Bol. Inst. Esp. Oceanogr. 18 (1-4). 2002: 393-400

BOLETÍN. INSTITUTO ESPAÑOL DE OCEANOGRAFÍA
ISSN: 0074-0195

Identification of a fibronectin-like molecule from a marine bivalve *Pecten maximus* (L., 1758) and its hyperaccumulation in the female compartment of the gonad

M.ª Paz[1], *M. F. Ruiz*[2], *L. Sánchez*[2] *and A. Mikhailov*[1]

[1] Institute of Health Sciences, University of A Coruña, Campus de Oza, Edificio El Fortín, As Xubias s/n, E-15006 A Coruña, Spain. E-mail: mariapaz@mail2.udc.es
[2] Centre for Biological Research, Velázquez, 147, E-28002 Madrid, Spain

Received July 2001. Accepted February 2002.

ABSTRACT

The present paper is the first report on the existence in marine scallops of a protein with properties similar to those of mammalian fibronectin (FN), henceforth called a scallop fibronectin-like polypeptide (sFN-LP). We describe the results of the biochemical and immunochemical identification of sFN-LP in mature gonad tissue of the simultaneous hermaphroditic species *Pecten maximus* (L., 1758). sFN-LP is similar to mammalian FN in the following respects: (1) it is a dimeric polypeptide with subunits of approx. 200 kDa; (2) it is recognised by antibodies against human FN on Western-blots; and (3) it is located in the basement membranes of follicles, the connective-tissue matrix, and the integument of the gonad. In the *P. maximus* female gonad compartment, sFN-LP is highly accumulated in the connective-tissue fibrilar network but is not detected in oocytes. In the male gonad tissue, only trace amounts of sFN-LP were detected.

Keywords: Fibronectin, scallops, gonad, sex differentiation.

RESUMEN

Identificación de una molécula similar a la fibronectina en el bivalvo marino* Pecten maximus *(L., 1758) y su hiperacumulación en el compartimento femenino de la gónada

Ésta es la primera demostración de la existencia en los pectínidos marinos de una proteína con propiedades similares a la fibronectina humana, a la que se ha denominado scallop fibronectin-like polypeptide *(sFN-LP). Se describen los resultados obtenidos de la identificación bioquímica e inmunoquímica de sFN-LP en el tejido gonadal maduro de la especie hermafrodita simultánea* Pecten maximus *(L., 1758). sFN-LP es similar a la FN humana en los siguientes aspectos: (1) es un polipéptido dimérico con subunidades y peso molecular de alrededor de 200 kDa; (2) en* Western-blot *es reconocido por anticuerpos contra la FN humana; (3) se localiza en la membrana basal de los folículos, en la matriz del tejido conectivo y en el tegumento de la gónada. En el compartimento gonadal femenino de* P. maximus*, sFN-LP se acumula en gran cantidad en la red fibrilar de tejido conectivo, pero no se detecta en los oocitos. En el tejido gonadal masculino se detectaron cantidades traza de sFN-LP.*

Palabras clave: *Fibronectina, pectínidos, gónada, diferenciación sexual.*

INTRODUCTION

Fibronectin (FN) is the most intensively studied extra-cellular protein, with available data regarding its structure and functions in vertebrates (Hynes, 1990; Schwarzbauer, 1991a,b). This large dimeric glycoprotein consists of two nearly identical polypeptide subunits (molecular weight 220-250 kDa) connected by disulfide chains. Heterogeneity among FN subunits isolated from different sources arises, at least in part, from alternative splicing of the primary FN transcript. The FN molecule contains several domains with different binding activities specific for collagen, fibrinogen, heparin, as well as a cell domain (figure 1). FN is present in soluble form in body fluids, and in insoluble form on cell surfaces, in extra-cellular matrices, and in basal membranes.

In vertebrates, FN seems to play an important role in a variety of morphogenetic processes, mediating cell adhesion, cell migration, and signal transduction. Studies on mammalian development have provided initial evidence of a requirement for FN in the early differentiation of the epithelial and interstitial compartments of the gonads (Paranko *et al.*, 1983). In the adult state, FN has been found in both female (Zhao and Luck, 1995; Colman-Lerner *et al.*, 1999) and male (Skinner *et al.*, 1989; Schaller, Glander and Dethloff, 1993) reproductive-tract tissues, as well as in ejaculated sperm cells (Fusi and Bronson, 1992; Pinke *et al.*, 1997), seminal plasma (Wennemuth *et al.*, 1997), and ovarian follicular fluid (Tsuiki, Preyer and Hung, 1988). Moreover, it has been demonstrated that FN is a useful marker for follicle development and oocyte maturation (Hung, Tsuiki and Yemini, 1989; Gentry, Zareie and Liptrap, 1996).

In invertebrates, FN-like polypeptides have been detected in insects (Gratecos *et al.*, 1988), sea urchins (Iwata and Nakano, 1983; DeSimone, Spiegel and Spiegel, 1985), bivalve molluscs (Suzuki and Funakoshi, 1992; Di Rosa *et al.*, 1994; Panara *et al.*, 1996), medusas (Schage, 1988), and sponges (Müller, 1997). Whereas it has been shown that these FN-like polypeptides play a role in promoting cell adherence and cell spreading, their expression patterns in the reproductive tissue and sex cells had yet to be studied.

As outlined in our previous publications, the development and functional maturation of the tubular gonad in bivalve molluscs, as well as the activation of spermatogenesis or oogenesis, can result in the induction of sex-cell-dependent protein expression in the corresponding reproductive-tract

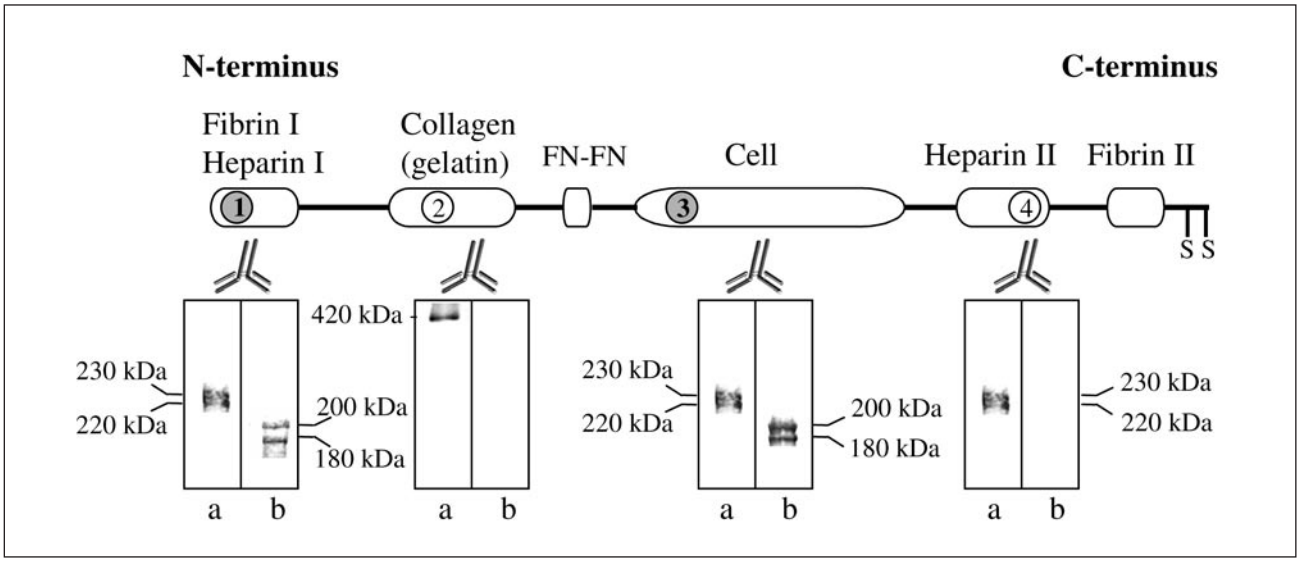

Figure 1. Immunochemical comparison of vertebrate FN and *P. maximus* sFN-PL. (Top): Schematic map of structural and functional organization of vertebrate FN domains for binding fibrin, collagen, fibronectin, cells, and heparin, as well as the N-terminus and C-terminus, are indicated. The pair of carboxyl terminal cysteins required for dimer formation is shown as SS; (cycles 1-4): FN epitopes recognised by the anti-human FN monoclones used; (dark cycles: 1 and 3): human FN epitopes similar to those of the *P. maximus* sFN-LP. (Bottom): Representative Western-blot analysis of the human FN sample; (a) an sFN-LP-containing fraction; (b) of the *P. maximus* female gonad sediment; (cycle 2): to detect the corresponding epitopes of human FN, SDS-PAGE was used under non-reducing conditions; (180 to 420 kDa): MW values of the polypeptide bands cross-reacting with the antibodies used

somatic tissues (for reviews, Mikhailov and Torrado, 1999, 2000). Particularly, the common gonad (i.e., ovotestis) of *Pecten maximus* (L., 1758) is characterized by a sperm-cell-dependent pattern of esterase-like expression restricted mainly to its male compartment (Torrado *et al.*, 2000; Paz, Torrado and Mikhailov, 2001). For the present study, we developed a complementary approach for further analysis of sex cell-dependent protein expression in the *P. maximus* gonad using the FN signal, which could be related to functional differentiation and maturation of the female gonad compartment. In addition, to the best of our knowledge, our study is the first report on FN-like polypeptides in marine scallops.

MATERIALS AND METHODS

Specimens and tissue separations

Sexually mature adult *P. maximus* scallops were purchased from commercial suppliers in A Coruña (Galicia, northwest Spain) in the spring and summer throughout 1999 and 2001. Using a scalpel, molluscs were opened and a small portion of gonad tissue was microscopically examined to detect sperm cells and oocytes. The sperm, oocytes and gonad duct fluids were obtained in accordance with procedures described previously (Torrado and Mikhailov, 1998, 2000). Then, female and male gonad portions and other tissues were microsurgically dissected out from the animals and immediately frozen at –80 ºC. Sperm cells and oocytes were washed several times in ice-cold sterile seawater and frozen at –80 ºC.

Extraction procedures

Weighted tissue/cell samples were subjected to the following extraction procedure: 1) extraction by two volumes of 100 mM Tris-EDTA buffer, pH 7.0, at 4 ºC for 1 h; 2) centrifugation at 30 000 g for 1 h at 4 ºC; 3) the supernatant was saved and the sediment was washed several times with a Tris-EDTA buffer and then re-extracted by the same buffer containing 1 % Triton X-100 (Merck) or 12M urea (Merck) for 30 min at 8 ºC; 4) centrifugation at 30 000 g for 1 h at 8 ºC; 5) the resulting supernatants and sediments were saved and processed as described below in the section on SDS-PAGE and Western-blot procedures. All extraction solutions contained a mixture of the following protease inhibitors (Sigma): 1 µg/ml pepstatin, 2 µg/ml aprotinin, 2 µg/ml leupeptin, and 100 µg/ml phenyl methylsulfonyl fluoride (PMSF). Protein concentration was measured (Ultrospec 1 000E spectrophotometer, Pharmacia) according to the Bradford method using bovine serum albumin (Sigma) as a standard.

Antibodies

For immunoblot and immunocytochemical analyses, the following primary antibodies were used: 1) rabbit polyclonal antibodies raised against human FN (Sigma); 2) mouse monoclonal antibody (MAB) directed against human FN (Sigma); 3) MAB raised against human FN gelatin binding domain (Chemicon); 4) MAB raised against human FN C terminus (Chemicon); and 5) MAB directed against human FN N-terminus (Chemicon).

SDS polyacrylamide gel electrophoresis (SDS-PAGE) and Western-blot procedures

For all separations, the Mini-Protean II Electrophoretic Cell (Bio-Rad) was used, and the Mini Trans-Blot Cell (Bio-Rad) was used for Western-blot transfers. To prepare samples, equal volumes of protein extracts and 2X Laemmli sample buffer containing reducing agents (mercaptoethanol or dithiothreitol, Merck) were mixed and kept 1 h at room temperature. For some experiments, extracts were treated with the same buffer, but without reducing agents. Protein samples were separated by SDS-PAGE on 8 % acrylamide gels and electro transferred to nitrocellulose (Optitran) or nylon (Nytran, Schleicher and Schuell) membranes (Torrado and Mikhailov, 2000). The apparent molecular weights (MW) of the bands were determined by comparing low and high MW calibration protein kits (Pharmacia). For immunodetection, the blots were assayed as described in Mikhailov and Simirsky (1991). In all cases, highly purified human FN (Sigma) was used as a test antigen. Peroxidase-labelled anti-rabbit or anti-mouse immunoglobulins (Sigma) were used as the second-stage reagent, and diaminobenzidine (Sigma) was used to develop the blots. To deter-

mine sFN-LP levels in the female and male gonad portion of the *P. maximus* ovotestis, the variant (Browning and Nixon, 1998) of a quantitative Western-blot analysis was used. Band intensities were quantified by densitometry using a model GS-700 densitometer (Bio Rad) and Molecular Analysis software (Bio-Rad). For total carbohydrate identification, blots were treated with an Immun-Blot kit (Bio-Rad) for glycoprotein detection as described (Protocol 1A) by the manufacturer; chicken egg ovalbumin (Sigma) and rabbit liver carboxylesterase (Sigma) were used as a positive controls.

Immunofluorescence

Gonad-tissue fragments were fixed in Bouin's or 4 % formaldehyde (Merck) solutions, both prepared using filtered seawater. Standard histological techniques were used for sample dehydration, embedding in paraffin, sectioning at 5 mm, deparaffinization and rehydration. The slides were precoated with 3-aminopropyltriethoxysilane (Sigma). Prior to immunostaining, sections were treated with 0.4 % pepsin (Sigma) in 0.01 M HCl for 5 min at 37 ºC. The reaction was stopped by rinsing the sections in ice-cold 0.05 M Tris-HCl buffer (pH 8.0) containing 2 mg/ml pepstatin and 200 mg/ml PMSF. Then, sections were blocked with 20 % normal horse serum (Sigma) and assayed as described in Mikhailov and Simirsky (1991). FITC-labelled anti-rabbit or anti-mouse immunoglobulins (Sigma) were used as a secondary reagent. The sections were mounted in 100 mM Tris-HCl (pH 8.5):glycerol (1:9) containing 0.1 % p-phenilenediamine (Sigma) and examined under the Nikon Microphot microscope. In all cases, no specific immunofluorescent staining was observed when primary antibodies were omitted or replaced by non-immune gamma globulins.

RESULTS AND DISCUSSION

Immunochemical evidence of a FN-like protein in *P. maximus*

By using monoclonal antibodies against highly purified human FN and its different structural and functional domains, we demonstrated the presence of a similar protein in the *P. maximus* female gonad (figure 1), which we named scallop FN-like polypeptide (sFN-LP). It seems to be composed of disulfide-bonded monomers: when reduced with mercaptoethanol or dithiothreitol, it appeared as a doublet on Western-blot transfers from PAAG with a MW of 180 and 200 kDa. The same sFN-LP doublet was detected not only in the *P. maximus* gonad tissue but also in the gills, foot, auricle, and mantle (not shown). It is noteworthy that *P. maximus* sFN-LP is highly sensitive to proteolytic degradation, which could be visualised by the appearance of bands with lower MW values on Western-blot transfers treated with anti-FN antibodies. Even immediate treatment of the tissue in the Laemmli sample buffer, containing 4 % SDS and a mixture of protease inhibitors, did not completely prevent such degradation.

A sample of human FN was analysed in parallel with each of the monoclonal antibodies used, which were found to display a doublet on Western-blots that was characterised by slightly higher MW values (220- and 230-kDa bands) than those of sFN-LP (180- and 200 kDa bands). A fraction of this discrepancy may be due to the differential covalent addition of carbohydrates. It is generally accepted that glycosylation can increase the apparent MW of proteins. Human FN is a glycoprotein. In contrast, the *P. maximus* sFN-LP bands (on Western-blot transfers of the female gonad tissue) did not display any positive staining after treating the corresponding blots with the Immun-Blot kit for glycoprotein detection (not shown).

A comparison of epitope mapping results (figure 1) indicated that *P. maximus* sFN-LP should be distinguished from human FN. Only the epitopes located within the fibrin I domain and the cell domain (fourth type-III module) of human FN were detected in sFN LP. The monoclones raised against the human FN collagen/gelatin- and the human FN heparin II domain did not cross-react with the *P. maximus* gonad protein.

In the marine sponge *Geodia cydonium* (Jameson, 1811 as *Alcyonum*), the module type III characteristic of vertebrate FN was identified in a large putative multi-adhesive protein, additionally containing a cyctein-rich scavenger receptor and a complementary control protein domain (Pahler *et al.*, 1998). It seems, therefore, that scallop FN-LP contains at least two domains sharing properties similar to those of its human FN homologue. This may

also mean that other structural blocks of sFN-LP are similar to those of the *G. cydonium* putative multi adhesive protein. It is interesting to note that a similar, but not identical to human FN, protein designated sea-urchin fibronectin has been partially purified from the ovary of sea urchins (Iwata and Nakano, 1983).

Levels of FN-LP expression in the *P. maximus* ovotestis

For the reasons cited above, FN was chosen as a representative indicator of female-dependent gonad-tissue differentiation. In order to investigate this possibility, a comparative study of sFN-LP levels in the female and male gonad compartment of the *P. maximus* ovotestis was carried out with the aid of SDS-PAGE followed by Western-blot with anti-human FN antibodies.

After centrifugation at 30 000 g, the female gonad-tissue homogenate was separated into three clearly distinct layers, namely: 1) the soluble transparent supernatant, 2) the insoluble orange-colored gel-like fraction, and 3) the insoluble membrane-containing sediment. Note that the orange-coloured gel-like fraction was a characteristic of the female gonad-tissue extract only, and was never observed in that of the male.

By analogy with vertebrate FN, which is detected in relatively large amounts in a soluble form in body fluids, we first searched for FN cross-reacting antigens in water-saline extracts of the *P. maximus* female gonad. Surprisingly, no cross-reactivity was found in saline-soluble gonad supernatants, nor in gonad duct fluids. In contrast, practically all FN cross-reactivity was detected in the urea-soluble fraction of the gonad-tissue sediment. In the orange-coloured fraction, weaker sFN-LP signals were observed. Note that extracts prepared from mature oocytes did not reveal any positive immuno-blot reactions with the anti-FN antibodies used (figure 2).

After extraction of the female gonad-tissue sediment with Triton X-100, no cross-reactivity was observed in the Triton-soluble fraction (containing cytoskeleton proteins), whereas the Triton-insoluble material (dissolved in the urea-containing buffer) displayed the typical sFN-LP immuno-signals (data not shown). Altogether, the results obtained indicate that in the *P. maximus* female gonad the majority of sFN-LP molecules are associated with insoluble membrane matrix components of the somatic tissue.

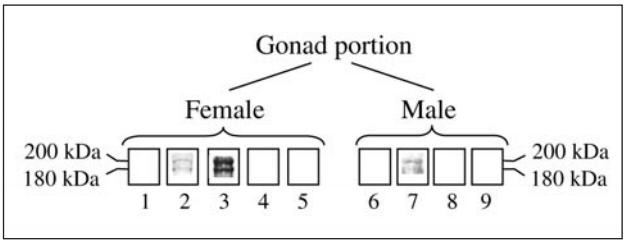

Figure 2. Representative Western-blot study of sFN-LP levels in the *P. maximus* ovotestis. In all cases, the mono-clone IST-3 (Sigma) raised against an epitope located within the fourth type-three repeat of human plasma FN, was used as the primary reagent. (lane 1): soluble fraction of the female gonad homogenate; (lane 2): orange-coloured, gel-like fraction of the female gonad; (lane 3): urea-soluble fraction of the female gonad sediment; (lane 4): duct luminal fluid of the female gonad; (lane 5): SDS-treated oocyte homogenate; (lane 6): soluble fraction of the male gonad homogenate; (lane 7): urea-soluble fraction of the male gonad sediment; (lane 8): duct luminal fluid of the male gonad; (lane 9): SDS-treated sperm cell homogenate; (180 and 200 kDa): MW values of sFN-LP

In the male gonad compartment, weak immuno-blot reactions were observed only in SDS- and urea-soluble fractions of the gonad-tissue sediment. No positive immuno-staining was detected on blots of either sperm cells or gonad duct fluids. These findings are consistent with reports of very low FN presentation on the sperm plasma membrane in humans (Fusi and Bronson, 1992; Pinke *et al.*, 1997).

Using semi-quantitative Western-blot techniques, we found that sFN-LP concentration in the *P. maximus* female gonad tissue is approximately 4 times higher than in male tissue. These results strongly suggest that regulation of sFN-LP expression in scallop ovotestis is sex-cell-dependent.

Immunofluorescence localization of sFN-LP in the *P. maximus* ovotestis

Localization of sFN-LP was simultaneously studied in cross-sections of whole ovotestis containing both female and male gonad tissues using immunofluorescent approaches with anti-human FN antibodies (figure 3). In the female gonad, sFN-LP was detected in the membrane of all oocyte-containing follicles and in the surrounding connective tissue, with patches of very strong immunofluorescence bordered by 3-5 follicles (figure 3d). A marked accumulation of sFN-LP was also observed

in the gonad somatic tissue adjacent to the digestive-tract wall, as well as in the integument. Low but detectable levels of sFN-LP staining were seen around gonad ducts. No specific fluorescence was found in oocytes.

In the male gonad compartment, immunostaining demonstrated a similar pattern for sFN-LP localization, but the intensity of the signals was weaker than in the female. Only the male gonad integument displayed a level of sFN-LP fluorescence comparable to that of the female gonad. Note that the patches of a very strong immunofluorescence characteristic of the female gonad tissue were not found in the connective tissue of the male gonad portion (figure 3d). No detectable immunofluorescenscence was observed in sperm cells.

Overall, the results of our immunofluorescent analyses correlated well with Western-blot data on the predominant accumulation of sFN-LP in the female gonad portion of *P. maximus* ovotestis. These observations suggest the intriguing possibility that sFN-LP expression in the ovotestis may be locally regulated at sites of female follicle formation.

CONCLUSIONS

No report has hitherto been published on the presence of FN-like polypeptides in marine scallops, although such polypeptides seem to have been identified in other species of bivalve molluscs (Suzuki and Funakoshi, 1992; Di Rosa *et al.*, 1994). Here, we present evidence confirming the existence in *P. maximus* of sFN-LP –a protein that displays several properties similar to those of human FN. A better understanding of the relationship between sFN-LP and its vertebrate analogues will be obtained from the cloning of the *P. maximus* gene encoding sFN-LP, which is currently in progress.

The high concentrations of sFN detected in the *P. maximus* female gonad compartment could be linked to its secretion by follicle cells and its consequent accumulation in adjacent somatic tissue. We suggest that sFN may be involved in processes related to female gonad structural remodelling, which depend on follicular development and oocyte maturation. These conclusions are consistent with data on FN expression patterns in the mammalian ovary. It has been demonstrated that granulose cells can synthesise and secrete FN. Moreover, FN concentrations increase during follicle development and correlate positively with follicular size and oocyte maturity (Hung, Tsuiki and Yemini, 1989). While we have no experimental evidence for this scenario in the *P. maximus* gonad, it forms the basis of our working hypothesis, in which signals coming from differentiating oocytes could provoke hyperproduction of sFN-LP by follicle cells.

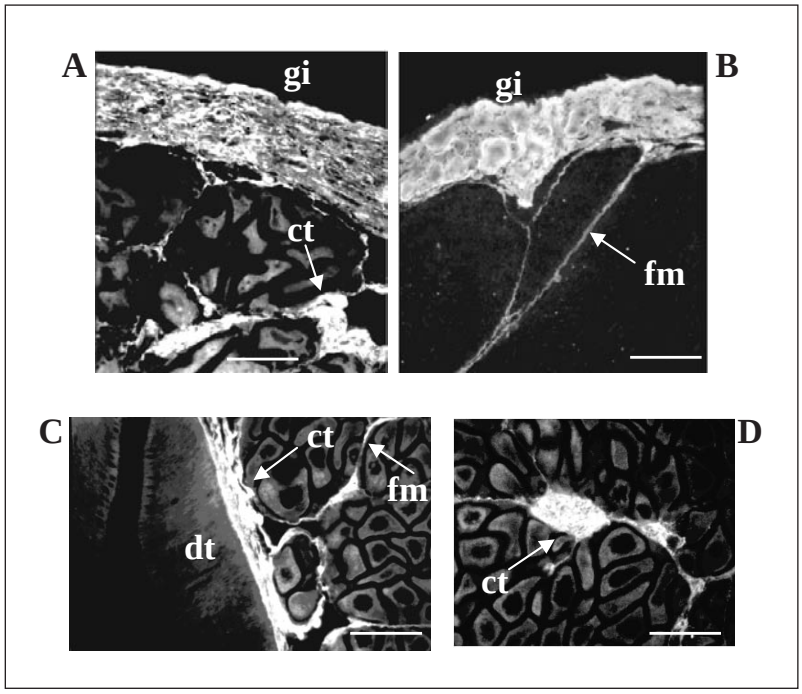

Figure 3. Distribution of sFN-LP immunosignals in cross-sections of the *P. maximus* ovotestis. Representative immunofluorescence micrographs of sections of female (A, C, D) and male (B) gonad portions stained with rabbit polyclonal anti-human FN antibodies and FITC-labelled anti-rabbit immunoglobulins. Scallop mature oocytes (but not sperm cells) were characterised by a yellow auto-fluorescence signal, whereas the gonad integument (gi), follicle membranes (fm), and connective-tissue (ct) structures displayed a bright green FN-specific immunofluorescence. (dt): digestive tract. Scale bar: 100 µm

ACKNOWLEDGEMENTS

This work was supported by the XUGA 16101A98 grant from the Galician Regional Government to A. M., and by a pre-doctoral fellowship from the Spanish Ministry of Education to M. P.

REFERENCES

Browning, M. D. and K. Nixon. 1998. The use of quantitative Western-blot analysis to determine expression of NMDA receptor subunits. *Neurotransmissions* 14: 18-21.

Colman-Lerner, A., M. L. Fishman, G. M. Lanuza, D. M. Bissell, A. R. Kornblihtt and J. L. Barañao. 1999. Evidence for a role of the alternativy spliced ED-I sequence of fibronectin during ovarian follicular development. *Endocrinology* 140: 2541-2548.

DeSimone, D. W., E. Spiegel and M. Spiegel. 1985. The biochemical identification of fibronectin in the sea urchin embryo. *Biochem. Byophys. Res. Commun.* 133: 183-188.

Di Rosa, I., S. Contenti, A. Fagotti, F. Simoncelli, G. B. Principato, F. Panara and R. Pascolini. 1994. Fibronectin from the hemolymph of the marine bivalve *Mytilus galloprovincialis*. Purification, immunological characterization and immunocytochemical localization. *Comp. Biochem. Physiol.* 107 (B): 625-632.

Fusi, F. M. and R. A. Bronson. 1992. Sperm surface fibronectin expression following capacitation. *J. Androl.* 13: 28-35.

Gentry, P. A., M. Zareie and R. M. Liptrap. 1996. Fibronectin concentrations correlate with ovarian follicular size and estradiol values in equine follicular fluid. *Animal Reproduction Science* 45: 91-102.

Gratecos, D., C. Naidet, M. Astier, J. P. Thiery and M. Sémériva. 1988. *Drosophila* fibronectin: a protein that shares properties similar to those of its mammalian homologue. *EMBO J.* 7: 215-223.

Hung, T. T., A. Tsuiki and M. Yemini. 1989. Fibronectin in reproduction. *Steroids* 54: 575-582.

Hynes, R. O. 1990. *Fibronectin*. Springer-Verlag. New York: 544 pp.

Iwata, M. and E. Nakano. 1983. Characterization of sea-urchin fibronectin. *Biochem. J.* 215: 205-208.

Mikhailov, A. T. and V. N. Simirsky. 1991. *Immunochemical methods in developmental biology. A manual*. Russ. Acad. Sci. Press. Moscow: 287 pp.

Mikhailov, A. T. and M. Torrado. 1999. Carboxylesterase overexpression in the male reproductive tract: a universal safeguarding mechanism? *Reprod. Fertil. Dev.* 11: 133-145.

Mikhailov, A. T. and M. Torrado. 2000. Carboxylesterases moonlight in the male reproductive tract: a functional shift pivotal for male fertility. *Frontiers in Bioscience* 5: 53-62.

Müller, W. E. C. 1997. Origin of metazoan adhesion molecules and adhesion receptors as deduced from cDNA analysis in the marine sponge *Geodia cydonium*: a review. *Cell Tissue Res.* 289: 383-395.

Pahler, S., B. Blumbach, I. Müller. and W. E. G. Müller. 1998. Putative multiadhesive protein from the marine sponge *Geodia cydonium*: cloning of the cDNA encoding a fibronectin-, an SRCR-, and a complement control protein module. *J. Exp. Zool.* 282: 332-343.

Panara, F., I. di Rosa, A. Fagotti, F. Simoncelli, C. Mangiabene, R. K. Pipe and R. Pascolini. 1996. Characterization and immunocytochemical localization of actin and fibronectin in haemocytes of the mussel *Mytilus galloprovincialis*. *Histochem. J.* 28: 123-131.

Paranko, J., L. J. Pelliniemi, A. Vaheri, J. M. Foidart and T. Lakkala-Paranko. 1983. Morphogenesis and fibronectin in sexual differentiation of rat embryonic gonads. *Differentiation* 23 (Suppl.): 72-81.

Paz, M., M. Torrado and A. T. Mikhailov. 2001. Germ cell contributions to the sexual polypeptide dimorphism of reproductive-tract tissues: lessons from hermaphroditic species of bivalve mollusks. In: *Convergencia entre Investigación y Empresa: Un reto para el Siglo XXI*. Proceedings of the Seventh National Congress on Aquaculture (May 19-21, 1999. Las Palmas de Gran Canaria, Las Palmas, Spain). H. Fernández-Palacios Barber and M. Izquierdo López (eds.): 525-531. Instituto Canario de Ciencias Marinas. Las Palmas de Gran Canaria (Las Palmas), Spain.

Pinke, L. A., D. J. Swanlund, H. C. Hensleigh, J. B. McCarthy, K. P. Roberts and J. L. Pryor. 1997. Analysis of fibronectin on human sperm. *Journal of Urology* 158: 936-941.

Schage, W. K. 1988. Isolation and characterization of a fibronectin from marine coelenterates. *Eur. J. Cell Biol.* 47: 395-405.

Schaller, J., H. J. Glander and J. Dethloff. 1993. Evidence of β1 integrins and fibronectin on spermatogenic cells in human testis. *Human Reproduction* 8: 1873-1878.

Schwarzbauer, J. E. 1991a. Alternative splicing of fibronectin: three variants, three functions. *BioEssays* 13: 527-533.

Schwarzbauer, J. E. 1991b. Fibronectin: from gene to protein. Curr. Opin. *Cell Biol.* 3: 786-791.

Skinner, M. K., B. Stallard, C. T. Anthony and M. D. Griswold. 1989. Cellular localization of fibronectin gene expression in the seminiferous tubule. *Mol. Cell. Endocrinol.* 66: 45-52.

Suzuki, T. and S. Funakoshi. 1992. Isolation of a fibronectin-like molecule from a marine bivalve, *Pinctada fucata*, and its secretion by amebocytes. *Zool. Sci.* 9: 541-550.

Torrado, M. and A. T. Mikhailov. 1998. Male-associated polypeptide (MAP) expression in different compartments of the reproductive system of the mussel *Mytilus galloprovincialis*: immunocytochemical and Western-blot study. *Cell Tissue Res.* 294: 537-547.

Torrado, M. and A. T. Mikhailov. 2000. Frog Lim-1-like protein is expressed predominantly in the nervous tissue, gonads, and early embryos of the bivalve mollusk *Mytilus galloprovincialis*. *Biol. Bull.* 199: 29-40.

Torrado, M., A. T. Mikhailov, M. Paz and L. I. Korochkin. 2000. Comparative analysis of male reproductive-tract carboxylesterase: Sex-dependent expression is shared by species with different patterns of reproduction and sexuality. En: *Recent Research Developments in Comparative*

Biochemistry and Physiology. S. G. Pandalai (ed.) vol. 1, part I: 39-50. Transworld Research Network. Trivadrum, India.

Tsuiki, A., J. Preyer and T. T. Hung. 1988. Fibronectin and glycosaminoglycans in human preovulatory follicular fluid and their correlation to follicular maturation. *Human Reproduction* 3: 425-429.

Wennemuth, G., P. J. Schiemann, W. Krause, A. M. Gressner and G. Aumüller. 1997. Influence of fibronectin on the motility of human spermatozoa. *Int. J. Androl.* 20: 10-16.

Zhao, Y. and M. R.Luck. 1995. Gene expression and protein distribution of collagen, fibronectin and laminin in bovine follicles and corpora lutea. *J. Reprod. Fertil.* 104: 115-123.