.M., Wallace,R.B., Reyes,A.A. and Bonner, J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3256-3260.

Schibler,U., Hagenbuchle,O., Wellauer,P.K. and Pittet,A.C. (1983) *Cell*, **33**, 501-508.

Seglen,P.O. (1973) *Exp. Cell Res.*, **76**, 25-30.

Simon,M.P., Besmond,C., Cottreau,D., Weber,A., Chaumet-Riffaud,P., Dreyfus, J.C., Sala-Trépat,J., Marie,J. and Kahn,A. (1983) *J. Biol. Chem.*, **258**, 14576-14584.

Sirica,A.E., Richards,W., Tsukada,C., Sattler,C. and Pitot,H.C. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 283-287.

Spiegelman,B.M. and Green,H. (1980) *J. Biol. Chem.*, **255**, 8811-8818.

Thiery,J.P., Delouvée,A., Gallin,W.J., Cunningham,B.A. and Edelman,G.M. (1984) *Dev. Biol.*, **102**, 61-78.

Thomas,P.S. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5201-5205.

Uzan,G., Frain,M., Park,I., Besmond,C., Maessens,G., Sala-Trépat,J., Zakin, M.M. and Kahn,A. (1984) *Biochem. Biophys. Res. Commun.*, **119**, 273-281.

Vaulont,S., Munnich,A., Marie,J., Reach,G., Pichard,A.L., Simon,M.P., Besmond,C., Barbry,P. and Kahn,A. (1984) *Biochem. Biophys. Res. Commun.*, **125**, 135-141.

Walsh,F.S. and Crumpton,M.J. (1977) *Nature*, **269**, 307-311.

Williams,G.M. and Gunn,J.M. (1974) *Exp. Cell Res.*, **89**, 139-142.

Received on 12 June 1985; revised on 11 July 1985