Journal of Supramolecular Structure
and Cellular Biochemistry 16:345–358 (1981)
Cellular Recognition 263–276

The Structure of Fibronectin and Its Role in Cellular Adhesion

Steven K. Akiyama, Kenneth M. Yamada, and Masao Hayashi

Section of Membrane Biochemistry, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Fibronectin is a large, adhesive glycoprotein which is found in a number of locations, most notably on cell surfaces, in extracellular matrixes, and in blood. Fibronectin has been detected in all vertebrates tested and in many invertebrates. Its presence in sponges is significant because this suggests that fibronectin may have appeared very early in evolution, possibly with the most primitive multicellular organisms. Cellular and plasma fibronectins have many striking similarities. However, the locations of the polypeptide chain differences between these two proteins indicate that plasma fibronectin cannot be derived from cellular fibronectin by means of simple post-translational proteolysis. Instead, these different types of fibronectin may be products of different genes or of differentially spliced messenger RNA molecules. Amniotic fluid fibronectin is possibly a third form of the protein. Cellular and plasma fibronectins are composed of at least six protease-resistant domains which contain specific binding sites for actin, gelatin, heparin, Staphylococcus aureus, transglutaminase, fibrin, DNA, and a cell surface receptor. The relative locations of these domains have been mapped in the primary structure of fibronectin. The cell surface receptor for fibronectin has not been positively identified, but may be a glycoprotein, a glycolipid, or a complex of the two. Although cell–substratum adhesion is mediated by fibronectin, the locations of the areas of closest approach of the cell to the substratum (the adhesion plaques) and fibronectin are not coincident under conditions of active cell growth. Under conditions of cell growth arrest in low serum concentrations, some fibronectin may become localized at the adhesion plaques. Models describing the domain structure of fibronectin and the molecular organization of the adhesion plaque area are presented.

Key words: fibronectin, evolution, proteolytic fragment, domain structure, receptor, glycoprotein, cellular adhesion, adhesion placque, cell surface protein

Fibronectins are high molecular weight, multifunctional, adhesive glycoproteins found on cell surfaces, in extracellular matrices, and in blood. Studies of the structure and function of fibronectin have expanded rapidly in the past few years, and since earlier work has been extensively reviewed in numerous previous publications [cf. 1–4], such an effort will not be repeated here. In this brief review, we shall concentrate on several recent important conceptual advances in the biochemistry of this glycoprotein. The reader should consult the recent general reviews for background and historical information.

Received April 14, 1981; accepted June 26, 1981.

0275-3723/81/1604-0345$04.00

EVOLUTION

Fibronectin has been localized in such a wide variety of mammalian and avian tissue and cell types that it can be considered a virtually ubiquitous structural and adhesive protein in these animals. Sources of fibronectin, besides cultured fibroblasts and blood, include amniotic fluid [5–7], platelets [8], endothelial cells [9–11], myoblasts [12,13], lens epithelial cells [14], cerebrospinal fluid [15], and seminal plasma [16]. Furthermore, fibronectin has been localized in a wide range of basement membranes [17] including tooth basement membrane [18], kidney mesangium [19], and glomerular basement membrane [20]. The biochemical similarities among the fibronectins from the different sources are quite striking, and the immunological cross-reactivities are well established [21–23].

Fibronectin, or an antigenically similar protein, has been detected by immunofluorescence techniques on cells from three lower invertebrates: the sea urchin (Sphaerachinus granularis) embryo [24], dissociated freshwater sponge (Ephydatia mulleri) cells [25], and dissociated seawater sponge (Microciona porifera) cells [26]. In most cases, fibronectin is localized to the external surfaces of the cells and the area between cells. Unlike vertebrate cell surface fibronectin, invertebrate fibronectin does not appear to be arrayed in fibrillar patterns. Populations of dissociated sponge cells do not diplay uniform staining, but instead, a fraction of both the freshwater and seawater sponge cells always exhibit no fibronectin. This may be attributable to the existence of different sponge cell types producing varying amounts of fibronectin or to sponge cells in different stages of development producing different amounts of fibronectin. Nevertheless, these results suggest that fibronectin on invertebrate cells may play a similar role to that on vertebrate cells—as a component of the extracellular matrix.

Radioimmunoassay determinations of fibronectin in animal tissue homogenates suggest that fibronectin is present in all vertebrates and urochordates and in most invertebrates [26]. Fibronectin apparently evolved with the earliest organisms displaying differentiated cells. Some animals, notably the horseshoe crab and the earthworm, have no fibronectin in their body tissues, although horseshoe crab hemolymph appears to contain substantial amounts. It must be emphasized, however, that negative results with the radioimmunoassay do not necessarily imply the complete absence of fibronectin. Such a result may be caused by lack of sensitivity or immunocrossreactivity under the conditions of the assay.

MAJOR FIBRONECTIN TYPES

Although fibronectin is distributed in a wide variety of animals and tissues, there are at least two major forms: plasma fibronectin, also known as cold insoluble globulin (or CIg), is found in blood, and cellular fibronectin, previously known as LETS (large, external, transformation-sensitive) protein or CSP (cell surface protein), is found on the surfaces of cultured cells. Plasma fibronectin is involved in cell–substratum adhesion, blood clotting, and reticuloendothelial system clearance of colloids. Cellular fibronectin seems to function in cell–cell adhesion, cell–substratum adhesion, cell motility, specific binding of macromolecules, and maintenance of a normal phenotype [1–4, 27–36].

Both types of fibronectin have closely similar molecular weights of $\approx$200–250,000 [2], similar amino acid compositions and peptide maps [37, 38], immuno-

logical cross-reactivities [54, 69], carbohydrate contents of ≈5% [38], and similar spectroscopic and hydrodynamic properties [37, 39, 40]. Additionally, the two forms of fibronectin both show biological activities for cell attachment to collagen, cell spreading, and binding to gelatin, heparin, actin, DNA, Staphylococcus aureus, transglutaminase, and the plasma membrane [1, 2, 42–44].

However, there are some significant differences between the two major types of fibronectin. Cellular fibronectin is an insoluble multimer under physiological conditions, whereas plasma fibronectin is a soluble dimer [37]. Cellular fibronectin is ≈50× more active in morphological restoration of transformed cells and 150–200× more active in hemagglutination [44]. Cellular fibronectin also contains much less fucose and possibly sialic acid than the plasma molecule [45, 46].

Chicken fibroblast fibronectin and chicken plasma fibronectin have at least three specific regions in their polypeptide chains that differ [42]. Figure 1 shows a sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis pattern of thermolysin digests of chicken cellular and plasma fibronectins. The two forms are cleaved in a homologous manner, but the 132,000- and 114,000-molecular weight (MW) fragments from plasma fibronectin are ≈10,000 daltons smaller than the analogous fragments from cellular fibronectin. These fragments are found on further analysis to contain two difference regions [42]. In contrast, the 44,500-MW fragment from plasma fibronectin is *larger* than the 43,200-MW fragment from the cellular protein. This fragment contains the third difference region. These differences are significant in that their internal locations on the polypeptide chain exclude the possibility that plasma fibronectin is a simple posttranslational proteolytic product of cellular fibronectin. Therefore, the existence of these two different types of fibronectin can be attributable either to difference genes or to different messenger RNA splicing mechanisms. Thus, although all fibronectins appear to be very similar in a number of important aspects, it must be emphasized that they are not identical.

A third possible form of fibronectin is found in amniotic fluid. This material is derived from amniotic tissue rather than maternal or other fetal tissues [5, 6]. It has immunological cross-reactivity with plasma fibronectin, although amniotic fluid fibronectin has a slightly higher monomer MW of 230,000 [5, 6]. This difference at least partially derives from its higher carbohydrate content of 9.5–9.6% compared with plasma (5.8%) and cellular (5.6%) fibronectins [7, 37]. Amniotic fluid fibronectin has similar amounts of mannose and sialic acid as plasma fibronectin, but contains more glucosamine, galactosamine, galactose, and fucose. This difference in glycosylation may possibly be related to differences in cell type or developmental stage [7]. Amniotic fluid and plasma fibronectins have the same amino acid compositions, and very similar, though not identical tryptic digestion maps. However, such differences in the peptide maps may be attributable to differential susceptibility to proteolysis resulting from dissimilar carbohydrate content [7, 47].

Amniotic fluid fibronectin has the same activity as plasma fibronectin in cell spreading assays [7]. Furthermore, amniotic fluid fibronectin has been localized to the extracellular and pericellular matrix of cultured amniotic fluid cells in fibrillar patterns that are similar to those of cellular fibronectin [6]. The biological significance of the difference in glycosylation of this form of fibronectin is not known, and its biochemical relationship to the plasma and cellular fibronectins is not yet clear.

DOMAIN STRUCTURE OF FIBRONECTIN

Fibronectin is an asymmetric glycoprotein with disulfide bonded A and B chains joined near the C-terminus [1–4]. The A and B chains are very similar but not identical polypeptides [48] which are organized into discrete functional domains. These domains have been characterized as specific protease-resistant fragments that bind to gelatin [7, 42, 49–53, 55–62], heparin [42, 57, 63, 64], actin [42, 62], DNA [42], S aureus [42, 43], fibrin [66], transglutaminase [43, 58, 67], and the plasma membrane [56–58]; they also have characteristic carbohydrates and free sulfhydryl groups. These domains, isolated or identified mainly by affinity chromatography of proteolytic fragments, are summarized in Table I. A model representing the assembly of these functional domains into a complete fibronectin monomer is shown in Figure 2, emphasizing the relative positions of the binding sites, carbohydrates, and the intrachain disulfide bonds within the primary structure.

The N-terminus has been studied in detail [50, 60, 68]. The terminal amino acid is a pyrolidone glutamic acid which can be removed by pyroglutamate ami-

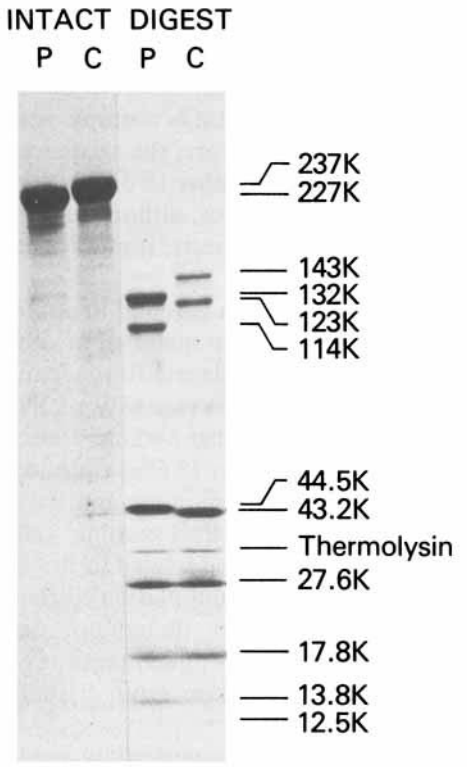

Fig. 1. SDS-polyacrylamide gel electrophoresis of proteolytic fragments of chicken plasma (p) and cellular (c) fibronectins. The fibronectins (1 mg/ml) were digested in 0.1 M NaCl, 10 mM $CaCl_2$, and 50 mM Tris-HCl (pH 7.0) at 30°C for 30 min by thermolysin at a concentration of 30 μg/ml. A composite gel system was employed using a 4% stacking gel with a 7.5–13.5% resolving gel as described in reference [42]. Each slot was loaded with 15 μg of protein. Numbers to the right of the gel indicate the molecular weights of the fibronectin fragments.

no-peptidase. The amino acid sequence is pGlu-Ala-Glx-Glx-Met-Val- which is identical to that of a 72,000-MW gelatin binding fragment formed by cathepsin D digestion [50]. This 72,000-MW fragment is further cleaved into a 29,000- and a 43,000-MW fragment by subsequent thrombin digestion. The smaller fragment retains the blocked N-terminal amino acid sequence, but does not bind to gelatin, whereas the 43,000-MW fragment binds to gelatin and has a different N-terminal amino acid sequence of Ala-Ala-. N-terminal fragments of MW 27–34,000 similar to the 29,000-MW cathepsin D thrombin fragment appear early in proteolytic digests by subtilisin, thermolysin, leukocyte elastase, and trypsin. The latter fragments can be purified either as a nongelatin binding fraction or with a DEAE column as a nonadsorbed fraction. This polypeptide region (designated as domain 1) contains binding sites for actin [42, 62], fibrin [66], heparin [42, 57], and S aureus [42, 43], and has many intrachain disulfide bonds but no carbohydrates or free sulfhydryls. It can be cross-linked to polyamines and collagen by transglutaminase [42, 58, 67].

The nearest neighbor to domain 1 is a region which binds exclusively to gelatin (domain 2). It has an N-terminal sequence of Ala-Ala-Val-Tyr-Gln-Pro-Gln-Pro-His-Pro-Glu-Pro-(Pro)-(Gly)-Try-Gly-His-( )-Val- [50, 59, 60], and has a MW of 30–45,000. Domain 2 can be produced by virtually all proteases. This region contains almost all of the total fibronectin carbohydrate and ≈ 25 half-cystines, but no free sulfhydryls [58].

Limited thermolysin digestion results in a 55- to 57,000-MW fragment which can bind to actin, DNA, gelatin, heparin, and S aureus; it can be subsequently

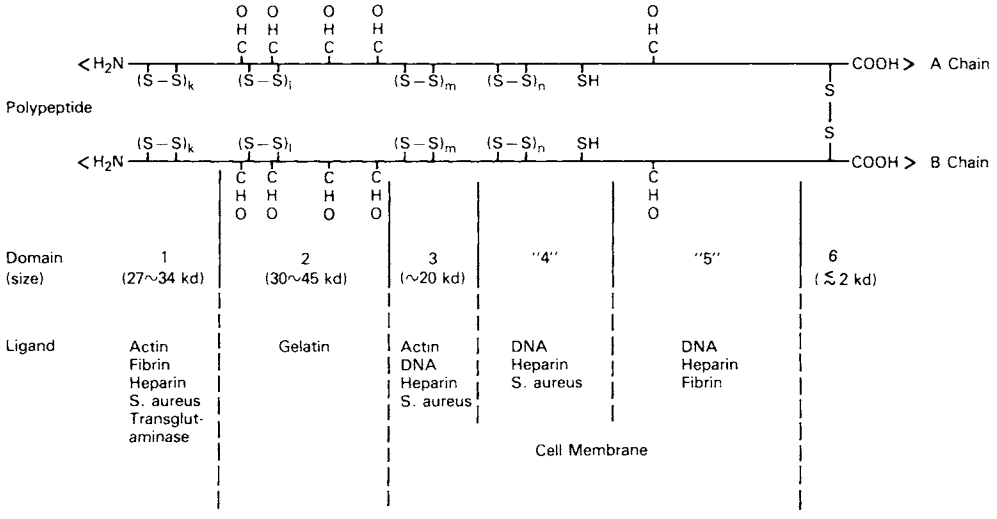

Fig. 2. Tentative model of the domain structure of fibronectin. This model was deduced from the data reported in the references cited in the text. CHO indicates the positions of the complex carbohydrate moieties which are attached to the polypeptide chain via asparaginyl residues. S-S and -SH indicate the relative positions of the disulfide bonds and a free sulfhydryl group, respectively. The domains are numbered as described in the text. The sizes of the domains represent the molecular weights of proteolyic-resistant fragments and, hence, vary according to the protease used. The ligands refer to the binding site specificities possessed by each domain.

TABLE I. Characteristics of the Functional Domains of the Fibronectins

Domain	Proteolytic fragments[a]	Domain of fragments[b]	Source of FN	Characteristics[c]	Ref.
Actin	Tryp 70k	1+2	Cf	Gelatin	62
	Subt 28.5k, 27k	1	Cf		62
	Therm 195–202k, 55.1–57.4k	2+3+4+5, 2+3	Cp & Cf	DNA, gelatin, heparin, S aureus	42
	Therm 33.7–27.6k	1	Cp & Cf	Heparin, S aureus	42
Cell membrane	Chym 205k	2+3+4+5	Cf	Gelatin, cell spreading & hemagglutination	56
	Chym 160k	(3)+4+5	Cf	Cell spreading	56
	Therm 140k, 150k	(3)+4+5	Hsp	Heparin, cell spreading	57
	LeEl 140k	(3)+4+5	Hp	Cell spreading, free SH	58
DNA	Therm 55.1–57.4k	2+3	Cp & Cf	Actin, gelatin, heparin, S aureus	42
	Therm 26.2–27k	4 or 5	Cp & Cf	Heparin, S aureus	42
	Therm 15k	4 or 5	Cp & Cf	Heparin	42
Fibrin	Tryp 200k	2+3+4+5	Hsp	Gelatin, A chain, cleaved to Therm 21k	66
	Tryp 32k	1	Hsp	Cleaved to Therm 24k	66
Gelatin	CathD 72k	1+2	Hp	Cleaved to Plas 42k, contains most CHO	49
	CathD 72k	1+2	Haf	N-terminal	51
	CathD 72k	1+2	Hp	N-terminal, pGlu-Ala-Glx-Glx-Met-Val- cleaved to Throm 43k with Ala-Ala- (& Throm 29k with pGlu-Ala- of domain 1)	50
	Tryp 200k	2+3+4+5	Hsf	Most of CHO, free SH	52
	Tryp 70k	1+2	Cf	Actin	62
	Tryp 30k	2	Hp	Ala-, Gly rich, Con A binding	53
	Tryp 30k	2	Haf & Hp	Con A	7
	Chym 205k	2+3+4+5	Cf	Cell spreading & hemagglutination	56
	Chym 40k, 79k	2, (1) + 2	Cp		55
	Chym 40k	2	Cf	Most of CHO	55, 56, 62
	Chym 42k	2	Hp		55, 56
	Therm 230–235k	2+3+4+5	Hp	Ala-Ala-Val-Tyr-	60
	Therm 40k	2+(3)	Hsp	CHO rich	57

	Therm 55.1–57.4k	2+3	Cp & Cf	Actin, DNA, heparin, S aureus, cleaved to Therm 44.5–43.2k then to Therm 30.8k	42
	LeEl 60k & 40k	2+(3), & 2	Hp	$(S–S)_{12-14}$, CHO rich	58
	Subt 50k	2+3	Hp	Cys rich, weak cell attachment, Val-Tyr-Gln-Pro-Gln-Pro-His-Pro-Gln-Pro-(Pro)-(Gly)-Tyr-Gly-His-( )-Val-. cleaved to Subt 30k with same N-terminus	59
	Subt 40k	2	Cf		62
	MCCh or CathG	1+2+3+4+5,	Hp		60
	200k, 70k or 40k	1+2 or 1			61
Heparin	Chym 205k	2+3+4+5	Cf	Gelatin, cell membrane, cleaved to Chym 160k then to Pron 50k	63
	Pron 50k	4	Cf	Glu–Asp–Asp–, one Cys, 2 Met	64
	Therm 140k, 150k	3+4+5	Hsp	Cell spreading	57
	Therm 32k	1	Hsp		57
	Therm (multiple peptides)		Cp & Cf		42
	CathD 70k	1+2	Hp	Gelatin, transglutaminase, cleaved to Plas 30k	103
S aureus	Tryp 27k	1	Hp	Transglutaminase	43
	Therm (Multiple peptides)		Cp & Cf		42
Transglutaminase	Tryp 27k	1	Hp	S aureus	43, 67
	CathD 70k	1+2	Hp		43, 67
	LeEl 29k	1	Hp		58

[a]Abbreviations: Proteases used for the generation of fragments are designated as Therm, thermolysin; Tryp, trypsin; Subt, subtilisin; Chym, chymotrypsin; LeEl, leukocyte elastase; Plasm, plasmin; MCCh, mast cell chymase; CathD, cathepsin D; CathG, Cathepsin G; Throm, Thrombin; and Pron, pronease. Sources of fibronectin are Hp, human plasma; Haf, human amniotic fluid; Hsp, hamster plasma; Hsf, hamster fibroblasts; Cp, chicken plasma; and Cf, chicken fibroblasts. Other abbrevations are FN, fibronectin; Con A, concanavalin A; CHO, carbohydrate.

[b]See Figure 2 for the location of the domains in the fibronectin molecule.

[c]Characteristics include binding activity to other ligands, amount of carbohydrate, presence of disulfides and free SH groups, further distinctive cleavage products which retain the same ligand binding activity, and N-terminal amino acid sequence.

cleaved to a 45- to 45,000-MW fragment which binds only gelatin [42]. Thus, domain 3 must be a very small (MW $\leq$20,000) region which can bind to actin, DNA, gelatin, heparin, and S aureaus. Domain 3 has not yet been isolated or well characterized, but seems to have few disulfides and no carbohydrates.

The C-terminal half of fibronectin is not well understood. To avoid confusion, the next two domains will be designated domain "4" and domain "5", where the quotation marks emphasize the tentative nature of any assignment. Both domains "4" and "5" bind to heparin and DNA and either domains 3, "4", or "5" may contain the fibronectin cell-binding site [42, 56, 57, 58, 65]. Domain "4" binds to S aureus whereas domain "5" does not. Domain "4" seems to have no carbohydrates, few disulfides, one free sulfhydryl, and includes a 50,000-MW heparin-binding fragment with an N-terminal sequence of Glu–Asp–Asp– [64]. Domain "5" has few carbohydrates, no free sulfhydryls, and has a 21,000-MW fibrin-binding fragment derived from the A chain [66]. Domains "4" and "5" have, however, not been extensively investigated and the possibility exists that, in the future, the region may be divided into three or more domains; the arrangement of this section of the fibronectin molecule in Figure 2 must, therefore, be regarded as provisional.

The C-terminus of the fibronectin molecule is contained in a very small fragment of MW $<$2000 (domain 6). The C-terminus itself appears to be blocked and its sequence is unknown. Domain 6 has not been positively identified but is believed to contain the interchain disulfide bond(s). Very mild digestion of fibronectin by any protease seems to remove this domain, as indicated by the fact that the migration of the remaining large fragment (MW $\approx$200,000) in SDS-gel electrophoresis has never been observed to be influenced by the presence or absence of dithiothreitol.

The three difference regions between chick cellular and plasma fibronectins discussed in the previous section have all been localized: in domain 2 (cellular fibronectin is 1000 MW smaller), in domain "4" (cellular fibronectin is 11,000 MW larger), and in domain "5" (cellular fibronectin is 1000 MW smaller) [42].

CELL SURFACE RECEPTOR

Fibronectin is a cell surface and extracellular matrix protein and not an integral membrane protein, as it can be detached without the use of detergents [1, 2, 30, 70]. Although arranged in immobilized fibrillar arrays across the cell surface, fibronectin does not form molecular barriers. Surface antigens, lipid probes, and fluorescent ganglioside analogs all have free mobility within the entire lipid phase of the cell surface [71], although large particles can be impeded [72].

Direct and indirect interactions between cytoskeletal elements and fibronectin on the cell surface have been suggested [1, 2, 73–76]. There appear to be areas of close transmembrane associations between fibronectin fibers and 5-nm microfilaments in dense zones just inside the plasma membrane [74]. Some immunofluorescence studies suggest a direct transmembrane linkage between fibronectin and actin-containing fibers [75, 76]. The directionality of the fibronectin–cytoskeletal interaction appears to be two way. Treatment of normal cells with proteases or antifibronectin antibodies causes cell rounding and a loss of cytoskeletal organization [22]. Disrupting the cytoskeleton with cytochalasin causes a release of cellular

fibronectin [76]. Conversely, the addition of fibronectin to transformed cells promotes the organization of microfilament bundles [1, 2, 36, 78].

All the observations cited previously suggest that fibronectin might bind at discrete points on the cell surface via a specific receptor. Although the exact nature of the fibronectin receptor is still unknown, it should be specific for fibronectin, anchored in the plasma membrane, and somehow directly or indirectly interact with cytoskeletal components. There are indications that the cell surface receptor for fibronectin is either a glycoprotein, or a glycolipid, is possibly both [79–84].

Fibronectin-coated latex beads bind to the surface of baby hamster kidney cells [79]. The rate of binding is temperature dependent, with much faster binding at 22°C than at 4°C; surprisingly, the binding can occur even in the absence of divalent cations. Binding of the beads to the cells is saturable and is competitively inhibited by attachment of the cells to fibronectin-coated dishes. Mild pretreatment of the cells with trypsin (0.01 mg/ml for 10 min) results in greatly reduced cell–bead binding. Also, when cells were treated with various plant lectins, eg, the presence of 50 μg/ml wheat germ agglutinin, but not concanavalin A, inhibited the binding of the coated beads to the cells. These findings have been interpreted as suggesting that the cell surface receptor for fibronectin is a glycoprotein [79, 80].

However, glycolipids have also been implicated as possible cell surface fibronectin receptors [81–84]. Gangliosides rich in sialic acid inhibit the binding of Chinese hamster ovary cells to collagen [81]. Of the six gangliosides tested, GT_1 and GD_{1a} most effectively inhibit cellular attachment in a concentration-dependent manner. Such effects cannot be attributed to cytotoxicity and are not produced by the ceramide portion of the gangliosides, individual sugars, or polysaccharides. The isolated, sialic acid-containing oligosaccharide portion of mixed gangliosides retains this inhibitory effect, whereas oxidation of the sialic acid portion of intact gangliosides diminishes it. Mixed brain gangliosides inhibit both the binding of fibronectin to human fibroblasts [83] and the spreading of baby hamster kidney cells on plastic [84]. A variety of other biological assays for fibronectin are inhibited by gangliosides [82]. These assays include fibronectin-mediated hemagglutination, cell spreading, and restoration of a normal morphological phenotype to transformed cells. Charged phospholipids such as phosphatidylserine and phosphatidylinositol are less inhibitory, whereas uncharged lipids and ceramides are not inhibitory. The extent of inhibition increases with the number of sialic acid residues per ganglioside. The specificity for the fibronectin–ganglioside interaction appears to involve the oligosaccharide moiety, but it is relative rather than absolute. Consequently, a cell with large amounts of GM_1 may be able to bind as much fibronectin as a cell with smaller amounts of GD_{1a} or GT_{1b}.

The interaction of fibronectin and the cytoskeleton is especially interesting on the ventral surfaces (bottoms) of fibroblasts at those areas of minimum distance between the cell and the substratum called adhesion plaques. Fibronectin is concentrated on the ventral surfaces of isolated cells [2, 85]. Although cell–substratum adhesion can be *mediated* by fibronectin [33], it has become apparent that, in general, the locations of fibronectin and those of adhesion plaques do not coincide. On freshly plated human fibroblasts, the locations of fibronectin immunostaining and adhesion plaques (focal contacts) as observed by reflection inter-

ference microscopy are not identical [86], although it could be argued that the Triton X-100 extraction used in this case was insufficient to allow penetration of the antifibronectin antibodies [87]. The adhesion plaques (focal contacts) of chick heart fibroblasts are also generally found to be devoid of fibronectin, although it can often be found in very close proximity to these loci [88]. Less than 10% of the adhesion plaques/cell show any substantial fibronectin localization and 7–12% show weak fibronectin localization. These results are independent of the method of fixation (3% glutaraldehyde or 4% formaldehyde) and method of permeabilization (1% Triton X-100 or 100% acetone). In addition, ultrathin frozen sections show no immunoelectron microscopic labeling for fibronectin at adhesion plaques although there is extensive labeling elsewhere on the cell surface [89]. As a control, labeling for concanavalin A is observable in the adhesion plaques, indicating such areas are accessible to antibodies [88, 89].

The organization of fibronectin on the cell surface closely resembles but does not necessarily coincide with that of microfilament bundles [1, 2, 73, 75, 90]. Vinculin is a 130,000-MW protein, which is located at the membrane attachment regions for actin fibers throughout the cell and near the ends of the fibronectin fibers on the dorsal surfaces (tops) of the cells [91, 92]. Fibronectin fibers have been found to be associated with vinculin in the adhesion plaques of growth-arrested cells cultured in very low concentrations of serum; however, this relationship seems to disappear in growing cells [93], where the distributions of fibronectin and vinculin seem not to be related in many cases.

The major features of the findings discussed in this section can be summarized in a highly schematic model (Fig. 3) for the possible molecular arrangement of the adhesion plaques of cultured fibroblasts. It must be emphasized that cell adhesion is a very complex event. There are at least two types of adhesion con-

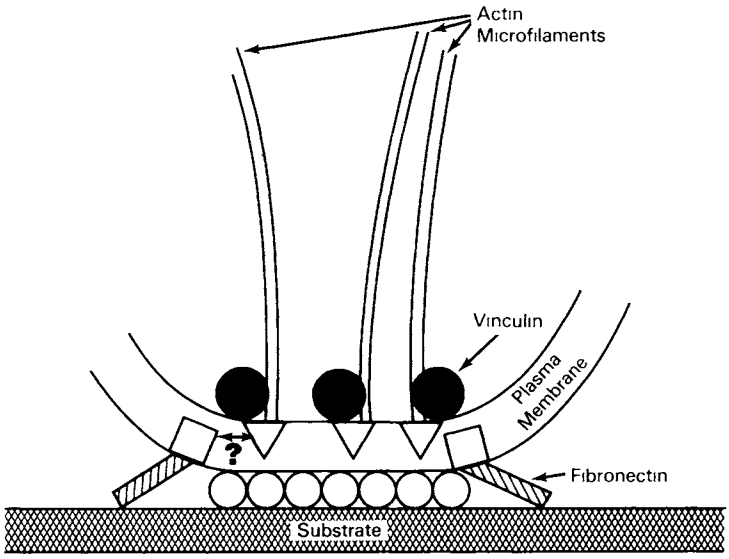

Fig. 3. Tentative schematic model for the adhesion plaque area of a cell. The symbol ● represents as yet unidentified serum proteins, □ represents the cellular fibronectin binding site, and ∇ represents the intramembrane anchoring site for microfilament bundles.

tacts of cells to substrata [94, 95] and multiple, specialized cell–cell adhesive structures [95–98]. These cell–substratum structures include both adhesion plaques (focal contacts) and broad areas of slightly more distant contact called close contacts. It is, therefore, likely that other proteins besides fibronectin will be implicated in cell adhesion.

Cells are thought to adhere to collagen or artificial substrates by means of intermediary proteins such as fibronectin or other serum and cellular proteins and macromolecules. The role of fibronectin in adhesion has been well documented [1–4, 98] and, although it is absent from adhesion plaques under some conditions, it is present in the close contact regions of cell–substratum adhesion [86, 87, 89]. In fact, cell spreading and locomotion can occur in the absence of focal contacts, presumably by means of close contacts [100, 101]. Thus in Figure 3, fibronectin is shown as being adjacent to, but not at the site of, the adhesion plaque. The molecules on the substrate under the plaque may be serum proteins or other cellular proteins besides fibronectin.

The model shows two key anchor points at the plasma membrane. The first involves vinculin and perhaps an integral membrane component anchoring actin microfilaments to the inner face of the plasma membrane. The other is the fibronectin receptor (either a glycoprotein, glycolipid, or both in a complex) anchoring fibronectin fibrils to the outside of the plasma membrane. These two anchor points may not be at superimposable locations at opposite sides of the plasma membrane, and the question mark emphasizes our current uncertainty about the relationship of the hypothesized points. In fact, vinculin and the fibronectin receptor are probably bound to separate membrane constituents, since their immunolocalization patterns can differ. The two anchor points may communicate via a hypothetical transmembrane element, although not necessarily by a direct physical association. Further analysis of this possible linkage will be of considerable interest.

PROSPECTS FOR THE FUTURE

A large number of advances have been made during the past few years in fibronectin research, especially in the areas of the structure and localization of fibronectin. However, much more research will be necessary to elucidate the molecular basis for the functionality of fibronectin. Characterization of the cellular binding site on fibronectin is an important and necessary next step. Although a cell binding domain seems to have been observed, it has neither been isolated nor examined in detail. Perhaps the use of antibody or glycolipid affinity chromatography will help to isolate the cellular binding domain(s). Furthermore, the DNA, transglutaminase, and other domains have yet to be positively identified. Moreover, the possible binding of certain ligands to the same sites should be examined. The C-terminal portion of the molecule is still ill defined, and positive localization and quantitation of the interchain disulfide bond(s) are still needed.

Isolation of the cell surface receptor for fibronectin is essential before questions about the molecular basis for cellular attachment and mobility can be answered. The relationship between fibronectin and the cytoskeleton must also be better elucidated. Since fibronectin seems to be a highly conserved protein, it would also be very interesting to isolate and/or localize fibronectins from various

invertebrate sources and compare their molecular characteristics, eg, domain structures.

Finally, the cell type that synthesizes plasma fibronectin should be identified, and the genetic or messenger RNA splicing mechanisms responsible for the production of cellular versus plasma fibronectins determined. It is probably safe to predict that the field of fibronectin research will continue to provide many more surprises and insights into the molecular basis of cell behavior.

ACKNOWLEDGMENTS

S.K. Akiyama is supported by a Human Cancer-Directed Fellowship DRG-443-F of the Damon Runyon-Walter Winchell Cancer Fund.

REFERENCES

1. Hynes RO, Destree AT, Perkins ME, Wagner DD: J Supramol Struct 11:95, 1979.
2. Yamada KM, Olden K: Nature (London) 273:179, 1978.
3. Vaheri A, Mosher DF: Biochim Biophys Acta 516:1, 1978.
4. Mosesson MW, Amrani DL: Blood 56:145, 1980.
5. Chen AB, Mosesson MW, Solish GI: Amer J Obstet Gynecol 7:958, 1976.
6. Crouch E, Balian G, Holbrook K, Duksin D, Bornstein P: J Cell Biol 78:701, 1978.
7. Ruoslahti E, Engvall E, Hayman EG, Spiro RG: Biochem J 193:295, 1981.
8. Ginsberg H, Painter RG, Birdwell C, Plow EF: J Supramol Struct 11:167, 1979.
9. Birdwell CR, Gospodarowicz D, Nicholson GL: Proc Natl Acad Sci 75:3273, 1978.
10. Macarak EJ, Kirby E, Kirk T, Kefalides NA: Proc Natl Acad Sci 75:2621, 1978.
11. Jaffe EA, Mosher DF: Exp Cell Res 147:1779, 1978.
12. Chen LB: Cell 10:393, 1977.
13. Furcht LT, Mosher DF, Wendelschafer-Crabb G: Cell 13:263, 1978.
14. Hughes RC, Mills G, Courtois Y: Biol Cellulaire 36:321, 1979.
15. Kuusela P, Vaheri A, Palo J, Ruoslahti E: J Lab Clin Med 92:595, 1978.
16. Vuento M, Salonen E, Koskimies A, Stenman U-H: Hoppe-Seyler's Z Physiol Chem 361:1453, 1980.
17. Linder E, Stenman S, Lehto V-P, Vaheri A: Ann NY Acad Sci 312:151, 1978.
18. Thesleff I, Barrach HJ, Foidart JM, Vaheri A, Pratt RM, Martin GR: Devel Biol 81:182, 1981.
19. Madri JA, Roll FJ, Furthmayer H, Foidart J-M: J Cell Biol 86:682, 1980.
20. Courtoy PJ, Kanwar YS, Hynes RO, Farquhar MG; J Cell Biol 87:691, 1980.
21. Kuusela P, Ruoslahti E, Engvall E, Vaheri A: Immunochemistry 13:649, 1976.
22. Yamada KM, Yamada SS, Pastan I: J Cell Biol 74:649, 1977.
23. Yamada KM: J Cell Biol 78:520, 1978.
24. Spiegel E, Burger M, Spiegel M: J Cell Biol 87:309, 1980.
25. Labat-Robert J, de Ceccatty MP, Robert L, Auger C, Lahias C, Garrone R: In Schauer R, Boer P, Buddecke E, Kramer MF, Vliegenthart JFG, Wiegandt H (eds): "Glycoconjugates. Proceedings of the Fifth International Symposium." Stuttgart, FGR: Georg Thiene Publishers, 1979, pp 431–432.
26. Akiyama SK, Johnson M, Yamada KM: manuscript in preparation.
27. Grinnell F, Feld MK: Cell 17:117, 1979.
28. Blumenstock FA, Saba TM, Weber P, Laffin R: J Biol Chem 253:4287, 1978.
29. Dessau W, Jilek F, Adelmann BC, Hörmann H; Biochim Biophys Acta 533:227, 1978.
30. Yamada KM, Yamada SS, Pastan I: Proc Natl Acad Sci 72:3158, 1975.
31. Klebe RJ: J Cell Physiol 86:231, 1975.
32. Ali IU, Hynes RO: Cell 14:439, 1978.
33. Yamada KM, Olden K, Pastan I: Ann NY Acad Sci 312:256, 1978.
34. Kleinman HK, McGoodwin EB, Klebe RJ: Biochem Biophys Res Comm 72:426, 1976.
35. Yamada KM, Yamada SS, Pastan I: Proc Natl Acad Sci USA 73:1217, 1976.

36. Ali IU, Mautner V, Lanza R, Hynes RO: Cell 11:115, 1977.
37. Yamada KM, Schlessinger DH, Kennedy DW, Pastan I: Biochemistry 16:5552, 1977.
38. Ruoslahti E, Pekkala A, Engvall E: FEBS Lett 107:51, 1979.
39. Alexander, Jr, SS, Colonna G, Yamada KM, Pastan I, Edelhoch H: J Biol Chem 253:5820, 1978.
40. Colonna G, Alexander, Jr, SS, Yamada KM, Pastan I, Edelhoch H: J Biol Chem 253:7787, 1978.
41. Klebe RJ: Nature (London) 250:248, 1974.
42. Hayashi M, Yamada KM: J Biol Chem (in press).
43. Mosher DF, Proctor RA: Science 209:927, 1980.
44. Yamada KM, Kennedy DW: J Cell Biol 80:492, 1979.
45. Takasaki S, Yamashita K, Suzuki K, Iwanaga S, Kobata A: J Biol Chem 254:8548, 1979.
46. Fukuda M, Hakomori S: J Biol Chem 254:5442, 1979.
47. Olden K, Pratt RM, Yamada KM: Proc Natl Acad Sci 76:3343, 1979.
48. Kurkinen M, Vartio T, Vaheri A; Biochim Biophys Acta 624:490, 1980.
49. Balian G, Click EM, Crouch E, Davidson J, Bornstein P: J Biol Chem 254: 1529, 1979.
50. Furie MB, Frey AB, Rifkin DB: J Biol Chem 255:4391, 1980.
51. Balian G, Click EM, Bornstein P: J Biol Chem 255:3234, 1980.
52. Wagner DD, Hynes RO: J Biol Chem 254:6746, 1979.
53. Ruoslahti E, Hayman EG, Kuusela P, Shively JE, Engvall EE: J Biol Chem 254:6054, 1979.
54. Ruoslahti E, Vaheri A: J Exp Med 141:497, 1975.
55. Hahn L-HE, Yamada KM: Proc Nat Acad Sci 76:1160, 1979.
56. Hahn L-HE, Yamada KM: Cell 18:1043, 1979.
57. Sekiguchi K, Hakomori S: Proc Natl Acad Sci 77:2661, 1980.
58. McDonald JA, Kelly DG: J Biol Chem 255:8848, 1980.
59. Gold LI, Pardo-Garcia A, Frangione B, Franklin EC, Pearlstein E: Proc Nat Acad Sci 76:4803, 1979.
60. Furie MB, Rifkin DB: J Biol Chem 255:3134, 1980.
61. Vartio T, Seppä H, Vaheri A: J Biol Chem 256:471, 1981.
62. Keski-Oja J, Yamada KM: Biochem J 193:615, 1981.
63. Yamada KM, Kennedy DW, Kimata K, Pratt RM: J Biol Chem 255:6055, 1980.
64. Hayashi M, Schlesinger DH, Kennedy DW, Yamada KM: J Biol Chem 255: 10017, 1980.
65. Sekiguchi K, Hakomori S: Biochem Biophys Res Comm 97:709, 1980.
66. Mosher DF, Schad PE, Kleinman HK: J Clin Invest 64:781, 1979.
67. Mosher DF, Schad PE, Vann JM: J Biol Chem 255:1181, 1980.
68. Mosesson MW, Chen AB, Huseby RM: Biochim Biophys Acta 386:5091, 1980.
69. Mosesson MW: Thromb Hemostasis 38:742, 1977.
70. Graham JM, Hynes RO, Davidson EA, Bainton DF: Cell 4:353, 1975.
71. Schlessinger J, Barak LS, Hammes GG, Yamada KM, Pastan I, Webb WW, Elson EL: Proc Natl Acad Sci 74:2909, 1977.
72. Albrecht-Buehler G, Chen LB: Nature (London) 266:454, 1977.
73. Wartiovaara J, Linder E, Ruoslahti E, Vaheri A: J Exp Med 140:1522, 1974.
74. Singer II: Cell 16:675, 1979.
75. Heggeness MH, Ash JF, Singer SJ: Ann NY Acad Sci 312:414, 1978.
76. Hynes RO, Destree AT: Cell 15:875, 1978.
77. Ali IU, Hynes RO: Biochim Biophys Acta 471:16, 1977.
78. Willingham MC, Yamada KM, Yamada SS, Pouyssegur J, Pastan I: Cell 10: 375, 1977.
79. Grinnell F: J Cell Biol 83:50a, 1979.
80. Grinnell F: J Cell Biol 86:104, 1980.
81. Kleinman HK, Martin GR, Fishman PH: Proc Natl Acad Sci 76:3367, 1979.
82. Yamada KM, Kennedy DW, Grotendorst GR, Momoi T: J Cell Physiol, in press.
83. Rennard SI, Wind ML, Hewitt AT, Kleinman HK: Arch Biochem Biophys 206:205, 1981.
84. Yamada KM, Olden K, Hahn L-HE: In Subtelny S, Wessells NK (eds): "The Cell Surface: Mediator of Developmental Processes." New York: Academic Press, 1979, pp 43–77.
85. Culp LA: Biochemistry 15:4094, 1976.
86. Birchmeier C, Kreis TE, Eppenberger HM, Winterhalter KH, Birchmeier W: Proc Nat Acad Sci 77:4108, 1980.
87. Grinnell F: Cell Biol Int Reports 4:1031, 1980.
88. Fox CH, Cottler-Fox MH, Yamada KM: Exp Cell Res 130:477, 1980.

89. Chen W-T, Singer SJ: Proc Natl Acad Sci USA 77:7318, 1980.
90. Mautner VM, Hynes RO: J Cell Biol 75:743, 1977.
91. Geiger B: Cell 18:193, 1979.
92. Burridge K, Feramisco JR: Cell 19:587, 1980.
93. Singer II, Paradiso PR: Cell 24:481, 1981.
94. Rees DA, Lloyd C, Thom D: Nature (London) 267:124, 1977.
95. Heath JP, Dunn GA: J Cell Sci 29:197, 1978.
96. Culp LA: Curr Topics Membr Transp 11:327, 1978.
97. Rees DA, Badley RA, Lloyd CW, Thom D, Smith CG: Soc Exp Biol Symp 32:241, 1978.
98. Grinnell F: Int Rev Cytol 53:65, 1978.
99. Orly J, Sato G: Cell 17:295, 1979.
100. Thom D, Powell AJ, Rees DA: J Cell Sci 35:281, 1979.
101. Couchman JR, Rees DA: J Cell Sci 39:149, 1979.