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Organization of the Coelomic Lining and a Juxtaposed Nerve Plexus in the Suckered Tube Feet of *Parastichopus californicus* (Echinodermata: Holothuroida)

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ABSTRACT The coelomic lining of the water-vascular canal in a suckered tube foot from the sea cucumber, *Parastichopus californicus*, is a pseudostratified myoepithelium consisting of flagellated adluminal cells and myofilament-bearing retractor cells. The bodies of adluminal cells flank the water-vascular canal and send basal processes between the underlying retractor cells to confront the podial connective tissue. Retractor cells have a contractile apparatus of unregistered thick and thin myofilaments. The contractile apparatus is confined to the medullary sarcoplasm and oriented parallel to the primary axis of a tube foot. The bodies and processes of retractor cells intermingle with the basal processes of adluminal cells at the basal lamina of the coelomic lining. A ganglionated nerve plexus in the podial connective tissue approximates the basal lamina. Neuronal connectives link the ganglia to one another and to the nerve plexus in deep sectors of the podial epidermis. External laminae enveloping the ganglia and connectives in the podial connective tissue are continuous with the basal lamina of the epidermis. The adventitial nerve plexus, since it merges with the epidermal nerve plexus, is a component of the ectoneural division of the echinoderm nervous system. J. Morphol. 267:41–49, 2006.

KEY WORDS: adventitial nerve plexus; coelomic lining; epidermal nerve plexus; sea cucumber; tube foot

The tube feet of echinoderms are multifunctional appendages responsible for locomotion and adhesion, food gathering, sensation, and respiration (Nichols, 1967). Classical descriptions of podial morphology indicate a tetralaminar organization, consisting of an (outer) epidermis, a connective tissue layer, a layer of retractor muscle, and an (inner) coelomic epithelium (Coleman, 1969; Vickery and McClintock, 2000). Ultrastructural studies on the locomotor tube feet of modern (Wood and Cavey, 1981) and primitive (Cavey and Wood, 1991) sea stars have demonstrated that the coelomic epithelium and the retractor muscle should be unified into a single entity. Tube feet should, thus, be regarded as trilaminar appendages, consisting of an outer epidermis, a middle layer of connective tissue, and an inner coelomic lining.

Flagellated adluminal cells (alternatively called peritoneocytes) and myofilament-bearing retractor cells (alternatively called myocytes) populate the coelomic lining and directly or indirectly confront a basal lamina at the interface between coelomic lining and podial connective tissue. Since all adluminal and retractor cells anchor to the same basal lamina, the coelomic lining qualifies as a pseudostratified epithelium or, considering the prevalence of microfilaments in the adluminal cells and myofilaments in the retractor cells, a pseudostratified myoepithelium. This myoepithelial nature of the coelomic lining in the tube feet of sea stars has been confirmed in the tube feet of echinoids, ophiuroids, holothuroids, and crinoids (Rieger and Lombardi, 1987). It is also consistent with observations of other sectors of the water-vascular system and with observations of the coelomic linings outside the water-vascular system (Harrison and Chia, 1994).

The connective tissue layer along the shaft of a locomotor tube foot is generally regarded as aneural (Florey and Cahill, 1977). The closest neurons reside in the epidermal nerve plexus (basiepithelial nerve plexus). A suckered tube foot from the sea cucumber, *Parastichopus californicus*, does not conform to this customary pattern. In the connective tissue layer of a tube foot there exists a ganglionated nerve plexus linked to the epidermal nerve plexus. The goals of this report are to update the description of the coelomic lining in a suckered tube foot of *P. californicus* and to illustrate the adventitial nerve plexus, including its somata and neurites, and the connectives

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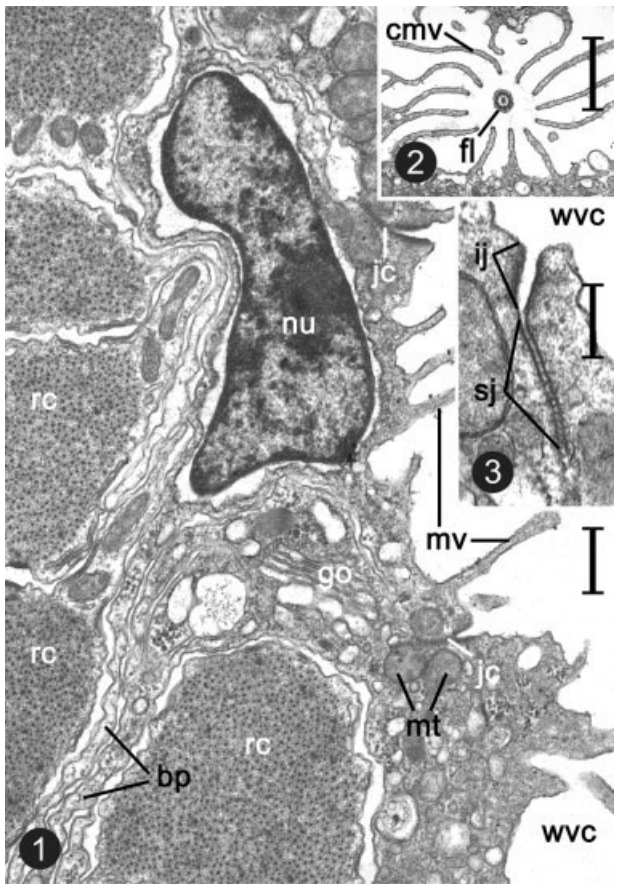

Fig. 1. *Parastichopus californicus*. Ultrathin transverse section of the coelomic lining (apical region). Adluminal cells (go, Golgi body; jc, junctional complex; mt, mitochondrion; mv, microvillus; nu, nucleus) send basal processes (bp) between the underlying retractor cells (rc). No basal lamina segregates the adluminal cells from the retractor cells, and the retractor cells lack connective tissue coverings. wvc, water-vascular canal. Scale bar = 0.5 μm.

Fig. 2. Ultrathin horizontal section of the apex of an adluminal cell (cmv, coronal microvillus; fl, flagellum). Scale bar = 1 μm.

Fig. 3. Ultrathin vertical section of a junctional complex (ij, intermediate junction; sj, septate junction) between adluminal cells. wvc, water-vascular canal. Scale bar = 0.25 μm.

that link the ganglia to each other and to the epidermal nerve plexus. Portions of this study have appeared in abstracts (Cavey and Jang, 1986; Cavey, 2001).

MATERIALS AND METHODS

Adult specimens of *Parastichopus californicus* were purchased from WestWind SeaLab Supplies (Victoria, BC, Canada) and maintained in aquaria supplied with recirculating artificial seawater at 10°C. The five ambulacra of this sea cucumber are represented by two fields of papillate tube feet (the bivium) and three fields of suckered tube feet (the trivium). Intact animals were gently placed in a chilled solution of equal volumes of 0.37 M magnesium chloride and artificial seawater (Strathmann, 1987). Animals were kept in this mild anesthetic solution for 10–15 min, allowing moderate protraction of the tube feet in the trivium. Ligatures were placed on tube feet near their bases and the tube feet were amputated with razor blades.

Tube feet were immersed in a primary fixative containing 2.5% glutaraldehyde, 0.2 M Millonig's phosphate buffer at pH 7.4, and 0.14 M sodium chloride (Cloney and Florey, 1968). After 5 min of immersion the tube feet were cut into short cylindrical segments with razor blades and the segments were transferred to a container of fresh fixative for 60–90 min at room temperature. Without rinsing, the primary fixative was replaced with a secondary fixative consisting of 2% osmium tetroxide in 1.25% sodium bicarbonate buffer, pH 7.2 (Wood and Luft, 1965). The segments were osmicated for 60–90 min at 0–4°C and then rinsed with demineralized water for 10–15 sec at room temperature. Specimens were dehydrated in graded solutions of ethanol, passed through propylene oxide, and infiltrated and embedded in a 7:3 mixture of Epon A:Epon B (Luft, 1961). Specimens were embedded at 60°C for 18 h in a laboratory oven.

Light microscopic sections (1 μm in thickness) were cut with glass knives on a Sorvall MT-2B ultramicrotome. The sections were affixed to glass slides, stained with an alkaline solution of azure II and methylene blue (Richardson et al., 1960), and mounted in high viscosity immersion oil. Sections were viewed and photographed with a Nikon Optiphot compound microscope equipped with planachromatic objective lenses and a Nikon HFX-IIA photomicrographic attachment. Photomicrographs were made on Kodak High Contrast Copy or Technical Pan 2415 film. The photomicroscope was calibrated with a stage micrometer (100 lines/mm).

Specimens were further polymerized at 60°C for 6 h before ultramicrotomy. Electron microscopic sections (70 nm in thickness) were cut with diamond knives on a Sorvall MT-6000 ultramicrotome, collected on unsupported copper grids, and stained serially with aqueous solutions of uranyl acetate (saturated) and lead citrate (Reynolds, 1963). Sections were viewed and photographed with a JEOL JEM-100S transmission electron microscope operated at 60 kV or a Hitachi H-7000 transmission electron microscope operated at 75 kV. Electron micrographs were made on Kodak 4489 electron microscope film. Transmission electron microscopes were calibrated with a carbon replica of a diffraction grating (2,158 lines/mm).

Negatives produced with the compound microscope and the transmission electron microscope were subsequently digitized with a Polaroid SprintScan 45i film scanner set for a resolution of 600 dpi. Scanned images were stored as TIFF files without compression and adjusted and printed with Photoshop CS 8.0 for Windows software (Adobe Systems, San Jose, CA). Measurements on the digital images were made with SigmaScan Pro 5.0 for Windows software (Systat, Point Richmond, CA). Each diameter, length, or width is expressed as a mean ± standard deviation and derives from a sample of 50 measurements.

RESULTS

Organization of a Suckered Tube Foot and Its Coelomic Lining

Collagenous connective tissue constitutes the middle layer of a suckered tube foot from the trivium of *Parastichopus californicus*. The connective tissue layer is adjoined externally by a covering epithelium (the epidermis) and internally by a lining epithelium (the coelomic lining of the water-vascular canal). A nerve plexus resides in deep sectors of the epidermis.

Two principal cell types populate the coelomic lining. The bodies of the *adluminal cells* line the water-vascular canal (Fig. 1) and overlie multiple layers of longitudinally oriented *retractor cells* (Fig. 4). Basal processes emerge from the bodies of adluminal cells and collect into small bundles that infiltrate be-

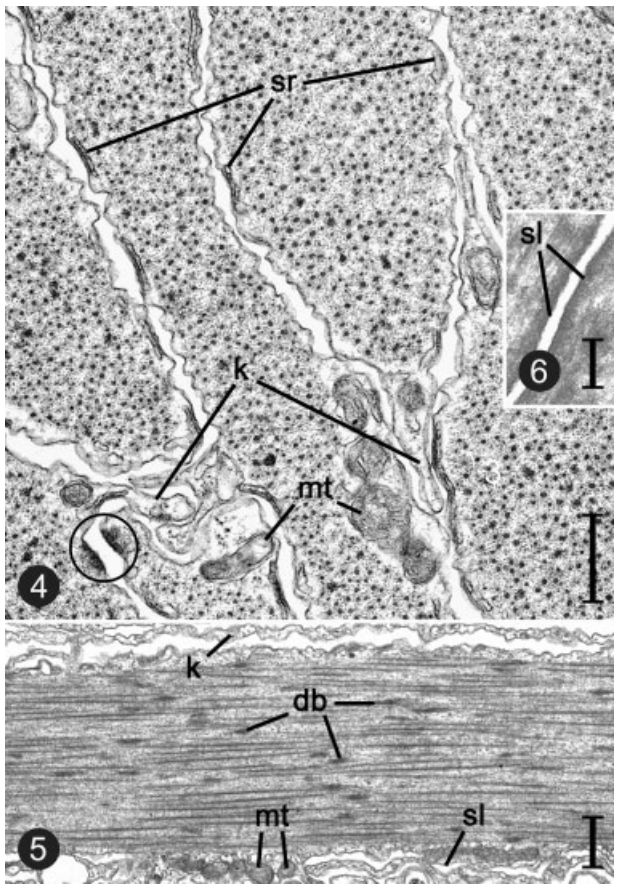

Fig. 4. *Parastichopus californicus*. Ultrathin transverse section of the coelomic lining (intermediate region). Retractor cells (k, keel; mt, mitochondrion; sr, sarcoplasmic reticulum) orient parallel to the primary axis of a tube foot. Ultrastructural technique has caused the sarcolemmata in a symmetric intermediate junction (circle) to retract. Scale bar = 0.5 μm.

Fig. 5. Ultrathin longitudinal section of the coelomic lining (intermediate region). The contractile apparatus of a retractor cell (k, keel; mt, mitochondrion; sl, sarcolemma) is an unregistered array of thick and thin myofilaments punctuated by dense bodies (db). Scale bar = 0.5 μm.

Fig. 6. Ultrathin section of a symmetric intermediate junction between retractor cells. Thin myofilaments associate with filamentous mats beneath the junctional sarcolemmata (sl). Scale bar = 0.25 μm.

tween the underlying retractor cells (Fig. 1). The terminus of a basal process is a tapered or bulbous pedicel on the basal lamina that isolates the coelomic lining from the podial connective tissue (Figs. 7, 8). There is no basal lamina segregating adluminal cells from retractor cells, and the retractor cells are devoid of connective tissue coverings (Fig. 1). Laminar branching of the retractor cells is prevalent. Bodies or branches of the retractor cells also anchor to the basal lamina of the coelomic lining, intermingling with the pedicels of adluminal cells (Figs. 7, 8). The coelomic lining thus qualifies as a pseudostratified epithelium. Due to the presence of contractile filaments (microfilaments in the adluminal cells and myofilaments in the retractor cells), the coelomic lining could also be described as a pseudostratified myoepithelium. Shallow indentations appear sporadically on the deep surface of the myoepithelium and they carry along adjoining sectors of the basal lamina (Fig. 8).

Cytology of the Cells in the Coelomic Lining

The nucleus of an adluminal cell resides in the cell body (Fig. 1). The nucleus is typically oblong and bounded by an envelope with relatively few pores. Condensed chromatin rests on the inner (nucleoplasmic) surface of the inner nuclear membrane, and polyribosomes adhere to the outer (cytoplasmic) surface of the outer nuclear membrane. The outer nuclear membrane is directly continuous with cisternae of the granular (rough) endoplasmic reticulum. Free ribosomes, glycogen granules and rosettes, short cisternae of agranular (smooth) endoplasmic reticulum, and small, spherical mitochondria appear in the cytoplasm around the nucleus. Several Golgi bodies also occupy the perinuclear cytoplasm. Signs of heterophagy and autophagy are abundant in a cell body, so Golgi activity may involve the production of primary lysosomes.

A pair of centrioles is found in the apical cytoplasm of each adluminal cell and one member of the pair serves as the basal body of a flagellum. The flagellum emerges from an apical depression in the cell body and extends into the water-vascular canal. Tall, ridge-like microvilli radiate outward from the site where a flagellum emerges (Fig. 2). These *coronal microvilli* contrast sharply with the short, pleomorphic microvilli found elsewhere on the cell apex (Fig. 1). The adluminal cell in this coelomic lining conforms to the monociliated cell type of Rieger and Lombardi (1987).

A simple junctional complex is apparent on the sides of the adluminal cells (Figs. 1, 3). Two intercellular junctions, an (apical) intermediate and a (subapical) septate junction, constitute the complex. Both junctions are zonular in configuration. The intermediate junction appears to be the more labile of the two, with apposed membranes tending to pull apart. The smallest measurement for width of the intercellular cleft in an intermediate junction is 49.9 nm. A fine filamentous meshwork adheres to the cytoplasmic surface of each junctional membrane, and cytoplasmic microfilaments (5.9 ± 0.9 nm in diameter) enter this meshwork. Bundles of microfilaments appear in the perinuclear cytoplasm, and also extend into the basolateral folds of a cell and into its basal process. The septate junction, unlike the intermediate junction, has an intercellular cleft of relatively uniform width (14.7 ± 1.6 nm). Spacing of the intercellular septa is 32.2 ± 3.5 nm, but the distance between septa can be quite variable (ranging from 25.6 to 37.8 nm).

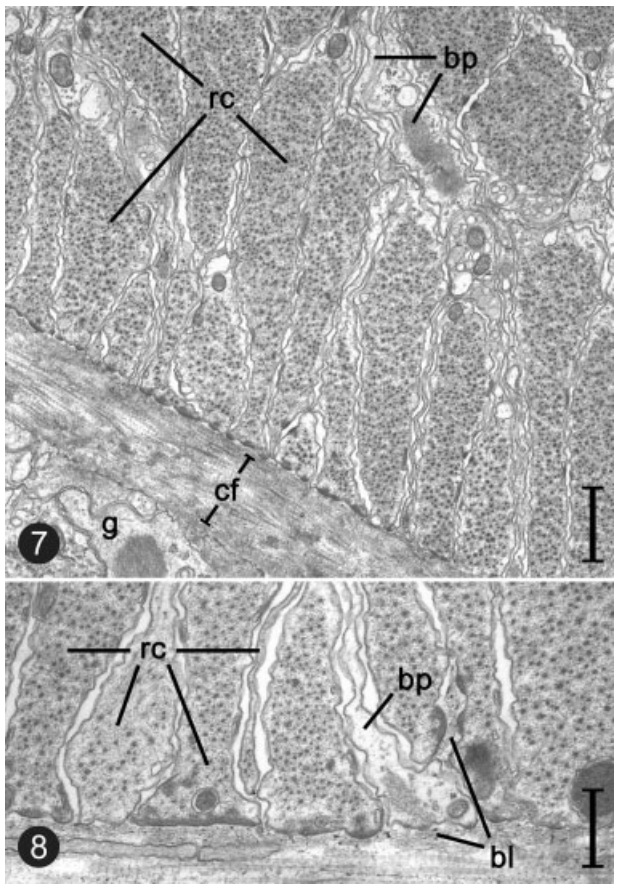

Fig. 7. *Parastichopus californicus*. Ultrathin transverse section of the coelomic lining (basal region) showing the interface of myoepithelium (bp, basal process of adluminal cell; rc, retractor cell) and podial connective tissue (cf, collagen fibrils). A ganglion (g) of the adventitial plexus appears in the lower left corner of the field. Scale bar = 1 μm.

Fig. 8. Ultrathin transverse section of the basal processes of adluminal cells (bp) and the processes and bodies of retractor cells (rc) aligned on the basal lamina (bl) of the coelomic lining. An incursion of the basal lamina into a shallow indentation of the myoepithelium is apparent. Scale bar = 0.5 μm.

Basolateral surfaces of the adluminal cells are evaginated and interdigitated, but there are no apparent junctions between the plasmalemmata in this region. It is curious that communicating (gap) junctions appear to be absent entirely. Basal processes of the adluminal cells can be distinguished from the processes of retractor cells that may contain just thin myofilaments, because the thin myofilaments have a slightly greater diameter than the microfilaments. There are also structural differences between the pedicels of adluminal cells and the terminals of retractor cells (Fig. 8). In the former, focal filamentous mats appear in the cytoplasm beneath the plasmalemma and microfilaments project into these mats. The subsarcolemmal mats in cell bodies/terminals of the retractor cells can be thick and expansive, resembling the filamentous mats in the intermediate junctions between retractor cells (see below) and, like them, associated with thin myofilaments of the contractile apparatus. These associations are the *anchoring junctions* of Wood and Cavey (1981) and the "type V" junctions of Dolder (1972). A narrow electron-lucent zone appears between the cells and cell terminals and the basal lamina.

The retractor cells consist largely of a contractile apparatus that is a longitudinal array of unregistered thick and thin myofilaments (Figs. 4, 5). The thick myofilaments are fusiform in shape, averaging 36.5 ± 2.3 nm in diameter at their midpoints and 12.5 ± 2.4 nm in diameter near their tapered ends. Axial periodicity is sometimes evident along the thick myofilaments; the major period is 46.6 ± 2.2 nm. The thin myofilaments are 6.6 ± 0.9 nm in diameter; accurate measurements of length cannot be obtained because the filaments frequently deviate from the plane of an electron microscopic section. Thin myofilaments affiliate with filamentous patches both within the contractile apparatus and applied to the undersurface of the sarcolemma. The patches within the contractile apparatus may equate with the dense bodies in vertebrate smooth muscle cells and fulfill the role of Z-lines in striated muscle cells. The patches applied to the sarcolemma may equate with the filamentous mats of intermediate junctions, and thus represent sites where tension is transferred from the contractile apparatus to the cell membrane (Fig. 6). Many of the subsarcolemmal patches are solitary (*asymmetric intermediate junctions* of Wood and Cavey [1981] or "type II" junctions of Dolder [1972]), while others show alignment between cells and give the impression of an organized intercellular contact (*symmetric intermediate junctions* of Wood and Cavey [1981] or "type I" junctions of Dolder [1972]). Most symmetric and asymmetric intermediate junctions occur on the lateral surfaces of the retractor cells and, thus, orient parallel to the direction of generated tension. The width of the cleft between aligned patches is quite labile, so reliable measurements are difficult to obtain. The addition of tannic acid to the primary fixative has been recommended as a way to stabilize the width of the cleft (Wood and Cavey, 1981). There is no morphological evidence of communicating junctions between the retractor cells. There are no junctions whatsoever between the retractor cells and the overlying adluminal cells or their basal processes, accounting for the relative ease in manually separating these two elements of the coelomic lining (Nichols, 1959).

A simple network of agranular cisternae is sandwiched between the contractile apparatus and the sarcolemma (Fig. 4). Flattened tubular cisternae in this sarcoplasmic reticulum are predominantly oriented, like the myofilaments, in the longitudinal (primary) axis of a cell. Lateral/oblique connections are apparent between the longitudinal cisternal sectors. Simple mitochondria and glycogen granules

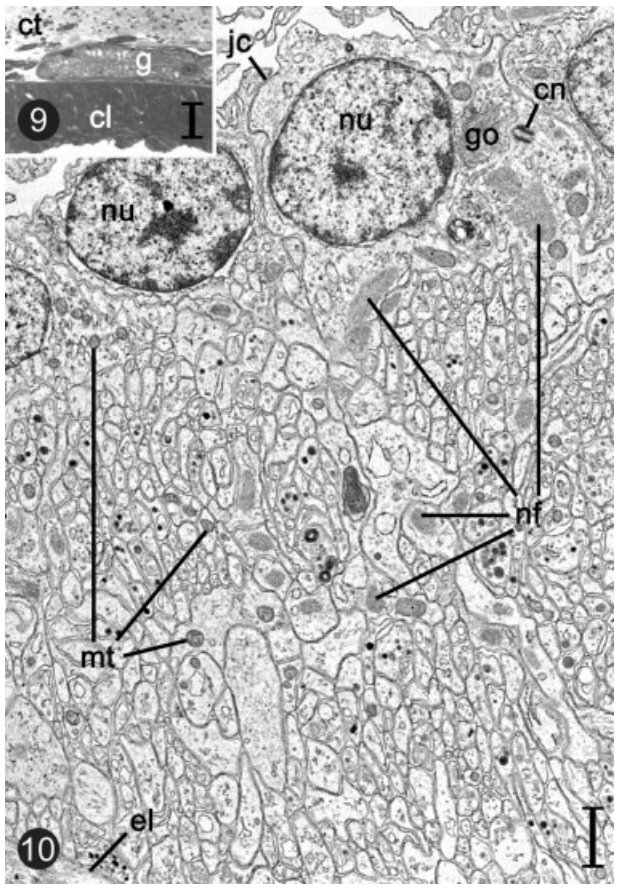

Fig. 9. *Parastichopus californicus*. Thin transverse section of the coelomic lining (cl) and a ganglion (g) in the adjacent nerve plexus. Darkly staining strands in the podial connective tissue (ct) are connectives that link ganglia to one another and to the epidermal nerve plexus. Scale bar = 10 μm.

Fig. 10. Ultrathin transverse section of a ganglion (el, external lamina) in the adventitial nerve plexus. Neuronal somata (cn, centriole; go, Golgi body; mt, mitochondrion; nf, neurofibril; nu, nucleus), restricted to the periphery of a ganglion, are sometimes joined by rudimentary junctional complexes (jc) that are similar in composition to those between adluminal cells (see Fig. 3). Numerous neurites emerge from the somata and branch prolifically in the core of the ganglion (see also Figs. 11, 12). Scale bar = 1 μm.

share the cortical sarcoplasm with the cisternae. Mitochondria are often wider than the sarcoplasmic zone between the contractile apparatus and the sarcolemma, resulting in deflection of the cell membrane. Occasionally, the outer cisternal membranes become rigidly parallel to overlying sectors of the sarcolemma. The sarcoplasmic cleft between membranes measures 11.1 ± 1.3 nm in width. These appositions represent structural (and possibly functional) contacts between the two membrane systems. Such *peripheral couplings* likely occur at sites of cisternal confluence, and an electron-dense plaque situates in the sarcoplasmic cleft, lying equidistant from the apposed membranes.

The surfaces of neighboring retractor cells tend to evaginate and interdigitate with each other and with the bodies and basal processes of adluminal cells (Figs. 4, 5); these laminar evaginations, referred to as *keels* in the tube feet of sea stars (Wood and Cavey, 1981; Cavey and Wood, 1991), correlate with the degree of podial extension, being sparse in cells of protracted tube feet and conspicuous in cells of retracted tube feet (see also Ubukata and Takahashi, 2001).

An ovoid nucleus resides in the cortical sarcoplasm near the midpoint of a retractor cell; it situates just outside the limits of the contractile apparatus, typically at the base of a keel. A pair of centrioles flanks one pole of the nucleus, and some cells even exhibit a short cilium (see also Rieger and Lombardi, 1987). Mitochondria in the vicinity of the nucleus are relatively small and spherical-to-ovoid in shape. Sarcoplasmic reticulum in the vicinity of the nucleus includes both agranular and granular forms. Multiple Golgi bodies flank the nucleus of a retractor cell, and the presence of vesicles and vacuoles with electron-dense contents is suggestive of endocytosis. Free ribosomes and multigranular glycogen rosettes are observed in the perinuclear sarcoplasm, as well as in the sarcoplasm of the keels.

Ganglionated Plexus in the Podial Connective Tissue

A major difference between the suckered tube feet of *Parastichopus californicus* and the locomotor tube feet of a sea star or a sea urchin is the existence of interconnected ganglia in the connective tissue next to the coelomic lining. These ganglia are readily detected in light microscopic sections (Fig. 9), lying as close as 1.0 μm to the cells and cell terminals at the basal lamina of the coelomic lining (Fig. 7). A narrow cleft, occupied by cross-striated collagen fibrils, separates the basal lamina of the coelomic lining from the external lamina that envelops the ganglia and their connectives (Fig. 10). Most ganglia are oblong in shape and gently curved, conforming to the contour of the undersurface of the coelomic lining (Fig. 9). Both ganglionic size and interganglionic spacing are subject to wide variation.

Each ganglion consists of a few neuronal somata and an impressive number of neurites (Fig. 10), a description that holds as well for ganglia in other echinoderms (Pentreath and Cobb, 1972). Neurite branching is conspicuous and likely explains the massive numbers observed in ultrathin sections (Fig. 12). Neuronal somata tend to localize at the edges of a ganglion (Fig. 10). There are no discernible neuroglial cells within the limits of a ganglion or within the interganglionic connectives. There are no connective tissue investments associated with the neuronal somata or the neurites.

The soma of a ganglionic neuron contains a large spherical or slightly ovoid nucleus with one or two prominent nucleoli and a modest amount of condensed chromatin applied to the inner surface of the

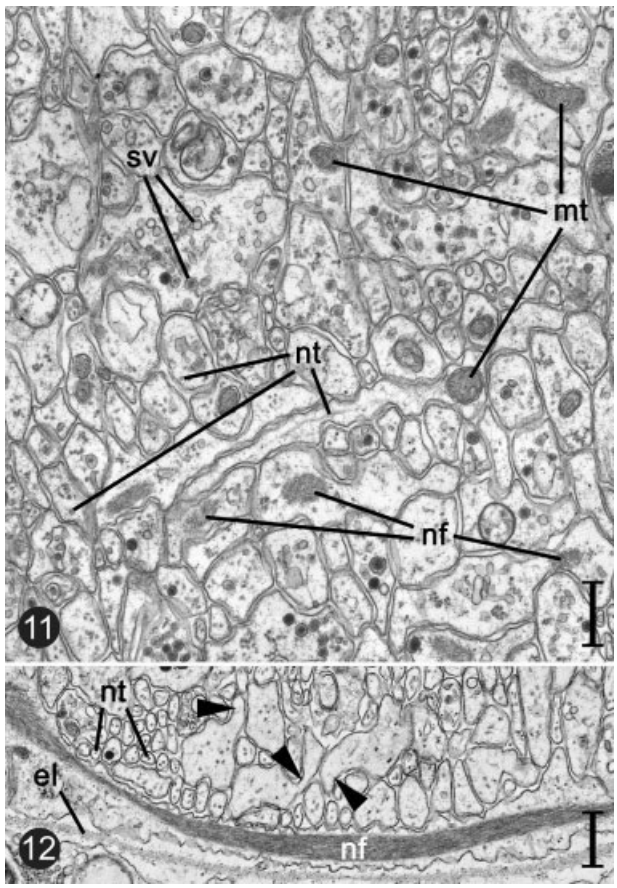

Fig. 11. *Parastichopus californicus*. Ultrathin transverse section of the core of a ganglion from the adventitial nerve plexus. Neurites (gly, glycogen rosettes; mt, mitochondrion; nf, neurofibril; nt, neurotubule) contain both clear and dense-cored synaptic vesicles (sv). Scale bar = 0.5 μm.

Fig. 12. Ultrathin section of neurites in a ganglion from the adventitial nerve plexus. A prominent neurite bearing a neurofibril (nf) leaves a neuronal soma and projects over the surface of the ganglion (el, external lamina; nt, neurotubule). Observe the blunt branches (arrowheads) of the larger neurites. Scale bar = 0.5 μm.

inner membrane of the envelope (Fig. 10). Perinuclear cytoplasm is the site of small, spherical mitochondria, one or more Golgi bodies, a pair of centrioles, and neurofibrils. The neurofilaments (intermediate filaments) of a neurofibril have a diameter of 10.5 ± 2.1 nm. Evaginations of the outer membrane of a nuclear envelope are continuous with granular and agranular cisternae of the endoplasmic reticulum. Both the Golgi cisternae and the nearby vesicles contain electron-dense material, possibly indicating that the organelle is synthetically active. A small number of free ribosomes, glycogen granules and rosettes, and neurotubules occur in the perikaryon.

Neurofibrils from the neuronal soma project into the neurites (Figs. 10–12). Mitochondria with poorly developed cristae are also evident in the cytoplasm of the neurites, as are sparse neurotubules (microtubules) with a diameter of 26.8 ± 2.4 nm. Electron-dense granules of various sizes appear in the neurites (Fig. 10). These granules are membrane-bounded, and some are comparable in size to those in coelomocytes that migrate throughout the inner and middle layers of a tube foot. The electron-dense granules are distinct from the clear and dense-cored synaptic vesicles in the neurites (Fig. 11).

As indicated above, fascicles of neurites interconnect the ganglia that ring the coelomic lining, and similar, but smaller fascicles project across the podial connective tissue toward the epidermis. The somata of putative neurons often appear in the

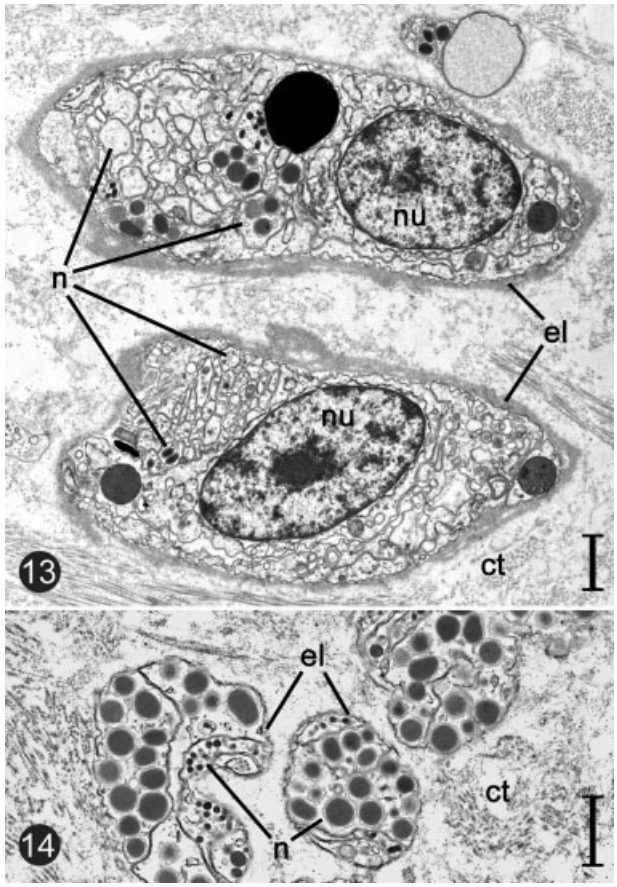

Fig. 13. *Parastichopus californicus*. Ultrathin transverse section of the connectives between ganglia of the adventitial nerve plexus. Neuronal somata (nu, nucleus) can be found in the connectives, as well as neurites (n) similar to those in the ganglionic cores. Membrane-bounded, electron-dense granules of various sizes occupy some neurites. An external lamina (el) surrounds each connective, segregating it from the podial connective tissue (ct). Scale bar = 1 μm.

Fig. 14. Ultrathin transverse section of connectives spanning from ganglia in the adventitial nerve plexus to the epidermal nerve plexus. Such connectives are smaller in diameter than those interlinking ganglia of the adventitial plexus, and they seldom incorporate neuronal somata. Membrane-bounded, electron-dense granules are commonly observed in the neurites (n). An external lamina (el) surrounds each connective, isolating it from the podial connective tissue (ct). Scale bar = 1 μm.

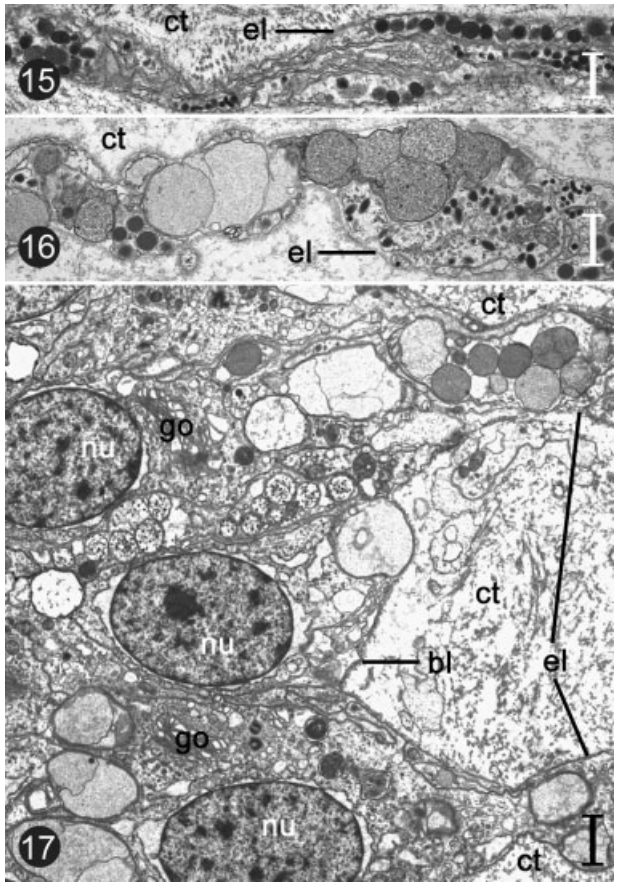

Fig. 15. *Parastichopus californicus*. Ultrathin longitudinal section of a fascicle of neurites (el, external lamina) bearing membrane-bounded, electron-dense granules. ct, podial connective tissue. Scale bar = 0.5 μm.

Fig. 16. Ultrathin longitudinal section of neurites carrying membrane-bounded, electron-dense granules as well as vacuoles with flocculent contents. Such vacuoles are increasingly prominent in connectives nearer the epidermal nerve plexus. ct, podial connective tissue; el, external lamina. Scale bar = 1 μm.

Fig. 17. Ultrathin vertical section of the junction between the epidermal nerve plexus (left side of the micrograph) and two connectives (right side of the micrograph) linking it to the adventitial nerve plexus. External laminae (el) of the connectives merge with the basal lamina (bl) beneath the epidermal cells (go, Golgi body; nu, nucleus). ct, podial connective tissue. Scale bar = 1 μm.

larger connectives that interlink ganglia in the adventitial nerve plexus (Fig. 13), but they are seldom observed in the smaller connectives spanning between the adventitial and epidermal plexuses (Figs. 14–16). Ganglia and their connectives are enclosed by a continuous external lamina (basal lamina-like material that completely or almost completely encases a cell or a group of cells and segregates it from the connective tissue). At the junction with epidermis, the external laminae of the connectives merge with the basal lamina of the epidermis. Neuronal somata within the epidermal plexus tend to be slightly more electron dense than those in the connectives or in the ganglia of the adventitial plexus. Large electron-dense granules are common in the somata and neurites of the epidermal plexus, as are large, membrane-bounded vacuoles with flocculent contents in various stages of condensation (Figs. 14, 15). The vacuoles with flocculent contents are especially prevalent in those somata and neurites close to the epidermis–connective tissue junction (Figs. 16, 17), and they bear a striking resemblance to vacuoles in the spherule cells of the connective tissue (Smiley, 1994). Similar vacuoles are also found within the connectives linking the two nerve plexuses, but they have rarely been encountered in intraganglionic somata or in neurites near the coelomic lining or in the interganglionic connectives.

DISCUSSION

Explanation of excitation–contraction coupling in the coelomic lining of a locomotor tube foot is complicated by a number of morphological peculiarities. There are, for example, no nerve endings among adluminal and retractor cells of the coelomic lining or in the outlying connective tissue along the podial shaft in sea stars (Wood and Cavey, 1981; Cavey and Wood, 1991; Chia and Koss, 1994) and sea urchins (Florey and Cahill, 1977; Cavey and Märkel, 1994). In sea stars and sea urchins, the closest neurons to the coelomic lining belong to the epidermal nerve plexus; in a sea urchin, diffusion of neurotransmitters from neuronal somata in the epidermis, possibly with the alteration of connective tissue consistency, has been suggested as a possible mechanism of excitation–contraction coupling (Florey and Cahill, 1977, 1980). Based on diffusion distances and the number of layers of retractor cells to be innervated, especially in the larger tube feet of sea stars, there are potential shortcomings in such an explanation. Furthermore, motor neurons of the hyponeural division, not those of the ectoneural division, are usually implicated in the innervation of echinoderm skeletal muscles (Cobb, 1987).

The search for direct nervous innervation of the retractor cells by elements of the hyponeural division has been conducted in both sea urchins (Märkel and Röser, 1991, 1992) and sea stars (Cavey, 1998b). In both groups the proximal retractor cells are closely approached by branches of the radial nerves, but no synapses are formed. The extreme difficulty in finding morphological evidence of direct nervous innervation must be attributed to relatively inaccessible sites where neurons approximate the ends of retractor cells. These sites, the test of the sea urchin and the ambulacrum of the sea star, are heavily impregnated with calcium salts and pose a number of preparative challenges when trying to eliminate the calcium without damaging the soft tissues. Application of EDTA to echinoid tissues (Märkel and Röser, 1983) and ascorbic acid to asteroid tissues (Dietrich and Fontaine, 1975; Cavey and Yeung, 1991; Cavey, 1998a) have begun to reveal areas

heretofore inaccessible to histology and ultrastructure.

Conceding the likelihood of direct nervous innervation of the proximal retractor cells in the coelomic lining, another problem in explaining excitation–contraction coupling is an absence of communicating junctions between retractor cells of the coelomic lining (Wood and Cavey, 1981; Cavey and Wood, 1981, 1991). If dealing with small numbers of retractor cells that span the entire length of a tube foot, the need for communicating junctions is moot. If dealing with large numbers of retractor cells and/or retractor cells that only span part of the length of a tube foot, the absence of communicating junctions is problematic. How might the innervated retractor cells elicit the excitation of neighboring cells that lack direct innervation? One suggestion has been the participation of the symmetric intermediate junctions and the phenomenon of transcellular activation, wherein sarcolemmal stretch (physical deformation) is the mechanism for recruiting retractor cells that are not innervated directly. The idea of a mechanical spread of excitation was, in fact, proposed by Bargmann and Behrens (1963) in their early ultrastructural study of sea-star ampullae.

One hypothesis on excitation–contraction coupling in the sea-star tube foot proposes a direct innervation of at least some of the retractor cells and the diffusion of neurotransmitters from the epidermal nerve plexus to control delicate or subtle movements of the appendage by localized excitation of cells and by adjustments to the consistency of podial connective tissue (Cavey, 1998b). The notion that ectoneural cells participate in the motor control of tube feet gains support from two observations in the present study, namely, the linkage of epidermal and adventitial nerve nets and the proximity of the adventitial net to the coelomic lining. Why, in the sea-cucumber tube foot, is there a need for greater intimacy between ectoneural neurites and retractor cells? Proximity between neurons and retractor cells should facilitate better control of tube foot movements by improved targeting of neurotransmitter. Unfortunately, the larger tube feet of the sea stars and the longer tube feet of the sea urchins have repertoires of motor behavior that equal or exceed those of the tube feet of sea cucumbers. Why does such proximity between the ectoneural division of the nervous system and the coelomic lining not exist in the tube feet of either asteroids or echinoids?

Morphological observations, to be reported shortly, indicate that the locomotor tube feet of the brittle star share some resemblances to the trivial tube feet of the sea cucumber. In *Ophiopholis aculeata*, a nerve plexus has also been found close to the coelomic lining (Cavey and Laudel, 1990). This network lacks the prominent ganglia described in the adventitial plexus of *Parastichopus californicus*, but it still represents an extension of the epidermal plexus that apposes the coelomic lining. Similar findings are mentioned by Byrne (1994) in another ophiuroid, *Ophionereis schayeri*.

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