Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 755–759, February 1983
Cell Biology

Degradation and resynthesis of gap junction protein in plasma membranes of regenerating liver after partial hepatectomy or cholestasis

(quantitative immunoblot/bile duct ligation/recanalization/cell–cell communication/metabolic cooperation)

OTTO TRAUB, PETRA MARIA DRÜGE, AND KLAUS WILLECKE

Institut für Zellbiologie (Tumorforschung), Universität Essen, Hufelandstrasse 55, 4300 Essen 1, Federal Republic of Germany

Communicated by Arthur B. Pardee, October 12, 1982

ABSTRACT **Changes in the total amount of the gap junction protein (M_r 26,000) after partial hepatectomy or bile duct ligation and recanalization were investigated in rat liver membranes by quantitative immunoblot with rabbit antiserum to the M_r 26,000 protein. The loss and reappearance of the M_r 26,000 protein roughly paralleled loss and reappearance of gap junction plaques analyzed previously under similar physiological conditions by freeze-fracture of hepatocyte surfaces. The total amount of the hepatic M_r 26,000 protein in liver plasma membranes and the total area of the hepatocyte surface occupied by gap junction plaques appeared to be proportional under these conditions. However, at the minimum, 28–35 hr after partial hepatectomy we still find about 15% of the M_r 26,000 protein, in contrast to <1% of gap junction plaques, determined by morphometric analysis. This discrepancy is probably due to the fact that very small gap junction plaques, single connexons, and free M_r 26,000 gap junction subunits are missed by the morphometric analysis. At the times of the minimal amount of the M_r 26,000 protein in hepatic plasma membranes after partial hepatectomy or bile duct ligation we found that crude hepatic lysosomal membranes of these rats contained less M_r 26,000 protein than lysosomal membranes of nonoperated control animals. Thus, we conclude that the decrease and increase of the total amount of the M_r 26,000 protein cannot be explained only by dispersal and reuse of gap junction subunits but are largely due to degradation and resynthesis of the M_r 26,000 protein. No significant change in the amount of the M_r 21,000 protein that had been isolated with gap junction plaques was observed in liver plasma membranes after partial hepatectomy. This confirms our previous conclusion that the M_r 26,000 and M_r 21,000 proteins are independent of each other.**

Gap junctions form channels between contiguous cells in organs or in tissue culture and are thought to be involved in metabolic cooperation, tissue homeostasis, or transmission of regulatory signals (1, 2). The gap junctions of mouse or rat liver are relatively well characterized (3, 4), although some controversy remains regarding the exact molecular weight of the subunit protein which is readily cleaved by proteases during isolation. Recently, the partial sequence of the M_r 26,000–28,000 subunit protein (5) isolated from purified rat liver gap junction plaques has been determined (6). Using electron microscopy, Revel and co-workers found that the total area of rat liver gap junction plaques on the surface of hepatocytes decreased and increased after partial hepatectomy (7–9). Later it was shown by electrophoretic analysis that the M_r 26,000 gap junction protein could not be detected in preparations of gap junction plaques when these structures were absent from the surface of hepatocytes (5). However, these authors could not eliminate the possibility that gap junction protein could have become soluble in the nonionic detergent used for the preparation of gap junction plaques, thereby escaping its detection. Using an antiserum that specifically recognized the M_r 26,000 gap junction protein in liver plasma membranes (10, 11), we decided to measure the total amount of the gap junction M_r 26,000 protein after partial hepatectomy in rat liver to compare the results with the extensive morphometric studies of Revel and co-workers.

Furthermore, Metz *et al.* had found by freeze-fracture analysis that gap junction plaques also disappeared from the surface of hepatocytes after bile duct ligation and reappeared after recanalization (12–14). Because the liver gap junction protein had not been investigated biochemically after cholestasis we carried out an immunological quantitation of the M_r 26,000 liver protein after bile duct ligation and recanalization.

METHODS AND MATERIALS

Animals and Operations. For partial hepatectomy and bile duct ligation male BDIX rats, 23–25 days of age, were used. Partial hepatectomies (two-thirds removal) were performed exactly as described by Higgins and Anderson (ref. 15; cf. refs. 7–9). Regenerated livers were perfused with 10 ml of an ice-cold 0.9% NaCl solution. The livers were excised, frozen immediately, and stored at −70°C. For ligation the bile duct was mobilized from the vena porta between liver and gut. A 2.5- to 3-mm piece of silicone tubing (inner diameter, 1 mm; outer diameter, 2 mm) was tied firmly to the bile duct at two sites by using threads of silk (type 5-0). Peritoneum and skin were sutured separately with silk. Forty-eight hours after ligation a bubble-like swelling of 3–4 mm in diameter in the bile duct proximal to the ligation was observed in all animals. At this time recanalization of the bile duct was performed: the silicone tubing was cut lengthwise with a pointed pair of scissors, thereby also cutting the silk threads. After removal of the silicone tubing the peritoneum was sutured with silk and the skin was closed with Michel clamps.

Incorporation of [^{3}H]Thymidine and Isolation of DNA. All hepatectomized animals were labeled by intraperitoneal injection of [*methyl*-^{3}H]thymidine (New England Nuclear, specific activity = 6.7 Ci/mmol; 1 Ci = 3.7×10^{10} Bq; 6 μCi/g of body weight, dissolved in 0.9% NaCl solution) for 70 min just before the animals were killed and the livers were removed. [^{3}H]-Thymidine incorporation was always performed between 10 and 11 a.m. to minimize changes in DNA labeling due to the diurnal cycle of the rats (16).

The frozen liver tissue was thawed and homogenized in a glass homogenizer by using the tightly fitting Teflon pestle S (Braun, Melsungen, Federal Republic of Germany). Half of the homogenate was used for preparation of plasma membranes (4) and subsequent immunoblot (see below). The other half of the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

homogenate was centrifuged in sucrose-containing buffer (17). The nuclei were purified by several centrifugations in the presence of Triton X-100 (18), treated with RNase T1, and lysed in $NaDodSO_4$. The DNA was purified according to standard conditions.

Immunoblot of Gap Junction Proteins. The conditions that have been described in our previous paper (10) were standardized. Throughout all experiments we used 12.5% polyacrylamide gels, prepared according to Laemmli (19), for separation of liver membrane proteins. The blotting time was always 24 hr. Four-fold concentrated electrophoresis buffer (0.1 M Tris/0.77 M glycine, pH 8.3/0.4% $NaDodSO_4$) was used throughout. After transfer and incubation with 1% bovine serum albumin, the nitrocellulose paper was incubated with rabbit antisera (1:25 dilution) for 12 hr at room temperature. New batches of rabbit antiserum to the M_r 26,000 protein and rabbit antisera to the M_r 21,000 protein were prepared in specific pathogen-free rabbits (Hoechst) maintained under germfree conditions throughout the immunization time. The preparation of antigens and the amounts used per injection were identical to those previously described (10). After three injections at days 1, 35, and 49, a titer in the enzyme immunoassay was reached after 2 months that was similar to that measured previously after 5 months (10).

Bilirubin Analysis During Bile Duct Ligation and Recanalization. Approximately 1.2 ml of blood was drawn from the vena porta into an Eppendorf tube and was centrifuged after 45 min at 1,000 × *g* for 10 min. The supernatant serum was used for spectrophotometric bilirubin analysis following the protocol of a commercial test kit (Testomar Bilirubin, Behringwerke, Marburg, Federal Republic of Germany). The liver then was perfused with 10 ml of ice-cold 0.9% NaCl solution, divided into three portions, and frozen immediately at −70°C.

RESULTS

Quantitation of the M_r 26,000 Protein in Liver Membranes by Immunoblot. The immunoblot conditions with rabbit antiserum to the M_r 26,000 protein and ^{125}I-labeled protein A (^{125}I-protein A) for autoradiographic detection of the M_r 26,000 protein in purified gap junction plaques or liver membranes have been described (10). We noticed that under the previously described conditions of capillary blot (24 hr) the M_r 26,000 protein band was transferred onto nitrocellulose paper only to about 50%. The immunoblot technique allowed the detection of at least 5 ng of gap junction protein. Furthermore, between 10 and 1,000 ng of gap junction protein we observed a proportional increase in the intensity of the spots over the M_r 26,000 protein on the autoradiographs. This is illustrated in Fig. 1*A*. The intensities of the spots over the M_r 26,000 protein on the autoradiographs were scanned in a densitometer. When the data were plotted on a half-logarithmic scale a linear calibration curve was obtained between 10 and 1,000 ng (Fig. 1*B*). Alternatively, the radioactive spots (1 cm^2 each) were cut out from the nitrocellulose paper and the radioactivity was counted in a gamma-scintillation spectrophotometer. In this case a linear calibration curve was obtained between 10 and 500 ng of gap junction protein when the data were plotted on a double linear scale. Both methods of calibrating the data gave similar results.

Analysis of Gap Junction Protein in Rat Liver Plasma Membranes After Partial Hepatectomy. Using equal amounts of rat and mouse liver membrane proteins for quantitative immunoblot we estimated that the anti-mouse liver M_r 26,000 antiserum reacted with rat liver M_r 26,000 protein to about 70% of its reaction with M_r 26,000 protein from mouse liver. This result showed that the anti-mouse liver M_r 26,000 antiserum could be used to quantitate the M_r 26,000 protein in regenerating rat liver. The autoradiograph of Fig. 1*C* illustrates that the total amount of the M_r 26,000 protein decreased to relatively low levels 36 hr after partial hepatectomy and increased again at 48 hr. At the time of the minimal amount of the M_r 26,000 protein no immunoreactive protein bands of smaller molecular weight were detected on the corresponding gels. In Fig. 1*D* the Coomassie blue-stained polyacrylamide gel demonstrates that equal amounts of liver plasma membrane proteins were applied onto the gel. The M_r 26,000 gap junction protein cannot be seen under these conditions.

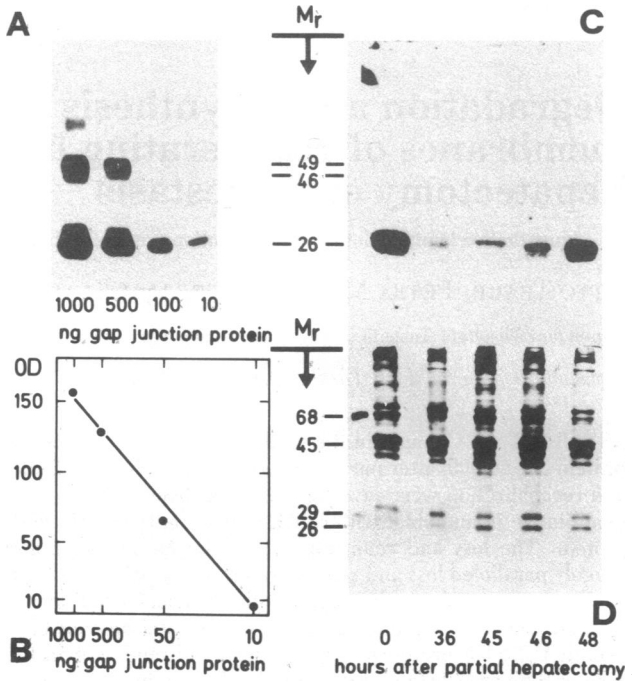

FIG. 1. (*A*) Autoradiograph obtained after quantitative immunoblot of hepatic gap junction protein. Increasing amounts of M_r 26,000 protein purified as hepatic gap junction plaques (4) were electrophoresed on $NaDodSO_4$/polyacrylamide gels, transferred onto nitrocellulose paper, and subjected to immunoblot by using rabbit antiserum to the M_r 26,000 protein (10). The antigen–antibody complex was detected by binding to ^{125}I-protein A and autoradiography. Molecular weights are shown as $M_r \times 10^{-3}$. (*B*) Densitometric evaluation of quantitative immunoblot. The density of the spots over the M_r 26,000 protein on the autoradiograph shown in *A* was traced by a densitometer. After subtraction of the background the intensities were integrated and plotted against the amount of gap junction protein used for electrophoresis and quantitative immunoblot. (*C*) Autoradiograph of immunoblot with antiserum to the M_r 26,000 protein and purified liver plasma membranes after partial hepatectomy. Liver plasma membranes were purified from hepatectomized rats. Fifty micrograms of membrane protein was electrophoresed on $NaDodSO_4$/polyacrylamide gels and subjected to immunoblot by using rabbit antiserum to the M_r 26,000 protein. The antigen–antibody complexes were detected by binding to ^{125}I-protein A and autoradiography. Molecular weights are shown as $M_r \times 10^{-3}$. (*D*) Coomassie blue staining of the polyacrylamide gel used for preparing the autoradiograph shown in *C*. The lane on the left-hand side indicates the position of reference proteins of known molecular weight ($M_r \times 10^{-3}$) (10, 11).

Fig. 2*A* shows the changes in the amounts of the M_r 26,000 protein analyzed by quantitative immunoblot of the M_r 26,000 protein during liver regeneration. About 17 hr after partial hepatectomy the amount of M_r 26,000 protein in the plasma membranes of the remaining liver lobes started to fall and reached a minimum of about 15% of its initial level after 28–35 hr. Between 18 and 24 hr after partial hepatectomy the concentration of the M_r 26,000 protein decreased with a $t_{1/2}$ of 3–6 hr. About 48 hr after hepatectomy we again found in liver plasma mem-

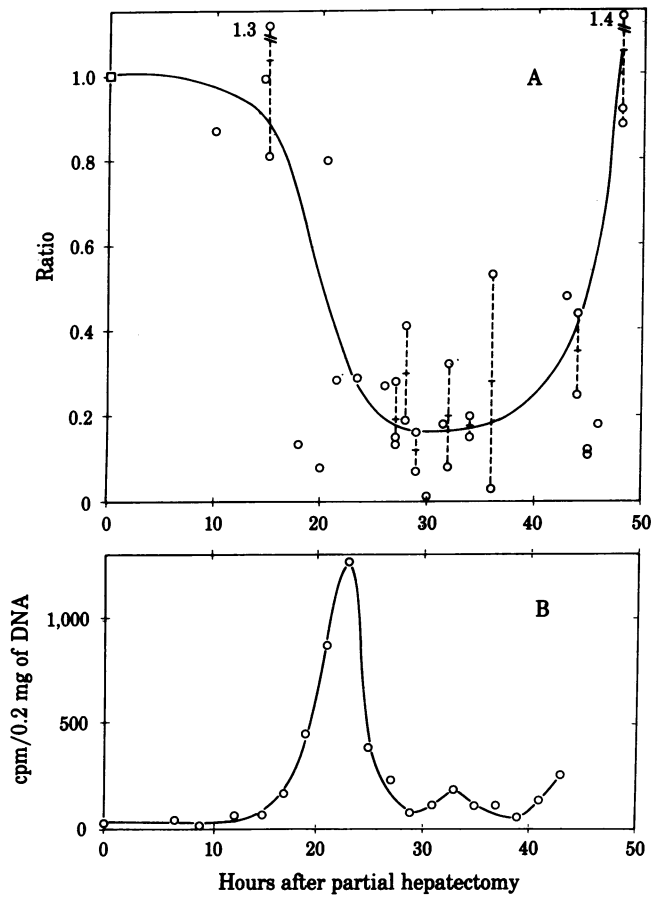

FIG. 2. (*A*) Amounts of M_r 26,000 protein determined by quantitative immunoblot in rat liver plasma membranes after partial hepatectomy. The graph was constructed from the results of electrophoresis on slab gels, each involving purified hepatic plasma membranes from five rats after partial hepatectomy and from one nonoperated (control) rat. In addition, 50, 100, 250, and 500 ng of purified hepatic gap junction plaques were applied onto each slab gel to allow a quantitative comparison of each immunoblot. The amounts of M_r 26,000 protein were visualized after quantitative immunoblot by autoradiography, as shown in Fig. 1*C*, and evaluated by densitometry, as shown in Fig. 1*B*. Circles connected by dashed lines represent data from different rats but identical time points. In this case the calculated average is indicated by a horizontal bar on the dashed lines. Ratio indicates amount of M_r 26,000 protein relative to mean control value. (*B*) Incorporation of [^{3}H]thymidine into DNA of livers of hepatectomized rats. [^{3}H]Thymidine was injected intraperitoneally 70 min before the hepatectomized rats were killed. Most of the rat livers studied in *A* and some additional rat livers were investigated. To account for the diurnal cycle, the animals always were killed between 10 and 11 a.m. DNA was isolated and the radioactivity was counted as described in *Methods and Materials*.

branes the initial amount of the M_r 26,000 protein, arbitrarily set to 100%. We estimated that this corresponded to about 750 ng of M_r 26,000 protein per 50 μg of liver plasma membrane protein. In Fig. 2*B* the [^{3}H]thymidine pulse incorporation into the DNA of the regenerating liver is shown. Maximal incorporation was observed 22 and 33 hr after partial hepatectomy. These results are very similar to those published by Bucher for weanling rats (16).

In Fig. 3 plasma membranes from regenerating liver were used for quantitative immunoblot with the anti-mouse liver M_r 21,000 antiserum (10). The M_r 21,000 protein had been isolated by Henderson *et al.* (4) together with the M_r 26,000 protein in preparations of gap junction plaques from mouse liver. Lane A in Fig. 3 illustrates that our newly prepared anti-mouse liver

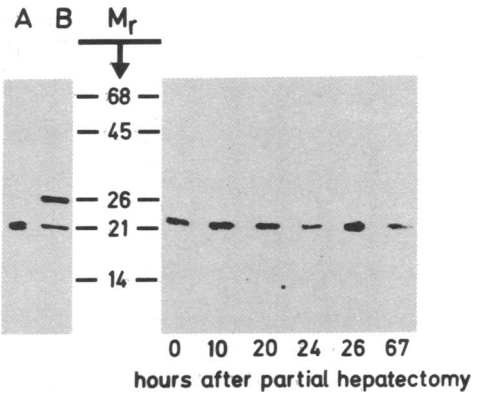

FIG. 3. Autoradiograph of immunoblot with antiserum to the M_r 21,000 protein and purified liver plasma membranes after partial hepatectomy. The conditions were as outlined in Fig. 1*C*, except that antiserum to the M_r 21,000 protein was used. For comparison, lane B shows a Coomassie blue-stained gel after electrophoresis of purified hepatic gap junction plaques and lane A shows the corresponding autoradiograph after immunoblot with antiserum to the M_r 21,000 protein. Note that this antiserum to the M_r 21,000 protein does not crossreact with the M_r 26,000 protein (cf. ref. 10). The positions of reference proteins are indicated: bovine serum albumin (M_r 68,000), ovalbumin (M_r 45,000), and ribonuclease A (M_r 14,000). Molecular weights are shown as $M_r \times 10^{-3}$.

M_r 21,000 antiserum crossreacted with the rat liver M_r 21,000 protein but did not react with the M_r 26,000 protein in the same preparations. No significant change of the M_r 21,000 protein concentration in rat liver plasma membranes was detected during liver regeneration at times when the M_r 26,000 protein decreased and increased again (cf. Fig. 3 and additional measurements on different autoradiographs). These results confirm and extend our previous conclusion (10) that the M_r 21,000 protein must be independent of the M_r 26,000 protein and could be a contaminant of the mouse liver gap junction preparations. Recently we have located the M_r 26,000 protein in purified as well as in native mouse liver gap junction plaques using immunoelectron microscopy (unpublished data).

Analysis of the M_r 26,000 Gap Junction Protein After Bile Duct Ligation and Recanalization. About 40 hr after bile duct ligation the concentration of the M_r 26,000 protein in rat liver plasma membranes decreased with a $t_{1/2}$ of 4–6 hr, reaching a minimum after 48 hr and 2 hr of recanalization (Fig. 4*A*). At this time we found in purified plasma membranes (collected at the 42–50.5% interface of the sucrose gradient) (4, 10) only 1% of the initial level of the M_r 26,000 protein.

Unexpectedly, we found in four rats another minimum in the amount of the M_r 26,000 protein about 24 hr after bile duct ligation. It is possible that in these animals the concentration of the M_r 26,000 protein would have dropped further during the next hours to the low level observed in other rats at 48 hr after bile duct ligation. After opening the ligation at 48 hr, the amount of the M_r 26,000 protein increased again and reached the normal level (100%) about 30 hr later. Fig. 4*B* shows the increase and decrease of the bilirubin level in the serum during bile duct ligation and recanalization. The concentration of bilirubin in the serum indicates the extent of regurgitation of bile from the canaliculi into the liver sinusoids.

In Table 1 the relative amounts of the M_r 26,000 protein in two crude membrane preparations are compared by quantitative immunoblot. We determined in nuclei and crude plasma membranes (8,600 $\times$ *g* pellet) 29 hr after partial hepatectomy or 48 hr after bile duct ligation and 2 hr after recanalization 10–20% of the M_r 26,000 protein present in the 8,600 $\times$ *g* pellet from control liver. The apparent discrepancy between this min-

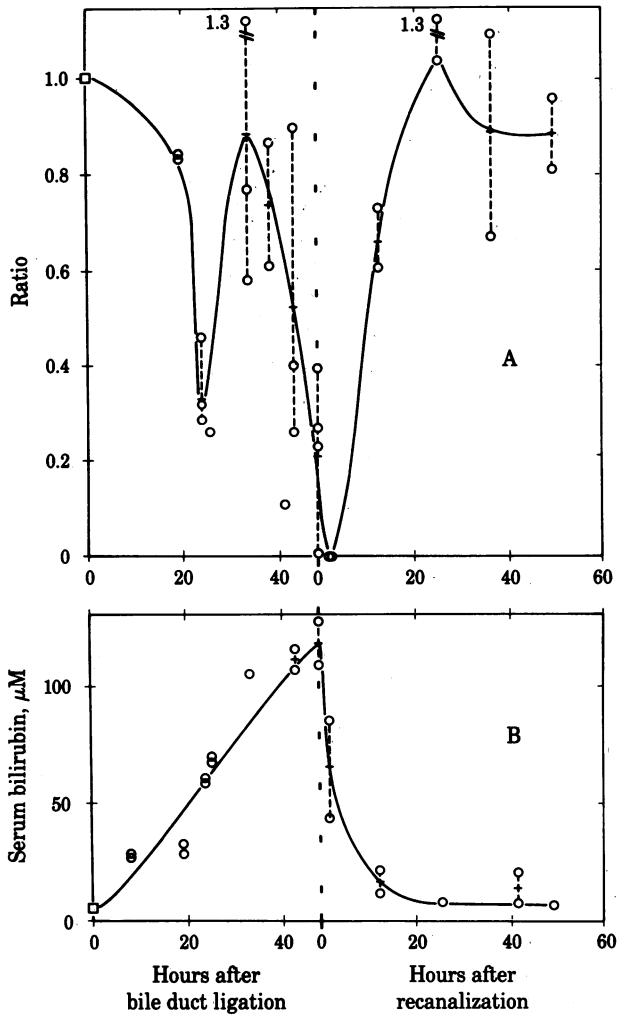

FIG. 4. (A) Amounts of M_r 26,000 protein determined by quantitative immunoblot in rat liver plasma membranes after bile duct ligation and recanalization. The graph was constructed from the results of slab gel electrophoresis, each involving purified hepatic plasma membranes from five rats after bile duct ligation or recanalization and from one nonoperated (control) rat. Autoradiography, quantitative immunoblot, and densitometric evaluation of the data were as outlined in the legend of Fig. 2A. Points connected by dashed lines represent data from different rats but identical time points (see Fig. 2A). Ratio indicates amount of M_r 26,000 protein relative to mean control value. (B) Amounts of bilirubin determined in the serum of rats after bile duct ligation and recanalization. Samples of blood were withdrawn from most of the rats used in A and from some additional rats just before the livers were removed. The serum was used for bilirubin analysis as described in *Methods and Materials*.

imal amount of M_r 26,000 protein after cholestasis and the minimal amount (about 1%) measured after cholestasis in purified plasma membranes (see Fig. 4A) can be solved by our finding that at the same time point about 10–20% of the M_r 26,000 protein is present in the pellet and the 67–54% interface of the sucrose gradient (4, 10) used for plasma membrane purification. This residual amount of the M_r 26,000 protein could not be seen when only the plasma membranes purified at the 42–50.5% interface of the sucrose gradient were analyzed. Table 1 shows further that crude lysosomal membranes (100,000 × g pellet), taken from livers at the times of the minimal amounts of the M_r 26,000 protein after partial hepatectomy or cholestasis, contained less M_r 26,000 protein than the lysosomal membranes isolated from nonoperated control livers. This result excludes the possibility that the decrease and increase of the M_r 26,000 protein in liver plasma membranes after hepatectomy or bile duct ligation can be caused only by internalization and reuse of the M_r 26,000 protein stored in endocytotic vesicles.

Table 1. Estimation of relative amounts of the M_r 26,000 protein present in two crude membrane fractions from the same rat livers

Physiological condition	Amount of M_r 26,000 protein, %	
	8,600 × g pellet	100,000 × g pellet
Partial hepatectomy*	10–20	3
Bile duct ligation and recanalization†	10–20	3
Control (no operation)	90	10

One-third of the rat livers were homogenized in 1 mM sodium bicarbonate (pH 7.4) and were centrifuged according to Henderson *et al.* (4). The 8,600 × g pellets contained nuclei and crude plasma membranes. The supernatants containing mitochondria, lysosomes, and microsomes were centrifuged at 100,000 × g for 60 min at 4°C. The resulting pellets were suspended in 1 mM EDTA (pH 7.4). Aliquots were taken for protein determination (20) or $NaDodSO_4$/polyacrylamide gel electrophoresis and quantitative immunoblot by using the antiserum to the M_r 26,000 protein (10). The total amount of M_r 26,000 protein present in control liver was set at 100%. Data of two different experiments were averaged.

* Twenty-nine hours after partial hepatectomy.

† Forty-eight hours after bile duct ligation and 2 hr after recanalization.

DISCUSSION

The results of this study suggest that the gap junction protein is degraded and resynthesized after partial hepatectomy or temporary bile duct ligation. We can largely exclude dispersal and reassembly of gap junction particles under these conditions, as had been previously discussed after freeze-fracture analysis (8, 13). There are some interesting points of comparison between the results of the quantitative analysis of gap junction plaques (8, 13) and of the M_r 26,000 protein reported in this paper.

Twenty-eight to 35 hr after partial hepatectomy we determined about 15% residual M_r 26,000 protein, whereas Meyer *et al.* (9) found <1% residual gap junction plaques when compared to the initial level (100%). As discussed by these authors, the morphometric analysis does not allow the detection of very small gap junction plaques or free M_r 26,000 protein not yet aggregated into a connexon. In the livers of two rats we found the minimal amount of the M_r 26,000 protein already at 18 and 20 hr after partial hepatectomy (see Fig. 2A). This could mean that in some rats the M_r 26,000 protein is degraded faster than in the majority of the other rats, perhaps depending on the nutritional state of the animals. In contrast to Metz and Bressler's morphometric investigation of gap junction plaques (13), we did not find a sudden increase in the minimal amount (10–20%) of the M_r 26,000 protein 48 hr after bile duct ligation and 2 hr after recanalization. No quantitative morphometric data of gap junction plaques relating to the time 24 hr after bile duct ligation have been reported (13, 21). Thus, we cannot correlate the decreased amount of the M_r 26,000 protein that we found at this time with changes in the amount of gap junction plaques.

Although the decrease of the M_r 26,000 protein occurred with similar $t_{1/2}$ of 3–6 hr after both partial hepatectomy as well as bile duct ligation, the molecular mechanisms possibly are different. After partial hepatectomy there was a coincidence of the maximal incorporation of thymidine into DNA and the strong decrease of the M_r 26,000 protein (see Fig. 2). If the biosynthesis of the gap junction protein occurred in the G_1 phase of hepatocytes, this could explain why the total amount of the M_r 26,000 protein decreased as more and more cells enter the S phase, provided that the $t_{1/2}$ of the M_r 26,000 protein were short

relative to the length of the S phase. Fallon and Goodenough (22) have reported a $t_{1/2}$ of 5 hr for the main protein component (M_r 21,000!) of mouse liver gap junctions, whereas Yancey *et al.* (23) estimated the $t_{1/2}$ of the liver M_r 26,000 protein to be <10 hr. By using the quantitative immunoblot, it should be possible to analyze whether degradation and biosynthesis of the M_r 26,000 protein is dependent on the phase of the cell cycle in hepatocytes. At present, alternative explanations for degradation and resynthesis of the M_r 26,000 protein after partial hepatectomy cannot be excluded.

On the other hand, after cholestasis due to bile duct ligation, the increased pressure in the bile canaliculi leads to regurgitation of the bile into the liver sinusoids (24). Possibly the corresponding hemichannels of gap junction plaques on contiguous hepatocytes can no longer couple under these conditions, due to decreased cellular adhesion. This condition may lead to degradation of the M_r 26,000 protein (see the hypothetical model of connexon channel formation described in ref. 1). Alternatively, the increased concentration of bile acids in hepatocytes (25) may inhibit the biosynthesis of the M_r 26,000 protein. These and other hypotheses are now amenable to experimental analysis by using the antiserum to the M_r 26,000 protein.

We thank the following colleagues: Dr. K. Littmann (Department of Surgery, Universität Essen), who introduced us to the operation techniques of partial hepatectomy and bile duct ligation with young rats; Dr. O. Ahrens of this institute, who constructed the densitometer and automated image analysis system used in this study for quantitative evaluation of the M_r 26,000 protein on autoradiographs; and Dr. P. Nehls of this institute, who provided advice for isolation of DNA from small amounts of rat liver. The technical assistance of Mrs. Beate Karow is gratefully acknowledged. This work was supported by a grant from the Ministerium für Wissenschaft und Forschung (Düsseldorf) and in part by grants from the Deutsche Forschungsgemeinschaft (SFB 102) and Fonds der Chemischen Industrie to K.W.

1. Loewenstein, W. R. (1981) *Physiol. Rev.* **61**, 829–913.
2. Hooper, M. L. & Subak-Sharpe, J. H. (1980) *Int. Rev. Cytol.* **69**, 46–104.
3. Hertzberg, E. L., Anderson, D. J., Friedlander, M. & Gilula, N. B. (1982) *J. Cell Biol.* **92**, 53–59.
4. Henderson, B., Eibl, H. & Weber, K. (1979) *J. Mol. Biol.* **132**, 193–218.
5. Finbow, M., Yancey, S. B., Johnson, R. & Revel, J.-P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 970–974.
6. Nicholson, B. J., Hunkapiller, M. W., Grim, L. B., Hood, L. E. & Revel, J.-P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7594–7598.
7. Yee, A. G. & Revel, J.-P. (1978) *J. Cell Biol.* **78**, 554–564.
8. Yancey, S. B., Easter, B. & Revel, J.-P. (1979) *J. Ultrastruct. Res.* **67**, 229–242.
9. Meyer, D. J., Yancey, S. B., Revel, J.-P. & Peskoff, A. (1981) *J. Cell Biol.* **91**, 503–523.
10. Traub, O., Janßen-Timmen, U., Drüge, P. M., Demietzel, R. & Willecke, K. (1982) *J. Cell Biochem.* **19**, 27–44.
11. Willecke, K., Traub, O., Janßen-Timmen, U., Drüge, P. M. & Dermietzel, R. (1982) in *Biochemistry of Differentiation and Morphogenesis*, ed. Jaenicke, L. (Springer, Berlin), in press.
12. Metz, J., Aoki, A., Merlo, M. & Forssmann, W. G. (1977) *Cell Tissue Res.* **182**, 299–310.
13. Metz, J. & Bressler, D. (1979) *Cell Tissue Res.* **199**, 257–270.
14. Metz, J. (1982) in *Basic and Clinical Hepatology*, eds. Motta, P. M. & Didio, L. J. A. (Nijhoff, The Hague, The Netherlands), pp. 51–68.
15. Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12**, 186–202.
16. Bucher, N. L. R. (1963) *Int. Rev. Cytol.* **15**, 245–300.
17. Teng, C. S., Teng, C. T. & Allfrey, V. G. (1971) *J. Biol. Chem.* **246**, 3597–3609.
18. Blobel, G. & Potter, R. V. (1966) *Science* **154**, 1662–1665.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–686.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 266–275.
21. Mutoh, H. (1981) *Arch. Histol. Jpn.* **44**, 345–367.
22. Fallon, R. & Goodenough, D. A. (1981) *J. Cell Biol.* **90**, 521–526.
23. Yancey, S. B., Nicholson, B. J. & Revel, J.-P. (1981) *J. Supramol. Struct. Cell Biochem.* **16**, 221–232.
24. Popper, H. (1977) in *Liver and Bile*, eds. Bianchi, L., Gerok, W. & Schlinger, K. (MTP, Lancaster, England), pp. 189–201.
25. Greim, H., Trülzsch, D., Roboz, J., Dressler, K., Czygan, P., Hutterer, F., Schaffner, F. & Popper, H. (1972) *Gastroenterology* **63**, 837–845.