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Fibronectin isoforms in plasma membrane domains of normal and regenerating rat liver

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Plasma membrane fractions were obtained from the three surface domains of normal and regenerating adult rat livers. It was shown by immunoblotting that sinusoidal plasma membranes contained the characteristic 220 and 210 kDa fibronectin doublet, whereas bile canalicular plasma membranes contained a 220 kDa component. In lateral plasma membranes, 180, 190 and 220 kDa fibronectin isoforms were present. Fibronectin in the sinusoidal and canalicular plasma membranes was shown, by detergent/aqueous phase partitioning, to be more hydrophilic than isoforms in lateral plasma membranes. Changes in the distribution of fibronectin between plasma membrane domains occurred during liver regeneration and their significance, especially in relation to cell division, is discussed.

Plasma membrane domain; Fibronectin; Liver regeneration

1. INTRODUCTION

The hepatocyte is a polarised cell with a plasma membrane differentiated into three major functional domains [1]. The extracellular matrix components that are associated with the hepatocyte's plasma membrane may vary according to whether the space of Disse, the bile canaliculus or an attached cell constitute the immediate environment. During liver regeneration, especially when hepatocytes enter into a proliferative stage [2], the nature of the interactions between hepatocytes and extracellular matrix components also changes [3–5]. This is illustrated by the decreased interaction of fibronectin and laminin with dividing hepatocytes [6].

Here, we have studied the association between fibronectin and subcellular fractions containing the functionally specialised liver plasma membrane domains and identified, using antibodies to fibronectin, various molecular mass isoforms of fibronectin associated with these membranes. The investigation was extended to the study of fibronectin expression in regenerating livers to determine whether it was differentially regulated at specific stages when the hepatocytes divide.

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2. EXPERIMENTAL

Rat liver plasma membranes from the blood-sinusoidal, bile canalicular and lateral domains were isolated and characterised as in [7]. Phenylmethylsulphonyl fluoride (3 mM) and aprotonin (0.5 mg/ml) were included in the media. For two-phase partitioning studies [8], membranes were solubilised at 4°C in 10 mM Tris-HCl, 150 mM NaCl (pH 7.4) containing 2% precondensed Triton X-114 (1 mg protein/ml buffer), cleared by centrifugation (13000 × *g* for 5 min; Eppendorf) and the supernatants adjusted to 30°C for 3 min. Centrifugation (6000 × *g* for 2 min in a swing-out rotor) at 20°C yielded two phases. The upper (aqueous) phase was transferred to another tube and washed with fresh 1% Triton X-114 and the centrifugation step repeated. The detergent and aqueous phases were pooled separately and aliquots analysed by electrophoresis in 8% SDS-polyacrylamide gels [9] ensuring that identical amounts of protein were loaded. Samples were transferred electrophoretically onto 0.1 μm nitrocellulose filters (Schleicher & Schuell, D-3354,

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Dassel, FRG) and these were incubated in a rabbit anti-rat fibronectin antiserum, followed by ^{125}I-protein A [10]. The antiserum [11] was purified as follows. Normal rat plasma (1 ml), treated with citrate, was passed through a gelatin-Sepharose column equilibrated in phosphate-buffered saline, pH 7.4. The fibronectin-free plasma fraction was collected, dialysed vs 0.1 M $NaHCO_3$, and coupled to CNBr-activated Sepharose 4B; the rabbit anti-fibronectin antiserum (1 ml) was passed through this column to provide a serum free of antibodies to non-fibronectin plasma components. Autoradiographs (Kodak X-AR films) of gels loaded with identical amounts of protein and exposed for the same time periods were analysed by densitometry (Joyce-Loebl chromoscan 3).

Partial hepatectomy of male Sprague-Dawley rats (250–300 g) was carried out as described by Higgins and Anderson [12] and plasma membranes prepared from 10 livers 12 and 24 h after the operation.

3. RESULTS

3.1. *Distribution of fibronectin isoforms in liver plasma membrane domains*

The distribution of fibronectin in plasma membrane subfractions originating from the sinusoidal, canalicular and lateral domains was probed using anti-fibronectin antibodies. The immunoblots in fig.1a demonstrate that various molecular mass forms of fibronectin were present in fractions face domains. Sinusoidal plasma membranes contained the two-fibronectin doublet (210 and 220 kDa), whereas the canalicular plasma membranes contained only a 220 kDa component. Lateral plasma membranes showed a more com-

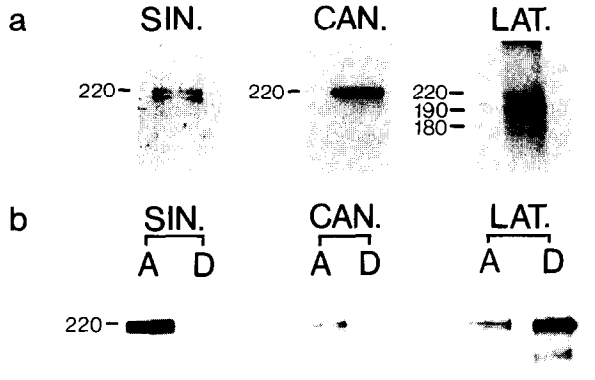

Fig.1. Immunoblots of rat liver plasma membrane subfractions originating from the blood-sinusoidal (SIN), bile canalicular (CAN) and lateral (LAT) domains of the hepatocyte surface. Proteins (50 μg per channel) were separated by electrophoresis in polyacrylamide gels, transferred to nitrocellulose filters, exposed to a rat anti-fibronectin antiserum and autoradiographs prepared. In (a), plasma membranes were electrophoresed. In (b), membranes were subjected to two-phase partitioning in Triton X-114 and the aqueous (A) and detergent (D) phases analysed separately. Molecular masses (in kDa) of the fibronectin variants are shown.

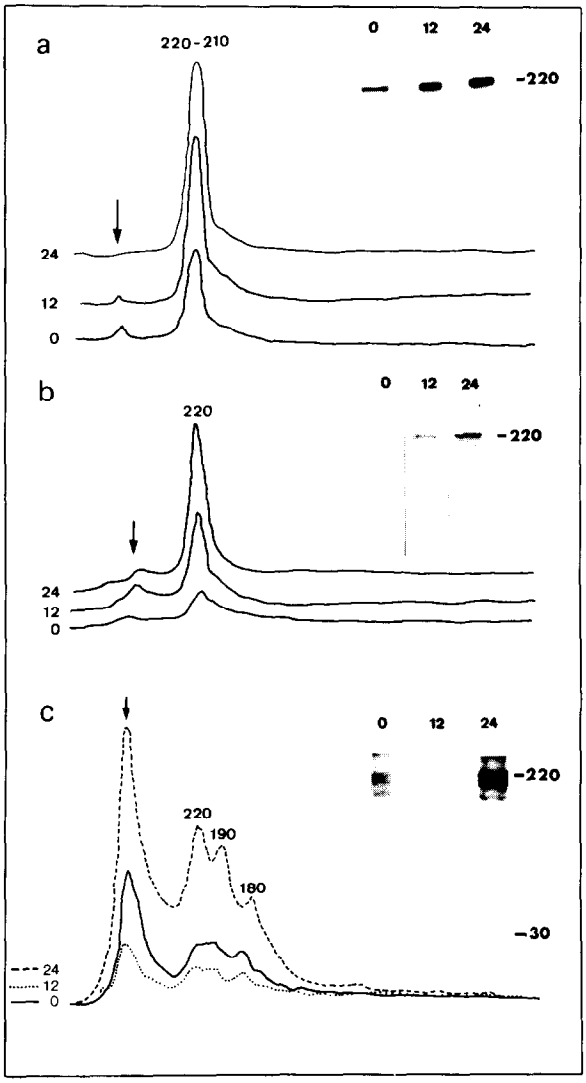

Fig.2. Densitometric tracings of autoradiographs of immunoblots of rat liver plasma membrane subfractions originating from the blood-sinusoidal (a), bile canalicular (b) and lateral (c) domains of the hepatocyte surface. Plasma membranes were prepared from rat control livers and at 12 and 24 h periods after hepatectomy. Molecular masses (kDa) are indicated. Proteins (30 μg per channel) were separated, transferred to nitrocellulose filters, and autoradiography carried out. Densitometry indicated the relative amounts of the isoforms; arrows indicate top of the gel.

plex pattern, with three isoforms identified (180, 190, 220 kDa). No other components of lower molecular mass that would suggest that proteolysis had occurred were detected.

The nature of the association of fibronectin isoforms with plasma membrane fractions was investigated by partitioning membrane proteins solubilised in Triton X-114 into detergent and aqueous phases. Fig.1b shows that fibronectin present in sinusoidal and canalicular plasma membrane fractions partitioned completely into the aqueous phase. In contrast, analysis of plasma membranes derived from the lateral domain showed that, although some of the 220 kDa component partitioned into the aqueous phase, most of this form, as well as two lower molecular mass isoforms, were recovered in the detergent phase. This result emphasised the variation in hydrophilicity of the fibronectin isoforms associated with membranes derived from lateral plasma membranes.

3.2. *Fibronectin expression in plasma membranes prepared from regenerating liver*

The expression of fibronectin in liver plasma membrane subfractions at intervals of 12 and 24 h after hepatectomy was investigated. Fig.2 shows that expression of the major form of fibronectin (220 kDa) increased in sinusoidal and canalicular plasma membrane domains. However, fibronectin, including its lower molecular mass isoforms, decreased at 12 h after hepatectomy and then increased dramatically at 24 h. Densitometric comparison of these differences showed that 12 h after partial hepatectomy, an approx. 2-fold increase in fibronectin occurred in sinusoidal and canalicular plasma membranes, whereas in lateral plasma membranes it decreased by approx. one-fifth (fig.2). 24 h after hepatectomy an approx. 3-fold increase relative to normal liver was recorded in all three plasma membrane domains.

4. DISCUSSION

The results show that there is an uneven distribution of fibronectin in the subcellular fractions corresponding to the three major domains of the hepatocyte. The two most common fibronectins reported are the secreted (plasma) and membrane-bound (cellular) forms [13]. It was shown that cultured hepatocytes synthesize and secrete plasma and cellular fibronectin [18], although the main form is the latter [14]. The identification of fibronectin isoforms of lower molecular mass in plasma membrane subfractions, originating from surface areas interacting with neighbouring hepatocytes, is a novel finding. The 180 and 190 kDa isoforms described here are unlikely to be products of proteolytic breakdown, since all plasma membrane fractions were prepared simultaneously from the same liver homogenate, and inhibitors of proteolysis were included in the media used in subcellular fractionation.

Since post-translational modifications alone probably cannot account for the differences between these fibronectin isoforms, one explanation for the differential expression and topographical distribution of fibronectin variants in the plasma membrane domains is that they arise by translation from different mRNAs by alternative splicing of a complex exon [16,18,23]. Indeed, up to three different mRNAs encoding for fibronectin have been isolated from rat liver [17,22,23]. The differences between these are located in the vicinity of the cell and heparin-binding domains and are likely to account not only for the two subunits of plasma fibronectin [22] but also for other properties, such as self-association and interaction with other molecules or with cells [16]. It is possible, therefore, that the different fibronectin isoforms now identified in the three plasma membrane domains of rat liver plasma membrane have arisen from multiple mRNA transcripts.

The three major plasma membrane domains of the hepatocyte fulfill different and well-documented functions [1]. The 220 kDa fibronectin detected in bile canalicular plasma membranes may have originated from the sinusoidal pole of the hepatocyte by endocytosis and transport through the cell, although its function in the membrane surrounding the bile spaces is obscure. Of greater interest were the isoforms present in the lateral plasma membrane of normal and regenerating liver. Unlike the fibronectin present in sinusoidal and canalicular plasma membranes, these isoforms were present after two-phase partitioning in the detergent-enriched phase, thus emphasising that they were more hydrophobic than their higher molecular mass counterparts. The use of the Triton X-114-aqueous two-phase technique

to distinguish between integral and peripheral membrane proteins is a well-established procedure [24]. This method exploits the preferential binding of Triton X-114 to hydrophobic integral proteins, thereby producing mixed micelles that partition into a detergent-enriched phase that can be separated from an aqueous phase above 20°C. Although the technique has been applied successfully to separate many integral membrane proteins, some exceptions have been reported. For example, acetylcholine receptor subunits [25] and chromaffin granule ATPases [26] behave anomalously. Such exceptions are usually explained by the presence of hydrophilic domains, as occur in the proteins forming aqueous channels across membranes, or by poor insertion into the membrane of F_1 subunits of ATPases as opposed to transport ATPases. It is possible that the hydrophobic isoforms of fibronectin may lack hydrophilic amino acid sequences that could lead to conformational modifications that feature in their functioning in the confined intercellular spaces present between hepatocytes.

Fibronectin is implicated in a number of properties related to cell-cell adhesion, cell-basement membrane and cell-extracellular matrix attachment and other interactions underlying cell division and migration. The fibronectin isoforms identified here in lateral plasma membranes may represent intermediates functioning in cell-cell adhesion processes occurring at intercellular junctions present in this plasma membrane domain. The displacement and endocytosis of fibronectin at areas of fibroblast adhesion substratum have been studied using morphological approaches [19–21], and the present work indicates that isoforms of varying size and hydrophobicity may be implicated in these processes.

In conclusion, the results show that fibronectin is differentially expressed in the three functional domains of the plasma membrane of hepatocytes. Further modulation of expression appears to occur during regeneration after partial hepatectomy.

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