CELL MEMBRANES

Methods and Reviews

Volume 3

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PREFACE

This volume assembles reviews on topics in two major related areas. One of these concerns the interactions of cells with substrata and with other cells, which are mediated by the extracellular matrix and soluble molecules. As described in this volume, these interactions are responsible for controlling cell functions ranging from embryogenesis and neural development to blood clotting. Moreover, important properties of the extracellular matrix can be modulated by the interdependent actions of tumor cells and fibroblasts. The other major area of interest concerns the response of cells to extracellular signals. Recent work has begun to reveal how a remarkable diversity of cellular functions, including neuronal, proliferative, membrane–cytoskeletal, and many other kinds of responses, are elicited through the mediation of a relatively small and interdependent set of second messenger systems. These include both changes in cytoplasmic ionic balances and activation of various kinds of protein kinases. Both subjects are covered in this volume. The two areas are linked by the common theme of cellular response to an external environment that is sensed through cellular interactions with informational molecules, which are soluble agents, as well as those that are components of insoluble matrices. It is only recently that we have come to appreciate the complex interplay between the matrix surrounding a cell and the cell's response to hormones and growth factors. Thus, we have tried to select examples in which this type of extracellular integration may play a role.

We thank all of the contributors for providing these excellent reviews and for doing so in a timely fashion.

Elliot L. Elson
William A. Frazier
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St. Louis, Missouri

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REGULATION OF DEVELOPMENT BY THE EXTRACELLULAR MATRIX

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1. INTRODUCTION

In the vertebrates, cells are considerably displaced during the processes leading to the shaping of the embryo. Extensive morphogenetic transformations, including changes of cell shape, cell migrations, distortions, remodelings, and dissociations of sheets of cells, are essential in establishing the basic structures of the embryo. These movements allow cells of different areas of the embryo to interact transiently, a necessary step for the transduction of inductive signals. Such events are responsible for the segregation of embryonic cells into the endoderm, mesoderm, and ectoderm, and then each layer becomes, in turn, regionalized.

At any time, cells can be engaged in one or more of the five primary processes of division, migration, adhesion, differentiation, and death. Division, differentiation, and death of cells are necessary for the construction of the body plan but are far from sufficient, since they cannot account for the transient interactions of cells and their final localization. Therefore, these processes can be considered as secondary compared with cell migration and adhesion in the shaping of the embryo.

Cell adhesion and migration are influenced in part by the relationships of the cells to their immediate environment and particularly by the interactions with the adhesive molecules of the extracellular matrix (ECM). The early appearance

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of fibronectin and laminin during embryogenesis and their further distribution as ubiquitous components of the ECM are essential for many different morphogenetic mechanisms. In this chapter, we will outline some of the biochemical properties and potential functions of fibronectin and laminin. We will then concentrate on two major morphogenetic events that occur during embryogenesis: cell migration and epithelium–mesenchyme interconversion.

2. MOLECULAR ANALYSIS, LOCALIZATION, AND FUNCTIONS OF CELL-TO-SUBSTRATE ADHESION MOLECULES

A common feature of fibronectin and laminin is that they are composed of separable functional domains, each specialized for specific binding activities. These domains are largely responsible for the different functions of the molecules.

2.1. Laminin

2.1.1. Structure of Laminin

Laminin (LN) is a high-molecular-weight glycoprotein (950 Kd) with a cross-shaped structure composed of a long arm and three short arms, as seen on electron microscopic images (Timpl *et al.*, 1979; Engel *et al.*, 1981) (Figure 1).The functional domains of LN consist of binding domains for cells, heparin, and type IV collagen (Terranova *et al.*, 1980; Rao *et al.*, 1982). The major heparin-binding site is located in the globular terminal domain in the long arm. In contrast, the cell-binding domain and the collagen-binding domain have not been precisely mapped; the cell-binding domain is probably present in the center of the molecule and extends along the short arms, whereas the collagen-binding domains seem located at the ends of the short arms (Rao *et al.*, 1983; Timpl *et al.*, 1983).

LN contains three distinct polypeptide chains of 200 Kd (B2 chain), 230 Kd (B1 chain), and 420 Kd (A chain). The precise arrangement of these chains is not known. However, preliminary results obtained from the cloning of the genes coding for the different chains suggest that the three chains are associated in the central part of the long arm into a coiled-coil alpha helix (Barlow *et al.*, 1984). In addition, regions with repeated cysteine residues are observed in the short arms (Ott *et al.*, 1982). LN does not form fibrils but can self-assemble into large complexes through its globular end domains (Yurchenko *et al.*, 1985).

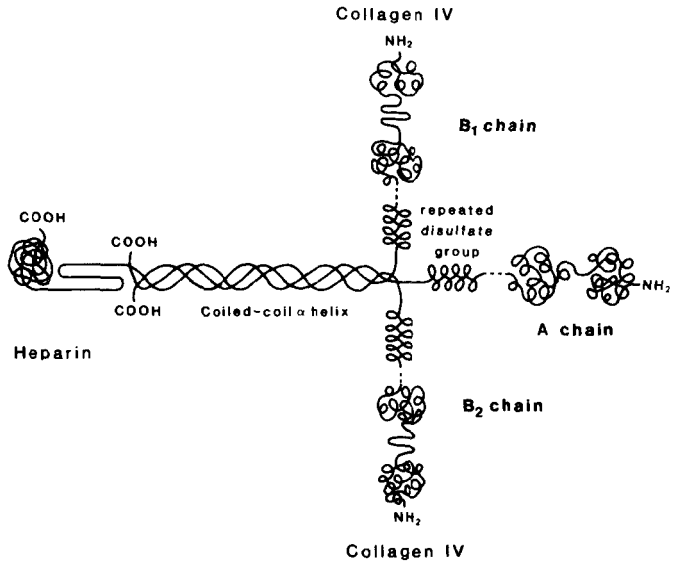

FIGURE 1. Schematic representation of LN: LN is a cross-shaped molecule composed of three chains (A, B_1, and B_2 chains). The chains are associated in the center of the cross into a coiled-coil alpha helix. Two globular domains on each B chain and three on the A chain are supposed to contain the binding sites for heparin and type IV collagen. The cell-binding site is suspected to distribute in the center of the cross, but it has not been precisely mapped. Finally, particular regions very rich in a repeated disulfate group have been represented in the center of the cross; they probably participate in the typical shape of the molecule.

2.1.2. Receptors for Laminin

A high-affinity receptor for LN ($K_D = 10^{-9}$ M) has been isolated from carcinoma and sarcoma cells, as well as from myoblasts (Rao *et al.*, 1983; Malinoff and Wicha, 1983; Lesot *et al.*, 1983; S. S. Brown *et al.*, 1983). It consists of a disulfide-linked glycoprotein complex that contains subunits with apparent molecular weights of 68 Kd. However, it remains to be determined whether all cell types interact with LN through the same receptor. A low-affinity receptor ($K_D = 10^{-6}$ M) has also been isolated that is, in fact, the receptor for FN (Horwitz *et al.*, 1986). Although the high-affinity receptor could participate in the permanent anchorage of epithelial cells, the low-affinity receptor might participate in dynamic processes during embryogenesis such as cell migration and tissue remodeling (see Section 3.4 and 4). Finally, LN can interact indirectly with the cell membrane through its heparin-binding domain (Edgar *et al.*, 1984).

2.1.3. Distribution and Functions of Laminin

LN appears very early during development, one polypeptide chain being synthesized during oogenesis whereas the second and third polypeptides appear in the 2- to 8-cell-stage mouse embryo. LN is finally expressed on the surface of blastomeres at the 16-cell stage (Leivo *et al.*, 1980; Cooper and McQueen, 1983). Later on in development, LN is present in the basal lamina of epithelia, but its precise distribution in the different compartments of the basal lamina is still controversial.

The primary role of LN is to mediate the adhesion of epithelial and endothelial cells to type IV collagen (Terranova *et al.*, 1980; Carlson *et al.*, 1981; Palotie *et al.*, 1983; Donaldson and Mahan, 1984). However, fibroblastic cells can also interact with LN (Couchman *et al.*, 1983), probably through the FN receptor. LN is also found on the surface of Schwann cells along the nerves (Cornbrooks *et al.*, 1983; Duband and Thiery, 1987), and transiently in the central nervous system of the mouse (Liesi, 1985). In this respect, LN seems to play an important role during the ontogeny of the nervous system (see Section 3.4).

2.2. Fibronectin

2.2.1. Structure of Fibronectin

Fibronectin (FN) constitutes a class of high-molecular-weight glycoproteins that consist of two similar, but not identical, polypeptide subunits of 220–250 Kd linked by disulfide bonds (for reviews, see Ruoslahti *et al.*, 1981; Hynes and Yamada, 1982; Yamada, 1983; Yamada *et al.*, 1985) (Figure 2). FN is present in large amounts in the plasma (0.3 mg/ml) as a soluble dimer (plasma FN), around mesenchymal cells in 10-nm fibrils, and on the basal surface of epithelia (cellular FN). Plasma FN is synthesized by hepatocytes and endothelial cells, whereas cellular FN is produced by a large variety of cell types (reviewed by Hynes and Yamada, 1982).

The primary structure of FN has now been completely established (Petersen *et al.*, 1983; Kornblihtt *et al.*, 1983, 1984a,b, 1985). Each FN chain contains approximately 2350 amino acids and contains three different types of internal repeating units called type I, II, and III sequence homologies.

Different forms of FN have been identified, and, in fact, each FN dimer consists of a characteristic set of distinguishable subunits differing slightly in apparent molecular weight. These differences are due in part to the variability

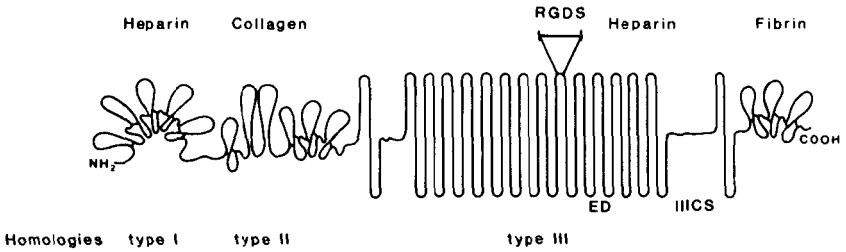

FIGURE 2. Schematic representation of one of the polypeptide chains of FN. The secondary structure of FN can be obtained from the arrangements of the different types of homologies (type I, II, and III). The structure of the homologies derives mostly from the sequence of the gene obtained in mammals. There are 12 type I homologies and 2 type II homologies, each being encoded by one exon; in contrast, each of the 16 type III homologies is probably generated by 2 exons. The binding domains mainly determined by partial proteolytic cleavage of the molecule correspond roughly to the association of several homologies. The cell-binding site (RGDS) has been precisely mapped; it is located on the top of the tenth type III homology. In fact, this domain corresponds to the insert of the DNA sequence coding for the RGDS peptide into a type III homology. Finally, the regions concerned by splicing, termed ED (extra domain) and IIICS, are indicated on the molecule.

of internal amino acid sequences. The various FN molecules derive from a single large gene covering 48 kb; a common mRNA precursor may undergo alternative splicing (Hirano *et al.*, 1983; Schwarzbauer *et al.*, 1983; Vibe-Pedersen *et al.*, 1984). The splicing events concern two DNA sequences coding for the extra domain (ED) and the IIICS domain. Complete and incomplete splicing in the IIICS region generates five different mRNA sequences; two mRNA populations are generated by the excision of the entire ED exon in a fraction of the primary transcripts (Kornblihtt *et al.*, 1983, 1985, Schwarzbauer *et al.*, 1983). The combination of the two splicing events could provide as many as ten different mature messages from a single gene. Interestingly, the ED sequence is totally deleted in hepatocyte mRNAs that code for plasma FN, whereas it is maintained in a fraction of mRNAs from other cell types (Kornblihtt *et al.*, 1983, 1984a).

The functional domains of FN comprise binding sites for a variety of molecules such as collagen, heparin, fibrin, actin, and possibly DNA (for reviews see Furcht, 1983; Yamada, 1983). The cell-binding sequence has been precisely mapped. It consists of a peptide in which the very hydrophilic sequence Arg-Gly-Asp-Ser (RGDS) is absolutely required for the adhesion of cells to FN (Pierschbacher and Ruoslahti, 1984a; Yamada and Kennedy, 1984). Variants of this sequence have been tested for their ability to inhibit binding of cells to FN or binding of the FN cell-binding region to the cell surface. Most substitutions, except at the carboxyl terminus, inactivate the peptide; in fact, RGDA, RGDV,

and RGDT sequences also retain binding properties (Piersbacher and Ruoslahti, 1984b; Yamada and Kennedy, 1985). Specific spacing between the two charged amino acids (Arg and Asp) is required; these charges must be located at a specific distance from the peptide backbone (Yamada and Kennedy, 1985). The RGDS sequence is found only once on the molecule, and is probably externally exposed on the top of a type III domain loop (Pierschbacher *et al.*, 1985). The RGDS sequence is found in several other molecules, such as fibrinogen, the receptor for phage on *Escherichia coli*, and the coat protein of Sindbis virus. The RGDA sequence is present in discoidin I, thrombin, collagen alpha 1(I), and alpha 2(I) chains, whereas the RGDV sequence is found in vitronectin (Pierschbacher and Ruoslahti, 1984b; Pierschbacher *et al.*, 1985; Dufour *et al.*, 1987). Interestingly, most of these molecules are known to have cell-binding properties. The wide distribution of the RGD sequence suggests that it constitutes an ancient recognition signal common to many proteins. However, it should be noted that the specificity of binding to the receptor may be ensured by the sequences surrounding the tetrapeptide, since adjacent sequences can also modify its binding specificity (Yamada and Kennedy, 1985).

In contrast to plasma FN, cellular FN self-associates to form polymers and frequently codistributes with collagens in fibers (Furcht *et al.*, 1980). So far, the sequences responsible for fibrillogenesis have not been yet completely identified. A possible candidate is the 90-amino-acid peptide coded by the ED sequence (Schwarzbauer *et al.*, 1983; Kornblihtt *et al.*, 1983, 1984a) but no "polymerization sequence" has been detected in the ED region. The frequent codistribution of FN and collagens in fibers suggests that collagen could constitute a framework for FN polymerization (Furcht *et al.*, 1980). However, this statement is contradicted by *in vitro* experiments which suggest that it is FN that is responsible for the organization of collagen fibers and not the opposite (McDonald *et al.*, 1982). Recently, a 70-Kd domain of FN located in the amino-terminus part of the molecule has been shown to participate to FN fibrillogenesis; this domain does not appear to contain neither the ED nor the cell-binding domain (McKeown-Longo and Mosher, 1985).

2.2.2. Receptors for Fibronectin

The modest affinity of FN for the cell membrane ($K_D = 10^{-6}$ M; Akiyama and Yamada, 1985) has greatly hampered the isolation of FN receptor(s). Recently, two different approaches—one using monoclonal antibodies that interfere with cell attachment (Greve and Gottlieb, 1982; P. J. Brown and Juliano, 1985; Chen *et al.*, 1985a, Damsky *et al.*, 1983, 1985; Knudsen *et al.*, 1985) and the

other based on affinity between the cell-binding sequence of FN and solubilized membrane proteins (Akiyama *et al.*, 1985)—have lead to the identification, in the chick, of a 140-Kd complex composed of three glycoproteins of approximately 120, 140, and 160 Kd, involved in the interaction of cells with FN. Similarly, a glycoprotein of 140 Kd has also been isolated from mammalian cells (Pytela *et al.*, 1985). This 140-Kd complex inhibits the attachment and spreading of fibroblasts into FN-coated substrates (Akiyama *et al.*, 1985). This complex is located on fibroblasts, myoblasts, and other cell types at the cell-to-substratum contact sites and codistributes with FN fibers and stress fibers (Chen *et al.*, 1985a,b; Damsky *et al.*, 1985). The proteins of the 140-Kd complex are integral membrane proteins that are linked to talin in the internal side of the membrane (Horwitz *et al.*, 1986). They thus must serve as a bridge between FN and the cytoskeleton. Interestingly, the 140-Kd complex is phosphorylated in transformed cells resulting in the disruption of the coupling between the complex and talin molecules (Hirst *et al.*, 1986). The arrangement of the three proteins within the cell membrane and the mode of interaction with FN remains to be determined. However, the three proteins are required to provide full binding with FN molecules (Buck *et al.*, 1986). The number of receptor molecules on fibroblasts has been estimated to approximately 3×10^5 molecules/cell (Akiyama and Yamada, 1985; Akiyama *et al.*, 1985). Finally, as already mentioned, this complex has also been shown to act as a low-affinity receptor for LN (Horwitz *et al.*, 1986). Even though the 140-Kd complex is now considered as the leading candidate for a functional FN receptor, one cannot neglect other possible receptors, such as a 47-Kd glycoprotein (Aplin *et al.*, 1981), cell-surface heparan sulfate (Laterra *et al.*, 1983), and gangliosides (Kleinman *et al.*, 1979; Spiegel *et al.*, 1985). These various receptors could each be of importance at different stages of development as a cell exhibits different behaviors. Interestingly, platelets interact with FN through a specific receptor, which is a different member of a class of 140-Kd-like complexes. This receptor is in fact the IIb–IIIa glycoprotein complex on the platelet surface that also binds fibrinogen and von Willebrand factor (Gardner and Hynes, 1985).

2.2.3. Functions of Fibronectin

The multiple cellular responses to FN have somewhat obscured its primary function. In addition, the behavior of a cell depends on the synthesis of FN, on the relative concentration of FN in the milieu, and on the mode of interaction of FN with the cell membrane (Couchman *et al.*, 1982; see Sections 3.2.2 and 3.2.3). It seems that the primary role of cellular FN is to promote cell-to-substrate