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The obturaculum of *Riftia pachyptila* (Annelida, Vestimentifera): Ultrastructure and function of the obturacular muscles and extracellular matrix

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Abstract: The Vestimentifera have in their branchial plume a centralized structure, the obturaculum, supporting the respiratory lamellae and enabling the worm to close its tube. We have studied its structure in *Riftia pachyptila*, focusing on the relations between its main parts: epidermis, muscles and extracellular matrix (ECM). The epidermis is supported by a thick collagenous layer, through which cell processes extend into the ECM. These processes contain rough endoplasmic reticulum (RER), and they often surround the subepidermal obturacular muscles. Each muscle is a bundle of 10-35 smooth muscle fibres, embedded in the ECM. The apical part, made of thin (and probably juvenile) muscle fibres, is present in each muscle. The thick myofilaments range from 20 to 150 nm in diameter, and show a cross-banded periodicity of 12-14 nm similar to paramyosin. Each thick myofilament is surrounded by 12 to 18 thin myofilaments about 5-7 nm in diameter. The nucleus is located laterally in the fibres, and generally surrounded by swollen cisterns of RER. The large obturacular matrix contains collagen fibrils, with a diameter of 30 nm and a striation every 64 nm. It also contains proteoglycans and cells, in groups of two or more, surrounded by a thin basal lamina, often lined with bundles of collagen fibrils. These cells often contain swollen cisterns of RER, suggesting they are equivalent to fibroblasts synthesizing the surrounding collagen fibrils. We discuss whether the obturaculum presents the features of a primitive cartilage, or those of a "catch" type connective tissue.

Résumé : *L'obturacle de* Riftia pachyptila *(Annelida, Vestimentifera) : Ultrastructure et fonction des muscles obturaculaires et de la matrice.* Les Vestimentifères ont dans leur panache branchial une structure centrale, l'obturacle, supportant les lamelles respiratoires et permettant au ver d'obturer son tube. Nous avons étudié sa structure chez *Riftia pachyptila*, en focalisant sur les relations entre ses principaux éléments : épiderme, muscles et matrice extracellulaire (ECM). L'épiderme repose sur une épaisse matrice collagénique, à travers laquelle il se prolonge dans l'ECM par des processus riches en réticulum endoplasmique rugueux (RER), qui entourent souvent les muscles obturaculaires sous-épidermiques. Chaque muscle est un faisceau de 10-35 fibres lisses incluses dans l'ECM. La partie apicale composée de fibres minces (probablement juvéniles) est présente dans chaque muscle. Les myofilaments épais mesurent de 20 à 150 nm de diamètre et montrent une striation transversale de période 12-14 nm, comme la paramyosine, Chaque myofilament épais est entouré de

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12 à 18 myofilaments fins d'environ 5 à 7 nm de diamètre. Le noyau est situé latéralement dans les fibres et généralement entouré de citernes renflées de RER. La grande matrice obturaculaire contient des fibrilles de collagène qui présentent un diamètre de 30 nm et une striation périodique de 64 nm. Elle contient aussi des protéoglycanes et des cellules groupées par deux ou plusieurs cellules, entourées d'une fine lame basale souvent bordée par des faisceaux de fibres de collagène. Ces cellules contiennent souvent des citernes renflées de RER, ce qui suggère qu'elles sont équivalentes à des fibroblastes synthétisant les fibrilles de collagène bordantes. Les caractéristiques ultrastructurales de la matrice obturaculaire, cartilage primitif ou tissu conjonctif de type "catch" sont discutées.

Keywords: Vestimentifera, obturaculum, smooth muscles, fibroblasts, collagen, extracellular matrix

Introduction

The obturaculum of the vestimentiferan tube-worms is, at the anterior end of the body, the central structure of the branchial plume that supports numerous respiratory lamellae. The terminal obturacular flaps, are slightly enlarged, devoid of branchial lamellae, and close the tube when the animal withdraws. The obturaculum is a characteristic organ of the Vestimentifera, and has not been observed in Pogonophora nor in Polychaeta. This organ is composed of two medially apposed symmetrical halves. Each half is composed of a large extracellular matrix (ECM) containing peripheral bundles of muscles, and covered by a simple epidermal layer that secretes an overlying cuticle. The general anatomy of *Riftia pachyptila* Jones 1981, especially the vascular and nervous systems, has been described in detail (Jones, 1981a, b, 1985, 1988, Gardiner & Jones, 1993), and the ultrastructure of the whole body integument (epidermis and cuticle) has been studied by Gardiner and Jones (1993). According to Jones (1985), longitudinal muscles internal to the epidermis, run throughout the whole length of each obturacular half forming about 250 peripheral rings, which led Jones to call them "ring muscles". The morphology of these muscles is well documented at the light microscopic level (Jones, 1981a, b, 1985, 1988 ; Gardiner & Jones, 1993), but nothing was known of their ultrastructure.

In the obturaculum of *Riftia pachyptila*, the main component of the obturacular ECM is collagen, which represents about 80% of the total volume of this organ (Hamraoui 1994, 1995). The interstitial and cuticular collagens have been thoroughly investigated at the molecular level (Gaill et al., 1991a, b 1994 ; Hamraoui 1994 ; Hamraoui et al., 1998). The ultrastructure and the immunological characterization of the constituents of the obturaculum in *Riftia pachyptila*, as well as the spatial interactions between epidermis, ECM and muscles needed to be examined and are the focus of the present work. Some comparisons with another hydrothermal tube-worm, *Tevnia jerichonana* Jones 1985 are also presented.

Materials and methods

1.Collection of animals

Riftia pachyptila and *Tevnia jerichonana* were collected during several cruises at 13°N in the East Pacific Rise (2600 m depth), with the submersibles *Alvin* (HERO IV, 1994) or *Nautile* (HOT,1996 and HOPE, 1999). Our study was realised with six individuals of *R. pachyptila* (from 6 to 18 cm total body length) and two small specimens of *T. jerichonana* having a branchial organ ranging from 7.5 to 15 mm in length.

2. Scanning electron microscopy (SEM)

Samples were fixed on board and stored in 10% neutral formalin in filtered sea water. Cross-sections of the obturaculum were later dehydrated with a graded series of ethanol, followed by the critical-point drying technique. They were sputter-coated with gold in an Edwards A306 evaporator, and examined with a JEOL JSM-840A SEM.

3.Transmission electron microscopy (TEM)

Prior to fixation, the osmolarity of each fixative was checked on board the ship with a Vapor Pressure Osmometer VAPRO and if necessary adjusted to about 1100 mosm with a few drops of 4M NaCl solution. Obturacular tissues were prefixed in paraformaldehyde (1% in filtered seawater) then cut in 1-2 mm sized pieces. The fixatives were either Glutaraldehyde 4% with 10% sucrose, in 0.1 M sodium cacodylate buffer pH 7.4 (GLUCAS), or a mixture of sucrose-picric acid-paraformaldehyde-glutaraldehyde (SPAFG) in 0.1 M phosphate buffer pH 7.3 (see Westheide & Purschke, 1988). Tissues were fixed at room temperature for, respectively, two (GLUCAS) and three hours (SPAFG). Due to the requirements of the ship scheduling, tissues were stored in respectively cacodylate or phosphate buffer for one month prior to postfixation. Tissues were postfixed for one hour with 1% osmium tetroxide in 0.1 M cacodylate buffer. After several rinses in 0.2 M cacodylate buffer, they were dehydrated in a graded ethanol series and embedded in Araldite or in Epon. Semi-thin sections (0.5-1 µm thick) were cut on a Reichert-Jung or on a Leica ultramicrotome, then stained with Azur II- and Methylene-blue (1v/1v) or with 1% Toluidine blue in 1% sodium borate. The light

microscopical observations and photomicrographs were made on a Nikon Optiphot or on a Leitz Laborlux D. Ultrathin sections (60 nm), obtained with a Leica ultramicrotome, were stained with 2.5% uranyl acetate in alcoholic solution followed by 0.2% lead citrate, and observed with a Philips 201 TEM or a Jeol SX 1200.

4. Antibodies and Immunofluorescence microscopy

Polyclonal antibodies raised specifically against collagens from *Riftia pachyptila*, against actin from *Loligo sp.*, against myosin from *Hediste (Nereis*) *sp.*, and against holothurian proteoglycans were kindly provided to one of us (L.H.) by Professor R. Timpl from the Department of Protein Chemistry Max Planck Institute (Planegg-Martinsried/ Germany). Small samples of tissues were dissected on freshly collected animals, rinsed in PBS, immediately embedded in OCT compounds (Tissue Tek, Miles Laboratoires, Inc., Naperville IL), and stored in liquid nitrogen. Immunofluorescence microscopy was performed on 6-8 µm frozen sections, as described previously (Hamraoui, 1994).

5. Extraction of obturacular collagen fibrils

Frozen samples (-20°C) of obturaculum from *Riftia* and *Tevnia* were thawed under running tap water, and kept at room temperature during all the following extraction steps. Small pieces (5 mm^3) of the obturacular matrix were dissected and ground a few seconds with a Polytron in 200 ml of extraction buffer (Tris-HCl pH=8.0 containing 0.5 M NaCl, 0.05 M Na-EDTA and 0.2 M 2-mercaptoethanol). The collagen extraction was proceeded in this buffer under mild stirring for 48 hours. The resulting solution was filtered on a nylon cloth, and the filtrate was centrifuged at 8000 g for one hour. The pellet was resuspended with the help of a teflon homogenizer in 20 ml of extraction buffer. The volume was raised to 200 ml and centrifuged at 10,000 g for one hour. Resuspension of the pellet followed by centrifugation was repeated three times. The final pellet was resuspended in 200 ml extraction buffer, and filtered on a nylon cloth. The final solution contained interstitial collagen fibrils and could be kept several weeks at +4°C. For observations under transmission electron microscopy one drop of the extracted collagen fibre solution was placed for 1 or 2 min on a carbonfilm-covered grid, then dried with a filter paper and contrasted with 2.5% uranyl acetate in alcoholic solution.

6. Immunogold labelling of extracted collagen fibrils.

Extracted collagen fibrils (200 µl) were centrifuged at 10,000 g for 2 min. The supernatant was discarded and the pellet was resuspended in phosphate buffered saline and left for 15 min. A renewed centrifugation was done at 10,000 g for 2 min, followed by 3 x 15 min rinses in PBS. The primary antibody incubation was performed on the pellet with 500 µl of the antibody against *Riftia* interstitial collagen, diluted 1:200 in PBS containing 0.2% BSA and 0.025% Tween 20. All incubations were left for one hour at room temperature. After rinsing four times in PBS containing 0.025% Tween 20, we added 500 µl of anti IgG conjugated with 10 nm gold particles diluted 1:20. The pellet was rinsed 4 x 15 min in PBS and resuspended in 200 µl PBS. One drop of suspension was loaded on a carbonfilm-covered grid, dried, contrasted with 2.5% uranyl acetate and observed by TEM.

Results

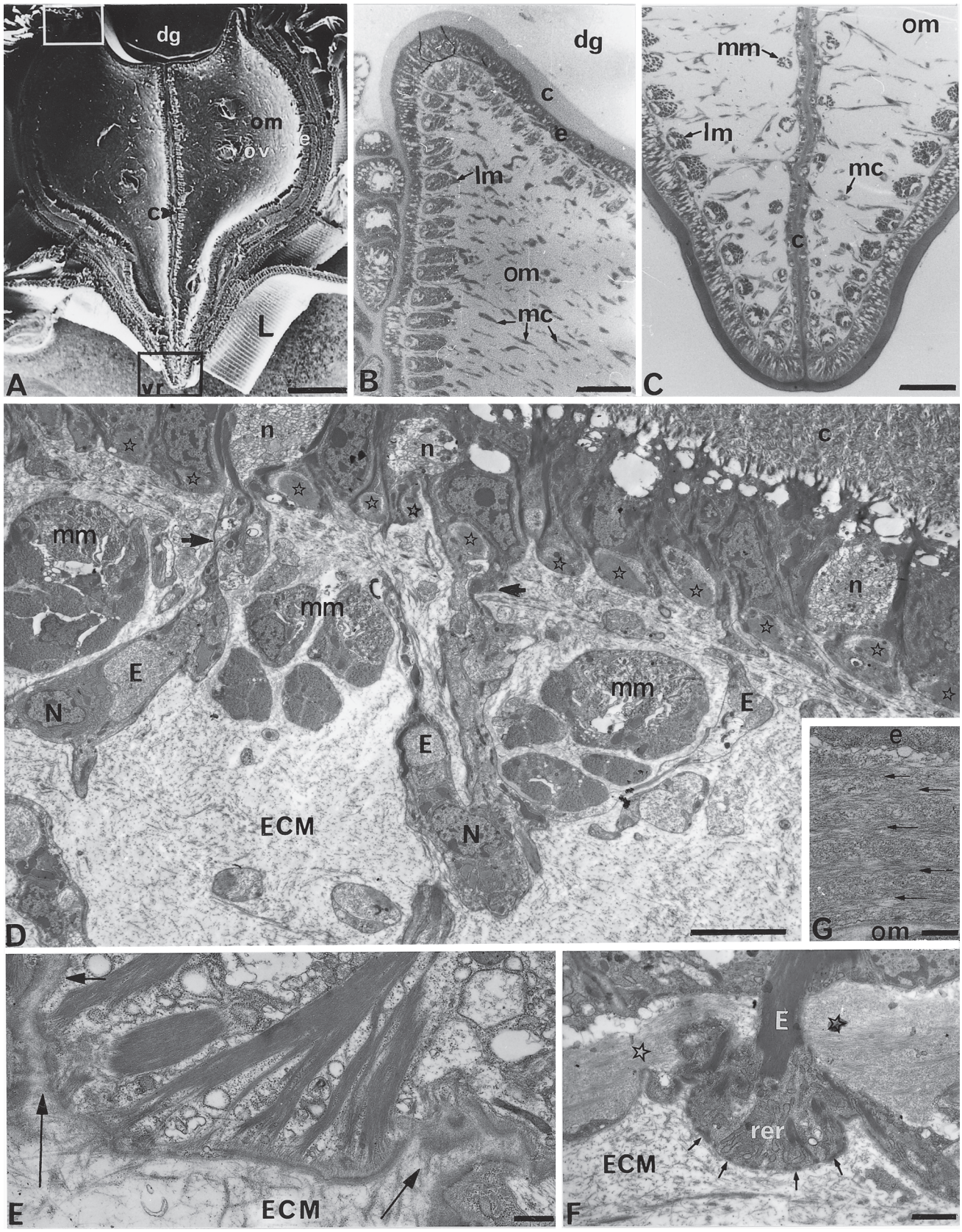
Morphology of the obturaculum of Riftia pachyptila *and* Tevnia jerichonana

The obturaculum of *R. pachyptila* is a long, robust and elongate organ comprising two symmetrical halves forming a characteristic heart-shaped cross-section (Fig.1A). The obturaculum of *T. jerichonana* is much shorter and Y-shaped in cross-section, due to a deeper dorsal groove. Each obturacular half (Fig. 1B, C) comprises an external cuticle covering the epidermis, a layer of peripheral longitudinal muscles and an extended central obturacular matrix. This matrix contains numerous mixed cell- and fibril-strands, oriented perpendicularly to the peripheral epidermis. These cell- and fibril-strands cross the obturacular matrix and join the base of the epidermis (Fig. 1C). A sinuous blind-ending obturacular blood vessel runs in the obturacular matrix (Fig. 1A). The obturaculum of *R. pachyptila* (Fig. 1B) and *T. jerichonana* (Fig. 1C) have a similar organization, except that the cell- and fibril-strands appear less abundant in the obturacular matrix of *Tevnia*.

Epidermis

The epidermis of *R. pachyptila* comprises four cell types: supporting cells, secretory, sensory and basal cells, already described by Gardiner and Jones (1993) by TEM. The obturacular epidermis (Fig. 1D) rests on a thin basal lamina (Figs 2A, B), and the tonofilaments of the supporting cells are attached to the basal plasma membrane by discrete hemi-desmosomes facing condensations of the collagen fibrils from the ECM (Fig. 1E).

A dense, fibrous and discontinuous structure supports the epidermis. This structure, called here cutis, comprises regular fibre bundles, about 1.25 µm in thickness (up to 4 µm on some sections) arranged in discontinuous segments of 1.9 - 2.8 µm in length (Fig. 1D). It comprises 7-11 alternating layers of collagen fibres oriented in various directions from one layer to the next (Fig. 1G). The spaces between the bundles vary from 1.9 to 3 µm, so that in several places epidermal cell processes extend through these spaces into the ECM, on a distance up to 25-30 µm (Figs 1D, F, 2A,C). In return the matrix sends finger-like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extensions between the basal parts of epidermal cells (Figs 1D, E, 2A).

Epidermal cell processes, containing tonofilaments and cytoplasm with swollen cisterns of rough endoplasmic reticulum (RER), cross the cutis and extend into the ECM to which they attach via hemidesmosomes (Fig. 1F). Processes occur generally from the supporting cells flanking the nerves in the epidermis (Figs 1D, 2A), and extend into the ECM between two successive muscles (Fig. 1D, 2A), often deeply enough to surround the basal part of the muscles (Fig. 1D). These cellular extensions sometimes comprise two apposed cell types: one, which contains microtubules and small dark vesicles, has the features of a nerve (Fig. 2B), the other, an epidermal secretory cell body, contains long mitochondria and a nucleus surrounded by swollen cisterns of RER in the enlarged terminal end (Fig. 1D, 2A). Some epidermal projections also cover the apical part of muscles located beneath the epidermis (Fig. 2C, D), or touch the lateral muscular cell bodies containing RER (Fig. 2E).

Figure 1. Cross sections of the obturaculum.

A. SEM view of the heart-shaped obturaculum of *Riftia pachyptila.* Laterally the organ supports branchial filaments fused into lamellae. The area in the upper framed box is detailed in 1B. The lower box indicates the corresponding position of 1C. (See abbreviations below).

B. Light micrograph of the obturaculum of *Riftia pachyptila* at the level of the area located in the upper framed box of 1A. Regularly spaced subepidermal longitudinal muscles are located laterally. Mixed strands of matrix cells and fibrils run through the obturacular matrix.

C. Light micrograph of the two obturacular halves of *Tevnia jerichonana,* in an area corresponding to the lower framed box of 1A, showing the two apposed cuticles and the median muscles, less developed than the lateral ones.

D-G: Ultrastructure of the obturaculum of *Riftia pachyptila*.

D. TEM view of the median part. The epidermis, covered by a cuticle is basally supported by a discontinuous cutis (☆) Beneath the cutis, the large obturacular extracellular matrix surrounds the median muscles. Epidermal cells, close to cross sectioned nerves, send long cytoplasmic processes into the ECM. The processes, often thin at the base of the epidermis (arrows), expand beyond the obturacular muscles, that they often surround.

E. Detail of the contact zone between epidermis and ECM. At the base of the epidermis, tonofilaments in the supporting cells join hemidesmosomes along the plasma membrane, facing collagen fibril condensations in the ECM. Arrows point to invaginations of ECM between epidermal cells.

F. A short epidermal extension, supported by tonofilaments, crosses the cutis (☆) and contains a rough endoplasmic reticulum. Small arrows indicate hemi-desmosomes.

G. Detail of the cutis beneath the epidermis. Note the alternate directions of the collagen fibres layers in a plywood pattern. Arrows indicate collagen fibres cut longitudinally.

(*c*) cuticle; (*dg*) dorsal groove; (*e*) epidermis; (*E*) extended epidermal process; (*ECM*) extracellular matrix; (*L*) branchial lamellae; (*lm*) lateral muscles; (*mc*) matrix cells; (*mm*) median muscles; (*n*) nerve; (*N*) nucleus; (*om*) obturacular matrix; (*ov*) obturacular vessel; (*rer*) rough endoplasmic reticulum; (*vr*) ventral ridge.

Figure 1. Coupes transversales de l'obturacle.

A. Vue en MEB de l'obturacle de *Riftia pachyptila.* Latéralement l'organe porte des filaments branchiaux fusionnés en lamelles. L'aire délimitée dans le cadre supérieur est détaillée en 1B. Le cadre inférieur indique un emplacement équivalent à celui de 1C.

B. Photographie en microscopie photonique de l'obturacle de *Riftia pachyptila* au niveau de l'aire localisée dans le cadre supérieur de 1A. Des muscles longitudinaux sous-épidermiques, régulièrement espacés, sont situés latéralement. Des cordons mixtes de cellules de la matrice et de fibrilles parcourent la matrice obturaculaire.

C. Photographie en microscopie photonique des deux moitiés obturaculaires de *Tevnia jerichonana,* dans une zone équivalente au cadre inférieur de 1A, montrant les deux cuticules apposées dans le plan sagittal et les muscles médians, moins développés que les latéraux.

D-G: Ultrastructure de l'obturacle de *Riftia pachyptila*.

D. Vue en MET de l'obturacle (région médiane). L'épiderme, recouvert de cuticule, repose basalement sur un cutis (☆) discontinu. Sous le cutis, la grande matrice extracellulaire obturaculaire entoure les muscles médians. Des cellules épidermiques, avoisinant les nerfs coupés transversalement, envoient de longs processus cytoplasmiques dans l'ECM, souvent fins juste sous l'épiderme (flèches), puis élargis sous les muscles obturaculaires qu'ils entourent fréquemment.

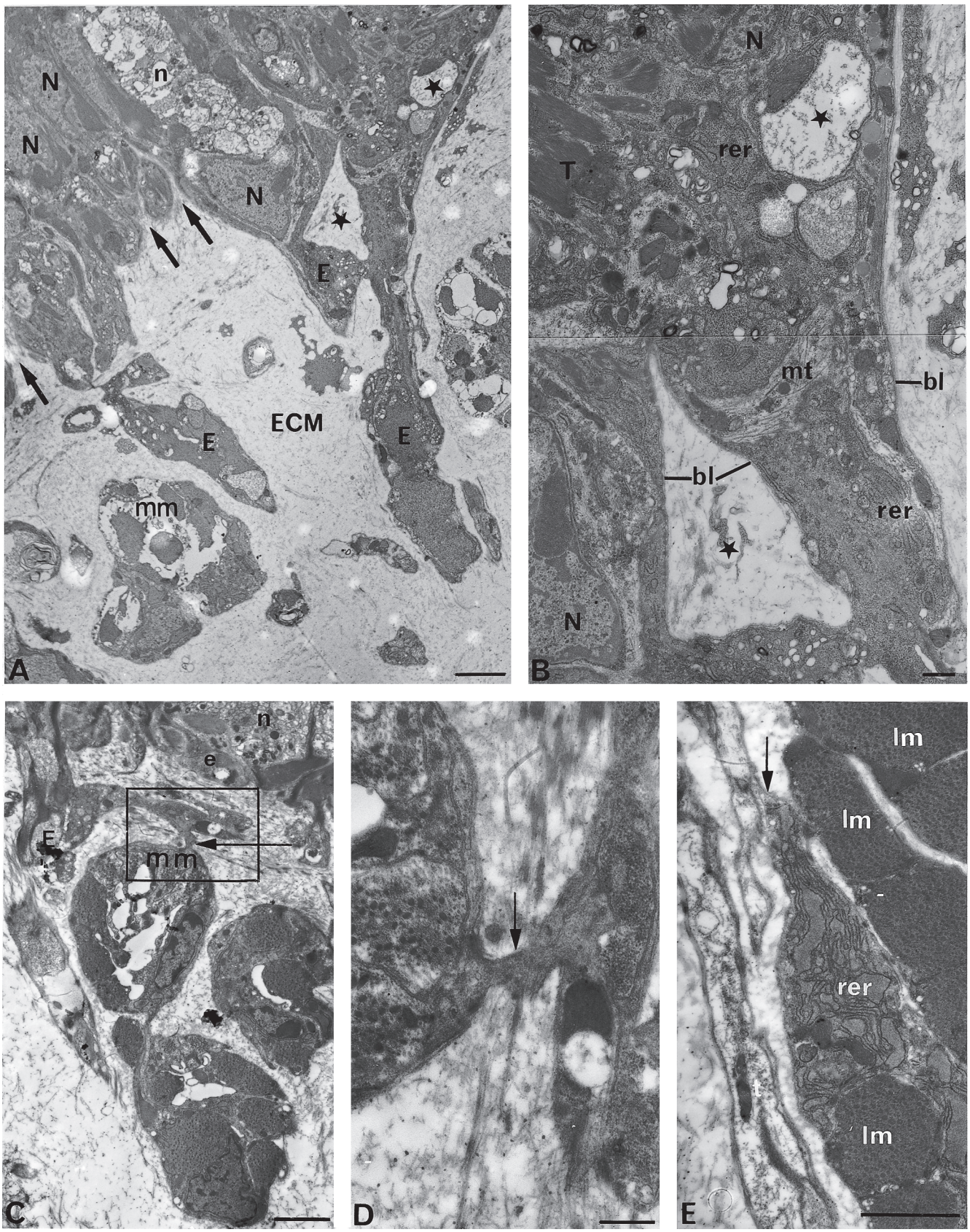
E. Détail du contact épiderme-ECM. A la base de l'épiderme, les tonofilaments des cellules de soutien rejoignent des hémidesmosomes, le long de la membrane plasmique, faisant face à des densifications de fibrilles de collagène dans l'ECM. Les flèches montrent les invaginations de l'ECM entre les cellules épidermiques.

F. Courte extension épidermique, soutenue par des tonofilaments, traversant le cutis (☆) et contenant du réticulum endoplasmique rugueux. De petites flèches indiquent les hémidesmosomes.

G. Détail du cutis sous l'épiderme. Noter les directions alternées, selon le mode contre-plaqué, des couches de fibres de collagène. Les flèches indiquent les fibres de collagène coupées longitudinalement.

(*c*) cuticule ; (*dg*) sillon dorsal ; (*E*) expansion épidermique ; (*e*) épiderme ; (*ECM*) matrice extracellulaire ; (*L*) lamelles branchiales; (*lm*) muscles latéraux ; (*mc*) cellules de la matrice ; (*mm*) muscles médians ; (*n*) nerf ; (*N*) noyau ; (*om*) matrice obturaculaire ; (*ov*) vaisseau obturaculaire ; (*rer*) réticulum endoplasmique rugueux ; (*vr*) crête ventrale.

Scale bars (Echelles) : A = 1 mm ; B, C = 50 µm ; D = 5 µm ; E = 0.5 µm ; F = 1 µm ; G = 0.5 µm.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Obturacular muscles

General arrangement

Light microscopic observations of cross-sections of the obturaculum show that the longitudinal "ring muscles" (Jones, 1985) are more numerous and less spaced in *Riftia pachyptila* than in *Tevnia jerichonana* (Figs 1B, C), but that they have in both species the same arrangement forming bundles around a small apical cavity. Each muscle is actually a loose bundle of 10 to 35 muscle fibres in *R. pachyptila* (Fig. 3A). We use the terms defined by Jones (1985): "lateral muscles" for the large peripheral bundles located along the lateral sides of the obturaculum, and "median muscles" for the smaller bundles located along the sagittal plane (Fig. 1C).

Ultrastructure

Observed in transverse sections, each lateral muscle (Fig. 3A) comprises 20-35 large clustered fibres embedded in the ECM, while the median muscles (Fig. 3B) have only about ten fibres. Tight collagen fibrils from the ECM penetrate between the muscle fibres forming an endomysium (Fig. 3A, B).

Muscle fibres (Figs 3A, C, E) round or oval in cross section, ranging from 2 to 6 µm in diameter contain thick and thin myofilaments randomly distributed up to the edge of the fibres (Figs 2E, 3C, E), without any partition into A- and I-bands. The thick myofilaments have diameters ranging from 40 to 150 nm. The ratio of thin to thick myofilaments could not be calculated because the high electron density of the sarcoplasm, prevents a clear counting of the thin myofilaments. The nucleus and one or two large mitochondria (1-2 µm in diameter) are present at the periphery of the cross-sectioned fibres (Fig. 3C).

On both the lateral and the median muscles there is, in each muscle, an apical area which appears different from the main part of the muscle, having fibres arranged around a cavity, already visible at the light microscopic level (Fig. 1B, C). This apical part, located beneath the epidermis (Figs 3C, D), comprises 6 to 12 thin fibres (about 0.5 µm wide), sending long and slender sarcoplasmic extensions (total fibre height about 4 - 6 µm) towards a central cavity which corresponds to an intercellular space between the fibres. A few mitochondria are located at the fibres' periphery, but they are mainly located in the slender sarcoplasmic extensions devoid of contractile elements. The nuclei of the apical fibres are located near the central cavity (Fig. 3D). Continuous with the nuclear membrane, swollen RER cisterns, containing an electron dense material, are distributed around the nucleus (Fig. 3E) and invade the myoplasm (Fig. 3G). Thick and thin myofilaments are grouped in the centre of each fibre, while the edges contain a rather electron-lucent sarcoplasm. The myofilaments do not show any ordered arrangement with A and I bands (Fig. 3E). The thick myofilaments, round or oval in shape, range from 20 to 100 nm, and the thin filaments are about 5-7 nm in diameter. There are often 14 (range 12-18) thin myofilaments arranged around the thick ones (Fig. 3F).

Figure 2. Cross sections of the obturaculum of *Riftia pachyptila* showing relations between epidermis, extracellular matrix and muscles.

A. Epidermal extensions into the ECM (✱) in between the median muscles. Arrows point to penetrations of ECM between basal parts of epidermal cells.

B. Detail of epidermal extensions into the ECM. Supporting cells contain tonofilaments, numerous free ribosomes and cisterns of rough endoplasmic reticulum. A thin nerve process contains microtubules.

C. The median epidermis sends a basal process (arrow) in close contact with the apical part of a median muscle.

D. Detail of the framed box in 2C, focusing on an epidermal cell process (arrow) covering the apical part of a muscle.

E. Epidermal cell processes laterally adjacent (arrow) to lateral muscle fibres. One fibre contains swollen cisterns of rough endoplasmic reticulum.

(*bl*) basal lamina; (*e*) epidermis; (*E*) extended epidermal process; (*ECM*) (✱) extracellular matrix; (*lm*) lateral muscles; (*mm*) median muscles; (*mt*) microtubules; (*n*) nerve; (*N*) nucleus; (*rer*) rough endoplasmic reticulum; (*T*) tonofilaments.

Figure 2. Coupes transversales de l'obturacle de *Riftia pachyptila* montrant les relations entre épiderme, matrice extracellulaire (ECM) et muscles.

A. Extensions épidermiques dans l'ECM (✱) entre les muscles médians. Les flèches montrent les pénétrations de l'ECM entre les cellules épidermiques.

B. Détail des extensions épidermiques dans l'ECM. Les cellules de soutien contiennent des tonofilaments, de nombreux ribosomes libres et des citernes de réticulum endoplasmique rugueux. Une portion de nerf contient des microtubules.

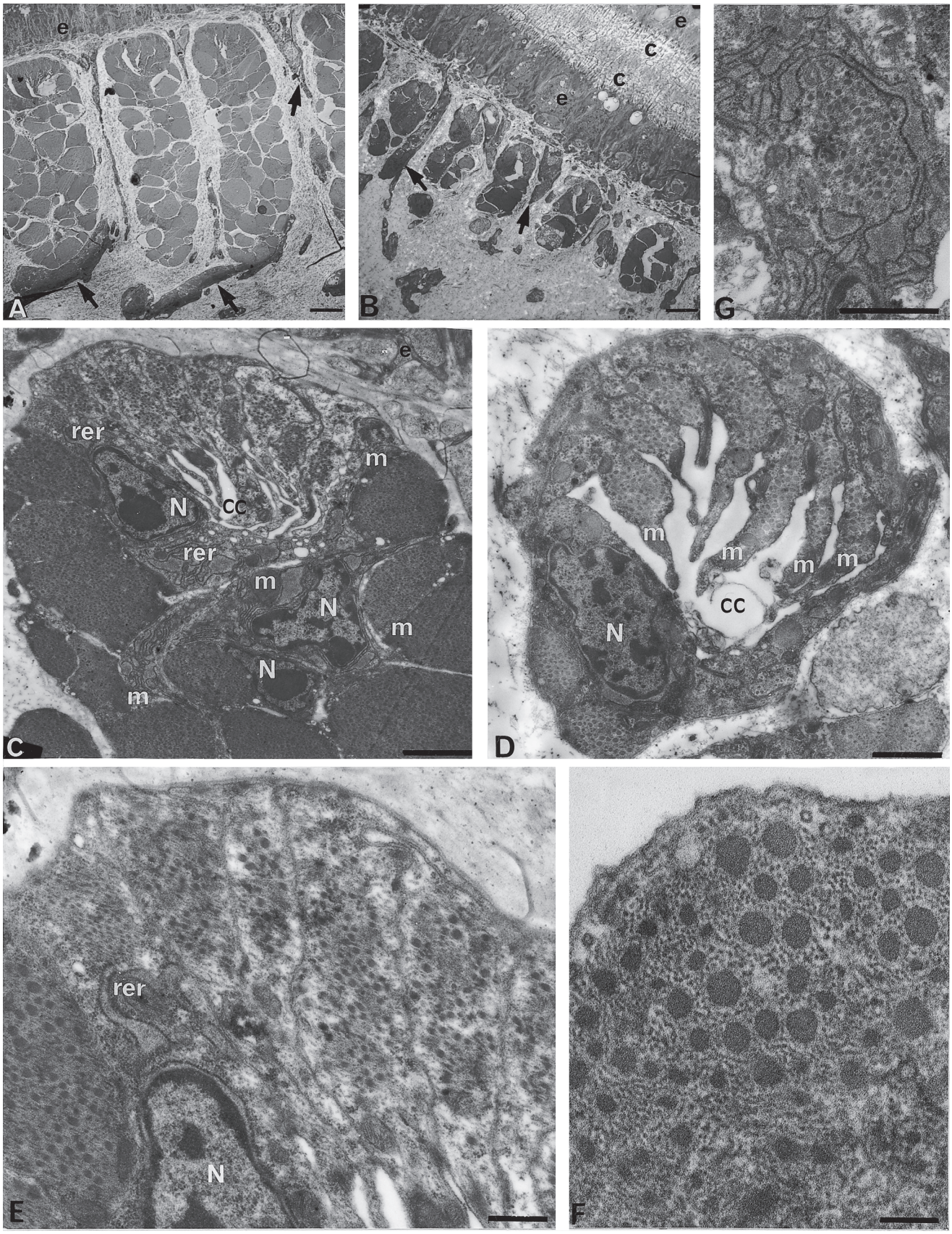
C. L'épiderme envoie un processus basal (flèche) en contact étroit avec la partie apicale d'un muscle obturaculaire.

D. Détail de l'encadré en 2C, focalisant sur le prolongement épidermique (flèche) couvrant la partie apicale du muscle.

E. Processus épidermique adjacent (flèche) à des fibres musculaires latérales. Une fibre musculaire contient des citernes renflées de réticulum endoplasmique rugueux.

(*bl*) lame basale ; (*e*) épiderme ; (*E*) expansion épidermique ; (*ECM*) (✱) matrice extracellulaire ; (*lm*) muscles latéraux ; (*mm*) muscles médians; (*mt*) microtubules ; (*n*) nerf ; (*N*) noyau ; (*rer*) réticulum endoplasmique rugueux ; (*T*) tonofilaments.

Scale bars (Echelles) : A= 2 µm ; B = 0.5 µm ; C = 2 µm ; D = 500 nm ; E = 2 µm.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Observed on longitudinal sections (Fig. 4), the thick myofilaments are generally oriented parallel to the long axis of the fibres (Fig. 4C, D), but sometimes also in more or less oblique directions or even with hairpin turns (Fig. 4A). Some lateral muscle fibres have their myofilaments ending in hemi-desmosomes facing denser collagen fibrils in the ECM (Fig. 4B). As in transverse sections, no clear A and I bands are distinguished. No Z-elements or dense bodies, to which the thin myofilament normally attach, could be seen, so that the sarcomeres are ill-defined. The thick myofilaments possess a transverse striation pattern with a periodicity of about 12-14 nm (Fig. 4E). In longitudinal section, the nucleus, with a large nucleolus, is elongate and measures about 6 µm in length by 0.7 µm in width (Fig. 4D). The nuclear membrane is often lined with ribosomes. Some clear vesicles, probably cisternae of the agranular sarcoplasmic reticulum, are visible along the sarcolemma which separates adjacent fibres (Fig. 4C).

Extracellular matrix of the obturaculum of Riftia pachyptila.

General arrangement

The main part of the obturaculum is occupied by an ECM containing cells and collagen fibrils. In the more central part of the obturaculum, on cross-sections, the matrix cells as well as the collagen fibrils may be cut transversally or longitudinally (Fig. 5B, C), which indicates that the cells and fibrils run in the matrix in various directions.

Ultrastructure of the matrix cells

The cells appear spindle-shaped, and their sections measure about 10 µm by 2 µm (Fig. 5A). They are generally grouped in twos (Fig. 5A, C), or even more (Fig. 5D) surrounded by a thin basal lamina. Each cell contains mitochondria (Fig. 5C, D) and the nucleus is often surrounded by a well developed RER, with swollen cisterns containing a moderately dense material (Fig. 5E). Typical interstitial collagen fibrils are often arranged in bundles parallel to the long axis of the matrix cells (Fig. 5B) and in close contact with their plasma membrane, suggesting that these cells produce collagen.

5. *Immunofluorescence labellings :*

The basal part of the epidermal cells shows a slight labelling with the antibody against *Nereis* myosin (Fig. 6A), while a thin subcuticular layer is clearly labeled apically with the antibody against *Loligo* actin (Fig. 6B). A bright staining of the muscles is observed with both antibodies (Figs 6A, B). The matrix cells contain a fine network labelled with actin antibody and a thicker one labelled with myosin antibody (results not shown), which indicates that their cytoskeletons contain actin- and myosin-like filaments.

Figure 3. Ultrastructure of the obturacular muscles of *Riftia pachyptila* in cross sections. **A, B.** Comparative views of the lateral **(A)** and the median **(B)** obturacular muscles observed at the same magnification. The muscles are embedded in the obturacular matrix and surrounded by epidermal extensions (arrows).

C. Apical part of a lateral muscle beneath the epidermis, showing two different types of muscle fibres. About ten small apical fibres with a moderate electron density, have narrow sarcoplasmic processes protruding in a central cavity. Below, larger fibres are more electron dense, owing to the presence of numerous myofilaments. Nuclei in several fibres are often surrounded by swollen cisterns of rough endoplasmic reticulum.

D. Apical part of a median muscle composed of about eight small fibres. The narrow sarcoplasmic projections protruding in a central cavity often contain mitochondria.

E. Detail of 3C. In both large and small fibres the myofilaments are randomly arranged.

F. High magnification of myofilaments showing variable diameters of the thick myosin filaments (20 to 70 nm) each surrounded by 12 to 18 thin actin filaments.

G. Detail of a muscle fibre showing the closeness between rough endoplasmic reticulum and myofilaments.

(*c*) cuticle; (*cc*) central cavity; (*e*) epidermis; (*m*) mitochondria; (*N*) nucleus; (*rer*) rough endoplasmic reticulum.

Figure 3. Ultrastructure des muscles obturaculaires de *Riftia pachyptila*, sur coupes transversales. **A, B.** Vues comparées des muscles latéraux **(A)** et médians **(B)** observés au même grandissement. Les muscles sont inclus dans la matrice obturaculaire et entourés par des extensions épidermiques (flèches).

C. Partie apicale d'un muscle latéral sous l'épiderme, montrant deux types différents de fibres musculaires. Environ dix petites fibres apicales de densité électronique modérée, ont d'étroits processus sarcoplasmiques en saillie dans une cavité centrale. Au-dessous, les grandes fibres circulaires ont une forte densité électronique en raison des nombreux myofilaments. Le noyau dans plusieurs fibres est entouré de citernes renflées de réticulum endoplasmique rugueux.

D. Partie apicale d'un muscle médian composé d'environ huit petites fibres. Les processus sarcoplasmiques étroits font saillie dans une cavité centrale et contiennent souvent des mitochondries.

E. Détail de 3C. Dans les deux types de fibres, les myofilaments ont une disposition désordonnée.

F. Fort grandissement des myofilaments montrant les diamètres variables des filaments de myosine épais (20 à 70 nm), chacun entouré par 12 à 18 fins filaments d'actine.

G. Détail d'une fibre musculaire montrant la proximité du réticulum endoplasmique rugueux et des myofilaments.

(*c*) cuticule ; (*cc*) cavité centrale ; (*e*) épiderme ; (*m*) mitochondrie ; (*N*) noyau ; (*rer*) réticulum endoplasmique rugueux.

Scale bars (Echelles) : A, B = 5 µm; C = 2 µm ; D = 1 µm E = 0.5 µm ; F = 0.1 µm ; G = 1 µm.

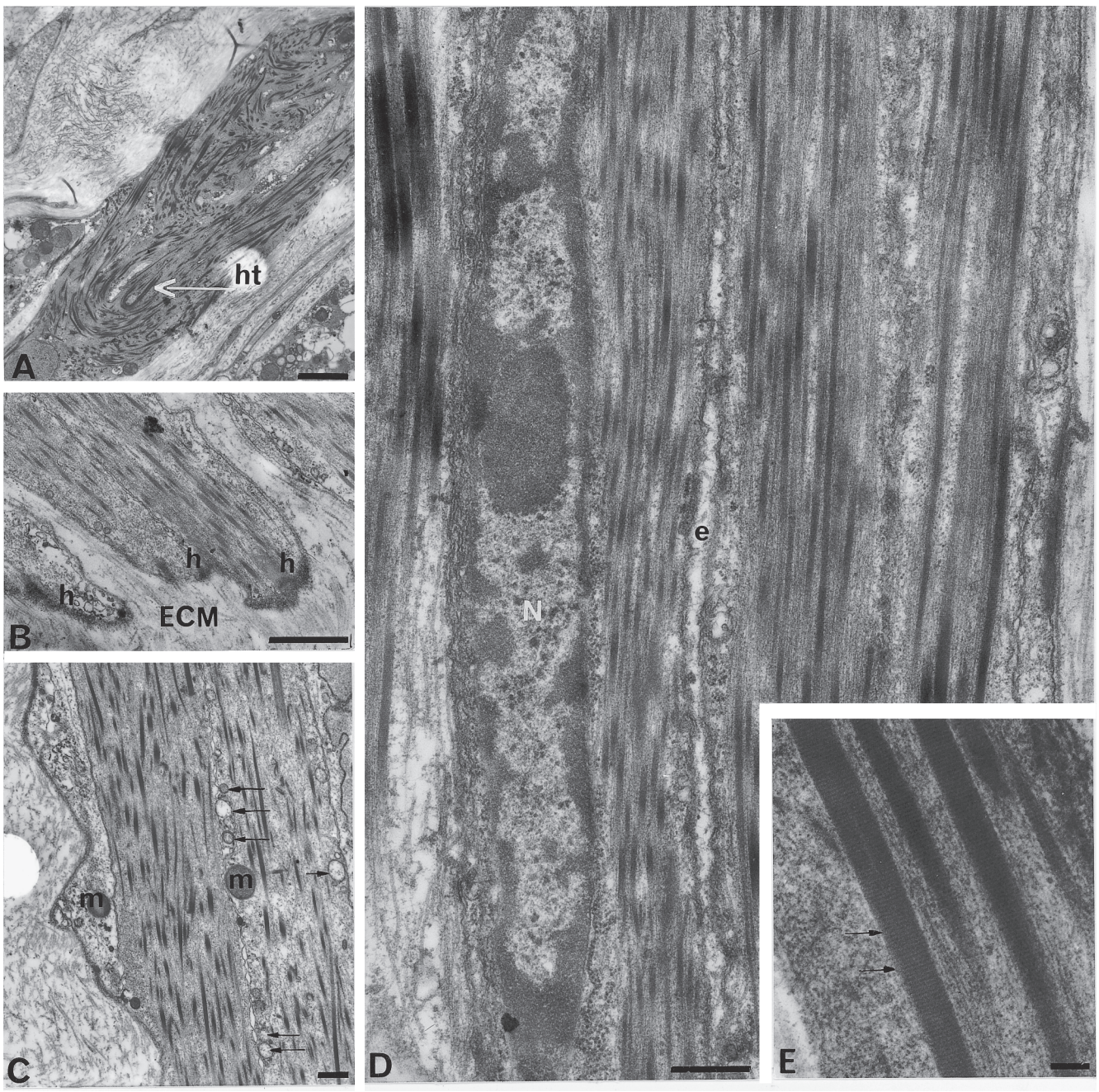


Figure 4. Ultrastructure of the obturacular muscles of *Riftia pachyptila*, in longitudinal sections.

A. Oblique section of the median muscle fibres, showing various orientations of the thick myofilaments, sometimes in hairpin turns.

B. Endings of lateral muscle fibres showing hemidesmosomes, facing condensations of collagen fibrils in the ECM.

C. Two fibres of median muscles bordered by clear sarcotubular vesicles (arrows), and mitochondria.

D. Higher magnification of fibres of a lateral muscle. Note the irregular arrangement of the thick myofilaments (no A- and I-bands), and the elongate nucleus. A thin collagenous connective sheet separates two fibres.

E. High magnification of thick myofilaments from a median muscle showing a cross banded striation pattern with a 12-14 nm periodicity (10 bands can be counted between the two arrows).

(*e*) endomysium; (*h*) hemidesmosomes; (*ht*) hairpin turn; (*m*) mitochondria; (*N*) nucleus.

Figure 4. Ultrastructure des muscles obturaculaires de *Riftia pachyptila*, sur des coupes longitudinales.

A. Section oblique des fibres d'un muscle médian, montrant les orientations diverses des myofilaments épais, parfois en épingle à cheveux.

B. Terminaisons de fibres d'un muscle latéral présentant des hémidesmosomes faisant face à des densifications de fibrilles de collagène dans la matrice extracellulaire.

C. Deux fibres d'un muscle médian bordées de vésicules sarcotubulaires claires (flèches).

D. Grandissement supérieur montrant les fibres d'un muscle latéral. Noter l'arrangement irrégulier des myofilaments épais (pas de bandes A, ni I) et le noyau allongé. Une fine lame conjonctive collagénique sépare les fibres.

E. Fort grandissement de myofilaments épais d'un muscle médian montrant une striation transversale de 12-14 nm de périodicité (on peut compter 10 bandes entre les deux flèches).

(*e*) endomysium ; (ECM) matrice extracellulaire ; (*h*) hémidesmosomes ; (*ht*) trajet en épingle à cheveux ; (*m*) mitochondries ; (*N*) noyau.

Scale bars (Echelles) : A = 2 µm; B = 1 µm ; C, D = 0.5 µm ; E = 0.1 µm.

The antibody against *Riftia* cuticular collagen exclusively stains the epidermal cuticle (Fig. 6C). A dense network of immunoreactive interstitial collagen fibrils underlines the epidermis and surrounds the obturacular muscles, while a loose network extends beneath the muscle layer in the obturacular ECM (Fig. 6D). These collagen fibrils form tracks between the muscular layer, underneath the epidermis, extending to the central obturacular matrix. In the centre of the obturaculum, immunoreactive interstitial collagen fibrils are seen in two perpendicular directions.

The antibody against holothurian proteoglycan labels the epicuticular and epidermal layers, as well as muscle bundles and stains clusters of aggregates in the obturacular matrix (Fig. 6E).

6. *Extracted collagen fibrils and immunogold labelling*

Extracted fibres from purified obturacular collagen appear as ribbon-shaped fibres of about 30 nm in diameter with a cross-banded periodicity of 64 nm in *Riftia* (Fig. 7A). They appear almost identical in *Tevnia* (Fig. 7B). They tend to aggregate in thicker bundles. The immunogold reaction with the antibody raised against the interstitial collagen of *Riftia* (Fig. 7C) details the labelling of the epitopes along two twisted fibres. Many gold particles are matching at the level of the dark bands of the supra-molecular structure, but no regular periodic pattern of the labelling is clearly visible, probably because the two fibres are more or less twisted around each other (Fig. 7C).

Discussion

Relations between epidermis, matrix and muscles in the obturaculum of Riftia.

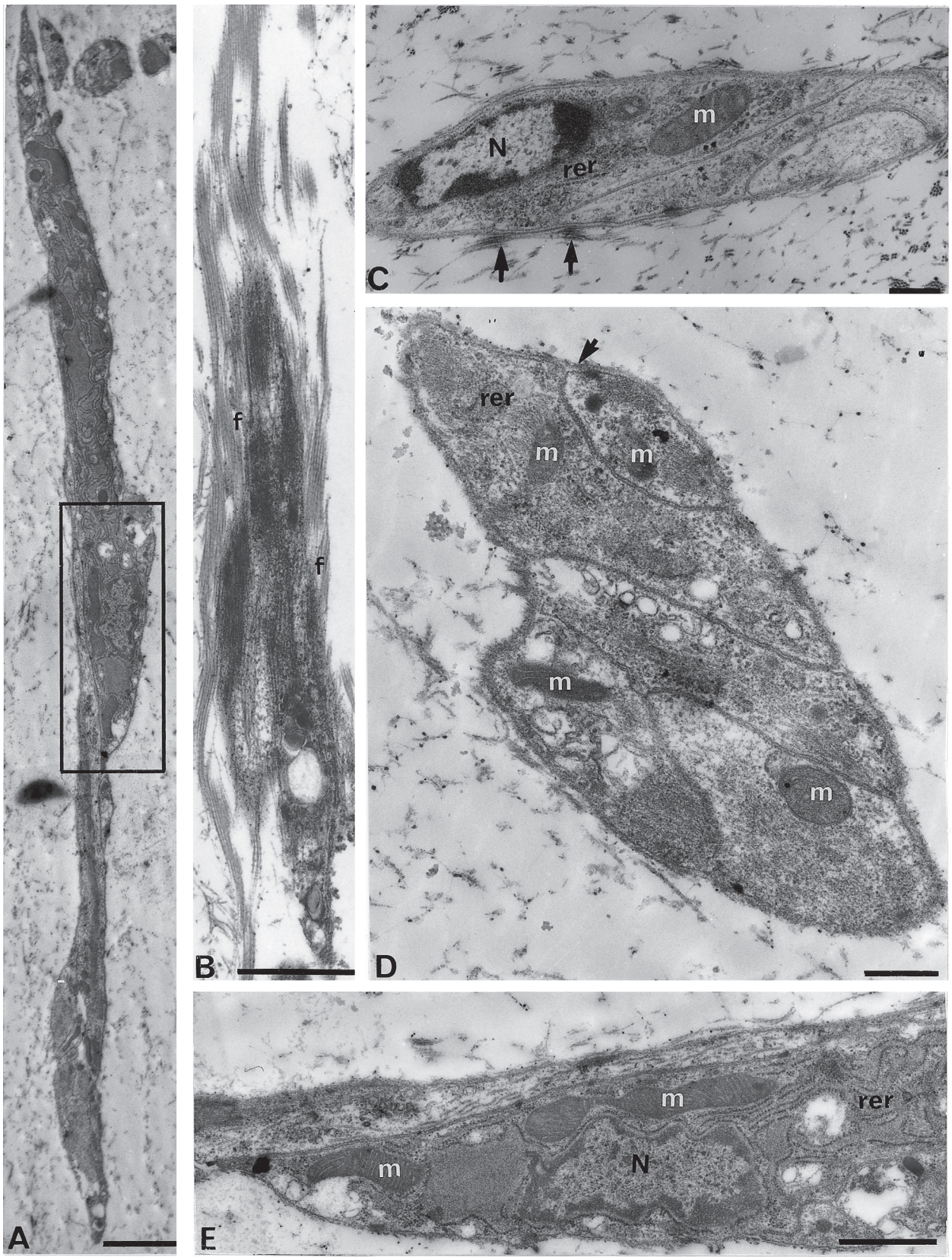
The epidermis rests on a thin basal lamina, also defined as "lamina densa" (Pedersen, 1991), that is lined with a robust and discontinuous fibrous structure, a "lamina fibroreticularis" according to Pedersen's terminology. This lamina fibroreticularis forms a framework through which epidermal cells extend into the ECM. Supportive epidermal cells and their epidermal extensions into the matrix show hemi-desmosomes along the basal lamina. The lamina fibroreticularis is composed of 7-11 layers of collagen fibres organized in a plywood substructure, and is very similar to the "cutis" described by Storch (1988) in the integument of various Polychaeta. In *Riftia,* the cutis has a thickness (up to 4 µm) similar to that of the ECM in the body wall of *Owenia fusiformis* (Fransen, 1980 *in* Gardiner 1992). Such a thickness, according to Gardiner (1992), might be related to the structural requirements of the tube-dwelling habits of *Owenia fusiformis*, thinner ECM being found in burrowing species. The cutis of *Riftia* is oriented perpendicularly to the longitudinal muscles and, may thus represent an antagonistic framework to the muscle system. We hypothesize that the cutis exerts a supportive function for the epidermal layer. The apparent discontinuities, might reflect a loose sinuous pattern allowing the epidermal and nerve processes to go through the cutis towards the muscles. Although we did not find any specialized junctions between muscles and epidermis, the cell to cell appositions suggest a functional coupling between epidermal projections and muscles.

The obturacular muscles

General arrangement

According to Jones (1985) the obturacular muscles constitute slender cylinders arranged in longitudinal rings running from the base of the obturaculum to its tip. However, the lateral fibres are more numerous than the median fibres, suggesting a loss of fibres somewhere in the ECM. We observed that some lateral muscle fibres stop in the matrix, their myofilaments ending in hemi-desmosomes facing denser collagen fibrils in the ECM.

Both lateral and median muscles are entirely embedded in the ECM with no distinct perimysium, while tight collagen fibrils running between the muscle fibres can be identified as endomysium. This disposition has also been described in leech body wall (De Eguileor et al., 1999). Lateral as well as median obturacular muscles show two different areas of fibres in *Riftia*: a main part with large fibres and an apical part with thin fibres grouped around a central cavity. In leeches there is also a similar arrangement with an external fibre different from the more numerous


A
B
f
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C
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rer
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fibres of the inner group (De Eguileor et al., 1998). The thin fibres in *Riftia*, which are small and always have abundant swollen cisterns of RER, lead us to suggest that they may represent an early stage in the development of muscle fibres. Another possibility is that they are a peculiar feature in muscles that maintain the tube closed for sustained periods; fibres with a slender tip are similarly observed in the opercular smooth muscles of *Pomatoceros lamarckii*, a serpulid polychaete (Bubel, 1983).

Obturacular muscles are smooth muscles

In the obturacular muscles of *Riftia* the myofilaments show no regular striation pattern, neither transverse nor oblique, and the thick and thin myofilaments are not disposed in separate A- and I-bands, as in striated muscles. Since a random arrangement of the myofilaments differentiates smooth muscles from striated muscles (Hanson and Lowy, 1961), we assert that the obturacular muscles of *Riftia* are smooth muscles.

Smooth muscles are rare in annelids; their bodywall muscles have generally been described as helical, i.e double-obliquely striated (for review on polychaete muscles see: Lanzavecchia et al., 1988 ; Gardiner 1992, and on invertebrate muscles Paniagua et al., 1996). Single-obliquely striated muscles have been reported once in the longitudinal fibres of *Magelona papillicornis* (Wissocq & Boilly, 1977). Fibres with an irregular myofilament organization are nevertheless present in annelids, in different regions of the body, and they are characterized as "highly polymorphous invertebrate smooth muscles" (Lanzavecchia et al., 1988).

Smooth muscles are more abundant in Molluscs, where they have been classified into various types according to the diameter of the thick filaments, the distribution pattern of the dense bodies, and the abundance of mitochondria and sarcoplasmic reticulum. Up to six different types of smooth muscles have been described in gastropods, but the various types do not correspond from one species to another (Plesh, 1977; Da Silva & Hodgson, 1987; Faccioni-Heuser et al., 1999), and do not correspond to the obturacular muscles in *Riftia* either.

An attempt to classify invertebrate and vertebrate smooth muscle cells has been done by Matsuno (1987), and more recently by Royuela et al., (2000). Matsuno established four different cell types, however, none of these types has the characteristics found in the obturacular muscles of *Riftia.* Additional criteria such as immunochemistry of muscle proteins, and calculations of myofilaments parameters are investigated by Royuela et al., (2000). In fact the diameters of the thick myofilaments of *Riftia* (20-100 nm and 40 to 150 nm) exceed both those recorded by Matsuno (1987) and by Royuela et al., (2000). The diameters are among the largest recorded, and are comparable to those found in the Oyster *Crassostrea virginica* opaque adductor (smooth) muscle (20-150 nm) (Lowy & Hanson, 1962 ; Morrison, 1996). It is interesting to mention that in relation to increasing thickness of thick myofilaments, the possible tension during muscular contraction increases, since the larger width of the thick myofilaments allows a greater number of cross-bridges with the thin myofilaments (Da Silva & Hodgson, 1987). The number of actin filaments (12-18) around the myosin filaments in *Riftia* is higher than that generally found in invertebrates (6-12) (Paniagua et al., 1996; Royuela et al., 2000). In addition the thick myofilaments of *Riftia* have a banded cross-striation with the same period (12-14 nm) as that found in the smooth muscles of Oyster (Hanson & Lowy, 1961) and *Pomatoceros lamarcki*, a Serpulid Polychaete (Bubel, 1983). This axial periodicity is referred to in the literature as

Figure 5. Ultrastructure of the matrix cells in the central part of the obturacular ECM of *Riftia pachyptila*.
A. Longitudinal section of two associated matrix cells showing their spindle-shaped form. The framed box is detailed in 5E.
B. Collagen fibrils from the ECM, parallel to the main cell-axis, surround a matrix cell.
C. Cross section of two matrix cells showing nucleus, rough endoplasmic reticulum, and mitochondrion. Surrounding collagen fibrils touch the basal lamina of the cells (arrows).
D. Cross section of five associated matrix cells surrounded by a thin basal lamina (arrow).
E. Detail of 5A showing the nucleus surrounded by swollen cisterns of rough endoplasmic reticulum and mitochondria.
(*f*) collagen fibrils; (*m*) mitochondria; (*N*) nucleus; (*rer*) rough endoplasmic reticulum.
Figure 5. Ultrastructure des cellules dans la partie centrale de la matrice extracellulaire obturaculaire de *Riftia pachyptila*.
A. Coupe longitudinale de deux cellules de la matrice, associées, montrant leur forme en fuseau. L'encadré est détaillé en 5E.
B. Les fibrilles de collagène de l'ECM extracellulaire, parallèles à l'axe cellulaire principal, recouvrent les cellules de la matrice.
C. Coupe transversale de deux cellules de l'ECM montrant noyau, réticulum endoplasmique rugueux et une mitochondrie. Des fibrilles de collagène de l'ECM s'accolent à la lame basale des cellules (flèches).
D. Coupe transversale de cinq cellules de la matrice, associées, entourées d'une fine lame basale (flèche).
E. Détail de 5A montrant le noyau entouré de citernes renflées de réticulum endoplasmique rugueux et de mitochondries.
(*f*) fibrilles de collagène ; (*m*) mitochondries; (*N*) noyau ; (*rer*) réticulum endoplasmique rugueux.
Scale bars (Echelles) : A = 2 µm ; B = 1 µm ; C, D = 0.5 µm ; E = 1 µm.

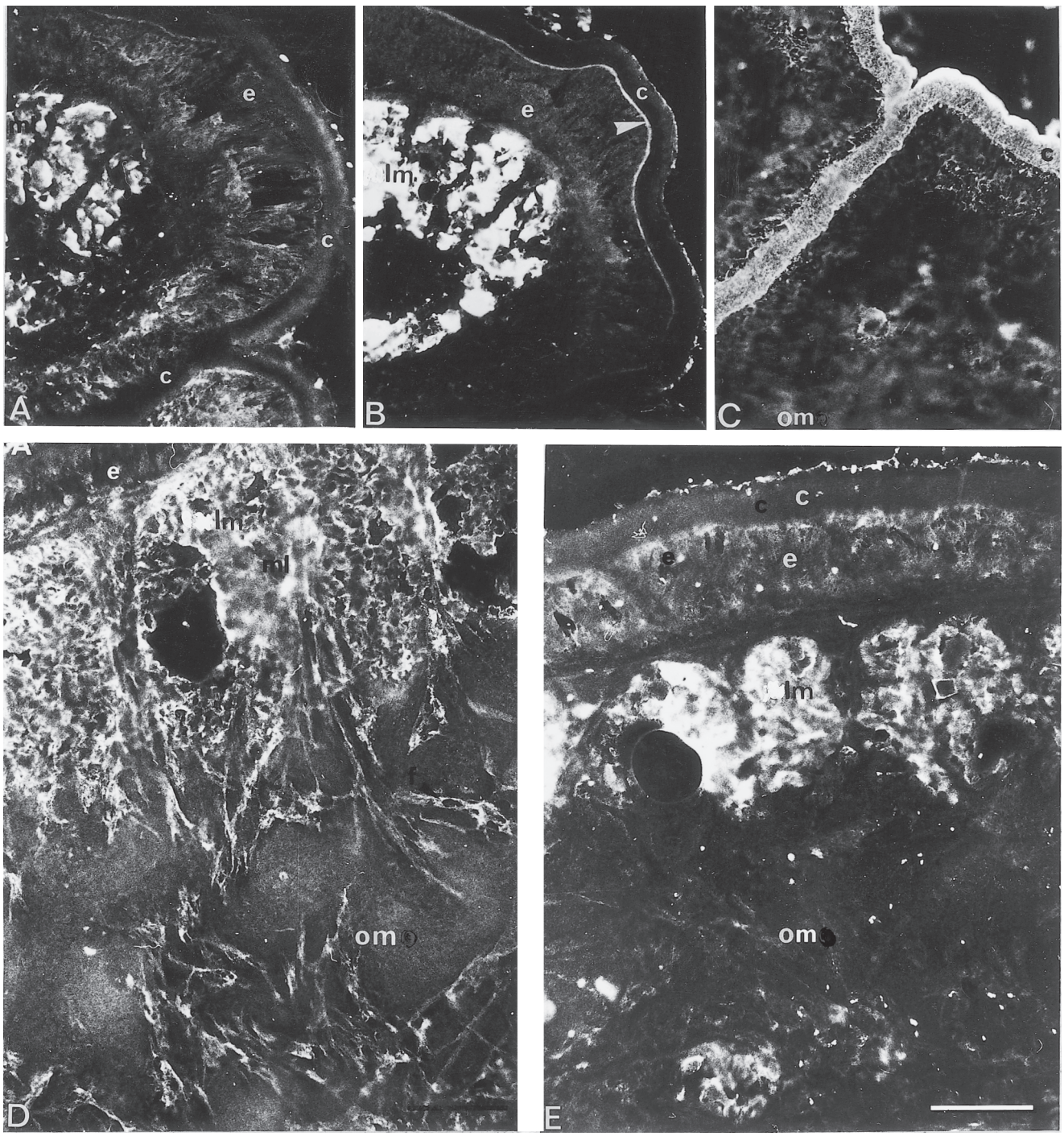

Figure 6. Immunofluorescent detection of various components in the obturaculum of *Riftia pachyptila.* A-E: same scale bar.

A. Immunoreaction with the antibody against *Nereis* myosin: the obturacular muscles are intensely labelled, while the epidermis has a faint positive reaction.

B. Immunoreaction with the antibody against *Loligo* actin: a very strong labelling of the obturacular muscles and, to a lesser extent, the very apex of the epidermis (arrowhead).

C. The immunoreaction with the antibody against *Riftia* cuticular collagen is restricted to the cuticle.

D. The immunoreaction with the antibody against *Riftia* interstitial collagen labels a dense network of fibrils underneath the epidermis, outlining the obturacular muscle fibres and crossing deeply into the obturacular matrix.

E. The immunoreaction with the antibody raised against holothurian proteoglycans labels the obturacular muscles nearly as strongly as myosin (in A). A faint staining is present at the periphery of epidermal cells and on the outside of the cuticle. Immunoreactive spots are numerous in the obturacular matrix.

(*c*) cuticle, (*e*) epidermis, (*lm*) lateral muscles, (*om*) obturacular matrix.

Figure 6. Détection par immunofluorescence de différents constituants de l'obturacle de*Riftia pachyptila.*

A. Réaction immunohistologique avec l'anticorps dirigé contre la myosine de *Nereis :* les muscles obturaculaires sont intensément marqués, tandis que l'épiderme présente une faible réaction positive.

B. Réaction immunohistologique avec l'anticorps dirigé contre l'actine de *Loligo* : marquage très intense des muscles obturaculaires et, dans une moindre mesure, de l'apex de l'épiderme (tête de flèche).

C. La réaction immunohistologique avec l'anticorps dirigé contre le collagène cuticulaire de *Riftia* est limitée à la cuticule.

D. Réaction immunohistologique avec l'anticorps dirigé contre le collagène interstitiel de *Riftia* : marquage d'un réseau dense de fibrilles sous l'épiderme entourant les fibres musculaires obturaculaires et traversant profondément la matrice obturaculaire.

E. La réaction immunohistologique avec l'anticorps dirigé contre les protéoglycanes d'holothurie marque les muscles obturaculaires presque aussi fortement que la myosine (en A). Un faible marquage est présent à la périphérie des cellules épidermiques et à l'extérieur de la cuticule. Des points immunoréactifs sont nombreux dans la matrice obturaculaire.

(*c*) cuticule ; (*e*) épiderme, (*lm*) muscles latéraux, (*om*) matrice obturaculaire.

Scale bar (Echelle) = 25 µm.

a core of paramyosin (or in some cases tropomyosin) surrounded by the myosin heads within the thick filaments (Lanzavecchia, 1977). However, Mill & Knapp (1970) have pointed out that it could also be the subunit repeat pattern of myosin filaments. It is noteworthy that this cross-striation is much more abundant in invertebrate smooth muscles than in striated muscles (Winkelmann, 1976).

The diameter of the thin filaments (5-7 nm) of *Riftia* is in accordance with that found in the literature for thin myofibrils in annelids (Paniagua et al., 1996, Royuela et al., 2000). We did not find, in *Riftia pachyptila*, clear Z-elements nor dense bodies, to which thin filaments attach. This absence has been previously reported in *Magelona* (Wissocq & Boilly, 1977) and *Pomatoceros* (Bubel, 1983), and attributed to the state of contraction of the muscles during fixation. This explanation for the absence of Z-elements may also be valid for *Riftia*. Thus, *Riftia* has no well defined sarcomeres, a characteristic also found in invertebrate smooth muscles (Royuela et al., 2000).

Overall, mitochondria are scanty in the obturacular muscle, an indication of low energy production as it is known in smooth muscles (Plesh, 1977). The sarcoplasmic reticulum (SR) is also scarcely distributed, with only a few vesicles observed along the sarcolemma. This implies a slow speed of muscle relaxation due to the limited rate at which calcium can be resequestered (Plesh, 1977). Glycogen is present at the periphery of the myoplasm (result not shown).

All these observations allow us to confirm that the obturacular muscles of *Riftia* are smooth muscles involved in slow but long lasting contractions with little energy expenditure. Smooth muscles have generally a single centrally located nucleus (Paniagua et al., 1996). However, nuclei in the fibres of *Riftia* are not centrally located, but on the side of the fibre. The presence in the myoplasma of expanded RER in the cell body is a feature only found in smooth muscles (Tandler, 1965, Plesh, 1977, Faccioni-Heuser et al., 1999). The large nucleolus in the nucleus, correlated with a large amount of ribosomes and the swollen cisterns of RER are signs of an active protein synthesis in the obturacular muscles. It is possible that this synthesis concerns the contractile proteins (actin and myosin), as suggested by Tandler (1965) for human smooth muscle.

The functional mechanism

Concerning the functional mechanism of the obturacular muscles, Jones (1985) wrote: "if the ring muscles relax and the blind-ended obturacular vessels straighten their linear undulations, by closure of their basal sphincters and by contraction of their vascular muscles, the branchial organ will elongate and extend the obturacular mass". Observations on live animals during the HOPE cruise (1999) do not support this interpretation. No longitudinal extension or contraction of the branchial organ, neither rapid nor slow, was perceptible, even when it was touched. The sinuous red obturacular vessel was clearly visible through the translucent cuticle, and its full elongation could never be observed *on videos* nor under normal pressure conditions (260 atmospheres) in the pressurized vessel IPOCAMP, used during the HOPE 99 cruise. In fact the quick withdrawal of *Riftia* into its tube, was caused by the fast contraction of the trunk body wall musculature, which instantly shortened the whole body length by about 15 to 20%. There was no accompanying change in the length of the obturaculum.

According to Jones (1985) there is no obvious antagonistic structure to the ring muscles, unless a hypothetical elasticity in the obturacular matrix of the obturaculum. We think that the thick basiepidermal framework formed by the collagenous cutis is a supportive tissue acting as an antagonist structure to the peripheral muscles.

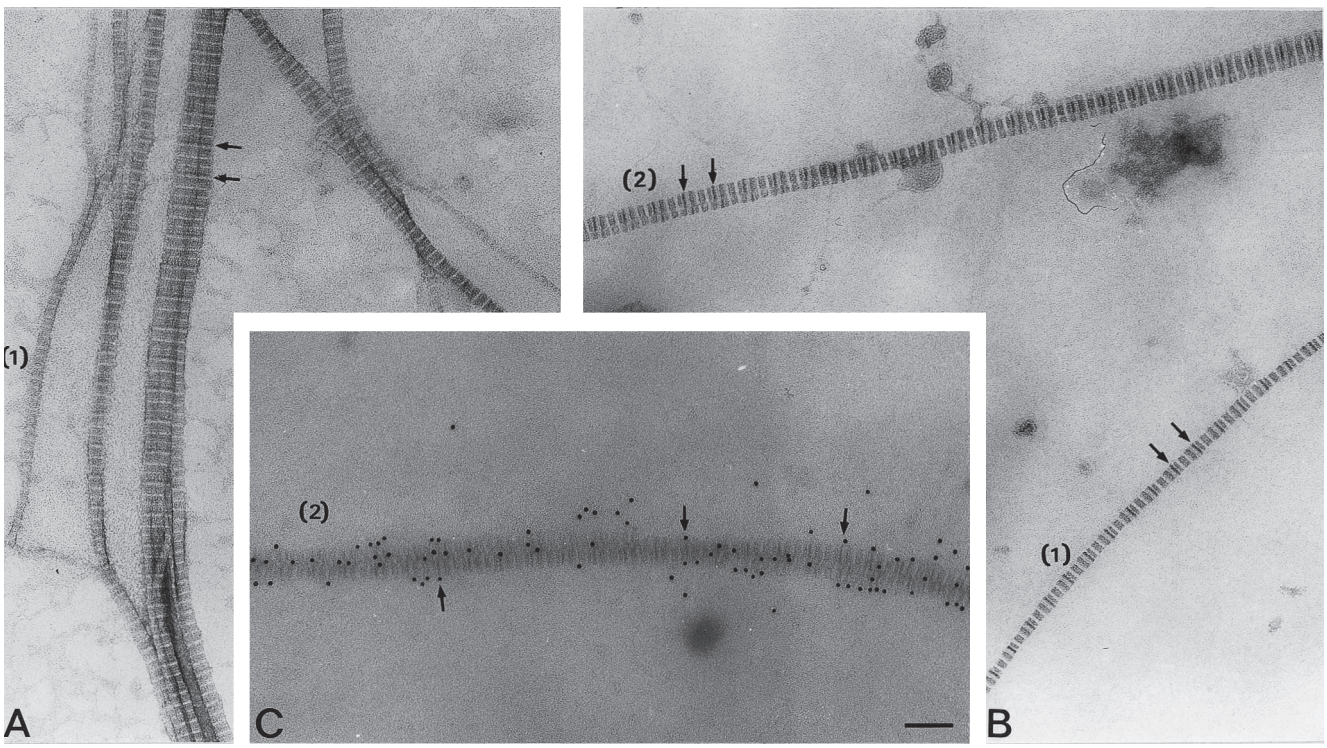

Figure 7. Interstitial collagen fibrils of *Riftia pachyptila* and *Tevnia jerichonana.*

A,B. Comparison of interstitial collagen fibrils extracted from the obturaculum of *Riftia* (A) and *Tevnia* (B). In both species each single fibre (1) measures ca. 30 nm in diameter; (2) indicates two joined fibrils. The striation of the fibrils presents the same regular pattern of transversal bands, with a periodicity of about 64 nm, measured between the arrow-doublets. Two successive black bands (arrows) are separated by three doublets of alternating grey and white bands.

C. Immunogold labelling of extracted fibrils of *Riftia* with an antibody against homologous interstitial collagen. The gold particles are generally localized at the level of the dark bands (arrows), but a regular labelling pattern is difficult to identify, since the two fibrils (2) are more or less twisted around each other.

Figure 7. Fibrilles de collagène interstitiel de *Riftia pachyptila* et de *Tevnia jerichonana.*

A, B. Comparaison des fibrilles de collagène interstitiel extraites de l'obturacle de *Riftia* (A) et de *Tevnia* (B). Dans les deux espèces chaque fibre (1) mesure ca. 30 nm de diamètre ; (2) indique deux fibrilles jointes. La striation des fibrilles présente le même patron régulier de bandes transversales, avec une periodicité d'environ 64 nm, mesurée entre les couples de flèches. Deux bandes noires (flèches) sont séparées par trois doublets de bandes alternées grises et blanches.

C. Immunomarquage à l'or des fibrilles de collagène extraites de *Riftia* avec un anticorps contre le collagène interstitiel homologue. Les particules d'or sont généralement localisées au niveau des bandes sombres (flèches), mais un motif de marquage régulier est difficile à repérer, car les fibrilles sont plus ou moins enroulées en spirale l'une autour de l'autre.

Scale bars (Echelles) : A, B = 150 nm ; C = 100 nm.

The smooth muscle of *Riftia* may be compared to the "paramyosin muscles" of the tube dwelling polychaete *Sabella penicillum*. This species belongs to the "group Sabellida", to which vestimentifera could be closely related, according to cladistic analyses based on morphological characters (Rouse & Fauchald, 1997). Smooth muscles, located at the base of the branchial crown of *Sabella*, are interposed between the longitudinal bodywall muscles and the branchial cartilage (Kryvi, 1975). They combine the ability to provide relatively free movement for the branchial crown, and also to maintain it firmly in position for sustained periods (Kryvi, 1975). Smooth muscles are known to be able to perform strong and prolonged contractions with little energy expenditure. Since we did not observe any length change of the branchial organ of *Riftia*, we suggest that the tension is produced without any effective shortening, a phenomenon known as isometric tone (Rosenbluth, 1972). Such a stretch-resistant state in which tension is maintained for long periods is known as "catch", and is widely described in molluscan smooth muscles (Castellani & Cohen, 1987). We hypothesize that in *Riftia*, the contracted state developed by the obturacular muscle fibres produces a catch tension allowing the terminal flaps of the organ to maintain the tube closed for long periods. In situ video recordings on *Riftia* during Hope 99 proved that periods of retraction within the tube can last for a long time,

and in other hydrothermal vestimentiferan species like *Ridgeia*, these periods have been measured to frequently exceed 30 min (Tunnicliffe et al., 1990).

The Obturacular matrix

The matrix cells

The matrix cells are in groups of two or more, and are enclosed within a basal lamina. Their extensive RER suggests that they are responsible for the synthesis of collagen fibrils and other matrix components such as proteoglycans, which are often packed around them. In both vertebrates and invertebrates, fibroblasts are generally isolated cells and are not surrounded by a basal lamina, unless they are considered as quiescent fibrocytes, such as the fibrocytes in fish scale pockets (Whitear et al., 1980). Nevertheless, in annelids, Hirudinea have fibroblasts surrounded by a basal lamina (Fernandez et al., 1992) with cytological features similar to those of the matrix cells of *Riftia,* but these fibroblasts are not grouped as in *Riftia.* The matrix cells also contain actin and myosin evidenced by immunofluorescence. This would confer cytoplasmic movements to the matrix cells.

Collagen

The collagen present in the obturacular matrix immunoreacts with the interstitial collagen that is different from the cuticular collagen, without cross-reactivity. As in the phylum Annelida, the cuticular collagen is composed of non-banded fibres organized in a plywood manner, while the interstitial collagen is made of cross-banded fibres (Murray & Tanzer, 1985). The antibodies raised against both collagen types obtained from *Riftia* cross-react also with the respective collagen types in *Tevnia***.** This indicates a close relationship between the collagens of these two vestimentiferan species, even though they belong to different families (respectively Riftiidae and Tevniidae). The interstitial collagen fibrils extracted from the obturacular matrix have a diameter of about 30 nm and a cross-banded periodicity of 64 nm. These fibres correspond to the fibre class described by Gaill et al. (1994) beneath the epidermis in the obturaculum of *Riftia*, with a periodicity of about 60 nm. A similar periodicity is found in Annelida, where isolated native fibres of banded collagen exhibit periodicities of 60-64 nm (Murray & Tanzer, 1985), or up to 67 nm in the oligochaete *Lumbricus* (Vitellaro-Zuccarello et al., 1985).

Proteoglycans

Other fibrils immunoreacting with the antibody against holothurian proteoglycans are revealed by immuno-fluorescence in the obturacular matrix. Gaill et al. (1994) have examined the presence of proteoglycans in extracted obturacular matrix of *Riftia*, and concluded that the obturaculum apparently contained a chondroitin sulfate proteoglycan. Immunoreactive proteoglycans are also present in the muscles, suggesting that the RER cisterns could also participate in the proteoglycan synthesis, or that the muscles bundles are linked together by proteoglycans at the level of the endomysium.

Conclusions

The obturacular matrix of *Riftia* (1) contains cells suspended in a relatively rigid matrix, (2) is rich in collagen fibrils, (3) contains acidic polysaccharides such as proteoglycans, carrying chondroitin sulfates. Thus, the obturacular matrix presents the three criteria that, according to Person & Mathews (1967), define cartilages in Invertebrates. Invertebrate cartilages never mineralize but allow some stiffness. Could the obturaculum of *Riftia* be considered as a primitive "cartilage"? Compared to the cartilages described in polychaetes, especially in the branchial crown of the Sabellidae (Person & Philpott, 1969 ; Kryvi, 1975), the obturacular matrix of *Riftia* has a quite different structure, the matrix cells being very scattered in the ECM.

In addition our study shows that the obturacular ring muscles of *Riftia,* are smooth muscles. They are antagonistic to the cutis, a compact supportive collagenic framework underlining the epidermis. We suggest that contraction of the smooth muscles produces a catch tension. It has been shown in other annelids that muscle cells may transmit their tension to the surrounding ECM via dense bodies (Rosenbluth, 1972; De Eguileor et al., 1999). In the case of *Riftia*, the tension could be transmitted, via hemi-desmosomes, to the surrounding obturacular matrix. These elements lead us to propose that the obturacular matrix might have properties similar to that of a catch connective tissue, first described in echinoderms (Motokawa 1984, Wilkie 1984). A catch connective tissue is able to change its mechanical properties (stiffness and elasticity) under muscular (Del Castillo & Smith, 1996) or nervous control (Wilkie, 1996). These functional properties would allow the obturaculum to be a plastic organ able to modulate its softness and stiffness.

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