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ABSTRACT

Most normal cells require attachment to extracellular matrix for survival, growth, migration and differentiation. In the liver, hepatocytes interact with a complex set of macromolecules, including interstitial and basement membrane components. Interactions of hepatocytes with extracellular matrix components occur throughout cell surface receptors that specifically recognize these proteins. These receptors include both integrins and non-integrin membrane-associated proteins. Integrins that belong to the β1 family are involved in the binding of hepatocytes to collagens, fibronectin, laminin and perlecan. Non-integrin receptors include the 32/67 kD laminin receptor, the 38/36 kD perlecan receptor and other versatile plasma membrane-associated proteins. Recently, it has been shown that these receptors may bind specific sites within matrix macromolecules. It may be hypothesized that both the composition of the extracellular matrix and the molecular structure of each component, modulate the behavior of hepatocytes in normal liver and in the course of hepatic fibrosis.

INTRODUCTION

Cell-matrix interactions are involved in most, if not all, of the key-events of cell life. Embryonic development, maintenance of tissue architecture, tissue repair, inflammation and tumor metastasis are some examples of biological processes in which extracellular matrix plays an important role. During the last ten years, this field has been intensively explored, leading to the discovery of cell surfaces receptors as mediators between extracellular macromolecules and the intracellular machinery. The molecular mechanisms involved in signal transduction are currently being investigated.

The liver is composed of several cell types which are organized within the hepatic lobule in a precise architecture. Hepatocytes, the major liver cell type which performs most hepatic functions, interact with both non-parenchymal cells and the extracellular matrix. Besides soluble factors, e.g. hormones and cytokines, structural requirements are involved in the stability and modulation of liver specific functions.

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At least three groups of specialized cell surface proteins maintain specific relationships between cells and either neighboring cells or extracellular matrix. These include cell adhesion molecules (CAMs), junction molecules and extracellular matrix receptors, i.e. integrins and non-integrin receptors.
This review will summarize our current knowledge on extracellular matrix receptors in the normal liver. Integrins, basement membrane structure and functions, laminin receptors, as well as the effects of extracellular matrix on hepatocyte behavior have been recently reviewed elsewhere (Abelda and Buck, 1990 ; Buck, 1987 ; Martin and Timpl, 1987 ; Mecham, 1991 ; Ruoslahti, 1991 ; and reviews by Schuppan et al. and Bissell in this book).

THE HEPATIC EXTRACELLULAR MATRIX

Normal adult liver contains only small amounts of extracellular matrix components. However, both immunofluorescent and immunoelectron studies have shown that hepatocytes interact with almost all the essential components of both interstitial extracellular matrix, e.g. collagens I, III, V and VI, and fibronectin, and basement membranes, e.g. collagen IV, laminin, entactin and the heparan sulfate proteoglycan, perlecan (Bianchi et al., 1984 ; Clément et al., 1984 ; Grimaud et al., 1980 ; Hahn et al., 1980 ; Martinez-Hernandez, 1984 ; Rescan et al. submitted). Furthermore, dramatic changes in both the amount and composition of the extracellular matrix occur in a variety of liver diseases leading to the development of hepatic fibrosis. For example during the "capillarization" process laminin, collagen IV and probably perlecan accumulate, thus forming a continuous basement membrane in the space of Disse. Recent immunoelectron studies have shown, in addition, that basement membrane components are much more abundant in fetal than in adult liver, particularly at the contact of hepatocyte microvilli (Rescan et al., 1989). Thus, the hepatic extracellular matrix must be considered not as a static network but rather as a dynamic structure which may change during the various physiopathological stages of the liver.
For a long time the origin of the hepatic extracellular matrix has been a matter of debate. Indeed, *in vitro* studies have shown that most liver cells, including hepatocytes (Diegelmann, 1986) and Ito cells (Friedman et al., 1985 ; Schafer et al., 1987 ; Weiner et al., 1990), may express matrix proteins when put in culture. During the last few years, it

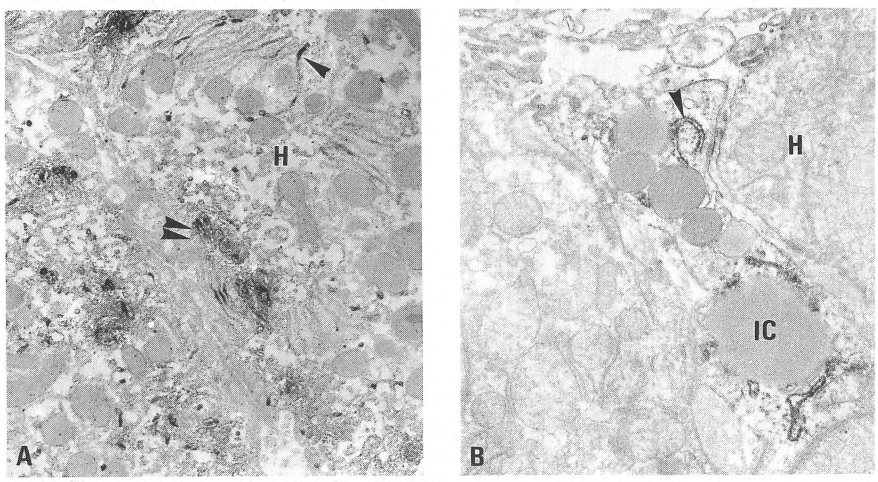

Figure 1 : Electron microscopy. Immunoperoxidase localization of fibronectin (A) and collagen IV (B) in normal adult rat liver. Fibronectin is abundant in the rough endoplasmic reticulum (arrowheads) and Golgi apparatus (double arrowheads) of hepatocytes (H), and collagen IV in the rough endoplasmic reticulum of an Ito cell (IC). (A : x 5,200; B : x 11,600)

became clear that the expression of matrix proteins at high levels by cultured liver cells are related to an adaptative process to survive *in vitro*. Accordingly, these findings indicate that liver cells have the capacity to express matrix genes in certain conditions, particularly when their environment is altered. Immunoelectron studies and more recently *in situ* hybridization have somewhat clarified the respective participation of each liver cell type in the formation of the hepatic extracellular matrix *in vivo* (Clément et al., 1984 ; 1986 ; 1988 ; Geerts et al., 1986 ; Milani et al., 1989a ; 1989b ; Takahara et al., 1989). Except for fibronectin adult normal hepatocytes are not a major source of extracellular matrix components (Figure 1). Rather, both endothelial and Ito cells have been shown to contain precursors of matrix proteins or their corresponding mRNAs. Interestingly, hepatocytes, in addition to sinusoidal cells, were found to produce extracellular matrix proteins, particularly basement membrane components, in alcoholic human livers (Clément et al., 1986) and in cholestatic rat livers (Abdel-Aziz et al., 1991), as well as during the perinatal period in both species (Rescan et al., 1989).

CELL SURFACE RECEPTORS FOR EXTRACELLULAR MATRIX

Adhesion molecules mediate interactions between cells and the extracellular milieu. Among these molecules, specific receptors allow cells to recognize extracellular matrix proteins. During the last few years the molecular mechanisms by which hepatocytes interact with extracellular matrix located in the space of Disse have been investigated. First lines of evidence that hepatocytes specifically bind individual extracellular matrix proteins came from *in vitro* studies (Rubin et al., 1981 ; Bissell et al., 1986). After their isolation from rat liver, hepatocytes can be set up on various substrata made of purified extracellular matrix components. As shown in figure 2, hepatocytes differently attach and spread on these substrata. Interestingly, the most efficient matrix molecules for

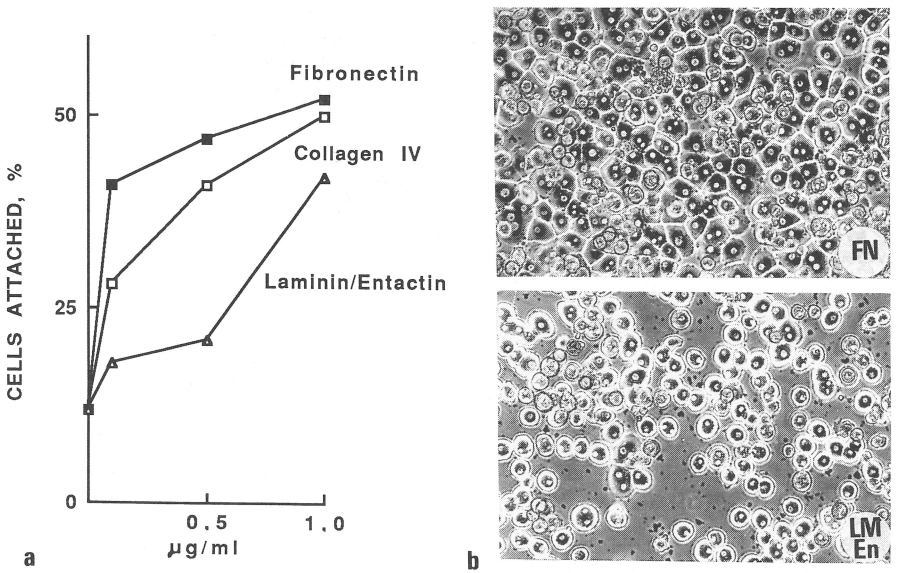

Figure 2 : Cell attachment of hepatocytes to various extracellular matrix components.
(a) : Various amounts of fibronectin, collagen IV and laminin/entactin complex were coated on 0.32-cm2 well tissue-culture plates. Then, freshly isolated hepatocytes (60,000 cells) were added in serum-free medium. After 30 min, plates were gently washed with phosphate buffer. The percentage of attached cells was determined using a lactacte dehydrogenase assay.
(b) : Phase-contrast microscopy. Hepatocytes were cultured on dishes coated with either fibronectin (FN) or laminin/entactin complex (LM/En) for 4 h. (x140)

attachment and spreading appear to be the less effective for maintenance of liver-specific functions, i.e. fibronectin and collagens vs laminin. These findings suggest that specific membrane proteins mediate hepatocyte interaction to distinct components of the hepatic extracellular matrix.

Integrins

Integrins are heterodimers consisting of noncovalently associated α and β subunits that mediate both cell-substratum and cell-cell adhesion. The integrin family is divided in at least three groups according to their common β chain. Most of integrins interacting with extracellular matrix components belong to the β1 - or VLA - subfamily. β1 integrins serve as receptors for fibronectin (α1β1 ; α3β1 ; α5β1 ; αvβ1), collagens (α1β1 ; α2β1 ; α3β1), and laminin (α1β1 ; α2β1 ; α3β1 ; α6β1). In addition, other integrins from the β3 (e.g. αvβ3) β4 (e.g. α6β4) and β5 subfamilies (e.g. αvβ5) have been shown to interact with matrix proteins. Since integrins were found to be present in every tissue or cells so far studied, it was expected to find integrins in the liver. Recently, Volpes et al (1991) have studied by immuno-histochemistry the distribution of the β1 chain and the variable α chain -1 to 6- of the VLA subfamily in normal, inflammatory and cholestatic human livers. The common β1 chain was always detected in portal spaces and in the sinusoids, while the pattern of staining for the α chains was different according to the structure analyzed and/or the pathological state of the liver. Specifically, only α1 and α5 chains were evidenced on both hepatocytes and sinusoidal cells in normal liver. By contrast, hepatocytes became positive for α2, α3, α5 and α6 chains in inflammatory and/or cholestatic livers.
Another strategy to identify integrins is to isolate these proteins from purified cell populations. The first identified integrin in hepatocytes was the fibronectin receptor. Johansson et al. (1987), have isolated a fibronectin receptor which specifically bound cell-binding fibronectin domain in a R-G-D-dependent manner. It appeared to consist of a β1 chain associated with an α chain distinct from that in fibroblastic cell lines. β1 integrins are also involved in the interactions of hepatocytes with perlecan (Rescan et al. submitted) and laminin, particularly the α1β1 integrin (see below).

Non-integrin matrix receptors

Extracellular matrix binding proteins that do not belong to the integrin family have been identified in hepatocytes. Rubin et al. (1986) have isolated rat liver glycoproteins with affinity for collagen I. They found that antibodies against the purified glycoproteins - Mr= 105 Kd, 115 Kd and 130 Kd- inhibited attachment of hepatocytes to collagen I, but not to either fibronectin or collagen IV. In chick liver, a major Mr=47,000 collagen binding protein that binds gelatin and native collagens I, III and IV has been localized in smooth muscle cells of the arterial wall and in perisinusoidal cells, but not in the hepatocyte (Saga et al., 1987). This glycoprotein is not likely to be a receptor surface protein, but may play a role in intracellular protein processing or translocation. More recently we have identified a Mr=80,000 protein on the surface of hepatocytes that binds collagen IV and also other basement membrane components, including laminin and perlecan (Clément and Yamada, 1990). The core protein of perlecan has been shown to directly interact with freshly isolated rat hepatocytes (Clément et al., 1989). A major Mr=36/38,000 and a Mr = 26,000 membrane proteins have been purified from cell surface iodinated hepatocytes using affinity chromatography column made with the core protein of perlecan. The 36/38 kD protein(s) was found on a variety of cells that contact basement membrane, including MDCK and NRK-52E kidney cells, mouse melanoma M2 cells and EHS tumor cells. Laminin binding protein(s) can be isolated from cell membranes by affinity chromatography on laminin-Sepharose. Thus, a major Mr=67,000 protein has been identified on a variety of cells, including tumor cells, muscle cells, macrophages, endothelial and epithelial cells and neuronal cells (for review, see Mecham, 1991). Wewer et al. (1986) have reported a partial cDNA for the human 67 kDa laminin binding protein. Subsequently, several groups have isolated full-length cDNA having a coding capacity for a 32 kDa, but not 67 kDa protein (Yow et al., 1988 ; Segui-Real et al., 1989). Recently, various proteins from isolated membranes of cell-surface iodinated hepatocytes were

identified using a laminin affinity column, including Mr=67,000 ; 45,000 ; and 32,000 proteins (Clément et al., 1990). These cell-surface proteins were recognized by antibodies made against a bacterial fusion protein coded for by the ß-galactosidase gene plus the 0.9 kb cDNA sequence encoding the nearly entire 32 kD laminin binding protein molecule (LBP-32). Northern-blot analysis revealed that hepatocytes contain 1.1 kb LBP-32 mRNAs (Rescan et al., 1990). Interestingly, the steady state LBP-32 mRNA level was much more higher in fetal and neoplastic hepatocytes as well as in hepatoma cell lines and in cultured adult hepatocytes, than in the normal adult liver (Rescan et al., 1990 ; 1991). This overexpression paralleled with the expression of B1 and/or B2 laminin chains. These findings suggest that the expression of both laminin chains and receptors in hepatocytes is related to changes of the normal phenotype and/or the pericellular environment.

INVOLVEMENT OF THE STRUCTURE OF LAMININ IN SPECIFIC INTERACTIONS WITH HEPATOCYTES

Laminin, the major component in basement membranes, was found to be composed of three genetically distinct chains, i.e. A (Mr=440,000), B1 (Mr=220,000) and B2 (Mr= 210,000) in murine Engelbreth-Holm-Swarm (EHS) tumor. Laminin of non-neoplastic origin may differ in either the A, B1 and B2 ratio or the presence of different chain(s). Thus, a variety of cells express A chain at very low level, if any. In addition, several normal tissues, including placenta, heart and neuromuscular junctions were found to contain laminin formed by the assembly of homologous but genetically distinct chains.
In the liver, although abundant in neighboring laminin-producing cells, i.e. sinusoidal endothelial and Ito cells, this glycoprotein is only sparsely deposited at the contact of hepatocytes (Clément et al., 1988 ; Hahn et al., 1980). Laminin extracted from the EHS tumor is a potent regulator of hepatocyte morphology and functions *in vitro* (Bissell et al., 1987). However, the relevance of these findings may depend on the actual structure of laminin in the space of Disse. Indeed, since this glycoprotein is a very large molecule it can be expected that several different cellular receptors on hepatocytes will bind to multiple cell adhesion sites on laminin, thus inducing specific regulatory signals.

Structure of laminin interacting with hepatocytes

Immunoelectron studies have clearly shown that in the normal adult liver, Ito cells is a major source of laminin that is deposited in the space of Disse (Clément et al., 1988). Recently, we have investigated the expression of the three chains of laminin in isolated rat lipocytes (Loréal et al., 1991). Both B1 and B2 chains, but not A were found in the medium of 5-day-old lipocyte primary cultures by western blotting and immunoprecipitation of radiolabeled proteins. An additional Mr=380,000 protein was found by immunoprecipitation only, while only one Mr=900,000 band was found under non-reducing conditions, thereby suggesting that lipocytes produce a variant form of laminin composed by the assembly of B1, B2 and the 380 kD polypeptide. Northern blots confirmed these data and showed that only B2 mRNAs were clearly detectable in freshly isolated lipocytes. Although both A and B1 genes could be transcribed at low levels and/or mRNAs rapidly processed, it is likely that Ito cells produce a variant form of laminin *in vivo*. Interestingly, Maher and MacGuire (1990) have recently shown that, in addition to Ito cells, endothelial cells isolated from normal adult rat liver contained high B2 chain mRNA levels.
Although not producing detectable laminin in the normal adult liver, hepatocytes have the capacity to express laminin genes (Clément et al., 1988 ; Rescan et al., 1989 ; 1990 ; 1991). By immunoelectron microscopy in both liver and cultured cells, immunoblotting and/or immunoprecipitation of proteins from media of cultured cells and Northern blotting, we have shown that hepatocytes express B1 chain of laminin in fetal liver, and both B1 and B2 chains in neoplastic hepatocytes from diethylnitrosamine-treated rat livers as well as in hepatoma cell lines. Interestingly, both rat and human hepatoma cells were found to synthesize a Mr=380,000 polypetide, not related to the A chain of laminin (Rescan et al., 1991). Finally, in normal adult hepatocyte primary cultures, B2 chain mRNAs were present as early as 4 hours after cell seeding, while the steady state B1

mRNA level remained very low during the first day in culture. Both B1 and B2 mRNA levels dramatically increased during the following 2 days. These findings clearly indicate that the expression of laminin chains by hepatocytes is not coordinated, depends on the maturation of the cells, and is related to changes of the normal phenotype and/or the pericellular environment.
Taken together, these data suggest that laminin probably exists in the space of Disse in a different form compared to that originally characterized from the EHS tumor.

Specific binding domains on laminin chains

Two complementary approaches have been designed to map laminin regions involved in biological activities, including proteolytic cleavage of the EHS laminin and synthetic peptides deduced from the cloned sequence of its three chains (Figure 3).

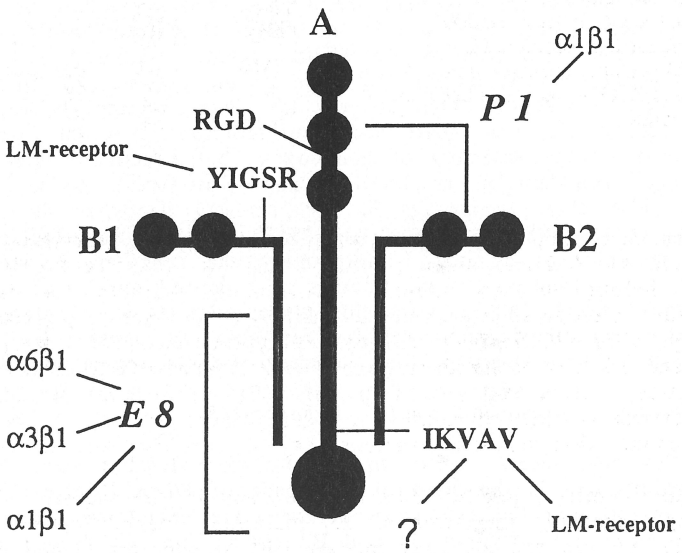

Figure 3 : Interactions of receptors with specific binding domains on EHS laminin chains

At least five active sites for cell adhesion have been identified using synthetic peptides, including YIGSR, PDGSR and F9 on the B1 chain (residues 929-933, 902-906 and 641-660, respectively) and CQFALRGDNP and CSRARKQAASIKVAVSADR on the A chain (residues 1115-1124 and 2091-2108, respectively). Interestingly, it has been shown that YIGSR and the sequence RYVVLPR within the F9 peptide inhibit metastasis (Graf et al., 1987 ; Skubitz et al., 1990), while SIKVAV-containing peptide promotes tumor cell invasion (Kanemoto et al., 1990). Rat hepatocytes bind to three different sites within the B1 and A chains of the EHS laminin, including YIGSR and RGD- and SIKVAV-containing peptides (Clément et al., 1990; Tashiro et al., 1989). SIKVAV-containing peptide was the most potent peptide, reaching 70% of the activity of laminin for hepatocyte adhesion. Affinity chromatography on peptide columns revealed that the SIKVAV-containing peptide specifically bound LBP-32 and related cell membrane proteins, as well as other minor membrane-associated proteins.

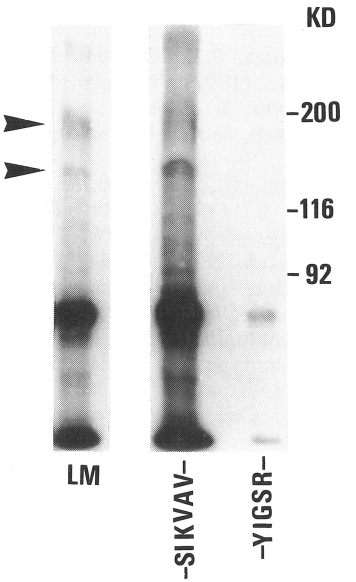

Figure 4 : Identification of cell surface proteins that bind laminin and related peptides in a divalent cation-dependent manner

125I-labeled purified cell membranes of normal adult hepatocytes were prepared in the presence of $MnCl_2$ and passed over columns made with either laminin (LM), SIKVAV or YIGSR. EDTA eluted a major Mr = 80,000 protein from these columns. In addition, Mr = 200,000 and 150,000 proteins (arrows) were eluted from laminin and SIKVAV columns but not from a YIGSR column.

Cell surface proteins that bind this peptide in a divalent cation-dependent manner were also identified (Figure 4). Although these proteins were not easily detectable, it is likely that proteins that belong to the integrin family may mediate the interaction of hepatocytes with this biologically active site. Other cell surface proteins were also identified interacting with YIGSR and RGD-containing peptides. This group of proteins (Mr = 80, 55, 38 and 36 kD) bound these peptides with lower activity than the others. In addition, YIGSR peptide elutes a Mr = 67 kD protein (LBP-32) from affinity column (Graf et al., 1987). It is noteworthy that polymeric and cyclic forms of YIGSR are required for activity, thus suggesting that tertiary structure may be critical for its recognition by receptor(s).

Another strategy to identify specific domains of laminin interacting with receptors consists in preparing proteolytic fragments of the molecule. Four different fragments of the EHS laminin -E5, E6, E8 and P1- may interact with hepatocytes (Timpl et al., 1983 ; Forsberg et al., 1990). Recently, $\alpha 1\beta 1$ integrins were found to recognize both the E8 and P1 fragments of laminin on hepatocytes (Forsberg et al., 1990). Also $\alpha 3\beta 1$ and $\alpha 6\beta 1$ integrins have been shown to interact with the E8 fragment (Gehlsen et al., 1989 ; Hall et al., 1990). Taken together these findings show that a specific domain of laminin may recognize different receptors and, on the other hand a same receptor may recognize different domains of laminin.

As above discussed, is likely that in any case normal hepatocytes do not interact with laminin formed by the assembly of A, B1 and B2 chains in a stochiometric complex. Whether analogues of laminin chains, e.g. S-laminin or merosin, might be present in the space of Disse remain to be elucidated. Since attachment of hepatocytes to a substratum made of EHS laminin in culture occurs, at least in part, via active sites within the A chain (Clément et al., 1990), conclusions from *in vitro* studies might be unrelevant compared to the normal situation *in vivo*. The presence of multiple binding proteins on the surface of hepatocytes which interact with active sites on laminin emphasizes the concept that the specificity of interactions of cells with extracellular matrix involves both specialized receptors and key-domains of macromolecules.

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Résumé

La plupart des cellules normales doivent s'attacher à une matrice extracellulaire pour leur survie, leur croissance, leur migration et leur différenciation. Dans le foie, les hépatocytes interagissent avec un assemblage complexe de macromolécules extracellulaires de type intersticiel ou de composants des lames basales. L'interaction des hépatocytes avec la matrice extracellulaire est possible grâce à des récepteurs membranaires qui reconnaissent spécifiquement chacune des protéines extracellulaires. Ces récepteurs sont des intégrines ou d'autres protéines associées aux membranes plasmiques. Les intégrines de la famille β_1 sont responsables des liaisons de l'hépatocyte avec les collagènes, la fibronectine, la laminine et le perlecan. Parmi les récepteurs n'appartenant pas à la super-famille des intégrines on trouve le récepteur à la laminine de 32/67 kD et le récepteur au perlecan de 38/36 kD. Récemment, il a été démontré que ces récepteurs interagissent avec les protéines extracellulaires via des sites spécifiques. Il est possible que, aussi bien la composition de la matrice extracellulaire hépatique que la structure de chacune des macromolécules qui la compose module le fonctionnement des hépatocytes dans le foie normal et cirrthotique.