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Mesogleal cells of the jellyfish *Aurelia aurita* are involved in the formation of mesogleal fibres

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Abstract

The extracellular matrix of the jellyfish *Aurelia aurita* (Scyphozoa, Cnidaria), known as the mesoglea, is populated by numerous mesogleal cells (Mc). We determined the pattern of the Mc and the mesoglea, raised polyclonal antibodies (RA47) against the major mesogleal protein pA47 (47 kDa) and checked their specificity. In the mesoglea, RA47 stains pA47 itself. In immunoblots of Mc, RA47 stains bands of 120 kDa and 80 kDa; weaker staining is observed at pA47. The same staining pattern is seen on blots of jellyfish epidermal cells and of whole *Hydra* (Hydrozoa) or isolated mesoglea of *Hydra*. Our data indicate that pA47 is synthesized by Mc and epidermal cells as high molecular precursors. Using immunostaining techniques, we showed Mc to be involved in the formation of mesogleal non-collagenous (called ‘‘elastic’’ in classic morphological studies) fibres. The biochemical and morphological data suggest that Mc originate from the epidermis.

Keywords: Extracellular matrix; Cnidaria; *Aurelia aurita*; Mesogleal cells; Mesoglea

1. Introduction

Comparative analysis of extracellular matrix (ECM) structure and synthesis gives information about the formation, function and evolution of ECM. The Cnidaria phylum represents one of the most interesting models for this kind of research due to its phylogenetic position. An extracellular substance, the mesoglea, is situated between epidermal and gastrodermal layers. The mesoglea is equivalent to the ECM of vertebrates and, as in vertebrates, it performs important functions (Chapman, 1974; Sarras and Deutzmann, 2001). Essentially it serves as a skeleton that maintains the specific animal body structure, and takes part in the transport and storage of nutrients (Chapman, 1966; Bouillon and Coppois, 1977; Weber and Schmid, 1985). The ECM is also involved in buoyancy regulation (Denton, 1963; Mackay, 1969). The forces generated by the contraction of the umbrella muscle system cause propulsive swimming of the animal at the medusa stage (Chapman, 1966; Gladfelter, 1972; Weber and Schmid, 1985; Demont and Gosline, 1988), while the elasticity of the ECM counteracts the muscle contraction (Bouillon and Coppois, 1977). The mesoglea plays a key role in the control of cell migration and morphogenetic processes (for review, see Schmid, 1992; Sarras et al., 1993; Frank and Rinkevich, 1999; Shimizu et al., 2002; Kleinman et al., 2003). Factors affecting the structural integrity of the ECM and interfering with cell–substrate interactions alter the state of cellular differentiation (for review, see Schmid, 1992; Schmid et al., 1993).

Two types of fibres in the jelly-like ground substance have been described in cnidarian ECM (Bouillon and Coppois, 1977). Evidence for the existence of collagen fibrils was reported in the late 1970s (Chapman, 1974; Bouillon and Coppois, 1977; Adams, 1978). A collagen fibre system lends resilience

Abbreviations: ECM, extracellular matrix; Mc, mesogleal cells; G, gastrodermal cells; E, epidermal cells; Mes, mesoglea; Abs, antibodies.

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to the mesoglea (Adams, 1978; Franc et al., 1984; Miura and Kimura, 1985; Napara et al., 1996a,b). Another type of fibril with a ramified and undulating appearance was revealed by electron microscopy (Chapman, 1959). It was found that these fibrils consisted of a central core encircled by a fine fibrillary meshwork. Because of their external similarities, these fibrils were designated "elastic" fibrils and were initially thought to consist of elastin (Bouillon and Vandermeerssche, 1956; Bouillon, 1959; Demont and Gosline, 1988). However, phylogenetic analysis showed that elastin was present only in vertebrates (for review, see Rosenbloom et al., 1993).

Some cnidarian ECM proteins have been characterized, e.g. several different types of fibrillary collagens (Franc et al., 1984; Miura and Kimura, 1985; Sarras et al., 1991; Tillet et al., 1996; Deutzmann et al., 2000). In *Hydra* sp., immunoreactive non-fibrillary collagen type IV, fibronectin, heparan sulfate and metalloproteinases have been reported (Sarras et al., 1991; Fowler et al., 2000; Leontovich et al., 2000). Laminin was identified by immunohistology and rotary shadowing in *Podocoryne carnea* (Beck et al., 1989) and cloned from *Hydra* sp. (Sarras et al., 1994; Zhang et al., 2002). Another protein involved in tightening the ECM network is fibrillin (for review, see Har-El and Tanzer, 1993). Fibrillin is a 330–340 kDa protein identified by monoclonal antibodies from a panel against ECM proteins. It can be concluded from a partial cDNA sequence of *Podocoryne carnea* fibrillin (Reber-Muller et al., 1995) that the fibrillin modules and their arrangements are highly conserved from Cnidaria to humans. Fibrillin as well as collagen must have developed early in metazoan evolution (Doolittle, 1992). Collagen provides rigidity and stiffness to the ECM, while fibrillin confers elasticity and resilience. These basic structural components evolved to provide a biomechanical basis of ECM architecture, allowing early cnidarians to exploit new opportunities.

The mesoglea of some species of the Scyphozoa and Anthozoa classes contains free motile cells – mesogleal cells (Chapman, 1966, 1974). The origin of these cells has not been elucidated and little is known about their functions. Their capacity for amoeboid movement and active phagocytosis has been described (Metchnikoff, 1880; Napara et al., 1994).

The mesoglea, with its mesogleal cells, has been shown to be similar to the connective tissues of more advanced animals (Zawarzin, 1945; Chapman, 1974). Its development has been studied by several methods, including autoradiography after ^{3}H-proline incorporation (Hausman and Burnett, 1971; Singer, 1974; Young, 1974; Franc et al., 1984). Even so, none of these methods has shed light on the possible role of mesogleal cells in mesoglea formation.

The "elastic" fibres of the Scyphozoan jellyfish *Aurelia aurita* contain lysine-rich proteins that bare heavily positive charge. "Elastic" fibre proteins are also enriched in cysteine and, consequently, in S–S bonds (Schmid et al., 1991; Napara et al., 1996a). *A. aurita* possesses numerous mesogleal cells at the medusa stage of the life cycle (Napara et al., 1994). Therefore, this species was chosen to determine whether mesogleal cells are involved in mesoglea development. We determined the protein pattern of mesogleal cells and the mesoglea, and raised antibodies against one of the major mesogleal proteins. Immunoblots and data from light and electron microscopic immunostaining demonstrate that mesogleal cells are involved in the formation of mesogleal "elastic" fibres.

2. Materials and methods

2.1. Animals

Jellyfish *A. aurita* were collected in the vicinity of the White Sea Biological Station of the Zoological Institute RAS "Kartesh" (Chupa Inlet, Kandalaksha Bay in the White Sea) during the summers of 1996–2003. To obtain mesogleal cells (Mc) from the growing medusae, pieces of about 0.5 cm^3 were cut out of the mesoglea and digested with collagenase (Sigma, Type II, C-6885, 1 mg collagenase per 1 ml mesoglea) at 18 °C for 2 h on a shaker. The cells were washed 3 times in filtered seawater (FSW) by mild centrifugation and the cell pellet was resuspended in FSW. The viability of the mesogleal cells was estimated by phase-contrast microscopy. The cell suspension was aliquoted at 9×10^6 cells per 0.1 ml and frozen. The cells were later used for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and antibody testing. Gastrodermal and epidermal tissue layers (G and E, respectively) were cut from the medusa body under a dissecting microscope. Pieces of these layers were also subjected to collagenase treatment to remove the remnants of adjacent mesoglea. For mesoglea (Mes) preparation, the tentacles and gastric pockets were removed from mature jellyfish and the mesoglea was isolated from the gastric channels and the epidermal epithelium under a dissecting microscope. The samples were used immediately or stored at −20 °C.

Hydra vulgaris animals were kindly donated by Dr. M. Samoilovich (Central Roentgeno-Radiological Research Institute, Russia) and stored alive in water or in a freezer at −20 °C. The *Hydra* sp. extracellular matrix was a kind gift from Dr. M. Sarras, Jr. (Rosalind Franklin University, USA).

2.2. Gels and antibodies

The protein composition of the preparations was determined using SDS-PAGE (Laemmli, 1970). Western blotting was performed according to Towbin et al. (1979) with modifications. Methanol-activated polyvinylidene difluoride (PVDF) membranes (0.45 μm pore size, Millipore Corporation, USA) and modified Laemmli's running buffer (25 mM Tris, 192 mM glycine, pH 8.3, 0.001% SDS, 10% methanol) were used for semi-dry protein transfer. Membranes with immobilized proteins were incubated in 20 mM Tris–HCl, pH 7.5, containing 0.02% Tween-20, 1% sodium caseinate and 3% bovine serum albumin. Antibody dilution and membrane washing were carried out in 20 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.02% Tween-20 (TBS-Tw). Bands were visualized by 3,3′-diaminobenzidine tetrahydrochloride staining of horseradish peroxidase-conjugated goat-anti-rabbit antibodies (ICN Biomedicals, USA).

Polyclonal antibodies (Ab) against the major mesoglea protein pA47 were raised as previously described (Podgornaya and Shaposhnikova, 1998). The 47 kDa proteins were cut out of the SDS-PAGE slab gels, homogenized in PBS and mixed with Freund's adjuvant (1:1); this mixture was injected intracutaneously into 2 male rabbits. After 4 weeks the animals received an intramuscular boost with the same mixture. A week later about 5 ml of blood was taken from the rabbits' ears. The serum was aliquoted and stored at −20 °C. Serum testing (Fig. 1) showed that it needed no further purification. The immune serum was designated RA47.

2.3. Histology

Pieces from a growing medusa body were fixed in Bouin solution for 24 h and then rinsed in 70% ethanol. The fixed samples were embedded in paraffin and sliced to give 5–7-μm thick sections. All traces of picric acid were washed out with 70% ethanol after the paraffin was removed, and the sections were washed 3 times with TBS-Tw, the basic solution for the whole procedure. The sections were preincubated with 3% skimmed milk in TBS-Tw for 1 h, washed 4 times for 10 min, incubated with RA47 at 1:2000 dilution for 1 h,

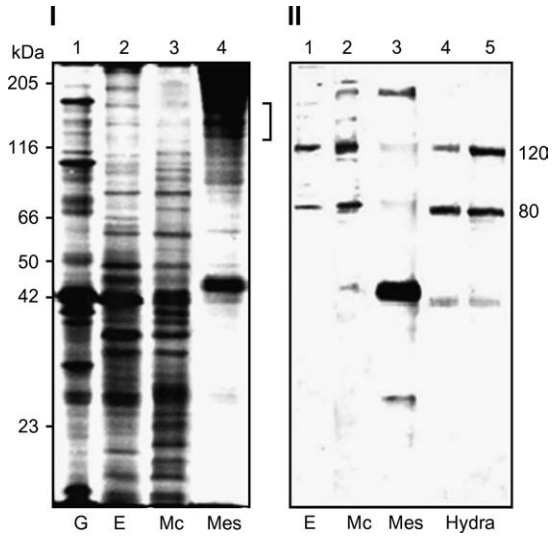

Fig. 1. Polypeptide composition (I) and immunoblot with RA47 (II). (I) Coomassie-stained 10% SDS-PAGE. 1 – gastrodermal cells (G); 2 – epidermal cells (E); 3 – mesogleal cells (Mc); 4 – mesoglea (Mes). In parentheses – 3 collagen lines on Mes indicated. Molecular masses of marker proteins (in kDa) are indicated on the left. (II) 1 – E; 2 – Mc; 3 – Mes. 1–3 correspond to lanes 2–4 in (I). Additionally loaded: 4 – *Hydra* ECM; 5 – the whole *Hydra*. The molecular masses of the stained polypeptides in addition to p47 are indicated on the right. Serum dilution 1:3000.

washed and incubated with alkaline phosphatase-conjugated goat-anti-rabbit Ig (GAR-AP, Sigma), and stained with BCIP-NBT (Sigma). All the procedures were carried out at room temperature (18 °C). The stained sections were mounted in 80% glycerol and examined under the light microscope. For immunofluorescence, the secondary Ab, goat-anti-rabbit, was FITC-conjugated (GAR-FITC, Sigma). These preparations were examined with a Zeiss Axioplan epifluorescence microscope equipped with a charge-coupled device (CCD) camera (Sony) controlled by KS100 software (Carl Zeiss, Germany). For controls, some sections were incubated with non-immune rabbit serum followed by all the subsequent steps. Images were prepared for publication using Adobe Photoshop 6.0 software.

For electron microscopy the pieces of mesoglea were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS) with 10% sucrose for 2 h at 10 °C, washed in PBS with 10% sucrose, postfixed for 1 h in phosphate-buffered 1% osmium tetroxide and dehydrated through a graded series of alcohol and acetone. Finally, the tissues were embedded in Epon 812. Ultrathin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate and examined with a transmission microscope (JEM 7A, Japan).

For immunostaining, the materials were fixed in 4% paraformaldehyde and 0.5% glutaraldehyde in PBS at 10 °C, washed in 0.05 M ammonium chloride in PBS and distilled water, and dehydrated before final embedding in LR-White resin (London Resin Company, Ltd). Non-specific binding was blocked by preincubating the thin sections with 10% normal bovine serum in TBS-Tw for 40 min. The sections were then incubated for 45 min with RA47 (diluted 1:300 in TBS-Tw), washed in TBS-Tw, incubated with 10 nm gold-conjugated secondary goat-anti-rabbit antibody (ICN) for 45 min and washed in TBS-Tw. For controls, some sections were incubated with non-immune rabbit serum followed by all the subsequent steps.

2.4. Spectrometry and sequencing

Several attempts were made to perform mass-spectrometry (MALDI; Pappin et al., 1993) of pA47 transferred to a PVDF membrane at the mass-spectrometry facility of Laval University (Quebec, Canada). The MALDI procedure for comparing pA80 with pA47 was carried out by the firm "PYNNY", CJSC, Moscow. The MALDI results were processed using the program MASCOT (http://www.matrixscience.com). N-terminal sequencing and internal fragment sequencing were done in the Protein Resource Center of The Rockefeller University, USA.

3. Results

Gastrodermal (G) and epidermal (E) cell layers were separated from adjacent mesoglea by collagenase digestion and used directly for SDS-PAGE (Fig. 1 I, G, E). The method for isolating mesogleal cells (Mc) is based on intense collagenase treatment of the mesogleal pieces followed by sedimentation and washing of the cells released from the ECM. The Mc remained viable and motile after isolation. We assume the difference in polypeptide composition of the cell types to be relevant in spite of the different methods of isolation. None of the three cell groups has an obvious major cell-type-specific protein. The major protein in each case is actin, 42 kDa, which is the main protein of most cells capable of active movement. The second main protein corresponds to histone H1 according to its apparent molecular mass (~30 kDa). Other cell polypeptides are in the mass range from hundreds to tens of kDa. The overall intensity and distribution of polypeptides are similar in E and Mc and both differ from G (Fig. 1 I, G vs E, Mc). This similarity in polypeptide pattern might indicate that Mc originates from the epidermis.

Mesoglea (Mes) contains some Mc, but the proteins of the mature medusa mesoglea preparation are not resolved by one-dimensional SDS-PAGE (Fig. 1 I, Mc, Mes). The major protein components of the mature medusa Mes are more likely to be ECM components. The three zones in the 150 kDa range correspond to the collagens known to be major ECM components in *Hydra* (Sarras and Deutzmann, 2001). The uppermost zones on the gel (>300 kDa) seem to correspond to fibrillin of molecular mass 330–340 kDa (Har-El and Tanzer, 1993; Reber-Muller et al., 1995), although other proteins could also produce zones in this area. The main Mes polypeptide is of molecular mass 47 kDa (pA47). A protein with similar molecular mass, 45 kDa, has been observed in *Hydra* ECM, but it did not react with Abs against any known vertebrate ECM proteins, and no suggestion was made about its nature (Sarras et al., 1991).

Polyclonal Abs against pA47 stain pA47 itself and some minor bands in the Mes preparations (Fig. 1 II, Mes). In Mc, the pA47 stains weakly. More prominent stained bands have apparent molecular masses of 120 kDa and 80 kDa. These latter bands are also stained in E though they are less prominent (Fig. 1 II, Mc vs E). The gastroderm preparation contains no polypeptides with corresponding antigenic determinants (data not shown).

We suppose that pA47 is synthesized as a high molecular mass precursor and undergoes restricted proteolysis during incorporation into the ECM. RA47 stains both the high molecular bands and 45 kDa band in ECM isolated from *Hydra*; the 45 kDa band is weaker in the whole animal (Fig. 1 II 4, 5). Hence, both animals, *Hydra* sp. and *A. aurita* medusa, synthesize the protein as a high molecular mass precursor, but post-translational modifications leading to the appearance of the mesoglea are more extensive in the medusa.

Immunofluorescence with RA47 shows that fibrils in the Mes and Mc are stained intensely (Fig. 2). The label looks like a circle or a patch with blurred boundaries on cross-sectioned fibrils (Fig. 2 I, arrows). The cells are characterized by similar blurred boundaries. Fig. 2 II reveals granules in the Mc of the growing medusa with very pronounced staining in paraffin sections. The cell contours are blurred and the cells look as though they are surrounded by weakly stained material. The ECM material itself is not heavily stained and fibres of other types are less obvious than those revealed by immunofluorescence (Fig. 2 I). This seems to be due to secretion of granular material from the Mc. In the epidermis, the antigen is distributed throughout the cell cytoplasm but immunostaining is most prominent in the apical parts of the cells (Fig. 2 IIa). The gastrodermis is not stained by RA47, in accordance with the biochemical data (not shown). Immunostaining together with biochemical data (Fig. 1) indicate that both the epidermal and mesogleal cells of *A. aurita* produce the main Mes protein, which is a component of the ''elastic'' fibres. Mc definitely participates in pA47 production and might play a role in pA47 modification during ''elastic'' fibre formation.

The relative sizes of Mc and ''elastic'' fibres in the Mes are shown in Fig. 3a. At high magnification, Mc has the typical appearance of a protein-synthesizing cell: the endoplasmic reticulum is well developed and there are several granules at different stages of maturation (Fig. 3b). The granules, which are located closer to the cell periphery, look smaller and clearer. The total number of granules in the Mc decreases as the medusa ages, at the end of its season of vegetation (data not shown). A longitudinal section of a typical-looking ''elastic'' fibre shows its difference from collagen fibres (Fig. 3c). The dense core previously revealed in ''elastic'' fibres (Bouillon, 1959) can be seen in cross-section (Fig. 3a).

Immunogold staining of such preparations shows that in Mc most of the label is present in granules of moderate electron density and at the ends of the endoplasmic reticulum tubes; some material on and outside the cell surface is also stained (Fig. 3d). Both the longitudinal section near the ''elastic'' fibre, with its characteristic turn (Fig. 3e), and the cross-section (Fig. 3f) show the label in the fibre and in material radiating from it. Collagen fibres are not stained (Fig. 3e,f).

The immunogold technique confirms that Mc synthesize and secrete the main Mes protein, which is included in the ''elastic'' fibres.

4. Discussion

The life cycle of the scyphozoan *A. aurita* is characterized by four main successive stages: planula larva, polyp, ephyra and medusa. When a planktonic larva settles on an appropriate substrate it undergoes metamorphosis into a polyp, the scyphistoma, which can multiply by asexual reproduction. Polyps form ephyra by strobilation. Ephyra can then metamorphose into the medusa, the sexual stage. It is distinguished from the polyp not only by a different shape and anatomical organization, but also by a set of somatic cells and sense organs that are absent in the polyp. The cycle is completed by the liberation of gametes, formation of a zygote and subsequent development of the planula (Hyman, 1940). In northern latitudes, medusae exist in the plankton only in summer and autumn (Grondahl, 1988).

One of the main differences in body plans between the medusa and the polyp is the appearance of a huge amount of mesoglea (ECM) between the two layers, epidermis and gastroderm. In some species mesoglea is populated with Mc, which are capable of amoeboid movement and can uptake foreign particles by phagocytosis (Metchnikoff, 1880; Napara et al., 1994). Recent cellular and molecular data show that some cells situated between ecto- and endodermal layers at the medusa stage, e.g. muscle cells, are derived from the entocodon, a mesoderm-like third cell layer, which separate from the ectoderm (for review, see Seipel and Schmid, 2005). Mc are believed to originate from the epidermis, though it has been suggested that they might arise from both layers (Ivanova-Kazas, 1995; Tardent, 1978). The similarity between the epidermal and Mc polypeptide patterns (Fig. 1 I) argues that Mc originate from the epidermal layer or its derivate.

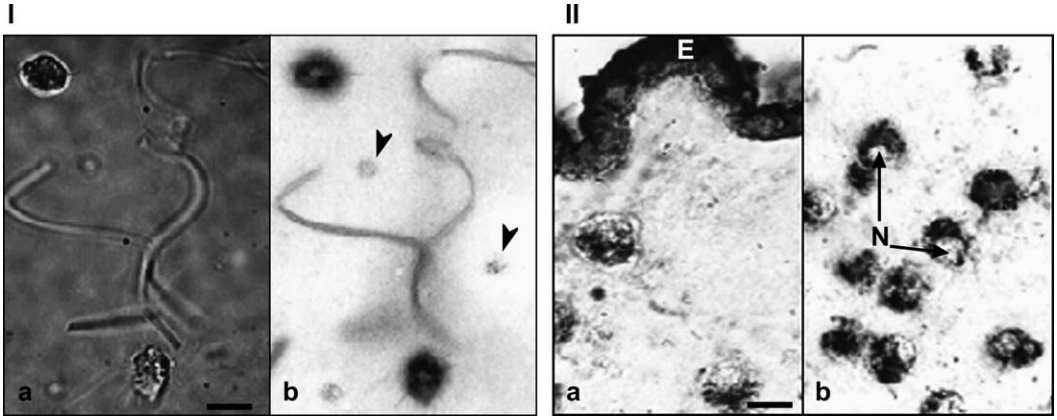

Fig. 2. Immunostaining of the mesoglea of a growing medusa (diameter 18 cm) on paraffin sections, serum dilution 1:2000. (I) Visualization with FITC: a – phase contrast; b – immunofluorescence in inverted grayscale. Cross-sectioned ''elastic'' fibres are marked by arrows. (II) Visualization with AP-NBT-BCIP: a – epidermis (E) and adjacent part of mesoglea; b – internal part of mesoglea, N – nuclei of mesogleal cells. Bar = 5 μm.

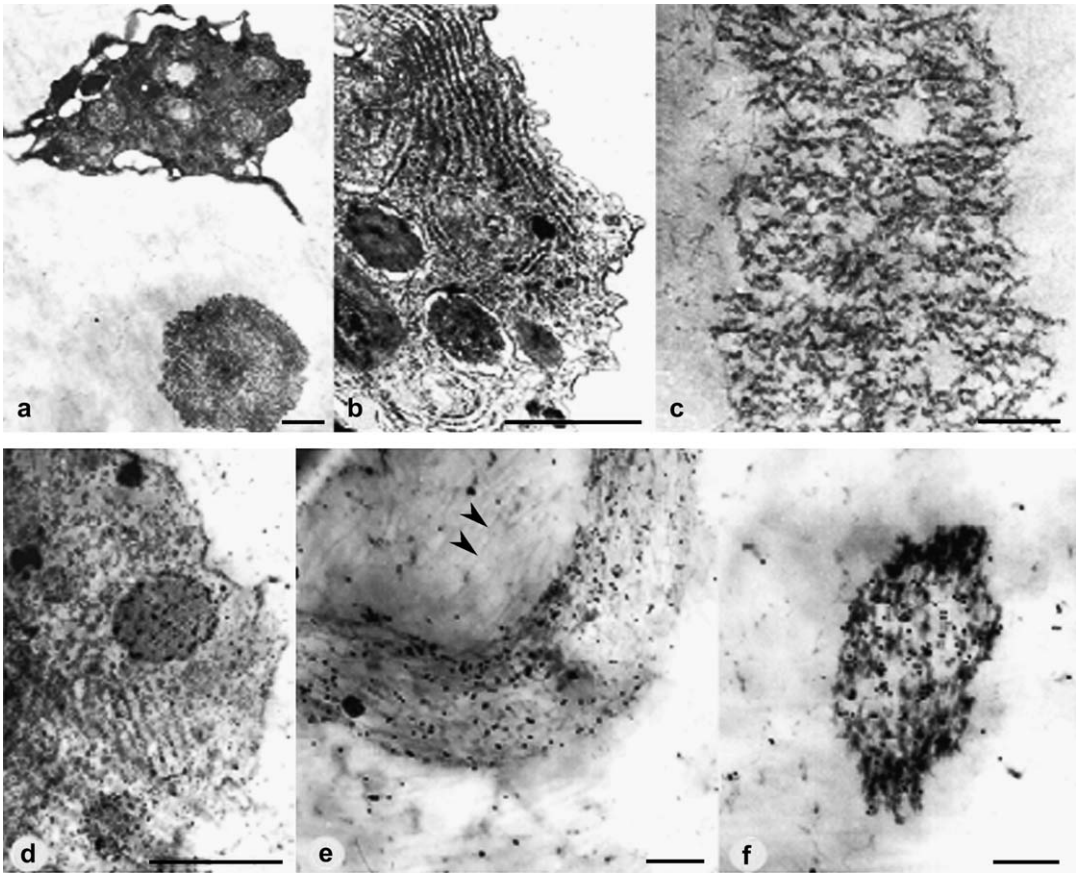

Fig. 3. Ultrastructure (a–c) and immunogold staining with RA47 in 1:300 diluted serum (d–f) of mesogleal cells and "elastic" fibres of mature medusa (diameter 26 cm). a – Mc and cross-section of the "elastic" fibre; b – Mc at high magnification; c – "elastic" fibre transverse section; d – Mc; e – longitudinal section of "elastic" fibre (arrows point to collagenous fibres); f – "elastic" fibre cross-section. Bar = 1 μm.

The Mc of scyphistoma have a low level of proliferation with mitotic index (MI) ≈0.1% (Napara and Chaga, 1992b). This situation changes dramatically during strobilation. The Mc in the upper part of the polyp, which transforms into the ephyra, become mitotically active (MI 2.2%) and look more like medusa Mc. In the young medusa, Mc remain mitotically active (MI 1.5%) and continue to phagocytose. Such proliferative activity makes the whole population self-supporting during medusa growth without the need for incorporation of cells from the established layers (Napara and Chaga, 1992a,b). Eviction of Mc from the epidermis probably occurs once during the first steps of ontogenesis.

Mc display synthetic activity in some species. For example, a labelled ^{3}H-proline precursor became incorporated into Mc and the label was subsequently translocated into the ECM in the actinia *Calliactis parasitica*; and the Mc of *A. aurita* actively incorporated ^{3}H-lysine (Young, 1974; Napara et al., 1996b). *A. aurita* medusa Mc are also typical protein-synthesizing cells (Fig. 3b). The material from the Mc granules is released by exocytosis. The granular material and ECM fibres share common cytochemical characteristics (Napara et al., 1996a). Thus, the Mc granules contain material for forming the ECM fibres. At least 3 types of granules could be identified morphologically in the Mc (Napara et al., 1996a). The Ab against pA47 stains granules inside the Mc as well as the "elastic" fibres of the mesoglea (Figs. 2, 3d–f). The staining of epidermis with RA47 and the absence of staining in the gastroderm also suggest that Mc originate from the epidermal layer.

In the mesoglea, pA47 is involved in the formation of undulating non-collagenous fibres. We did not observe any significant ramification, but they are definitely wavy (Figs. 2, 3). The undulated and ramified fibres in some cnidarian ECMs proved to be digestible with elastase only after oxidation with peracetic acid, and then they could be stained specifically for elastin (Bouillon and Coppois, 1977). This led to the conclusion that oxylatan fibrils were present and they were found to be morphologically similar to the elastic fibres. In invertebrates, proteins unrelated to elastin but with elastic properties have been reported: abductin in bivalvia, elastomer in the octopus, and resilin in arthropods (Rosenbloom et al., 1993). Despite the differences in apparent molecular mass, pA47 could be an ortholog of such a protein.

pA47 was subjected to the MALDI procedure and no analogue of it was found in the world databases. Maximal, but not reliable, similarity scores were obtained for the hypothetical *Caenorhabditis elegans* protein CBG 13184. Since pA47 could not be identified in the mass-spectra of its polypeptide profile, MALDI failed to answer the question of whether the 80 kDa protein is the precursor of pA47. The 80 kDa protein

spectra have even less similarity to the hypothetical proteins than pA47. Comparison of the pA47 and 80 kDa protein spectra shows that only 1/3 of the oligopeptides are similar. These two proteins share a common antigenic determinant (Fig. 1 II), so the degree of modification in pA47 processing could be quite high. We suggest that the degree of post-translational modification of the 120 and 80 kDa proteins is the main point of difference between the *Hydra* and *Aurelia* ECMs (Fig. 1 II). The same tendency, i.e. synthesis as high molecular weight precursors and subsequent modification, is seen in some ECM proteins, particularly collagens, vertebrate elastin and galaxin, a cnidarian ECM protein from the exoskeleton of the reef coral *Galaxea fascicularis* (Rucker, 1982; Har-El and Tanzer, 1993; Fukuda et al., 2003).

An attempt to determine the N-terminal sequence showed that the N-terminus was blocked, which also suggested post-translational protein modification during fibre formation (Figs. 1 II, 3d–f). The internal pA47 fragment was sequenced. A 7-residue sequence (YTFIENR) did not correspond to any protein or peptide sequence in the protein databases. A primer designed on the basis of this peptide, together with an arbitrary primer, was the foundation for cDNA library screening.

Our data indicate that pA47, which is involved in forming the ''elastic'' fibres of the mesoglea, is synthesized as high molecular mass precursor(s) by Mc and, to a lesser extent, the epidermis. Biochemical and morphological data suggest that Mc originate from the epidermis but not the gastroderm. Neither pA47 nor its precursors exist in the world protein databases; hence, gene cloning is necessary.

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References

Adams E. Invertebrate collagens. Science 1978;202:591–8.

Beck K, McCarthy RA, Chiquet M, Masuda-Nakagawa L, Schlage WK. Structure of the basement membrane protein laminin: variations on a theme. In: Aebi U, Engel J, editors. Cytoskeletal and extracellular proteins in biophysics, vol. 3. New York/Berlin: Springer-Verlag; 1989. p. 102–5.

Bouillon J. Nature et structure de la mesoglée des hydropolypes. Bull Biol Fr Belg 1959;1:64–71.

Bouillon J, Coppois G. Etude comparative de la mesoglée des cnidaires. Cah Biol Mar 1977;13:339–68.

Bouillon J, Vandermeerssche G. Structure et nature de la mesoglée des Hydro-et-Scyphomeduses. Ann Soc R Zool Belg 1956;87:9–25.

Chapman G. The mesoglea of *Pelagia noctiluca*. Q J Microsc Sci 1959;100: 599–610.

Chapman G. The structure and function of the mesoglea. In: Rees, editor. Cnidaria and their evolution. Symp Zool Soc Lond 1966;16:147–68.

Chapman DM. Cnidarian histology. In: Muskatine L, Lenhoff H, editors. Coelenterate biology. Reviews and new perspectives. NY, SF, London: Academic Press; 1974. p. 1–93.

Demont ME, Gosline JM. Mechanics of jet propulsion in the hydromedusan jellyfish *Polyorchis penicillatus*. J Exp Biol 1988;134:313–32.

Denton EJ. The buoyancy mechanism of sea creatures. Endeavour 1963;22: 85–7.

Deutzmann R, Fowler S, Zhang X, Boone K, Dexter S, Boot-Handford RP, et al. Molecular, biochemical and functional analysis of a novel and developmentally important fibrillar collagen (Hcol-I) in hydra. Development 2000;127:4669–80.

Doolittle RF. Reconstructing history with amino acid sequences. Protein Sci 1992;1:191–200.

Franc S, Franc JM, Garrone R. Fine structure and cellular origin of collagenous matrices in primitive animals: Porifera, Cnidaria and Ctenophora. Front Matrix Biol 1984;3:143–56.

Frank U, Rinkevich B. Scyphozoan jellyfish's mesoglea supports attachment, spreading and migration of anthozoans' cells in vitro. Cell Biol Int 1999; 23:307–11.

Fowler SJ, Jose S, Zhang X, Deutzmann R, Sarras Jr MP, Boot-Handford RP. Characterization of hydra type IV collagen. Type IV collagen essential for head regeneration and its expression is up-regulated upon exposure to glucose. J Biol Chem 2000;275:39589–99.

Fukuda I, Ooki S, Fujita T, Murayama E, Nagasawa H, Isa Y, et al. Molecular cloning of a cDNA encoding a soluble protein in the coral exoskeleton. Biochem Biophys Res Commun 2003;304:11–7.

Gladfelter WB. Structure and function of the locomotory system of *Polyorchis montereyensis* (Cnidaria, Hydrozoa). Helgol wiss Meeresunters 1972;23: 38–79.

Grondahl F. A comparative ecological study on the scyphozoans *Aurelia aurita*, *Cyanea capillata* and *C. lamarckii* in the Gullmar Fjord, western Sweden, 1982 to 1986. Mar Biol 1988;97:541–50.

Har-El R, Tanzer ML. Extracellular matrix. 3: evolution of the extracellular matrix in invertebrates. FASEB J 1993;7:1115–23.

Hausman RE, Burnett AL. The mesoglea of *Hydra*. IV. A qualitative radioautographic study of the protein component. J Exp Zool 1971;177:435–46.

Hyman LH. The invertebrates: Protozoa through Ctenophora. New York, London; 1940. 726 p.

Ivanova-Kazas OM. Evolutionary embryology of animals. St-Petersburg: Nauka; 1995. 565 p. [in Russian].

Kleinman HK, Philp D, Hoffman MP. Role of the extracellular matrix in morphogenesis. Curr Opin Biotechnol 2003;14:526–32.

Laemmli UK. Cleavage of structural protein during the assembly of the head of the bacteriophage T4. Nature 1970;4:680–2.

Leontovich AA, Zhang J, Shimokawa K, Nagase H, Sarras Jr MP. A novel hydra matrix metalloproteinase (HMMP) functions in extracellular matrix degradation, morphogenesis and the maintenance of differentiated cells in the foot process. Development 2000;127:907–20.

Mackay NC. Sulphate regulation in jellyfish. Comp Biochem Physiol 1969; 30:481–8.

Metchnikoff I. Uber die intracellulare Verdauung bei Colenteraten. Zool Anz 1880;3:261–3.

Miura S, Kimura S. Jellyfish mesoglea collagen. Characterization of molecules as $\alpha1\alpha2\alpha3$ heterotrimers. J Biol Chem 1985;260:15352–6.

Napara TO, Oskol'sky AA, Chaga OY. Mesogleal cells of *Aurelia aurita* (Cnidaria, Scyphozoa). I. Morphology of mesogleal cells and of the matrix of mesoglea. Tsitologiia 1994;36:623–30 [in Russian].

Napara TO, Chaga OY. Proliferative activity and growth of the mesogleal cell population in scyphomedusae *Aurelia aurita* (L.). I. Mesogleal cells of young and mature medusae. Tsitologiia 1992a;34(6):59–66 [in Russian].

Napara TO, Chaga OY. Proliferative activity and growth of the mesogleal cell population in scyphomedusae *Aurelia aurita* (L.). II. Mesogleal cells of strobilae and ephyrae. Tsitologiia 1992b;34(8):33–7 [in Russian].

Napara TO, Oskol'sky AA, Chaga OY. Mesogleal cells of *Aurelia aurita* (Cnidaria, Scyphozoa). II. Ultrastructural and histochemical analysis. Tsitologiia 1996a;38:456–64 [in Russian].

Napara TO, Oskol'sky AA, Shaposhnikova TG, Chaga OY. Mesogleal cells of *Aurelia aurita* (Cnidaria, Scyphozoa). III. An autoradiographic analysis of the synthesis of extracellular matrix of mesoglea. Tsitologiia 1996b;38: 465–74 [in Russian].

Pappin DJ, Hojrup P, Bleasby AJ. Rapid identification of proteins by peptide-mass fingerprinting. Curr Biol 1993;3:327–32.

Podgornaya OI, Shaposhnikova TG. Antibodies with the cell-type specifity to the morula cells from hemolymph of ascidian *Styela rustica*. Cell Struct Funct 1998;23:349–55.

Reber-Muller S, Spissinger T, Schuchert P, Spring J, Schmid V. An extracellular matrix protein of jellyfish homologous to mammalian fibrillins forms different fibrils depending on the life stage of animal. Dev Biol 1995;169: 662–72.

Rosenbloom J, Abrams WR, Mecham R. Extracellular matrix 4: the elastic fiber. FASEB J 1993;7:1208–18.

Rucker RB. Isolation of soluble elastin from copper-deficient chick aorta. In: Cunningham LW, Frederiksen DW, editors. Methods in enzymology 82, Part A 1982. p. 650–6.

Sarras Jr MP, Deutzmann R. *Hydra* and Niccolo Paganini (1782–1840) – two peas in a pod? The molecular basis of extracellular matrix structure in the invertebrate, *Hydra*. Bioessays 2001;23:716–24.

Sarras Jr MP, Madden ME, Zhang X, Gunwar S, Huff JK, Hudson BG. Extracellular matrix (mesoglea) of *Hydra vulgaris*. I. Isolation and characterization. Dev Biol 1991;148:481–94.

Sarras Jr MP, Zhang X, Huff JK, Accavitti MA, St John PL, Abrahamson DR. Extracellular matrix (mesoglea) of *Hydra vulgaris*. III. Formation and function during morphogenesis of hydra cell aggregates. Dev Biol 1993; 157:383–98.

Sarras Jr MP, Yan L, Grens A, Zhang X, Agbas A, St John PL, et al. Cloning and biological function of laminin in *Hydra*. Dev Biol 1994;164:312–24.

Seipel K, Schmid V. Evolution of striated muscle: jellyfish and the origin of triploblasty. Dev Biol 2005;282:14–26.

Schmid V. Transdifferentiation in medusae. Int Rev Cytol 1992;142:213–61.

Schmid V, Bally A, Beck K, Haller M, Schlage WK, Weber C. The extracellular matrix (mesoglea) of hydrozoan jellyfish and its ability to support cell adhesion and spreading. Hydrobiologia 1991;216/217: 3–10.

Schmid V, Baader C, Bucciarelli A, Reber-Muller S. Mechanochemical interactions between striated muscle cells of jellyfish and grafted extracellular matrix can induce and inhibit DNA replication and transdifferentiation in vitro. Dev Biol 1993;155:483–96.

Shimizu H, Zhang X, Zhang J, Leontovich A, Fei K, Yan L, et al. Epithelial morphogenesis in hydra requires de novo expression of extracellular matrix components and matrix metalloproteinases. Development 2002;129: 1521–32.

Singer I. An electron microscopic and autoradiographic study of mesogleal organization and collagen synthesis in the sea anemone *Aiptasia diaphana*. Cell Tissue Res 1974;149:537–54.

Tardent P. Coelenterata, Cnidaria. In: Seidel T, editor. Morphogenesis der Tiere. New York: Gustav Fischer Verlag; 1978. p. 71–391.

Tillet E, Franc JM, Franc S, Garrone R. The evolution of fibrillar collagens: a sea-pen collagen shares common features with vertebrate type V collagen. Comp Biochem Physiol B Comp Biochem Mol Biol 1996;113: 239–46.

Towbin H, Staechelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350–4.

Weber C, Schmid V. The fibrous system in the extracellular matrix of hydromedusae. Tissue Cell 1985;17:811–22.

Young JA. The nature of tissue regeneration after wounding in the sea anemone *Calliactis parasitica* (Couch). Mar Biol Assoc UK 1974;54:559–617.

Zawarzin AA. Essays on the evolution histology of blood and connective tissue. Moscow; 1945. 230 p. [in Russian].

Zhang X, Fei K, Agbas A, Yan L, Zhang J, O'Reilly B, et al. Structure and function of an early divergent form of laminin in hydra: a structurally conserved ECM component that is essential for epithelial morphogenesis. Dev Genes Evol 2002;212:159–72.