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The extracellular matrix (mesoglea) of hydrozoan jellyfish and its ability to support cell adhesion and spreading

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Key words: Hydrozoa, extracellular matrix, collagen, laminin, fibronectin, cell adhesion, mesoglea

Abstract

The outer mesoglea (extracellular matrix; ECM) of hydrozoan jellyfish was found to contain a species-specific meshwork of striated fibers of different diameters. In the mesoglea, molecules were identified which exhibit several features of well known vertebrate ECM: a laminin-like molecule which appears cross-shaped on electronmicrographs, a fibronectin-like molecule (both detectable by their immunoreactivity at the exumbrella side) and a species-specific collagen consisting of 3 different α-chains of which at least 2 can be decorated with con A. The α-chains are linked by disulfide bridges. Acetic acid extraction of the mesoglea and subsequent salt precipitation yields fibrils which appear banded in the electron microscope and support species-specific adhesion and spreading of isolated tissue. These precipitated fibrils are mainly composed of the disulfide-linked collagen.

Introduction

Within the last two decades it has become clear that the extracellular matrix (ECM) not only serves as a mechanical support to maintain tissue shape and integrity but also carries information for the proper function of organ systems for cellular determination, differentiation and morphogenesis. In our search for the mechanisms responsible for maintenance of the differentiated state of striated muscle tissue of jellyfish, the structural organization of the ECM (mesoglea) to which this tissue adheres, is of special interest. All methods which successfully alter the state of cellular differentiation (transdifferentiation) in this tissue, either directly (collagenase, pronase, trypsin, hyaluronidase) or indirectly (tumor promoters, diacylglycerol), seem to affect the structural integrity of the ECM.

In contrast to other organisms, the ECM (mesoglea) of jellyfish is their most prominent structure. It essentially shapes the body of the medusa and serves different functions, e.g., as a substratum for cell movement (Haynes *et al.*, 1968; Day & Lenhoff, 1981) and migration (Shostak *et al.*, 1965), the control of morphogenetic processes (Schmid *et al.*, 1976; Schmid, 1978), and the regulation of buoyancy (Denton, 1963; Chapman, 1966; Mackay, 1969).

Furthermore, the structural organization of the mesoglea and its elasticity allows it to act antagonistically to the deformative forces generated by the contraction of the muscle system of the umbrella (bell).

The components which build this multifunctional extracellular structure have been of interest for a long time. The pioneering work of Bouillon & Vandermeersche (1956), Chapman (1966),

Gladfelter (1972) and Weber & Schmid (1985) have demonstrated that the mesoglea consists of a system of branching fibrils embedded in a massive, highly hydrated ground substance. The mesoglea of the Cnidaria is thought to contain proteoglycans (Lowell & Haynes, 1968), collagen-like and other structural proteins (Chapman, 1966; Haynes *et al.*, 1968; for review see Adams, 1978 and Franc, 1985).

The present paper summarizes our recent investigations on some of the structural elements of the mesoglea and their putative role in cell adhesion and spreading.

Material and methods

The anthomedusan *Podocoryne carnea* M. Sars was reared in the laboratory (Schmid, 1979); all the other jellyfish were either caught and processed at the Friday Harbor Laboratories or transported from there to Basel and kept alive until use.

Collagen
Mesoglea was isolated from animals in several washes with distilled water or with Ca-Mg-free seawater in the presence of protease inhibitors. Collagen was extracted with guanidine-HCl or acetic acid (Schmid & Bally, 1988). Banded fibrils were precipitated by adding 1 M NaCl to the acetic acid extract. These fibrils could directly be tested for their ability to support cell adhesion and spreading as outlined by Schmid & Bally (1988).

Antibody
A monoclonal antibody (mAb) not yet further characterized, but specific for the fibrous system of the mesoglea, was prepared from mice and used for immunocytochemistry as described by Weber *et al.* 1987).

Laminin
Extraction of laminin followed the protocol of Paulsson *et al.* (1987) for the extraction of the laminin-nidogen complex from the mouse EHS tumor but an extended set of protease inhibitors was used. The supernatant of the EDTA/NaCl-extract was applied on a Superose 6 column equilibrated in a neutral volatile buffer. Fractions corresponding to the elution volume of mouse laminin were treated for rotary shadowing electron microscopy as described by Engel & Furthmayr (1987).

Fibronectin (FN)
Affinity purified human plasma FN (HFN) was a gift from the lab of J. Engel (Biozentrum, Basel). Coelenterate FN (CFN) was purified from urea extracts by affinity chromatography and ion exchange chromatography by a semimicrotechnique. CFN was detected and quantified by a high-sensitivity ELISA using antibodies against HFN. SDS-electrophoresis (PAGE) and Western blotting followed the standard procedures. For immunohistology, whole medusae were fixed in periodic acid/lysine/paraformaldehyde solution, dehydrated with ethanol, embedded in LR

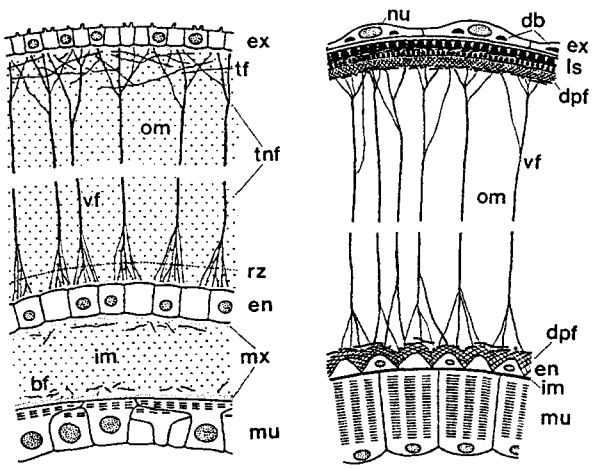

Fig. 1. Schematic representation of elements involved in the architecture of the fibrous system in the mesogleal ECM of *Polyorchis penicillatus* (left) and *Aglantha digitale* (right). The diagram does not show the actual sizes of the single elements. Bundles of fibrils (bf); dense body (db); dense plexus of fibrils (dpf); subumbrellar plate endoderm (en); exumbrella (ex); inner mesoglea (im); laminated structure (ls); swimming muscle (mu); dense matrix (mx); nucleus (nu); outer mesoglea (om); refractile zone (rz); plexus of tangential fibres (tf); three-dimensional network of fibrils (tnf); vertical fibre (vf). (From Weber & Schmid (1985) with permission of Longman Group Ltd.)

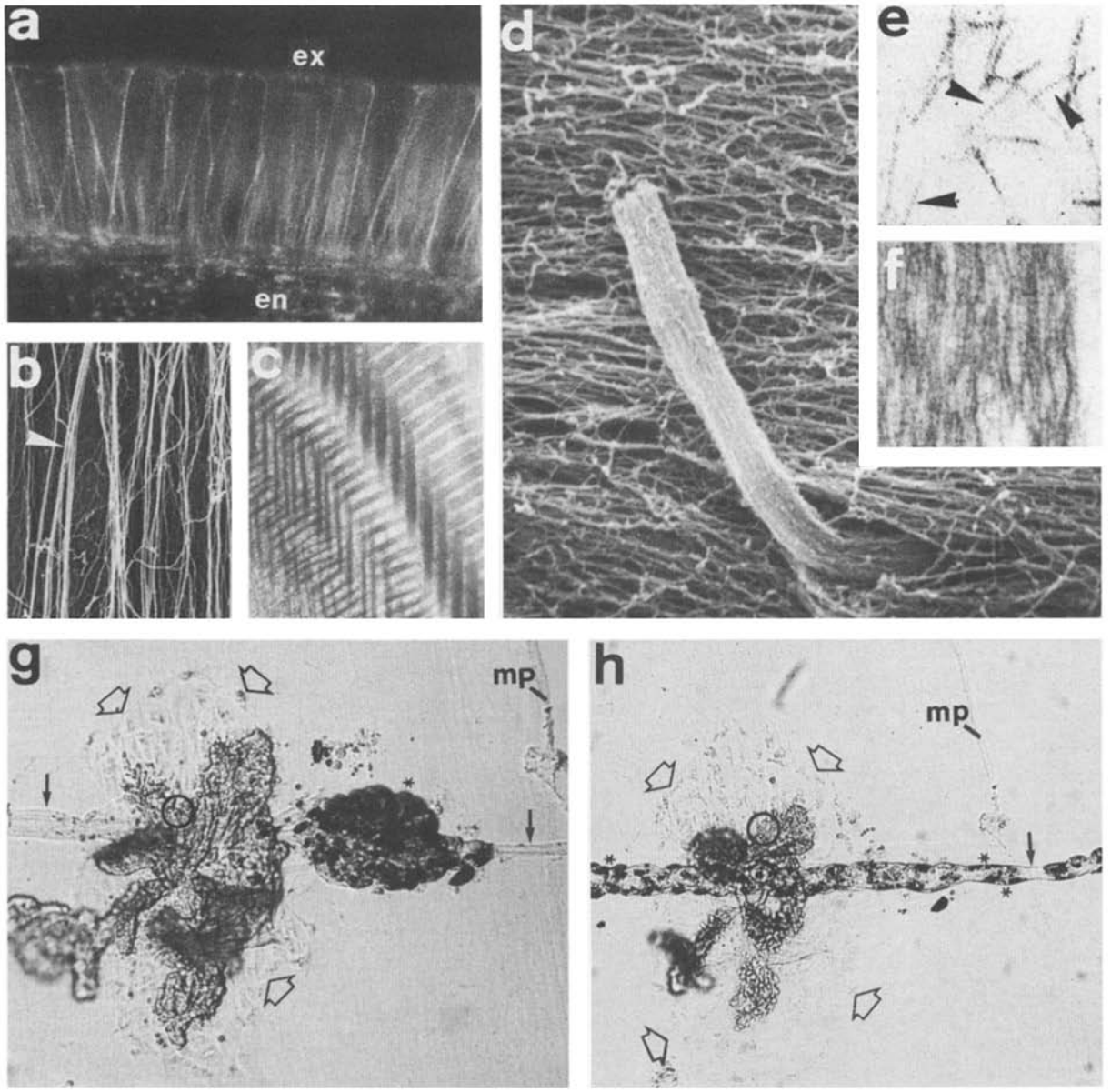

Fig. 2. a) Horizontal view of outer mesoglea immunolabeled with a mesoglea specific mAb (Weber *et al.*, 1987). The fibers traverse the ECM and run perpendicularly from the exumbrellar (ex) to the endodermal (en) side. × 750; *Podocoryne carnea*. b) Unidirectionally orientated fibrils branch (arrowhead) and are closely associated with a dense meshwork. × 260; SEM, *Aglantha digitale*. c) Tangential section through the laminated structure of *Aglantha digitale*. × 44 000; TEM. d) SEM micrograph of thick vertical fiber which penetrates the three-dimensional network of fibrils. × 12 000; *Polyorchis penicillatus*. e) TEM high magnification micrograph of fibrils which form the three-dimensional network. The fibrils are banded (arrowheads) and show a periodicity of 60–65 × 10^{-7} mm. × 107 000; *Polyorchis penicillatus*. f) Vertical fibre shows banded pattern. × 22 000; TEM, *Polyorchis penicillatus*. g–h) Species-specific cell adhesion and spreading on the ECMs of the polyp of *Podocoryne carnea* (large sheet, mp) and on the fine fibrillar precipitate (small solid arrows) of the acetic acid extract from *Polyorchis penicillatus*. The fibrillar precipitate of *Polyorchis* was air dried on the native mesoglea of *Podocoryne* and the striated muscle fragments of both species were grafted so that they would make contact with both types of ECMs. To distinguish between the tissues of the two species used, the cells of *Polyorchis* were stained with neutral red (dark cells, marked with *). g) 4 h after grafting the muscle tissue of *Podocoryne* (within circle) is unable to spread on the fibrillar precipitate (small arrows) of *Polyorchis* but easily does so (empty arrows) on the ECM of its own poly stage. × 80. h) same as g; 14 h after grafting the muscle tissue of *Polyorchis* has completely spread on the fiber of the precipitate and even undertunneled the *Podocoryne* muscle tissue. × 65.

White or Poly REC, and sections were incubated with antibodies (for details see Schlage, 1988).

For light and electron microscopy, standard procedures were followed (see also Weber & Schmid, 1985).

Results

With electron microscopy (TEM and SEM), phase contrast, dark field optics and immunohistology employing a mAb directed against an antigen associated with the mesogleal fibers the three-dimensional fibrous network of the mesoglea was demonstrated (Fig. 1, Fig. 2 a–f; Weber & Schmid, 1985). The structural organization of this network is species-specific (Fig. 1). Thick banded fibers (up to 1.8 μm diam.) run vertically between exumbrellar and subumbrellar tissues (Fig. 2a, b, d, f). At both ends, they impinge on a dense matrix which covers the tissue on the mesogleal side (Fig. 2c, d). This fiber system is embedded into a highly hydrated ground substance, presumably formed of proteoglycans, in which fine fibrils (periodicity of $60–65 \times 10^{-7}$ mm) form a three-dimensional network (Fig. 2e; Weber & Schmid, 1985). The periodicity of the striation pattern of these is in the same range as reported for those of vertebrate collagens (for review see Mayne & Burgeson, 1987).

From the mesoglea of several different cnidarian species a collagenous component can be extracted with acetic acid or guanidinium hydrochloride, and precipitated with salts. The precipitate forms banded fibrils which can sup-

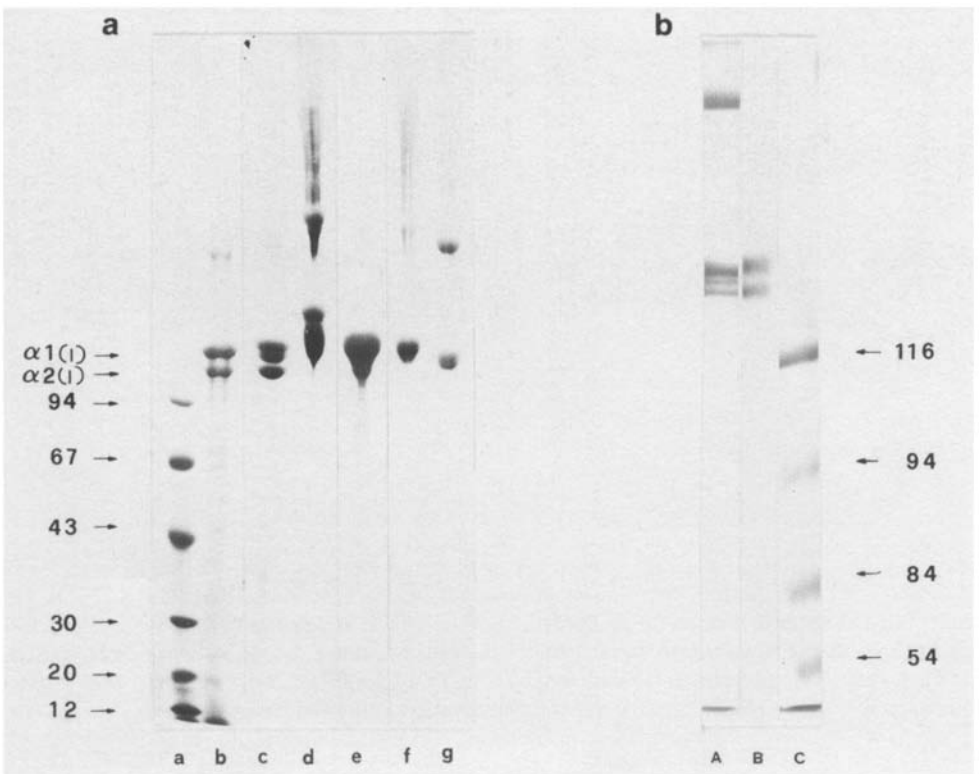

Fig. 3. a) 3–15% SDS-PAGE of pepsin solubilized collagen of different cnidarian species. Lanes: a, molecular weight markers (numbers give masses in kDa); b, Type I collage marker (rat tail); c, *Polyorchis penicillatus* (Eschscholtz); d, *Stomotoca atra* L. Agassiz; e, *Halistaura* sp; f, *Aequorea victoria*; g, *Abylopsis* (siphonophore). b) SDS-PAGE and Western blot of acetic extracted *Polyorchis penicillatus* collagen. Lanes: A, collagen banding pattern displaying 3 distinct α-chains on 5% gel; B, Peroxidase conjugated label marks the presence of mannose on 2 α-chains; C, molecular weight marker proteins.

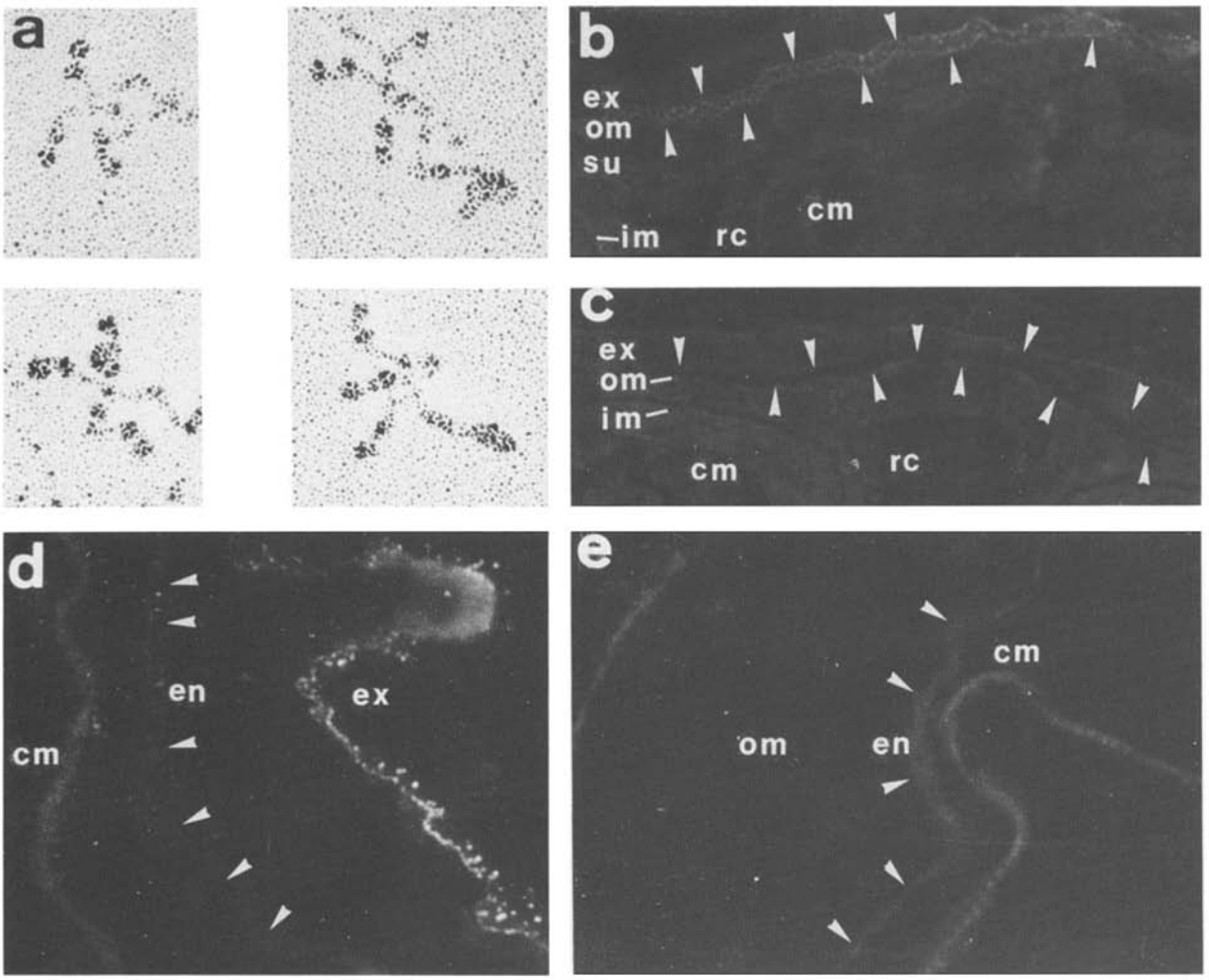

Fig. 4. a) Electron micrographs of rotary shadowed laminin-like molecules from medusae of *Podocoryne carnea*. × 250 000. b–e) Immunohistological localization of CFN from *Podocoryne medusa* buds (× 1 000; b and c) and medusae (× 1 300; d and e). b and D, FN-positive staining at the exumbrellar side of the outer mesoglea. c and e, negative control with FN-absorbed first antibody. Arrowheads: delineation of the outer mesoglea at the side of the subumbrellar plate endoderm (en); radial canal (rc); cross striated muscle epithelium (cm); other abbreviations as for Fig. 1.

port species-specific adhesion and spreading of medusa and polyp cells (Fig. 2g, h; Schmid & Bally, 1988). The precipitate was biochemically characterized and seems to be composed mainly of a collagen containing disulfide bridges with 3 different α-chains. The banding pattern reflects the different orders of association of the 3 α-chains (Schmid & Bally, 1988; Haller, 1989). Species-specificity in the collagen is demonstrated by a divergence in the electrophoretic mobility of the single α-chains (Fig. 3a). In Western blots, 2 of the 3 α-chains revealed the presence of mannose residues when tested with concanavalin A (Fig. 3b, Haller, 1989).

From entire medusae of *P. carnea*, a laminin-like molecule could be purified using a highly specific extraction procedure (Paulsson *et al.*, 1987). On electronmicrographs (Fig. 4a) it exhibits the shape of an asymmetric cross with 3 short arms of 36 ± 7 nm (SD) bearing terminal and inner globules and one long arm of 97 ± 9 nm in length with a thick end-globule. When analyzed by SDS-PAGE, this molecule comigrates with mouse laminin (M_r about 900 000); after reduc-

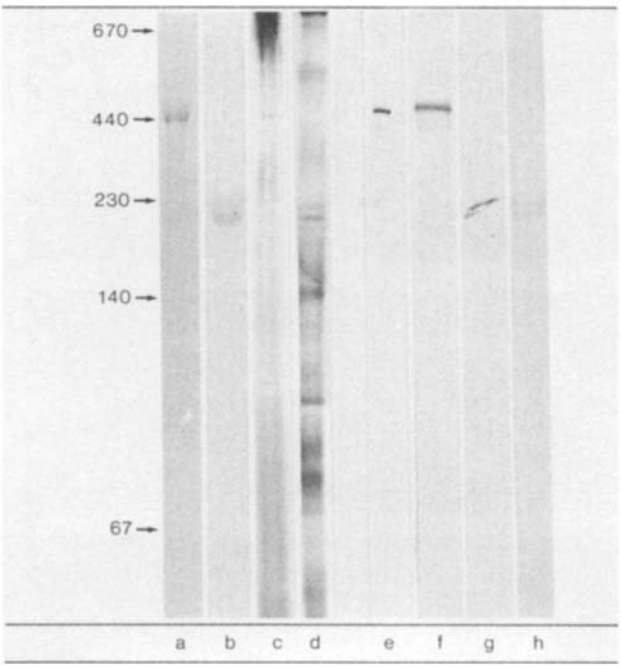

Fig. 5. SDS-PAGE and Western blot analysis of affinity-purified HFN and CFN. Lanes a to d stained slab gels. Lanes a, b: nonreduced and reduced human FN, Coomassie staining; lane c: *Podocoryne* FN, nonreduced, silver staining; lane d: *Aequorea* FN, reduced silver staining. Lanes e to h: immunoblots on nitrocellulose. Lane e: *Aequorea* FN, nonreduced; lane f: *Podocoryne* FN, nonreduced; lane g: *Aequorea* FN, reduced; lane h: *Podocoryne* FN, reduced. The numbers and arrows at the left margin denote the molecular masses in kDa and the position of the marker proteins (from Schlage (1988) with permission of Wissenschaftl. Verlagsges. mbH. Stuttgart).

tion, bands corresponding to M_r 340000 and 260000 could be detected (Beck *et al.*, 1989). In contrast to laminin-like molecules of other invertebrates, e.g., sea urchin and leech, no indications of the existence of a molecule related to nidogen could be found (Beck *et al.*, 1989).

A positive reaction with a polyclonal antibody against murine laminin was observed by immunofluorescence microscopy in medusa buds in the vicinity of the CFN localization using adjacent sections of the same specimen.

From *P. carnea* and *Aequorea victoria* (Murbach & Shearer), a fibronectin-like molecule was isolated which shares several properties with vertebrate FN (Schlage, 1988). It can be isolated and affinity purified by immobilized gelatin under equivalent conditions. The molecule cross-reacts with antibodies against human FN (10 ng ion-exchange purified CFN exhibits *ca* 0.3 ng of HFN equivalents in the ELISA, Fig. 5). The antigen has a similar size and dimeric structure. The molecule can be localized immunohistochemically in medusa buds and medusae where the exumbrellar tissue adheres to the mesoglea of the bell (Fig. 4b–e).

Discussion

In the outer mesoglea of hydrozoan jellyfish, an organized meshwork of bundles of banded collagen fibrils can be seen with the electron microscope. These fibers branch at both ends into a dense plexus of unorganized fibrils. The latter form a dense matrix to which the exumbrella and the subumbrellar-plate endoderm cells adhere (Fig. 2c, d; Gladfelter, 1972; Weber & Schmid, 1985). In a species-specific manner, however, cells can also adhere at any other place on the whole mesoglea, even in areas where cells normally do not occur (Schmid & Bally, 1988). The cell adhesion factor can be extracted from the mesoglea and salt-precipitated. The precipitated fibrils support species-specific cell adhesion and spreading (Schmid & Bally, 1988). In the only comparable study on cnidarians, no real species-specific affinity was noted in the freshwater *Hydra* (Day & Lenhoff, 1981). Together with the dense matrix of the highly hydrated ground substance (presumably proteoglycans) the organized architecture of the collagen fiber system lends the mesoglea the rigidity necessary to resist the deformation caused by the contraction of the muscle systems and thus acts like a vertebrate abductor muscle system.

Collagens have been identified biochemically in numerous cnidarians (Barzansky & Lenhoff, 1974; Barzansky *et al.*, 1975; Adams, 1978; Miura & Kimura, 1985; Schmid & Bally, 1988; Haller, 1989). They can be extracted and precipitated as banded fibrils which support species-specific cell adhesion. These fibrils are formed

mainly of disulfide bridges containing collagen (Schmid & Bally, 1988; Bally, unpubl. data). As known from other invertebrates, collagens are frequently glycosylated (Katzman & Kang, 1972). Possibly a species-specific glycosylation is responsible for the different banding patterns on SDS-PAGE (Fig. 3a). However, we do not yet know whether the species-specificity of the cell-matrix interaction (adhesion) resides in the collagen and its specific carbohydrate residues or in coprecipitating factors.

The studies on FN and laminin support the view that these important ECM glycoproteins have been highly conserved during evolution. This is especially true for laminin where reliable data are available for different phyla (Beck *et al.*, 1989). It remains to be demonstrated which functional homologies of FN and other ECM constituents, particularly laminin and collagen, exist in jellyfish, and what role they might play in the unparalleled transdifferentiation and regeneration potential of these animals (Schmid *et al.*, 1988).

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