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Three actin-binding proteins are developmentally regulated in rat liver plasma membranes

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Actin-binding membrane proteins (linking microfilaments to the cell membrane) are involved in cytoskeleton-membrane interactions which are supposed to undergo profound changes during cell proliferation and development. In this study 8 polypeptides were shown to bind F-actin directly in the liver cell membranes of mature rats. From these, the abundance of three polypeptides, of 130, 50 and 36 kDa, was observed to increase considerably during postnatal development, which indicates a developmental change in the cytoskeleton-membrane interactions.

Actin binding; Membrane protein; Cytoskeleton; Development

1. INTRODUCTION

The thinnest cytoskeletal structures called microfilaments are composed of actin, a highly conservative and abundant protein of all eukaryotic cells. Actin filaments localized in the cytoplasm and under the cell membrane may interact with the plasma membrane through actin-binding proteins of the membrane. Microfilament-plasma membrane linkages are supposed to be involved in such basic cell phenomena like cell adhesion, cell shape formation, capping, endocytosis and chemotaxis. In these F-actin–cell membrane interactions actin-binding proteins serve as part of bridges (or a kind of mediators) between the extracellular space and the intracellular cytoskeleton.

Although many actin-binding proteins of the cytoplasm have been described by now, only relatively few of them localized in the plasma membrane have been identified. The best characterized systems are the erythrocyte membrane [1–4] and the intestinal microvillar membrane [5–8], while as for other cell membranes this area is poorly defined. Furthermore, only very few developmental studies have been carried out [4,8].

Since membrane–cytoskeleton interactions are likely to undergo a gross alteration during development in all kinds of cells, we aimed at examining this question in a system not studied so far in this respect: in the liver cell membrane of rats, during postnatal development.

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2. MATERIALS AND METHODS

A crude cell membrane fraction was obtained from livers of CFY rats by the method of Maeda et al. [9]. The washing buffer contained 10 mM Tris-HCl (pH 7.3), 2 mM $MgCl_2$, 30 mM NaCl, 1 mM DTT, 100 U/ml aprotininum (protease inactivator). Further purification was carried out on Percoll gradient (Pharmacia) by centrifugation at 20000 × *g* for 30 min; the density of the Percoll suspension prepared with sucrose was 1.05 g/ml.

10% SDS-polyacrylamide gels were stained with Coomassie blue G-250 or with silver [10]. (Membrane samples were applied to gel electrophoresis without prior heating.) Na^+,K^+-ATPase, acidic phosphatase and glucose-6-phosphatase were assayed at 37°C as described [11–13].

Actin for chromatography was prepared from the acetone powder of rabbit skeletal muscle [14], and was further purified by gel filtration [15]. Coupling of F-actin to CNBr-activated Sepharose 4B (Pharmacia) resulted in the association of approx. 1.5 mg actin to a ml packed column. No ligand was attached to control columns. After the coupling procedure, the

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2-ml columns were washed with 20 vols of buffer solution containing 100 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM $MgCl_2$, 0.2 mM DTT, 100 U/ml aprotininum, and 2% (v/v) Triton X-100. About 8–10 mg plasma membrane protein was circulated through each column for 12–14 h at 4°C followed by column washes with 40 vols of detergent containing the solution described above. Elution was carried out with 1.5 vols of 1% SDS [16].

Membrane proteins separated by SDS-PAGE were transferred to nitrocellulose [17]. Protein transfer was carried out at 30 V (10 V/cm) for 1.5 h at 4°C. After quenching with 10% normal chicken serum in PBS containing 0.05% Tween 20 for 1 h, and subsequent washes with PBS-Tween, the blots were incubated with the peroxidase-conjugated reagents (diluted in F-buffer: 50 mM KCl, 20 mM Tris-HCl, pH 7, 2 mM $MgCl_2$, 1 mM ATP, containing 5% normal chicken serum, and 0.05% Tween 20) for 40 min. The blots were developed with a staining solution composed of 5 mg 3,3′-diaminobenzidine and 5 μl of 30% H_2O_2 in 10 ml F-buffer. Actin-peroxidase reagent (for sample blots) and ovalbumin-peroxidase reagent (for control blots) were prepared as described by Wilson and Nakane [18].

3. RESULTS

The simple but efficient protocol of Maeda et al. [9] supplemented with additional purification steps on Percoll gradient proved to result in relatively contaminant-free plasma membrane preparations. No impurities were detectable by electron microscopy in the negatively stained samples (not shown). Na^+,K^+-ATPase activity of the samples increased about 12-fold during the preparation, while enzyme activities characteristic for microsomal (glucose-6-phosphatase) and lysosomal contaminations (acidic phosphatase) did not rise considerably (table 1). The procedure of plasma membrane preparation was followed by SDS-gel electrophoresis. About 40 polypeptides of different molecular masses could be distinguished in the Coomassie blue-stained tracks of the cell membrane samples (figs 1 and 3, table 2).

For biochemical demonstration of the microfilament-linking proteins of our system, actin affinity chromatography and a blot overlay assay were performed. 17 polypeptides were discerned in the silver-stained gel profiles of the plasma membrane proteins eluted from the actin affinity column (fig.1). (The appearance of some of these polypeptides in the eluate of the control column is presumably due to aspecific binding to the column particles and/or to indirect actin binding through protein-protein associations.) Since actin remains bound to the actin affinity column after the final elution, the eluted polypeptide with

Table 1

Enzyme marker activities in adult rats

	Enzyme activity (μmol product/mg protein per h)	
	Liver homogenate	Plasma membrane
Na^+,K^+-ATPase	1.44 ± 0.60 (5)	17.53 ± 3.88 (5)
Glucose-6-phosphatase	5.16 ± 0.90 (5)	5.90 ± 1.22 (5)
Acidic phosphatase	2.65 ± 0.63 (3)	3.86 ± 0.97 (3)

Figures in parentheses indicate the number of assays performed

an apparent molecular mass of 43 kDa must belong to the cell membrane.

In the other experimental approach, plasma membrane proteins transferred from SDS-polyacrylamide gel to nitrocellulose were reacted with actin-peroxidase or ovalbumin-peroxidase conjugates. 13 specific bands were observed on the blot incubated with actin-peroxidase reagent. 8 of these polypeptides, with apparent molecular masses of 130, 54, 50, 43, 38, 36, 31 and 28 kDa,

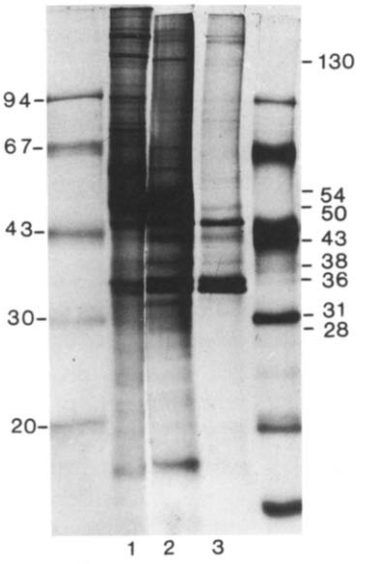

Fig.1. Detection of actin-binding proteins by actin affinity chromatography. Coomassie blue-stained SDS-PAGE profile of rat liver plasma membranes (lane 1), silver-stained tracks of eluates from actin affinity column (lane 2), and from ligand-free control column (lane 3). Numbers to the right of the figure designate the apparent molecular masses (in kDa) of the actin-binding polypeptides revealed also by the blot overlay assay.

Table 2

Polypeptide pattern of adult rat liver cell membranes

Apparent mol. mass (kDa)	Actin-binding polypeptides	Postnatal change in abundance
400		
300		
260		
200		
170		
(155)		
(140)		
(135)		
130	*	+
115		
100		
98		
95		
94		
(88)		
(84)		
(80)		
78		
75		
72		
70		
(65)		
(64)		
61		
60		
58		
55		
54	*	
52		
50	*	+
(48)		
45		
(43)	*	
(38)	*	
36	*	+
(31)	*	
(28)	*	
19		
17		
14		

Figures in parentheses relate to minor polypeptides; * marks actin-binding proteins, + indicates an increase in their abundance during postnatal development

were revealed also in the eluate of the actin affinity column (figs 1 and 2). Thus, these 8 polypeptides from the 40 distinguished membrane polypeptides are supposed to be direct microfilament-linking proteins (table 2).

In order to determine whether any change occurs in the scheme of actin-binding proteins during

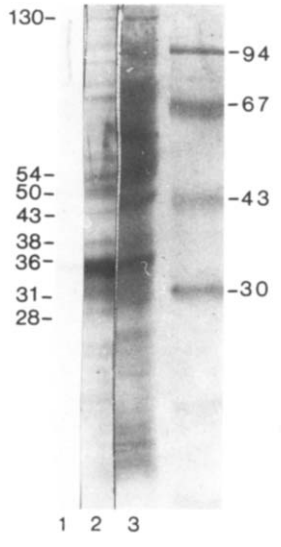

Fig.2. Detection of actin-binding proteins by blot overlay assay. Nitrocellulose strips were incubated with an actin-peroxidase reagent (lane 2), or with an ovalbumin-peroxidase reagent control (lane 1); transferred liver cell membrane proteins were stained with amido black (lane 3). Numbers to the left of the figure refer to the apparent kDa values of the actin-binding polypeptides shown also by affinity chromatography.

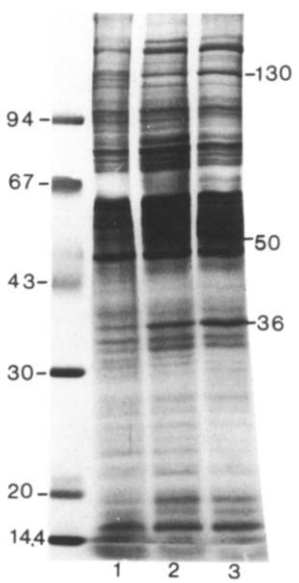

Fig.3. Comparison of SDS-PAGE polypeptide patterns of liver cell membranes from newborn (lane 1), 3-week-old (lane 2), and from mature (lane 3) rats. Molecular mass values to the right of the figure mark actin-binding polypeptides of different abundance in the three types of membranes (10% gel, stained with Coomassie blue).

postnatal development, liver cell membranes were prepared also from newborn and 3-week-old rats. Comparison of the gel profiles revealed several differences among the three age groups in the relative amounts of some membrane proteins (fig.3). Three main differences were related to actin-binding proteins. The relative levels of the 130, 50 and 36 kDa polypeptides were significantly lower in newborn rats than in mature ones. The plasma membrane scheme of the 3-week-old rats seemed to be closer to the adult pattern than to the neonatal one, considering the higher levels of the 130 and 36 kDa proteins; while the abundance of the 50 kDa polypeptide was still relatively low, like in the case of newborn rats. Taken together, these observations indicate an increase in the abundance of the 130, 50 and 36 kDa actin-binding proteins during postnatal development (table 2).

4. DISCUSSION

The present study has demonstrated that the cell membrane of liver cells in rats contains at least 8 actin-binding polypeptides, since from the 40 polypeptides discerned on SDS-polyacrylamide gel, 8 were shown to bind F-actin by both techniques used. In contrast to the findings presented here, Amrein-Gloor and Gazzotti [19] detected only three such proteins, with molecular masses of 240, 145 and 80 kDa, in rat liver cell membranes, performing a ^{125}I-actin blot overlay test. The low number of actin-linking proteins is apparently due to the 'overpurity' of their plasma membrane being probably devoid of several peripheral membrane proteins. On the other hand, the failure to detect the 240 kDa protein in the blot overlay assay of the present study presumably resulted from the lower efficiency of the blotting procedure to transfer proteins with high molecular masses, since a polypeptide of 260 kDa was shown by actin affinity chromatography. The 145 kDa polypeptide might be the same as the 130 kDa one detected by us.

The apparent molecular masses of the actin-binding polypeptides shown in the present paper suggest in some cases that they could be identical to proteins described previously in literature. The 130 kDa polypeptide may be identical to vinculin, an actin-binding membrane protein of focal adhesion plaques found in fibroblasts [20,21]; as well as in cardiac [22] and in smooth [23] muscle cells. The 36 kDa one is most probably the same as calpactin I heavy chain (equal to p36 or lipocortin II: [24]), since its instability during storage and its degradative derivatives of 34.5–34 kDa and 33 kDa [24] have also been observed in the present study (fig.1). The retention of this protein by the ligand-free Sepharose column is presumably a consequence of its close association with some phospholipids of the membrane sample (it belongs to the group of Ca^{2+}- and phospholipid-binding proteins: [25]), as lipids readily bind to the column particles [16]. The 54 kDa polypeptide may be related to the actin-bundling protein of 53–55 kDa observed in porcine brain [26] and in HeLa cells [27]. The 43 kDa protein could be actin (derived from the microfilament network underlying the cell membrane), or an actin-like protein.

The most interesting observation of this study is that some actin-binding membrane proteins appear to be developmentally regulated. According to our observations, the abundance of the 36 and 130 kDa polypeptides is increased from the lower neonatal level to the higher adult-type one during the first three weeks of postnatal development, while accumulation of the 50 kDa polypeptide appears to be initiated only after this time.

In summary, the present results suggest that the expression of actin-binding membrane polypeptides with molecular masses of 130, 50 and 36 kDa is under developmental control in the liver cells of rats.

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